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save the blue heart of europe

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Neretva Science Week 2022 – shedding light on the biodiversity of the upper Neretva River valley, Bosnia and Herzegovina

Gabriel SINGER[1], Ulrich EICHELMANN[2], Vera KNOOK[2], Steven WEISS[3], Vladimir TOPIĆ[4], Jelena IVANIĆ[4], Špela BORKO[2], Maja ZAGMAJSTER[5]

[1]Innsbruck University, Department of Ecology, Technikerstrasse 25, A-6020 Innsbruck, Austria
[2]Riverwatch, Society for the Protection of Rivers, Neustiftgasse 36, A-1070 Vienna, Austria
[3]University of Graz, Institute of Biology, Universitätsplatz 2, A-8010 Graz, Austria
[4]Center for Environment, Miše Stupara 5, 78 000 Banja Luka, Bosnia and Herzegovina
[5]University of Ljubljana, Biotechnical Faculty, Department of Biology, SubBioLab, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Abstract. Many rivers in the Balkans are at risk due to numerous planned hydropower plants. The *Save the Blue Heart of Europe* campaign and the *Scientists for Balkan Rivers* network are important initiatives trying to promote the protection of the region's intact rivers. In summer 2022, a *Science Week* was organised in the upper Neretva River valley. From 26.6. to 5.7.2022, a diverse team of in total nearly 60 scientists and students from seven countries were involved in fieldwork on about a dozen common field sites distributed along the upper Neretva River. The 13 contributions published in this special issue draw an impressive picture of high biodiversity in this understudied area. Altogether, more than 1000 species of animals and plants were documented, numerous of them protected, underscoring the extremely high conservation value of the various ecosystems in the upper Neretva river valley.

Key words: Save the Blue Heart of Europe, Scientists for Balkan Rivers, public outreach, scientific storytelling, hydropower, environmental protection, biodiversity conservation

Izvleček. Neretva Science Week 2022 – osvetlitev biotske raznovrstnosti doline gornje Neretve, Bosna in Hercegovina – Mnogo rek na Balkanu je ogroženih zaradi številnih načrtovanih hidroelektrarn. Kampanija *Rešimo modro srce Evrope* in pobuda *Znanstveniki za balkanske reke* sta pomembni iniciativi, ki skušata spodbujati zaščito neokrnjenih rek v regiji. Poleti 2022 je bil v zgornji dolini reke Neretve organiziran »Teden znanosti«. Od 26.6. do 5.7. 2022 je raznolika ekipa skoraj 60 znanstvenikov in študentov iz sedmih držav opravljala terensko delo na okoli ducat skupnih terenskih lokacijah, razporejenih vzdolž zgornjega toka reke Neretve. Trinajst prispevkov, objavljenih v tej posebni številki, priča o izjemno visoki biotski pestrosti v tem premalo raziskanem območju. Skupaj je bilo dokumentiranih več kot 1000 vrst živali in rastlin, številne med njimi so zaščitene, kar dokazuje izjemno varstveno vrednost ekosistemov v dolini zgornje Neretve.

Ključne besede: Modro srce Evrope, Znanstveniki za balkanske reke, ozaveščanje javnosti, znanstveno pripovedovanje, hidroenergija, varstvo okolja, varstvo biodiverzitete

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Apstrakt. Neretva Science Week 2022 – rasvjetljavanje biodiverziteta doline gornje rijeke Neretve, Bosna i Hercegovina – Mnoge rijeke na Balkanu su u opasnosti zbog brojnih planiranih hidroelektrana. Kampanja »Plavo srce Evrope« i Naučnici za rijeke Balkana su važne inicijative koje pokušavaju da promovišu zaštitu netaknutih rijeka u regionu. U ljeto 2022. godine u dolini gornjeg toka rijeke Neretve je organizovana »Sedmica nauke«. Od 26.6. do 5.7.2022 raznovrsan tim od ukupno 60 naučnika i studenata iz 7 zemalja je bio uključen u terenski rad na desetak zajedničkih terenskih lokacija raspoređenih duž gornjeg toka rijeke Neretve. 13 priloga objavljenih u ovom specijalnom izdanju daju impresivnu sliku visokog biodiverziteta u ovom nedovoljno proučavanom ekosistemu. Zajedno je dokumentovano više od 1000 vrsta životinja i biljaka, od kojih su mnoge zaštićene, što ukazuje na izuzetno visoku vrijednost očuvanja ovog ekosistema u cijelosti.

Ključne riječi: Plavo srce Evrope, Naučnici za rijeke Balkana, informisanje javnosti, naučno pripovijedanje, hidroenergija, zaštita životne sredine, očuvanje biodiverziteta

The *Save the Blue Heart of Europe* campaign, the *Scientists for Balkan Rivers* network, and the mission and scope of *Science Week* events on threatened Balkan rivers

On the Balkan Peninsula, one can still find many rivers flowing through near-pristine landscapes with alluvial forests, waterfalls and remote canyons. These ecosystems have survived the last decades of human development, which has plagued the rivers across most of the European continent and beyond.

However, Balkan rivers are at risk. More than 3,500 hydropower plants are planned in the Balkans (Balkanrivers 2023). If these plans are realised, hardly any river or stream will survive, resulting in severe consequences for all river-associated biodiversity, which – due to historical reasons acting across evolutionary time scales – is particularly high in the Balkans (Griffiths et al. 2014). The *Save the Blue Heart of Europe* campaign started more than ten years ago to save Balkan rivers and to stop all dam projects (Balkanrivers 2023). While this approach may seem naive, it follows a clear rationale: This part of Europe, with near-pristine rivers, can indeed be considered a »blue heart of Europe«, which needs protection for the sake and benefit of the entire continent - to safeguard a unique part of Europe’s biodiversity and to maintain rivers in a state that can guide restoration of degraded systems elsewhere. The campaign's vision is to prevent further destruction of rivers by dam constructions or water abstractions. To this aim, a network of NGOs, activists, lawyers, artists and scientists was created – at local, regional and international scale. This diverse network has managed to stop or helped to stop numerous planned dam projects. For instance, in 2023, the Federation of Bosnia and Herzegovina adapted electricity laws, stopping the issuing of permits for small (less than 10 MW) hydropower plants (OG BIH 2023) – a success that is especially due to the dedicated involvement of the Aarhus Center in Sarajevo (AARHUS 2023). In Albania, the campaign stopped 45 dam projects in the Vjosa River basin and catalysed the establishment of Europe's first Wild River National Park. A smaller, but impressive example happened recently at the Rupska River in Serbia, where the people of the small village of Dadince – supported by Blue Heart partners from Serbia and Bosnia and Herzegovina – convinced authorities, through enduring protest, not to build two dams.

A very important part of the campaign network is the initiative *Scientists for Balkan Rivers* (Balkanrivers 2023). A growing number of experts from Balkan countries and abroad are assessing the biodiversity and ecological processes in Balkan rivers through diverse activities including larger collaborative projects, targeted assessments of individual hydropower projects, and small individual projects by young researchers pursuing a Master's or PhD thesis. The activities themselves attract attention and put a spotlight on Balkan rivers, and – more importantly – the gathered data on biodiversity and the status of ecosystems represent firm support for advocating for the exceptional value of Balkan rivers. Findings are used as arguments by the *Lawyers for Rivers* (Euronatur 2023) in order to legally stop dam projects in the region or to push for a better protection status. Scientists and lawyers here work hand in hand.

Several *Science Weeks* have been organised on Balkan rivers since 2017. Even though these events last for only a few days (which is often acknowledged as merely allowing preliminary scientific work), they have three main objectives: First, scientists get an opportunity to do what they can do best – they (voluntarily) gather as much data as possible in this short time. Then, second, they put their discoveries into the hands of the media and involved journalists, often enough on-site and immediately. The field camp atmosphere provides a unique chance for the interaction of scientists with journalists, who are by far the more skilled storytellers that then actually put a public spotlight on an exceptional river system. Having journalists accompany scientists during fieldwork significantly contributes to the creation of good stories as well as of unique photo and film footage. Third, the events catalyse networking among dedicated scientists from various institutions and nations. This includes a capacity-building component by involving less experienced colleagues or students in strong international teams of knowledgeable experts, thereby empowering local scientists and strengthening their involvement in advocating for nature protection in their country. Over the years an additional benefit has emerged: The interaction with the local inhabitants makes it possible for the local communities to recognize their homeland as a natural heritage of international significance, and brings attention to alternative visions for development than hydropower.

The blueprint for Balkan River *Science Weeks* is the first *Vjosa Science Week 2017*. The results of this seminal event were published (Schiemer et al. 2018) and formed the scientific basis for lobbying for the protection of this unique riverscape (Schiemer et al. 2020). Follow-up activities included a scientific conference, the »Wild River Symposium« in Tirana in October 2019 (Balkanrivers 2023), and a second *Science Week* organised on a tributary of the Vjosa (Schiemer & Miho 2021). Taken together, these science-driven activities contributed markedly to the creation of the Vjosa Wild River National Park in March 2023 (https://www.vjosanationalpark.al/).

Why Neretva River

The Neretva River is 230 km long and flows through Bosnia and Herzegovina and Croatia before its confluence with the Adriatic Sea. While larger parts of the river network are already reservoirs, the Neretva and its tributaries upstream from Konjic are still free-flowing. Along the main course, from its source to the first reservoir near Konjic (Jablanica HPP), the Neretva River is 90 km long and drains a catchment area of approximately 1350 km^2. This area represents a rather untouched and sparsely populated landscape of Bosnia and Herzegovina, without heavy pollution, wastewater or heavy industry. However, the river is threatened by the partly ongoing and partly planned construction of a total of 24 new dams in this upper section of the Neretva River alone (Balkanrivers 2023). One of them, HPP Ulog, with a targeted capacity of 35 MW, is already under construction, with a planned dam height of up to 60 metres. Yet, there is still time to stop further development of the upper Neretva River: Two more large power plants, namely HPP Bjelimići and HPP Glavatičevo, are planned downstream of HPP Ulog. Additionally, several small hydroelectric power plants are planned both on the Neretva itself and on its tributaries, namely Hydro energetic system (HES) Gornja Neretva (7 dams), HES Jezernica (4 dams), and also on the Ljuta River (9 dams). The small hydropower plant Hotovlje on the stream Vrhovinska is already under operation. This situation made it crucial to provide evidence of what is at risk through further building of dams, and increase public visibility of the valuable ecosystems in this valley.

The overall approach of the *Neretva Science Week 2022*

We formulated two simple overarching objectives that all scientists, independent of their discipline and particular expertise, could follow. These were (i) to collect data on aquatic as well as terrestrial river-associated biodiversity and (ii) to assess the potential impacts of hydropower development specifically on threatened habitat types and protected species. We wanted to achieve local assessments of flora, fauna and habitats by knowledgeable specialists and integrate those into an expert-based opinion on the biodiversity and ecological integrity of the upper Neretva at a regional scale. Here, 'regional scale' means, for example, understanding the contributions of local karst phenomena to river network-scale heterogeneity and biodiversity, or assessing the potential effects of landscape and river network fragmentation through hydropower development.

The work covered a dozen field sites that had been chosen during a hiking and kayaking reconnaissance trip in April 2022, stretching from just below the source of the Neretva River to the town of Konjic (Fig. 1). Safety (e.g. designated landmine areas) and accessibility had to be considered for the selection of the sites in this exceptionally wild river network, yet the final set of field sites represented a respectable length continuum of the river up to a catchment size of 922 km^2 and covered important tributaries along the Neretva River's upper sections. Six sites were identified as main sampling sites and another six as additional sampling sites that were to be sampled when time permitted. We aimed at integrating data on terrestrial aquatic organisms; thus, colleagues working on terrestrial organism groups were asked to focus on »river-associated« habitats defined as maximally 500 m away from the river, thus transcending the extent of the riparian zone and potentially encompassing the entire floodplain on the valley floor. At many sites, however, the canyon-like character of the landscape restricted sampling to a rather narrow riparian zone before steep and drier hillslopes. Some groups had more time and found additional sites to visit, or sampled from boats to achieve a more continuous impression. Some groups augmented the overview of data with existing information stemming from prior research efforts.

An important factor was the effort made to bring journalists along during the fieldwork, allowing them to submerge in the Neretva landscape while being able to exchange knowledge with the scientists first-hand in the field. This resulted in some impactful reporting in various news outlets, as, on the one hand, journalists were directly and personally involved, and, on the other hand, scientists could make use of the immediate surroundings to provide a tangible and understandable expert opinion on otherwise abstract ecological mechanisms.

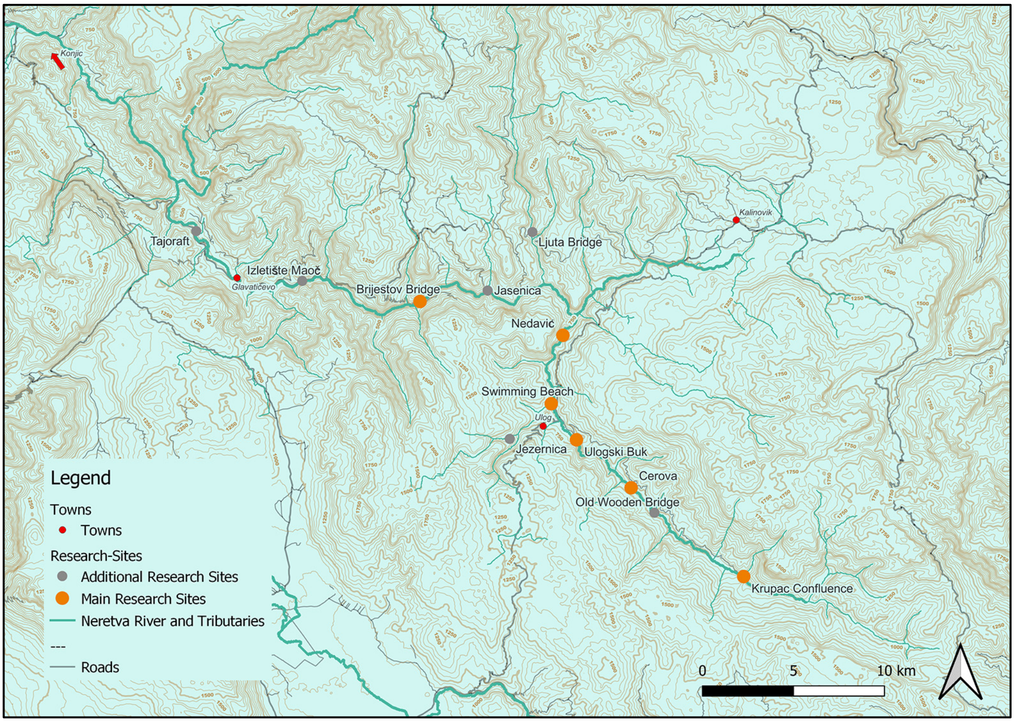

Figure 1. Map with main and additional research sites visited during the *Neretva Science Week 2022*.
Slika 1. Zemljevid z glavnimi in dodatnimi raziskovalnimi lokacijami, obiskanimi v *Tednu znanosti na Neretvi 2022*.
Slika 1. Mapa sa glavnim i dodatnim istraživačkim mjestima posjećenim tokom *Sedmice nauke na Neretvi 2022*.

The richness of findings from the *Neretva Science Week 2022*

From the 26.6. to 5.7.2022, a diverse team of in total 48 scientists and 11 students from seven countries were involved in fieldwork on the upper Neretva River (Fig. 2). They collected data aimed at characterising this pristine and highly threatened riverine ecosystem with a hitherto non-existing topical and taxonomical breadth, manpower and concentrated effort, and spatial resolution. Overall, all involved researchers were astonished by the pristine state of the surrounding forests and river bank vegetation, the amazing abundance of animals observed, and the unspoiled character of the diverse ecosystems found along the investigated river section (Fig. 3).

Figure 2. Attendants of the *Neretva Science Week 2022*.The number of experts brought together was as impressive as the river itself (photo: J. Lim).

Slika 2. Udeleženci *Tedna znanosti na Neretvi 2022*. Število zbranih strokovnjakov je bilo tako impresivno kot reka sama. (foto: J. Lim).

Slika 2. Učesnici *Sedmice nauke na Neretvi 2022*. Broj okupljenih stručnjaka bio je impresivan kao i sama rijeka (foto:J. Lim).

The 13 contributions published in this special issue of Natura Sloveniae, dedicated to results of the *Neretva Science Week 2022*, draw an impressive picture of high biodiversity in a precious vastly understudied ecosystem. The researchers present long lists of taxa with a high fraction of endemic species and several species new to science. They describe taxonomically specialised aspects of aquatic and terrestrial river-associated biodiversity. Finally, they report on the environmental conditions supporting this biodiversity and a selection of ecosystem functions this biodiversity may drive in the particular setting of this river. Notably, some data gathered during the *Neretva Science Week 2022* were published separately: Ivković et al. (2023) report on aquatic Empididae (Diptera) and a new species of crane fly is described in Kolcsár et al. (2023). Pešić et al. (2023) report the first dataset of COI sequences for aquatic mites in the region, based on material gathered during *Neretva Science Week 2022*. Altogether, more than 1000 species of animals and plants were documented, and dozens of them are protected under various laws. Ten species are protected by the EU Birds Directive (OJ EC 2009) and more than 30 by the EU Habitat Directive (OJ EC 1992). More than 50 species are strictly protected by Republika Srpska law, with an additional ten having the status »protected«. Many of the published and here presented results suggest the necessity for deeper exploration of specific taxonomic groups, yet they also form an important baseline, on which further studies may be built.

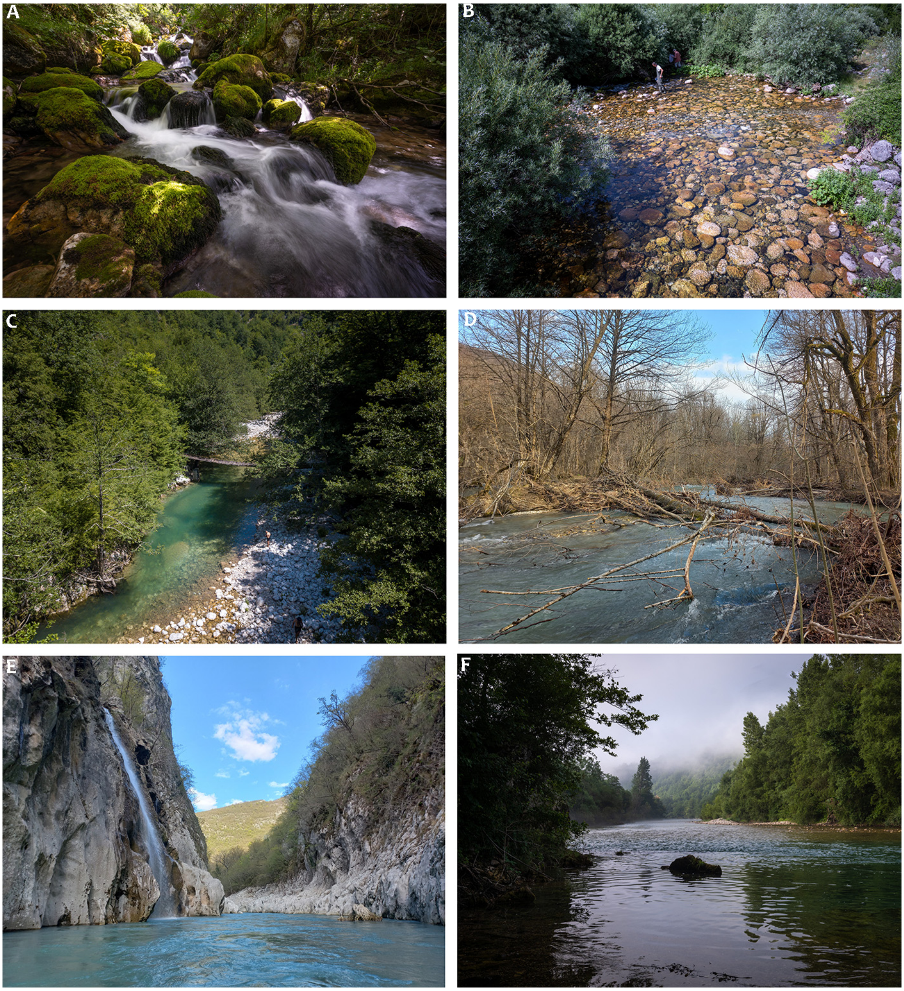

Figure 3. Varying habitats investigated along the upper Neretva River in summer 2022: A – Krupac tributary, the most upstream located research site; B – the Krupac tributary near its confluence with the Neretva; C – pool-riffle sequence at the 'Old Wooden Bridge' research site; D – alder forest floodplain between 'Cerova' and 'Ulogski buk' research sites, photo taken in April 2022 at higher water levels; E – canyon section with karst springs below 'Tajoraft' research site, photo taken in April 2022 at higher water levels; F – slow-flowing section near 'Izletište Maoč' research site, where the river has picked up in volume and is wider. Photos: Vladimir Tadić (A, B, C, F) and Vera Knook (D, E).

Slika 3. Različni habitati, raziskani vzdolž zgornjega toka reke Neretve: A – pritok Krupac, najvišje locirano raziskovano mesto; B – pritok Krupac ob izlivu v Neretvo; C – zaporedje tolmunov in brzic na raziskovani lokaciji 'Stari leseni most'; D – poplavno območje jelševega gozda med raziskovanima mestoma Cerova in Uloški buk, posneto aprila 2022 ob višjih vodostajih; E – kanjonski odsek s kraškimi izviri pod raziskovano lokacijo 'Tajoraft', posnet aprila 2022 ob višjih vodostajih; F – počasi tekoči del v bližini raziskovalne lokacije Izletište Maoč, kjer je Neretva že dobila volumen in širino. Foto: Vladimir Tadić (A, B, C, F) in Vera Knook (D, E).

Slika 3. Različita staništa su istražena duž gornjeg toka Neretve u ljeto 2022.: A – pritoka Krupac, najuzvodnija lokacija istraživanja; B – pritoka Krupac kod ušća u Neretvu; C – sekvenca brzaka i bazena na istraživačkom mjestu 'Stari drveni most'; D – poplavne šume johe između istražnih lokaliteta 'Cerova' i 'Ulogski buk', snimljeno u aprilu 2022. na većem vodostaju; E – kanjonski dio sa kraškim izvorima ispod istraživanog lokaliteta 'Tajoraft', snimljeno u april 2022. na većem vodostaju; F – sporo tekući dio kod istraživanog lokaliteta 'Izletište Maoč', gdje je rijeka postala većeg volumena i širine. Fotografije: Vladimir Tadić (A, B, C, F) i Vera Knook (D, E).

The scientists were accompanied by an almost equally sized diverse team of journalists, filmmakers and media content creators, who published impactful stories in close to 10 varying news outlets, including Science Magazine (Schiffman 2022), National Geographic (White 2022) and ARD Wien. A video summarizing the week's activities remains available on the internet (https://www.youtube.com/watch?v=R6IejDngw0A).

The initial findings as well as impressions of this diverse team of scientists were very consistent and underscored the remarkable state of the upper Neretva River. And while the researchers themselves were steadily surprised about the ecological richness of the valley, we also experienced first-hand how intensively the natural beauty of the place is used and enjoyed by the general public. On the weekend, hundreds of tourists visit the Konjic canyon by raft and evidently benefit from the ecologically intact river. Such intense use for touristic purposes may have its own harms and, indeed, asks for management. Yet, it also identifies the Neretva River as a rich recreational environment for humans, besides providing a habitat for a high level of biodiversity. Finding all this in one river valley underscores its extremely high conservation value in its entirety.

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All involvement of scientists in the *Neretva Science Week 2022*, including on-site participation as well as eventual preparatory work before the event and analysis work thereafter, happened on a completely voluntary and unpaid basis. This special issue of Natura Sloveniae is their contribution to saving the Neretva River from further damming – Thank you! Organising the Science Week would not have been possible without the support of Manfred-Hermsen-Stiftung (https://www.m-h-s.org/en/), a long-standing partner of the *Save the Blue Heart of Europe* campaign. Individual scientists used their own projects and funds for further analyses of the material they gathered, and these funds are acknowledged in the individual contributions. We would like to thank the team from Center for Environment for organisational support. The association of sports fishermen in Konjic and the fish association from Kalinovik welcomed us and supported the event. Finally, the support of the local community was crucial for the entire event. They are also the first ones to be directly impacted by the changes in their environment. We specifically thank Boban Škrkar and his family and friends from Ulog, who helped us with logistics, opened up their houses and gardens as places to stay, and provided meals to all participants.

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Clockwise from top-left: A word of welcome to the Neretva Science Week in Ulog by Ulrich Eichelmann; Jelena Ivkovic welcomes the scientists in the name of the Center for Environment (CZZS); Gabriel Singer, lead scientist of the Neretva Science Week explains the scientific approach; Vladimir introduces Boban, the local host of the Neretva Science Week in Ulog. Photos: Vladimir Tadić

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Molluscs (Gastropoda and Bivalvia) of the upper Neretva River

Michael DUDA[1], Elisabeth HARING[2,3*]

[1]3rd Zoological Department, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria;
E-mail: michael.duda@nhm-wien.ac.at
[2]Central Research Laboratories, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria;
E-mail: elisabeth.haring@nhm-wien.ac.at
[3]Department of Evolutionary Biology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria
*Corresponding author

Abstract. During a survey taking place from 29.6.2022–2.7.2022 in Bosnia and Herzegovina in the river-floodplain system of the upper Neretva River around Ulog, 51 species of molluscs were found at 12 sampling sites. Besides manual sampling in the water bodies, the main sampling technique consisted of sieving of river deposits, to maximise the coverage of species diversity. The majority of the species were terrestrial gastropods (45 out of 51), most of them common species of forests and moist areas, but also of grassland. In the Neretva River itself and in adjacent waterbodies, six species of freshwater molluscs were found. Among them, at least three species of spring snails of the taxonomically challenging genera *Belgrandiella*, *Bythinella*, and *Paladilhiopsis* were found, the latter one only in river deposits. Future research should focus on truncatelloidean snails in the groundwater system of the upper course of the Neretva River. Furthermore, a broader investigation of the mollusc fauna of Bosnia and Herzegovina would help to clarify taxonomically unresolved questions of species of the Balkan region and possibly reveal species new for science.

Key words: Mollusca, Bosnia and Herzegovina, Neretva River, spring snails, Truncatellidoidea, Balkan region

Izvleček. Mehkužci (polži in školjke) zgornjega toka Neretve – Med raziskavo, ki je potekala od 24.6. do 2.7. 2022 v Bosni in Hercegovini v rečno-poplavnem sistemu gornje Neretve v okolici Uloga, smo na 12 lokacijah našli 51 vrst mehkužcev. Poleg ročnega vzorčenja v vodnih telesih je bila glavna tehnika vzorčenja sejanje rečnih nanosov, kar je razkrilo največjo vrstno raznolikost. Največ vrst je bilo kopenskih polžev (45 od 51), večinoma pogostih vrst v gozdovih in vlažnih območjih, a tudi traviščih. V sami reki Neretvi in v sosednjih vodnih telesih smo našli šest vrst sladkovodnih mehkužcev. Med njimi so bile najdene vsaj tri vrste izvirskih polžev taksonomsko zahtevnih rodov *Belgrandiella*, *Bythinella* in *Paladilhiopsis*, slednji le v rečnih nanosih. Prihodnje raziskave bi se morale osredotočiti na polže iz naddružine Truncatelloidea v podzemnem vodnem sistemu zgornjega toka reke Neretve. Poleg tega bi širša raziskava favne mehkužcev Bosne in Hercegovine pripomogla k razjasnitvi taksonomskih vprašanj vrst balkanske regije in morda razkrila nove vrste za znanost.

Ključne besede: Mollusca, Bosna in Hercegovina, reka Neretva, izvirski polži, Truncatellidoidea, Balkan

Apstrakt. Mekušci (Gastropoda i Bivalvia) gornjeg toka rijeke Neretve – Prilikom istraživanja koje je obavljeno od 24.6. – 2. 7. 2022. godine u Bosni i Hercegovini u riječno-plavnom sistemu rijeke gornje Neretve oko Uloga, na 12 mjesta uzorkovanja pronađena je 51 vrsta mekušaca. Osim ručnog uzorkovanja u vodnim tijelima, glavna tehnika uzorkovanja se sastojala od prosijavanja riječnih naslaga, što je rezultiralo maksimalnom raznovrsnošću vrsta. Većina vrsta su bili kopneni puževi (45 od 51), većinom uobičajene vrste šuma i vlažnih područja, ali i travnjaka. U samoj rijeci Neretvi i u susjednim vodnim tijelima pronađeno je šest vrsta slatkovodnih mekušaca. Među njima su pronađene najmanje tri vrste izvorskih puževa taksonomski zahtjevnih rodova *Belgrandiella*, *Bythinella* i *Paladilhiopsis*, drugi navedeni samo u riječnim nanosima. Buduća istraživanja bi se trebala fokusirati na puževe superfamilije Truncatelloidea u sistemu podzemnih voda gornjeg toka rijeke Neretve. Nadalje, šire istraživanje faune mekušaca Bosne i Hercegovine pomoglo bi da se razjasne taksonomski neriješena pitanja vrsta balkanskog regiona i eventualno otkriju vrste koje su nove za nauku.

Ključne riječi: mekušac, Bosna i Hercegovina, rijeka Neretva, izvorski puževi, Truncatellidoidea, Balkan

Introduction

First overviews on the mollusc fauna of Bosnia and Herzegovina were given by Jaeckel et al. (1958), Radoman (1983), and Mulaomerović & Glöer (2022). Welter-Schultes (2012) provided indicative distribution maps and Bank & Neubert (2017) gave country lists of European non-marine snails (except spring snails) and mussels. For Gastropoda, Karaman (2006) reported the number of taxa of Bosnia and Herzegovina as 331 (135 genera in 47 families). Dedov et al. (2021) provided a dataset of all Gastropod species described for Bosnia and Herzegovina. Central and Northern Bosnia as well as northern Herzegovina are especially poorly researched (Mulaomerović & Glöer 2022). Yet, several new species were described from this area during the last years (e.g., Glöer & Grego 2015; Grego & Glöer 2019; Grego 2020). A general overview on aquatic Gastropoda is given by Glöer (2019) and Glöer (2022a, 2022b). For Bivalvia only general information, provided by Welter-Schultes (2012), is presently available.

Here we report on a survey of the mollusc fauna during the Neretva Science Week 2022, which was part of the »Scientists for Balkan Rivers« project within the »Save the Blue Heart of Europe« campaign.

Materials and methods

Sampling took place at 12 localities between 29.6.2022 and 2.7.2022 (Tab. 1). Except for three springs located in the adjacent forests (localities No. 2, 3, 8; Tab. 1), the remaining sampling sites were in the water bodies themselves or close to the riverside.

Molluscs (living and/or their empty shells) were sampled by three techniques (see also Duda et al. 2018): (1) manual sampling in the water bodies as well as accompanying riverside areas was applied for larger species (>1 cm) and empty shells. (2) Dry sieving was applied to river deposits. This is a good method to detect small species and to survey malacologically unexplored landscapes (Čiliak & Šteffek 2011). For this task, soil or river deposits were gathered (altogether

~ 20 litres), sieved with 3.0 mm and 0.5 mm mesh width and examined for mollusc shells. (3) Furthermore, wet sieving (Horsák 2003) was used to extract wet empty shells and living snails from moist substrate, e.g., the bottom of springs. For this task, the material collected from wet places and water bodies was washed in a sieve with 0.5 mm mesh width to remove detritus and plants. By this technique, snails – living animals and water-filled empty shells remain at the bottom of the sieve, while other particles float to the top. Living molluscs collected were immediately preserved in 96% alcohol.

Table 1. Sampling sites. Elevation in meters above sea level.
Tabela 1. Mesta vzorčenja. Nadmorska višina v metrih nad morsko gladino.
Tabela 1. Mjesta uzorkovanja. Nadmorska visina u metrima iznad nivoa mora.

Nr.	Locality name	N	E	Elevation	Date
1	Neretva-Krupac Confluence	43°19.732′	18°25.533′	779	29.6.2022
2	Krupica spring 1	43°19.835′	18°25.712′	805	29.6.2022
3	Krupica spring 2	43°19.821′	18°25.681′	802	29.6.2022
4	Krupac side arm	43°19.781′	18°25.629′	791	29.6.2022
5	Neretva locality 2	43°19.640′	18°25.692′	786	29.6.2022
6	Cerova locality 2	43°22.564′	18°21.536′	708	30.6.2022
7	Cerova Old Wooden Bridge	43°22.776′	18°21.316′	710	30.6.2022
8	Nedavic spring	43°27.494′	18°19.399′	668	1.7.2022
9	Nedavic canyon	43°27.610′	18°19.306′	626	1.7.2022
10	Ulogski Bug	43°24.291′	18°19.456′	674	1.7.2022
11	Ulog swimming beach	43°25.450′	18°18.479′	639	1.7.2022
12	Prijestov bridge	43°28.940′	18°13.605′	419	2.7.2022

Results and discussion

In the present survey, the majority of snails detected were land gastropods. In total, 51 species could be detected in the samples of the 12 localities, among them 45 land gastropod species, five water snail species and one mussel species. Species numbers range from 1 to 28 per locality. The data are summarised in Tab. 2.

In the upper Neretva River, species diversity of aquatic molluscs was low, besides the snail species *Galba truncatula* and *Ancylus recurves*, one pea clam species (tiny freshwater mussels) was found: *Euglesa casertana*. The springs in the wooded areas accompanying the river system harbored representatives of three families of spring snails (genera *Paladilhiopsis*, *Belgrandiella*, *Bythinella*), which could not be identified at species level (Figs 1, 2, 3). They should be identified by experts for this group, of which only a few exist in the world. Spring snail specimens assigned to these genera were also found in the deposits collected in the river. Of the aquatic molluscs – five species were encountered in the water bodies themselves (*Belgrandiella* sp., *Bythinella* sp., *Euglesa casertana*, *Ancylus recurvus*, *Galba trunculata*), yet only the latter two of them were found alive. One species - *Paladilhiopsis* sp. – is stygobiotic and was only found in river deposits.

Table 2. Taxa of molluscs collected at sampling sites 1-12. e = empty shells, l = living animals. Numbers refer to localities in Tab. 1.
Tabela 2. Taksoni mehkužcev, zbrani na lokacijah 1-12. e = prazne lupine, l = žive živali. Številke se nanašajo na lokalitete v Tab. 1.
Tabela 2. Taksoni mekušca prikupljeni na mjestima uzorkovanja 1-12. e = prazne školjke, l = žive. Brojevi markiraju lokalitete kao u Tab. 1.

Family/species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Cochlostomatidae												
Cochlostoma scalariniforme (A.J. Wagner, 1906)							e					
Pomatiidae												
Pomatias elegans (O. F. Müller, 1774)									e			e
Hydrobiidae												
Belgrandiella sp.		e									e	
Moitessieriidae												
Paladilhiopsis sp.									e		e	
Bythinellidae												
Bythinella sp.	e	l	l	e	e			l	e		e	
Lymnaeidae												
Galba truncatula (O. F. Müller, 1774)	e			e							e	
Planorbidae												
Ancylus recurvus E. von Martens, 1873	l											e
Ellobiidae												
Carychium minimum O. F. Müller, 1774				e					e		e	
Carychium tridentatum (Risso, 1826)									e		e	
Cochlicopidae												
Cochlicopa lubricella (Porro, 1838)					e							
Orculidae												
Sphyradium doliolum (Bruguiere, 1792)				e		e	e		e		e	
Valloniidae												
Acanthinula aculeata (O. F. Müller, 1774)	e				e		e		e		e	
Vallonia costata (O. F. Müller, 1774)											e	
Vallonia pulchella (O. F. Müller, 1774)					e		e		e		e	
Pupilliidae												
Pupilla muscorum (Linnaeus, 1758)							e					
Vertingidae												
Vertigo pusilla O. F. Müller, 1774							e		e		e	
Vertigo pygmaea (Draparnaud, 1801)											e	
Truncatellinidae												
Columella edentula (Draparnaud, 1805)							e				e	
Truncatellina cylindrica (J.B. Férrussac, 1807)							e		e			

Family/species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Enidae												
Merdigera obscura (O. F. Müller, 1774)					e		e				e	
Zebrina detrita (O. F. Müller, 1774)										e		
Clausiliidae												
Herilla ziegleri cf. *limana* O. Boetger, 1909									e			
Cochlodina laminata (Montagu, 1803)					e	e	e		e		e	e
Macrogastra ventricosa (Draparnaud, 1801)						e	e				e	
Alinda biplicata (Montagu, 1803)											e	
Strigillarica vetusta (Rossmässler, 1836)						e	e				e	e
Clausiliidae sp. juv					e	e			e			
Punctidae												
Punctum pygmaeum (Draparnaud, 1801)							e		e			
Helicodiscidae												
Lucilla singleyana (Pilsbry, 1889)				e								
Discidae												
Discus perspectivus (M. Mühlfeld, 1816)						e	e		e	e	e	
Gastrodontidae												
Aegopinella minor (Stabile1864)									e			e
Perpolita hammonis (Strøm, 1765)		e										
Pristilomatidae												
Vitrea contracta (Westerlund, 1871)							e					
Vitrea illyrica (A. J. Wagner, 1907)							e					
Vitrea sp.									e		e	
Oxychilidae												
Daudebardia brevipes (Draparnaud, 1805)					e							
Daudebardia rufa (Draparnaud, 1805)					e						e	
Zonitidae												
Aegopis verticillus (A. Férrussac, 1819)			e		e						e	
Limacidae												
Limax cinereoniger Wolf, 1803											l	
Limacoidea sp.											l	
Euconulidae												
Euconulus fulvus (O. F. Müller, 1774)							e			e		
Euconulus praticola (Reinhard, 1883)									e			

Family/species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Geomitidridae												
Cernuella cf. ci*salpina* (Rossmässler, 1837)							e				e	e
Xerolenta obvia (Menke, 1828)	e					e	e				e	
Hygromiidae												
Monachoides incarnatus (O. F. Müller, 1774)						e	e			e	e	e
Monacha claustralis (Menke, 1828)					e							
Monacha frequens (Mousson, 1859)	e		e		l	e	e			e	e	
Helicidae												
Dinarica pouzolzi (Deshayes, 1832)	e		e							e		
Caucasotachea vindobonensis (C. Pfeiffer, 1828)	e					e	e		e			e
Helix secernenda Rossmässler, 1847	e											
Sphaeridae												
Euglesa casertana (Poli, 1791)				l			e					
Number of taxa - empty shells	**8**	**2**	**3**	**5**	**11**	**10**	**23**	**0**	**19**	**6**	**26**	**8**
Number of taxa - living	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**2**	**0**

Concerning land gastropods, a higher number of species could be found at rocks at the riverside and in accompanying forests as well as in the river deposits collected. In general, the land gastropods detected in the investigated area resemble the typical species known from this area, represented by forest-dwelling species (e.g., *Sphyradium doliolum, Discus perspectivus*), with some of them found outside the springs, as well as species typical for open landscape (e.g., *Pupilla muscorum*, *Xerolenta obvia*).

Some species could not be identified with certainty. Concerning *Cernuella* cf. *cisalpina*, which was detected at three sites by empty shells only, it is yet not clear if it is a separate species or part of an unresolved taxon complex with *Cernuella virgata* (Duda et al. 2022). Of *Vitrea* sp. only juvenile empty shells were found, and of *Limacoidea* sp. only one juvenile living specimen.

Regarding the trucatelloid species, future research must be conducted, if *Bythinella* sp. and *Belgrandiella* sp. found in our survey could represent *Belgrandiella goranii* and *Bythinella buranii* recently described by Mulaomerovic & Glöer (2022) or whether they represent different species. Regarding *Paladilhiopsis*, we follow current research (Hofman et al. 2018) and assume that this genus is more likely to occur in Bosnia and Herzegovina than the morphologically similar genus *Iglica* (the latter listed in Glöer (2022a) for this area). Furthermore, it is still unclear, whether the collected empty shells could represent one of the described species within this genus in the region (*P. solida* Kuščer, 1933 *or P. arion* Rysiewska & Osokowski, 2021), but most likely due to the different shell morphology it could instead belong to a new taxon. To clarify this question, more intense research would be required to assess the distribution ranges of the neighboring species with greater detail. Molecular genetic methods may help to differentiate the genera, although it should be mentioned that even current genetic methods may not lead to satisfying conclusions in all species of Truncatelloidea (Richling et al. 2017; Schubert et al. 2023).

Figure 1. Empty shell of *Paladilhiopsis* sp. from locality 9 (Nedavic canyon). Scale bar = 1 mm.
Slika 1. Prazna lupina *Paladilhiopsis* sp. iz lokalitete 9 (kanjon Nedavić). Merilce = 1 mm.
Slkika 1. Prazna ljuska *Paladilhiopsis* sp. sa lokaliteta 9 (kanjon Nedavić). Skala = 1 mm.

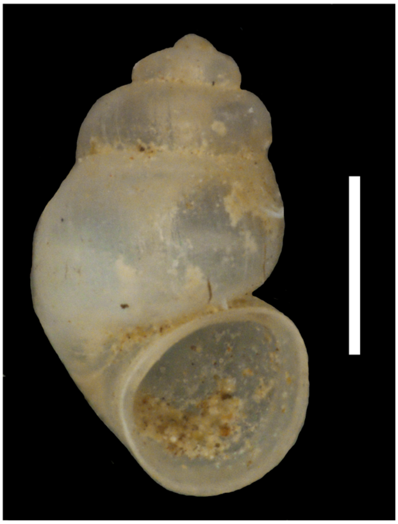

Figure 2. Empty shell of *Belgrandiella* sp. from locality 11 (Ulog swimming beach). Scale bar = 0.5 mm.
Slika 2. Prazna lupina *Belgrandiella* sp. iz lokalitete 11 (kopališče Ulog). Merilce = 0,5 mm.
Slika 2. Prazna ljuska *Belgrandiella* sp. sa lokaliteta 11 (plaža Ulog). Skala = 0,5 mm.

Figure 3. Empty shell of *Bythinella* sp. from locality 3 (Krupica spring 2). Scale bar = 1 mm.
Slika 3. Prazna lupina *Bythinella* sp. iz lokalitete 3 (izvir Krupice 2). merilce = 1 mm.
Slika 3. Prazna ljuska *Bythinella* sp. sa lokaliteta 3 (vrelo Krupca 2). Skala = 1 mm.

A rock-dwelling species, found alive on rocks at one site (locality No. 9, Nedavic canyon), was tentatively assigned to *Herilla bosniensis* (see also Knook et al. 2022) and deserves some comments (Fig. 4). Inspection of the collected specimens by Zoltan Fehér (Budapest, Hungary) showed that they in fact belong to *Herilla ziegleri*, most likely the subspecies *H. z. limana.* Several species of this genus were reported to occur in Bosnia (*H. bosniensis* (Vest, 1867); H. *ziegleri* (Küster, 1845); *H. pavlovici* (A.J.Wagner, 1914); *H. Illyrica* (Möllendorff, 1899); *H. jabucica* H. Nordsieck, 1971; *H. durmitoris* (Boettger, 1909)), yet almost no reference sequences were published in NCBI GenBank or BOLD database. The DNA barcoding sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1; data not shown) generated in the present study was compared with a single CO1 sequence of the genus *Herilla* available in GenBank (*Herilla ziegleri dacica*) and the p distance was 20.1%. This was in the same range as the distance to several other genera e.g., *Albinaria*, *Sericata*, *Inchoatia*, with p distances of around 19% (accessed on 22.1.2023). This genus deserves further investigation, including DNA sequence analyses of the various taxa over their distribution ranges. Presently, we consider snails of this population unidentified at subspecies level and we refer to it as *Herilla ziegleri* cf. *limana*. The BOLD Accession number of the CO1 sequence of this snail is ALNHM509-23.

Figure 4. Living specimen of *Herilla ziegleri* cf. *limana* on a rock in Nedavic canyon (locality 9).
Slika 4. Živi primerek *Herilla ziegleri* cf. *limana* na skali v kanjonu Nedavic (lokaliteta 9).
Slika 4. Živi primjerak *Herilla ziegleri* cf. *limana* na stijeni u kanjonu Nedavic (lokalitet 9).

Conclusions

Compared to the species diversity of land gastropods, the aquatic epigean mollusc fauna appears poor, yet this is typical for cool upper parts of rivers with fast-running water (Beran 2021). The occurrence of truncatelloid taxa (genera *Belgrandiella*, *Bythinella*, *Palhadilhiopsis*) detected alive in three springs raise interest for a thorough investigation of the crenobiont habitats (springs) of the area, including molecular genetic analyses. Given the high endemism of crenobiotic snails and their poor documentation in the Balkan region to date, a high number of unknown species may be expected. It is very likely that the spring snail species found during the Neretva Science Week 2022 represent endemics of Bosnia and Herzegovina. This is also true for some of the land gastropods. For most genera and species groups, comprehensive studies are needed to assess intra- and interspecific variation throughout the Balkan region and to detect possible cryptic species. The species diversity detected in the present survey in the surroundings of the upper Neretva underlines the high value of the region for biodiversity. The

need for protection of the upper Neretva is counteracted by the imminent anthropogenic threats, specifically the construction of hydropower plants and the accompanying draining infrastructure, as well as groundwater pollution and overexploitation.

Povzetek

Med raziskavo, ki je potekala od 24. do 26. junija 2022 v Bosni in Hercegovini v rečno-poplavnem sistemu zgornjega toka reke Neretve okoli Uloga, je bilo na 12 lokacijah najdenih 51 vrst mehkužcev. Da bi kar najbolje zajeli vrstno pestrost območja, smo uporabili dve tehniki: ročno vzorčenje v vodnih telesih in sejanje rečnih nanosov. Večina polžev, odkritih v tej raziskavi, so bili kopenski polži. Skupaj je bilo v vzorcih z 12 lokacij mogoče zaslediti 51 vrst, med njimi 45 vrst kopenskih polžev, pet vrst vodnih polžev in eno vrsto školjk. Število vrst se giblje od 1 do 28 na lokacijo. Pri kopenskih polžih je večje število vrst mogoče najti na skalah ob reki in v obvodnih gozdovih ter v zbranih rečnih nanosih. Na splošno so kopenski polži, odkriti na raziskanem območju, podobni tipičnim vrstam, znanih s tega območja, ki jih predstavljajo gozdne vrste (npr. *Sphyradium doliolum*, *Discus perspectivus*), nekatere izmed njih najdemo v bližini izvirov, pa tudi vrste, značilne za odprte pokrajine (npr. *Pupilla muscorum*, *Xerolenta* obvia) ali skalovje (*Herilla ziegleri* cf. *limana*).

V sami reki Neretvi in v sosednjih vodnih telesih je bila vrstna pestrost vodnih mehkužcev majhna. Poleg polžev *Galba truncatula* in *Ancylus recurvus* je bila najdena ena vrsta školjk *Euglesa casertana* (družina Sphaeriidae: drobne sladkovodne školjke). V izvirih v gozdnatih območjih, ki spremljajo rečni sistem, so bili zabeleženi predstavniki treh družin izvirskih polžev (rodovi *Paladilhiopsis*, *Belgrandiella*, *Bythinella*), ki pa jih ni bilo mogoče prepoznati do ravni vrste (Sl. 1., 2., 3). Izvirski primerki polžev, ki pripadajo tem rodovom, so bili najdeni tudi v usedlinah, zbranih v reki. Ena vrsta - *Paladilhiopsis* sp. – je stigobiont (tj. živi v podtalnici) in je bil najden samo v rečnih nanosih. Vrstna pestrost mehkužcev, ki smo jih našli med raziskovanjem gornje Neretve in okolice, podpira izjemno vrednost regije z vidika biotske raznovrstnosti. Prihodnje raziskave bi se morale osredotočiti na polže iz naddružineTruncatelloidea v podzemnem vodnem sistemu zgornjega toka reke Neretve. Poleg tega bi širša raziskava favne mehkužcev Bosne in Hercegovine pripomogla k razjasnitvi taksonomskih vprašanj o vrstah balkanske regije in morda razkrila nove vrste za znanost. To je pomembno z naravovarstvenega vidika, saj je regija močno antropogeno ogrožena, na primer gradnja hidroelektrarn in spremljajoče odvodne infrastrukture ter onesnaževanje in prekomerno izkoriščanje podzemne vode.

Sažetak

Prilikom istraživanja koje je obavljeno od 24. – 26. 6. 2022. godine u Bosni i Hercegovini u riječno-plavnom sistemu gornjeg toka rijeke Neretve oko Uloga, na 12 mjesta uzorkovanja pronađena je 51 vrsta mekušaca. Osim ručnog uzorkovanja u vodnim tijelima, glavna tehnika uzorkovanja se sastojala od prosijavanja riječnih nanosa, što je rezultiralo maksimalnim uspjehom u otkrivanju raznovrsnosti vrsta. Većina puževa otkrivenih u ovom istraživanju bili su kopneni puževi. Ukupno je u uzorcima sa 12 lokaliteta otkrivena 51 vrsta, među Kojima: 45 vrsta kopnenih puževa, pet vrsta vodenih puževa i jedna vrsta dagnji. Broj vrsta se kreće od 1 do 28 po lokalitetu. Što se tiče kopnenih puževa, veći broj vrsta može se naći na stijenama uz obalu rijeke i u okolnim šumama, kao i u sakupljenim riječnim naslagama. Uopšteno, kopneni puževi otkriveni na istraživanom području podsjećaju na tipične vrste poznate sa ovog područja, predstavljene vrstama koje žive u šumi (npr. *Sphyradium doliolum*, *Discus perspectivus*), neki od njih pronađeni u blizini izvora, kao i one tipične za otvorene pejzaže (npr. *Pupilla muscorum*, *Xerolenta obvia*) ili stijene (*Herilla ziegleri* cf. *limana*).

U samoj rijeci Neretvi i u susjednim vodnim tijelima diverzitet vrsta vodenih mekušaca je bio nizak. Pored puževa *Galba truncatula* i *Ancylus recurvus*, pronađena je i jedna vrsta školjkaša, *Euglesa casertana* (porodica Sphaeriidae: male slatkovodne školjke). Izvori u šumovitim predjelima koji prate riječni sistem bili su utočište predstavnika tri porodice izvorskih puževa (rodovi *Paladilhiopsis*, *Belgrandiella*, *Bythinella*), koji nisu mogli biti identifikovani na nivou vrste (sl. 1., 2., 3.). U naslagama sakupljenim u rijeci pronađeni su i primjerci izvorskih puževa koji pripadaju ovim rodovima. Jedna vrsta - *Paladilhiopsis* sp. – je stigobiont (tj. živi u podzemnim vodama) i pronađena je samo u riječnim naslagama. Raznolikost vrsta otkrivena u ovom istraživanju u okolini gornjeg toka Neretve naglašava visoku vrijednost biodiverziteta regije. Buduća istraživanja bi se trebala fokusirati na puževe superfamilije Truncatelloidea u sistemu podzemnih voda gornjeg toka rijeke Neretve. Takođe, šire istraživanje faune mekušaca Bosne i Hercegovine pomoglo bi da se razjasne taksonomski neriješena pitanja vrsta balkanskog regiona i eventualno otkriju vrste koje su nove za nauku. Ovo je važno sa stanovišta očuvanja budući da je regija pod velikim antropogenim uticajem zbog izgradnje hidroelektrana i prateće infrastrukture za odvodnjavanje, kao i zagađenjem i prekomjernom eksploatacijom podzemnih voda.

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Top to bottom: On the Krupac tributary within the thick forest, one finds waterfalls that form a natural barrier for fish migration; The Neretva area is very remote with few bridges upstream from Ulog, of which some are not in functioning shape anymore. Photos: Vladimir Tadić

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Aquatic insects (Ephemeroptera, Plecoptera, Trichoptera and Diptera: Tipuloidea) from the upper Neretva in Bosnia-Herzegovina

Wolfram GRAF[1], Ernst BAUERNFEIND[2], Marija IVKOVIĆ[3], Levente-Péter KOLCSÁR[4]

[1]BOKU, University of Natural Resources and Life Sciences Vienna, Institute of Hydrobiology and Aquatic Ecosystem Mangement, Austria; E-mail: wolfram.graf@boku.ac.at
[2]Natural History Museum, Vienna, 2. Zoology, Burgring 7, A-1010, Vienna, Austria
[3]Division of Zoology, Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia
[4]Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Ehime 790-8577, Japan

Abstract. Within the upper Neretva catchment in Bosnia several hydropower plants are projected. Between 28.6. and 1.7.2022 a baseline survey was initiated to screen the area regarding the diversity of aquatic insects and its linked conservation value. In total, 59 Trichoptera species, ten Plecoptera species, 19 Ephemeroptera and 16 Diptera (Tipuloidea) species were collected, of which *Baeoura* sp. is new to science. 35 species are endemics of the Balkans, most species are rheobiontic and need high water current. The most abundant taxa are species of the trichopteran genus *Agapetus* (*A. slavorum* and *A. ochripes*) – surface grazers sensitive to water fluctuations and therefore vulnerable to hydropower-induced water level changes. With estimated 40,000 attracted specimens per night, light traps were extraordinarily successful. The extremely high diversity, as well as the enormous abundance of aquatic insects, underline the importance of the upper Neretva as an unimpacted riverine system embedded in dense natural and near-natural forest.

Key words: biodiversity, conservation, dams, hydropower

Izvleček. Vodne žuželke (Ephemeroptera, Plecoptera, Trichoptera in Diptera: Tipuloidea) iz zgornje Neretve v Bosni in Hercegovini – V porečju zgornje Neretve v Bosni in Hercegovini je predvidenih več hidroelektrarn. Med 28. 6. in 1.7. 2022 smo opravili osnovne raziskave biotske raznovrstnosti vodnih žuželk in s tem povezane naravovarstvene vrednosti območja. Skupaj je bilo zbranih 59 vrst Trichoptera, 10 vrst Plecoptera, 19 vrst Ephemeroptera in 16 vrst Diptera (Tipuloidea), med drugim tudi nova vrsta za znanost iz rodu *Baeoura*. 35 vrst je balkanskih endemitov, večina vrst je reobiontov in potrebujejo hiter vodni tok. Najpogostejši taksoni so vrste iz rodu Trichoptera *Agapetus* (*A. slavorum* in *A. ochripes*) – površinski strgalci, ki so občutljivi za nihanje vode in zato tudi na spremembe vodnega stanja zaradi hidroelektrarn. Z ocenjeno 40.000 privabljenimi primerki na noč so bile svetlobne pasti izredno uspešne. Izjemno velika raznovrstnost in ogromna številčnost vodnih žuželk poudarjata pomen zgornje Neretve kot neokrnjenega rečnega sistema, obdanega z gostimi naravnimi in polnaravnimi gozdovi.

Ključne besede: biotska raznovrstnost, naravovarstvo, jezovi, hidroenergija

Apstrakt. Vodeni insekti (Ephemeroptera, Plecoptera, Trichoptera i Diptera: Tipuloidea) sa gornje Neretve u Bosni i Hercegovini – Unutar sliva gornje Neretve u Bosni i Hercegovini projektovano je nekoliko hidroelektrana. Između 28.6. i 1.7. 2022. godine započeto je osnovno istraživanje kako bi se područje pregledalo u pogledu raznovrsnosti vodenih insekata i njihove povezanosti sa očuvanjem vrijednosti područja. Ukupno je prikupljeno 59 vrsta Trichoptera, 10 vrsta Plecoptera, 19 vrsta Ephemeroptera i 16 vrsta Diptera (Tipuloidea), od kojih je *Baeoura* sp. nova u nauci. Trideset pet vrsta su endemi Balkana, većina vrsta su reobionti i potrebna im je jaka struja vode. Najzastupljeniji taksoni su vrste Trichoptera iz roda *Agapetus* (*A. slavorum* i *A. ochripes*) – površinski strugači osjetljivi na fluktuacije vode i samim tim osjetljive na promjene vodostaja uzrokovanog hidroelektranama. Sa procijenjenih 40.000 privučenih primjeraka po noći, svjetlosne zamke su bile izuzetno uspješne. Izuzetno velika raznovrsnost, kao i ogromna brojnost vodenih insekata, naglašavaju značaj gornjeg toka Neretve kao netaknutog riječnog sistema uklopljenog u gustu prirodnu i skoro prirodnu šumu.

Ključne riječi: biodiverzitet, očuvanje, brane, hidroenergija

Introduction

Due to historical environmental dynamics, the Western Balkan is a refuge and hot-spot of biodiversity regarding various groups of organisms, including a high percentage of endemic species (e.g. Graf et al. 2008). In contrast, conservation issues are largely neglected. In aquatic environments one of the most severe human activities is the increase in hydropower plant construction, which poses profound threats to freshwater biodiversity (Freyhof 2012; Schwarz 2012).

The Neretva river in Bosnia is famous amongst entomologists and several aquatic species are named after it like *Glossosoma neretvae* Marinkovic, 1988, *Rhithrogena neretvana* Tanasijević, 1985 and *Hydropsyche mostarensis* Klapálek, 1898, described from river's banks at Mostar. Most likely, further undescribed species might be hidden in its nowadays densely forested catchment. In this paper, the results of a snap-shot excursion are presented and effects of the planned hydropower plants on a natural system are shortly discussed.

Materials and methods

Sampling took place at the upper Neretva and its tributary, the Krupac, between 28.6. and 1.7.2022. Investigated sites (Fig. 1), coordinates and collecting methods used are provided in Tab. 1. During the day, aquatic insects were collected with sweeping nets for adult stages, a D-framed net was used to collect larval stages. On the 28. and 29.6. one light trap was operated at Ulogski Buk and at the site »Swimming Beach«, respectively. Both traps were exposed from dusk to dawn. At Ulogski Buk additionally one light trap was installed and specific specimens were picked from an illuminated sheet (Fig. 2). The collected material was conserved in 90% alcohol on site and further analysed in the lab. To get a rough estimation of the overall trapped material, all Trichopteran specimens from the light-trap at site »Swimming Beach« were subsampled and counted after identification. Ecological classifications and geographical distribution of species are based on Bauernfeind (2003), Bauernfeind & Soldán (2012), Graf et al. (2008), Neu et al. (2018), Oosterbroek (2023), the expert opinion of the authors and the data base freshwaterecology.info (https://www.freshwaterecology.info).

Table 1. Investigated sites, coordinates and methods used for collection of aquatic insects at the upper Neretva and its tributary the Krupac, Bosnia and Hercegovina.
Tabela 1. Lokacije, koordinate in uporabljene metode vzorčenja vodnih žuželk v gornji Neretvi in pritoku Krupac, Bosna in Hercegovina.
Tabela 1. Istražena mjesta, koordinate i metode za sakupljanje vodenih insekata na gornjoj Neretvi i njenoj pritoci Krupac, Bosna i Hercegovina.

Name of the site	Coordinates	Methods used	River
Krupac Confluence	43.32942°N, 18.42574°E	Sweeping net, larval collection	Krupac
Cerova	43.37887°N, 18.35621°E	Sweeping net, larval collection	Neretva
Ulogski Buk	43.40527°N, 18.32304°E	Light trap, Sweeping net, larval collection	Neretva
Swimming Beach	43.42414°N, 18.30837°E	Light trap, Sweeping net, larval collection	Neretva
Nedaviće	43.458°N, 18.32121°E	Sweeping net, larval collection	Neretva

Figure 1. Neretva River watershed and characteristics of some collection sites visited between 28.6. and 1.7.2022.
Slika 1. Povodje reke Neretve in značilnosti nekaterih lokacij vzorčenja, obiskanih med 28. 6. in 1. 7. 2022.
Slika 1. Sliv rijeke Neretve i karakteristike nekih mjesta za uzorkovanje posjećenih između 28. 6. i 1. 7. 2022. godine.

Results

During this short excursion 59 Trichoptera species, ten Plecoptera species, 19 Ephemeroptera species and 16 Diptera species were documented at five sites along the upper course of the Neretva (Tab. 2). Thirty-two species are endemic to the Balkans. The Trichoptera *Rhyacophila balcanica*, *Synagapetus iridipennis*, *Diplectrona atra*, *Drusus schmidi*, *Ecclisopteryx keroveci* and all Plecoptera species, with the exception of *Isoperla bosnica* and *Dinocras megacephala,* were exclusively found in the cold and spring-fed tributary Krupac. The enormous amount of light-trap material (Fig. 2) hampered exact and timely enumeration of species and sex ratios, but most dominant were both species of the genus *Agapetus* (*A. slavorum* and *A. ochripes*, which appeared roughly equal in numbers, Fig. 3) and representatives of the genus *Polycentropus* (*P. ieraptera* ssp., *P. schmidi* and *P. excisus*) as well as *Hydropsyche mostarensis* followed by *Sericostoma flavicorne* and to some extent, *Oecismus monedula*. During daytime, *Beraeamyia schmidi*, together with the Plecoptera *Chloroperla tripunctata* and *Isoperla bosnica*, were collected in high numbers from the riparian vegetation with sweep nets.

Regarding the distribution of species along the longitudinal gradient of the Neretva, the tributary Krupac is colonised by a distinct community, which differs greatly in species composition from that of the Neretva. The other visited sites along the Neretva (from Cerova to Nedaviće) seemed to host a similar benthic assemblage without remarkable changes in community structure.

Table 2. List of Trichoptera, Plecoptera, Ephemeroptera and Diptera (Tipuloidea) collected at five sites along the upper course of the Neretva River, ecological classification regarding water current and biogeographical distribution (r = rheobiontic species; e = Balkan endemic).

Tabela 2. Seznam vrst iz skupin Trichoptera, Plecoptera, Ephemeroptera in Diptera (Tipuloidea), zbranih na petih lokacijah vzdolž zgornjega toka reke Neretve, ekološka klasifikacija glede na hitrost vodnega toka in biogeografska razširjenost (r = reobiontska vrsta; e = balkanski endemit).

Tabela 2. Spisak Trichoptera, Plecoptera, Ephemeroptera i Diptera (Tipuloidea) sakupljenih na pet lokaliteta duž gornjeg toka rijeke Neretve, ekološka klasifikacija prema brzini struje vode i biogeografskoj rasprostranjenosti (r = reobiontske vrste; e = balkanski endem).

Taxonomic name/species	r	e
TRICHOPTERA		
Rhyacophila armeniaca Guérin, 1834	x	x
Rhyacophila balcanica Radovanovic, 1953	x	x
Rhyacophila fasciata viteceki Valladoid & Kucinic, 2020	x	x
Rhyacophila loxias Schmid, 1970	x	x
Rhyacophila morettina Botosaneanu, 1980	x	x
Rhyacophila nubila (Zetterstedt, 1840)	x	
Rhyacophila polonica Mclachlan, 1879	x	
Rhyacophila tristis Pictet, 1834	x	
Glossosoma bifidum Mclachlan, 1879	x	
Glossosoma boltini Curtis, 1834	x	
Glossosoma discophorum Klapálek, 1902	x	x
Glossosoma conformis Neboiss, 1963	x	
Agapetus ochripes Curtis, 1834	x	
Agapetus slavorum Botosaneanu, 1960	x	x
Synagapetus iridipennis Mclachlan, 1879	x	
Hydroptila tineoides Dalman, 1819		

Taxonomic name/species	**r**	**e**
Allotrichia pallicornis Eaton, 1873	x	
Ithytrichia lamellaris Eaton, 1873	x	
Wormaldia pulla Mclachlan, 1878	x	
Wormaldia subnigra McLachlan, 1865	x	x
Polycentropus flavomaculatus (Pictet, 1834)	x	
Polycentropus ierapetra dirfis Malicky, 1974	x	x
Polycentropus ierapetra slovenicus Malicky, 1998	x	x
Polycentropus excisus Klapálek, 1894	x	
Polycentropus schmidi Novak & Botosaneanu, 1965	x	
Psychomyia klapaleki Malicky, 1995	x	x
Psychomyia pusilla (Fabricius, 1781)	x	
Tinodes braueri Mclachlan, 1878	x	x
Tinodes rostocki Mclachlan, 1878	x	
Tinodes unicolor (Pictet, 1834)	x	
Tinodes waeneri (Linnaeus, 1758)		
Diplectrona atra Mclachlan, 1878	x	x
Hydropsyche incognita Pitsch, 1993	x	
Hydropsyche instabilis (Curtis, 1834)	x	
Hydropsyche mostarensis Klapálek, 1898	x	x
Brachycentrus montanus Klapálek, 1892	x	
Micrasema minimum Mclachlan, 1876	x	
Micrasema sericeum Klapálek, 1902	x	x
Thremma anomalum Mclachlan, 1876	x	x
Goera pilosa (Fabricius, 1775)	x	
Silo piceus (Brauer, 1857)	x	
*Crunoecia s*p.	x	
Lepidostoma basale (Kolenati, 1848)	x	
Drusus schmidi Botosaneanu, 1960	x	x
Ecclisopteryx keroveci Previšic, Graf & Vitecek, 2014	x	x
Limnephilus lunatus Curtis, 1834		
Limnephilus vittatus (Fabricius, 1798)		
Micropterna sequax (Mclachlan, 1875)	x	
Potamophylax pallidus Klapálek, 1899	x	x
Stenophylax mitis Mclachlan, 1875	x	
Oecismus monedula (Hagen, 1859)	x	
Sericostoma flavicorne Schneider, 1845	x	
Odontocerum albicorne (Scopoli, 1763)	x	
Beraeamyia schmidi Botosaneanu, 1960	x	x
Ernodes articularis Pictet, 1834	x	
Adicella filicornis Pictet, 1834	x	
Oecetis testacea Curtis, 1834		
Athripsodes albifrons (Linnaeus, 1758)	x	
Ceraclea dissimilis (Stephens, 1836)	x	
PLECOPTERA		
Chloroperla tripunctata (Scopoli, 1763)	x	
Isoperla bosnica Aubert, 1964	x	x
Isoperla tripartita gr. (nov.sp.?)	x	x
Dinocras megacephala (Klapálek, 1907)	x	
Perla marginata (Panzer, 1799)	x	
Brachyptera tristis (Klapálek, 1901)	x	x

Taxonomic name/species	**r**	**e**
Amphinemura sulcicollis (Stephens, 1836)	x	
Protonemura auberti Illies, 1954	x	
Protonemura intricata (Ris, 1902)	x	
Leuctra inermis Kempny, 1899	x	
EPHEMEROPTERA		
Siphlonurus (Siphlonurus) lacustris Eaton, 1870		
Baetis (Baetis) beskidensis Sowa, 1972	x	
Baetis (Baetis) cf. *lutheri* Müller-Liebenau, 1967	x	
Baetis (Rhodobaetis) rhodani (F.J. Pictet, 1843)	x	
Procloeon (Pseudocentroptilum) pennulatum (Eaton, 1870)	x	
Ecdyonurus (Helvetoraeticus) epeorides Demoulin, 1955	x	x
Ecdyonurus (Ecdyonurus) puma Jacob & Braasch, 1986	x	x
Electrogena mazedonica (Ikonomov, 1954)	x	x
Epeorus (Epeorus) assimilis Eaton, 1885	x	
Epeorus (Ironopsis) yougoslavicus (Šámal,1935)	x	x
Rhithrogena neretvana Tanasijević, 1985	x	x
Habroleptoides filipovicae Gaino & Sowa, 1985	x	x
Habrophlebia lauta Eaton, 1884		
Paraleptophlebia submarginata (Stephens, 1836)	x	
Ephemera (Ephemera) danica O.F. Müller, 1764		
Ephemerella ignita (Poda, 1761)	x	
Ephemerella mucronata (Bengtsson, 1909)	x	
Serratella ikonomovi (Puthz, 1971)	x	x
Caenis macrura Stephens, 1836	x	
DIPTERA: TIPULOIDEA		
Antocha alpigena (Mik, 1883)	x	
Baeoura neretvaensis Kolcsár & d'Oliveira, 2023	x*	x
Ellipteroides alboscutellatus (von Roser, 1840)		
Ellipteroides limbatus (von Roser, 1840)		
Eloeophila miliaria (Egger, 1863)		
Idiocera jucunda (Loew, 1873)	x*	
Idiocera lackschewitzi (Starý, 1977)	x*	
Molophilus crassipygus de Meijere, 1918		
Molophilus propinquus (Egger, 1863)		
Nephrotoma flavescens (Linnaeus, 1758)		
Nephrotoma quadrifaria (Meigen, 1804)		
Thaumastoptera calceata Mik, 1866		
Tipula fascipennis Meigen, 1818		
Tipula lateralis Meigen, 1804		
Tipula livida van der Wulp, 1859		
Tipula truncata Loew, 1873		

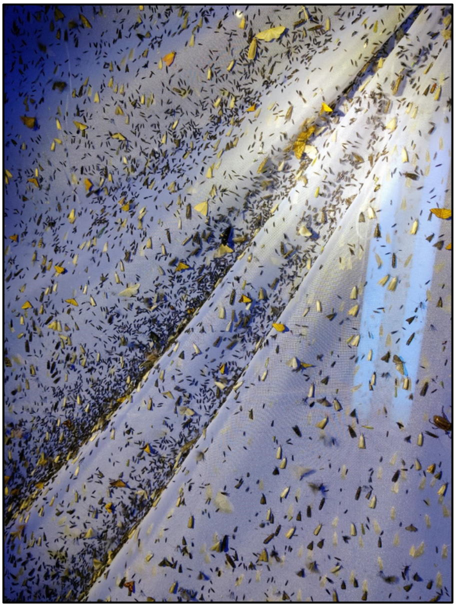

Figure 2. Impression from light-trapping at Ulog. The small black Trichoptera mainly belong to the genus *Agapetus*. Subsampling resulted in estimated 40,000 specimens of Trichoptera in one light trap at site »Swimming Beach«.
Slika 2. Primer svetlobne pasti v Ulogu. Majhne črne Trichoptere večinoma pripadajo rodu *Agapetus*. Rezultat podvzorčenja je bilo približno 40.000 primerkov Trichoptera v eni svetlobni pasti na lokaciji »Swimming Beach«.
Slika 2. Primjer sa svjetlosne zamke kod Uloga. Male crne trichoptera uglavnom pripadaju rodu *Agapetus*. Poduzorkovanje je rezultiralo sa oko 40.000 primjeraka Trichoptera u jednoj svjetlosnoj zamci na lokaciji »Plaža«.

Figure 3. Stony substrates covered by turtle-shaped cases of the dominant genus *Agapetus* at the Neretva
Slika 3. Kamnit substrat v Neretvi, prekrit z želvastimi ohišji prevladujočega rodu *Agapetus*.
Slika 3. Kamene podloge prekrivene kućicama oblika kornjače dominantnog roda *Agapetus* na Neretvi.

Discussion

In total, 103 species of Trichoptera, Plecoptera, Ephemeroptera and Diptera were found in a short, five-day survey conducted along the upper course of the Neretva River, the area where construction of hydropower plants is ongoing and planned. The majority (82 species) are rheobiontic species, indicating their vulnerability to damming. More than one third of all species (32) are endemic to the Balkans, many of which have a very restricted distribution. As the effects of the planned hydropower plants cannot be evaluated due to lack of information and as the spatial distribution of specific species is widely unknown, the loss of individual populations must be assumed to result in the potential extinction of specific species.

Allotrichia marinkovicae Malicky, 1977 for example - documented in the area by Stanic-Koštroman et al. (2015) - is known from very few sites worldwide (Neu et al. 2018) and the very rare Plecoptera *Isoperla bosnica* Aubert, 1964 is only known from the holotype male, collected in Bosnia (Murányi 2011).

The Ephemeroptera sample from the River Neretva is comparatively small, containing 188 specimens (135 larvae, 55 imagines and subimagines) from 19 taxa, reflecting approximately two thirds of the expected diversity for this river. The mayfly community observed indicates rhithralic conditions according to the rhithron-potamon-(Huet 1949; Illies 1961; Illies & Botosaneanu 1963; Bauernfeind et al. 2017); it should be remembered, however, that our knowledge of biocenoses and biocenotic regions in Mediterranean river systems is rather limited at present. Of the taxa collected, 37% are considered to represent taxa endemic to the Balkans, the rest of the species shows European or western Palaearctic distribution patterns (Eurosiberian faunal elements).

No recent checklist of Bosnian mayflies is available, but judging from Puthz (1980) and subsequently published scattered records (Tanasijević 1970, 1979, 1981a, 1981b, 1985a, 1985b; Bauernfeind 1991; Vilenica et al. 2018) approximately 45 taxa have been recorded so far from Bosnia and Herzegovina (including the present sample). Nomenclature follows Bauernfeind & Soldán (2012), the material has been deposited at the Natural History Museum Vienna.

Selected mayfly taxa deserve specific attention: *Baetis lutheri* is widely distributed throughout Europe, with closely related *B.* (*B.*) *mirkae* Soldán and Godunko, 2008 described from Cyprus (and reported subsequently also from the Island of Rhodos; Soldán & Godunko 2009). The present material (larvae) differs slightly from central European material and rather approaches *B. mirkae.* The material, however, is limited and differences may most probably be explained by intraspecific variation. Another taxon, *Habroleptoides filipovicae* Gaino & Sowa, 1985 was so far only known from the type locality (Vodice, Zlatibor Mountains, West Serbia) and from Greece (Vrisochori, Mount Tymphi, Pindos, 39.933331° N, 20.899991° E (Bauernfeind 2003)). The winged stages are easily recognized by the peculiar shape of the hind wings.

Crane flies (Tipuloidea) are a species-rich group, with over 1250 species in Europe (Oosterbroek 2023). Crane fly species occur in a wide range of habitats, but many are associated with aquatic and semiaquatic habitats or their surroundings. Biology and ecology of many species are still unknown. This is especially true for species known only from the Mediterranean area and the Balkans. The Western Balkans represent the least studied area in the case of crane flies in Europe (Kolcsár et al. 2023a). Our knowledge on crane flies from Bosnia-Herzegovina is rudimentary, as only a few studies dealt with Tipuloidea in the last century, and so far, only 166 species have been confirmed (Kolcsár et al. 2021, 2023a, 2023b; Oosterbroek 2023).

Although only 57 adult crane fly specimens were collected at the Neretva River, several interesting species were documented. In total 16 species were identified, from which the first occurrence data of eitght species in Bosnia-Herzegovina were recently published (Kolcsár et al. 2023a, 2023b). Generally, crane flies have a wider distribution range compared to other strictly aquatic groups like Ephemeroptera, Trichoptera or Plecoptera, and most of the identified species have a broader distribution area. However, a few rare and poorly known species, namely *Idiocera jucunda*, *Idiocera lackschewitzi* and *Eloeophila miliaria* were collected, which are associated with aquatic or semi-aquatic habitats. *Idiocera jucunda* was known only from a few specimens collected in Albania, Greece, Italy (Sicily), and North Macedonia. Two species of *Ellipteroides* (*E. alboscutellatus* and *E. limbatus*) and *Thaumastoptera calceata* are associated with riparian woodlands and *Antocha alpigena* is a typical rheobiontic species.

The most remarkable crane fly species is a new, small-sized limoniid crane fly named *Baeoura neretvaensis* Kolcsár & d'Oliveira, 2023, which was collected in high numbers along the Neretva River, representing 65% of the total specimens. This newly described species was also found in Montenegro and Slovenia, but only with a few individuals, showing that the Neretva River represents a good habitat for this interesting new taxon. For this reason, this new species was named after the River Neretva. Members of the genus *Baeoura* are very rare in Europe, and their distribution is mainly restricted to the Mediterranean region. The larval habitat of the European species is unknown; however, data of adults and literature data of an African species suggest that the larvae are rheobiont, and associated with streams and rivers with gravelly and sandy substrates (Kolcsár et al. 2023b).

Regarding Trichoptera, up to now 74 species are documented for the Neretva catchment. During the science week 59 species were found (Tab. 2), Stanic-Koštroman et al. (2015) listed 15 additional species from the lower Neretva.

Besides the high diversity, the abundance of aquatic insects is remarkable and outstandingly high (40,000 specimens trapped in a single night). Stanic-Koštroman et al. (2015) collected 4,813 specimens in total with light-traps at seven sites during two years (March 2002 to March 2003, every 15 days) in the lower part of the Neretva (below the reservoir Mostarsko jezero), where land-cover changes from natural dense forest to mainly agricultural areas. To what extent methodological differences or anthropogenic impacts like the damming of the river and the reservoir as well as the pulsed water release led to this density-loss can only be speculated, but the striking difference underlines the unique ecological condition of the upper Neretva. A vague indication of the effects of sudden water-level changes due to pulse releases might be the drastic reduction of the surface-dwelling grazer of the genus *Agapetus*. In the upper Neretva at least one third of the 40,000 collected specimens belonged to the species *A. slavorum* and *A. ochripes*. Below the dam, Stanic-Koštroman et al. (2015) collected in total three specimens of *A. ochripes* and none of *A. slavorum*.

Identification of one huge *Perla* female, about one third larger than the syntopic *Perla marginata* females, is still ongoing. Furthermore, *Isoperla* specimens from the tributary Krupac belonging to the *Isoperla tripartita* group represent most probably a new species, but this species-rich group is in urgent need of revision before any new taxon should be added.

Our results indicate an undisturbed riverine ecosystem with extremely high productivity. The high biomass transfer from the aquatic environment to the terrestrial area represents essential food resources not only for fishes but also for spiders, amphibians, bats and birds amongst others. The importance of these intact linkages between aquatic and terrestrial ecosystems has hardly been investigated, although they seem to be essential for a healthy and resilient environment.

Damming the Neretva would significantly change the benthic communities and especially decrease the number of rheobiontic species – which are the dominant benthic organisms based on our study - and consequently disturb the ecological processes permanently.

Dams have immense and severe consequences on important aspects of riverine ecosystems. They disrupt inherent processes like energy flow, nutrient cycling (Ru et al. 2020), sediment balancing, and connectivity (Poff & Hart 2002; Gilvear et al. 2016; Wohl 2019), and reduce hydrological dynamics (Graf 2006). This leads to serial discontinuity that severely impacts all sections of the river, not only the reservoir area but also up- and downstream sections (Schmutz & Moog 2018). Numerous studies underline large scale trends of community turnover, species loss and reduced abundance owing to habitat change, habitat fragmentation, and the disruption of the hydrological regime (e.g., Zwick 1992; Dynesius & Nilsson 1994; Monaghan et al. 2002; Nilsson et al. 2005; Liermann et al. 2012; Carvajal-Quintero et al. 2017).

Depending on operation regime, hydropower operations may result in abrupt changes in hydrology, according to power demands. This so-called hydropeaking frequently causes the drift (owing to increased hydraulic forces) and stranding (owing to the reduction of the wetted area) of fish and macroinvertebrates, considerably reducing biodiversity and biomass in the downstream sections of the river (Schülting et al. 2016; Greimel et al. 2018).

Changes in such crucial parameters as discharge, but also temperature, sediment distribution, nutrients, flow velocity etc. affect most riverine biota, especially macroinvertebrates. This potentially leads to a strong change of community composition.

The Balkans are considered to represent an aquatic biodiversity hotspot with high numbers of endemic species, but detailed information on the distribution of species are scarce and biological richness seems to be still underrated. Despite their importance for ecosystem services and intrinsic high value, natural river systems are under threat and frequently sacrificed for short-sighted economical projects like hydropower plants, while protection and conservation issues are largely neglected.

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Povzetek

Izjemno visoki raznovrstnost in številčnost vodnih žuželk poudarjata pomen zgornje Neretve kot neokrnjenega rečnega sistema, obdanega z gostimi naravnimi in skoraj naravnimi gozdovi. Skupaj je bilo zbranih 59 vrst Trichoptera, 10 vrst Plecoptera, 19 vrst Ephemeroptera in 16 vrst Diptera (Tipuloidea), med drugim tudi nova vrsta za znanost iz rodu *Baeoura*. 35 vrst je balkanskih endemitov, večina vrst je reobiontičnih in potrebujejo hiter vodni tok. Najpogostejši taksoni so vrste iz rodu Trichoptera *Agapetus* (*A. slavorum* in *A. ochripes*) – površinski strgalci, ki so občutljivi za nihanje vode in zato tudi na spremembe vodnega stanja zaradi hidroelektrarn.

Naši rezultati kažejo na neokrnjen rečni ekosistem z izjemno visoko produktivnostjo. Visok prenos biomase iz vodnega okolja na kopno je bistven vir hrane ne samo za ribe, temveč tudi za pajke, dvoživke, netopirje, ptice in druge živali. Pomen takšnih neokrnjenih povezav med vodnimi in kopenskimi ekosistemi je zelo slabo raziskan, pa čeprav se zdi, da so bistvenega pomena za zdravo in odporno okolje. Zajezitev Neretve bi bistveno spremenila bentoške združbe in predvsem zmanjšala število reobiontskih vrst – ki so po naši raziskavi dominantni bentoški organizmi – ter posledično trajno okrnila ekološke procese.

Balkan velja za vročo točko vodne biotske raznovrstnosti z velikim številom endemičnih vrst, vendar so podrobne informacije o razširjenosti vrst redke in zdi se, da je biološko bogastvo še vedno podcenjeno. Kljub pomembnosti za ekosistemske storitve in visoki intrinzični vrednosti so naravni rečni sistemi ogroženi in pogosto žrtvovani za kratkovidne ekonomske projekte, kot so hidroelektrarne, medtem ko so vprašanja zaščite in varstva ekosistemov večinoma zanemarjena.

Sažetak

Izuzetno velika raznovrsnost, kao i ogromno obilje vodenih insekata, naglašavaju značaj gornjeg toka Neretve kao netaknutog riječnog sistema uklopljenog u gustu prirodnu i skoro prirodnu šumu. Ukupno je prikupljeno 59 vrsta Trichoptera, 10 vrsta Plecoptera, 19 vrsta Ephemeroptera i 16 vrsta Diptera (Tipuloidea), od kojih je *Baeoura* sp. nova u nauci. Trideset pet vrsta su endemi Balkana, većina vrsta su reobionti i potrebna im je jaka struja vode. Najzastupljeniji taksoni su vrste trichopteran iz roda *Agapetus* (*A. slavorum* i *A. ochripes*) – površinski strugači osjetljivi na fluktuacije vode i samim tim osjetljive na promjene vodostaja uzrokovanog hidroelektranama.

Naši rezultati ukazuju na neporemećen riječni ekosistem sa izuzetno visokom produktivnošću. Visoki prenos biomase iz vodene sredine u kopneno područje predstavlja esencijalne prehrambene resurse ne samo za ribe već i za pauke, vodozemce, slijepe miševe i ptice, između ostalih. Važnost ovih netaknutih veza između vodenih i kopnenih ekosistema jedva da je istražena, iako se čini da su ključne za zdravu i otpornu sredinu. Pregrađivanje Neretve bi značajno promijenilo bentoske zajednice, a posebno smanjilo broj reobiontskih vrsta – koje su prema našem istraživanju dominantne bentoski organizmi – i posljedično trajno narušilo ekološke procese.

Smatra se da Balkan predstavlja žarište vodenog biodiverziteta sa velikim brojem endemskih vrsta, ali detaljne informacije o distribuciji vrsta su oskudne, a čini se da je biološko bogatstvo još uvijek podcijenjeno. Uprkos njihovoj važnosti za usluge ekosistema i visokoj stvarnoj vrijednosti, prirodni riječni sistemi su ugroženi i često žrtvovani za kratkovide ekonomske projekte poput hidroelektrana, dok se pitanja zaštite i očuvanja uglavnom zanemaruju.

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Contribution to the knowledge of water beetles sensu lato (Coleoptera) from the upper course of the Neretva River in Bosnia and Herzegovina

Michaela BROJER

2nd Zoological Department (Entomology), Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria;
E-mail: michaela.brojer@nhm-wien.ac.at

Abstract. Altogether, 57 species of water beetles sensu lato (Coleoptera) belonging to 11 families (Dytiscidae, Dryopidae, Elmidae, Georissidae, Gyrinidae, Hydraenidae, Helophoridae, Heteroceridae, Hydrophilidae, Limnichidae and Scirtidae) are presented based on a survey carried out in the upper section of the Neretva River. The Georissidae with two species, *Georissus costatus* Castelnau, 1840 and *G.* cf. *laesicollis* Germar, 1832, are reported for the first time from Bosnia and Herzegovina with certainty. Additional new records comprise *Hydraena vedrasi* Orchymont, 1931, *Limnebius paganettii* Ganglbauer, 1904, *Cercyon ustulatus* Preyssler, 1790, *Dryops lutulentus* (Erichson, 1847) and *Limnichus incanus* Kiesenwetter, 1851. The most speciose family collected were Hydraenidae with 18 species, seven endemic to the Balkans.

Key words: aquatic Coleoptera, new records, *Georissus costatus*, *Hydraena bosnica*, *H. vedrasi*, *Stenelmis puberula*

Izvleček. Prispevek k poznavanju vodnih hroščev sensu lato (Coleoptera) iz zgornjega toka reke Neretve v Bosni in Hercegovini – Skupno smo v raziskavi, opravljeni v zgornjem delu reke Neretve, našli 57 vrst vodnih hroščev sensu lato (Coleoptera), pripadajočih 11 družinam (Dytiscidae, Dryopidae, Elmidae, Georissidae, Gyrinidae, Hydraenidae, Helophoridae, Heteroceridae, Hydrophilidae, Limnichidae in Scirtidae). Prvič objavljamo zanesljiv podatek o najdbi družine Georissidae z dvema vrstama, *Georissus costatus* Castelnau, 1840 in *G.* cf. *laesicollis* Germar, 1832 v Bosni in Hercegovini. Dodatno navajamo nove podatke za *Hydraena vedrasi* Orchymont, 1931, *Limnebius paganettii* Ganglbauer, 1904, *Cercyon ustulatus* Preyssler, 1790, *Dryops lutulentus* (Erichson, 1847) in *Limnichus incanus* Kiesenwetter, 1851. Najbolj vrstno pestra družina so bili Hydraenidae z 18 vrstami, sedem endemičnih za Balkan.

Ključne besede: vodni hrošči (Coleoptera), novi podatki, *Georissus costatus*, *Hydraena bosnica*, *H. vedrasi*, *Stenelmis puberula*

Apstrakt. Doprinos poznavanju vodenih tvrdokrilaca sensu lato (Coleoptera) sa gornjeg toka rijeke Neretve u Bosni i Hercegovini – Tokom provedenog istraživanja u gornjem toku rijeke Neretve pronađeno je ukupno 57 vrsta vodenih tvrdokrilaca sensu lato (Coleoptera), koje pripadaju 11 porodica (Dytiscidae, Dryopidae, Elmidae, Georissidae, Gyrinidae, Hydraenidae, Helophoridae, Heteroceridae, Hydrophilidae, Limnichidae i Scirtidae). Po prvi put je u Bosni i Hercegovini zabilježena porodica Georissidae sa dvije vrste, *Georissus costatus* Castelnau, 1840 i *G.* cf. *laesicollis* Germar, 1832. Pored toga, izdvajamo nove podatke za *Hydraena vedrasi* Orchymont, 1931, *Limnebius paganettii* Ganglbauer, 1904, *Cercyon ustulatus* Preyssler, 1790, *Dryops lutulentus* (Erichson, 1847) i *Limnichus incanus* Kiesenwetter, 1851. Vrstama najbrojnija je porodica Hydraenidae sa ukupno 18 vrsta, sedam od njih su endemi Balkanskog poluostrva.

Ključne riječi: vodeni tvrdokrilci (Coleoptera), novi podaci, *Georissus costatus*, *Hydraena bosnica*, *H. vedrasi*, *Stenelmis puberula*

Introduction

Historically, the water beetle families Elmidae and Hydraenidae were well explored in Bosnia and Herzegovina (Apfelbeck 1894; Wanka 1908; Apfelbeck 1909; Reitter 1910; Pretner 1970; Berthélemy 1979). However, more recent publications on this region's water beetle fauna are relatively scarce. Modern faunistic surveys on aquatic Coleoptera, especially along the Neretva River could not be found. In biological water quality assessments and investigations on aquatic insect diversity undertaken in the Neretva River or its tributaries, aquatic Coleoptera species were not identified (Marković et al. 2022) or were omitted (Dedić et al. 2018). The whole Neretva River basin is recognized as a »Freshwater Key Biodiversity Area in the Mediterranean Basin Hotspot« (Darwall et al. 2014), but knowledge regarding aquatic Coleoptera of the upper course of the Neretva River is not available.

As Zaťovičová et al. (2004) pointed out, assemblages of aquatic Coleoptera examined by quantitative hydrobiological methods only show underrepresented diversity compared to results with additional qualitative sampling. Numerous microhabitats in streams constitute species reservoirs, but are mostly not examined by using standardised sampling techniques. Within rivers, beetles inhabit a wide range of habitats. Among others, substrate type, hydrogeology and current velocity are the upmost important abiotic factors influencing the diversity and distribution of water beetles in lotic habitats (Eyre et al. 1990; Bournaud et al. 1992).

Also, on a larger spatial scale, i.e., for Balkan countries, research on the water beetle fauna seems rather incomplete. This is suggested by numerous recent publications (e.g., Pešić & Pavićević 2005; Przewoźny et al. 2008, 2010; Pavićević & Pešić 2011; Mičetić Stanković et al. 2015; Scheers 2016) reporting the first records of particular water beetle species in various Balkan rivers, even of species rather well-known from other European countries. Notably detailed investigations have been carried out by Mičetić Stanković et al. (2018, 2019) of water beetle communities of karstic rivers, especially in Croatia.

The Neretva is the largest karst river in the Dinaric Alps with its source at Držirep village at an altitude of about 1,200 m a.s.l.. The headwaters are dominated by limestone and dolomite. The upper course is characterized by a nivo-pluvial flow regime, high water level fluctuations, very low water temperatures and natural waterflow. Subsequently, the river passes through deep canyons before being dammed by a series of hydroelectric-power plants. Finally it forms the Neretva Delta where it enters the Adriatic Sea (Skoulikidis et al. 2022).

The main goal of this study was to obtain a detailed overview of the water beetle species assemblage of the upper course of the Neretva River. Coleoptera treated within this survey encompass »True Water Beetles« as well as so-called »False Water Beetles« and »Shore Beetles« sensu Jäch (1998).

Materials and methods

The present study was conducted during the Neretva Science Week (29.6.–3.7.2022), organized by the Scientists for Balkan Rivers Network within the Save the Blue Heart of Europe campaign.

Following the aim to obtain a comprehensive overview of water beetles along the upper stretch of the Neretva River, sampling was carried out qualitatively by using a handnet (mesh width 500 µm) covering various habitats (riffles, pools, detritus, moss, dead wood, rootlets, bank substrates, boulders protruding above water's surface, rocks, dead wood) at each of nine localities in total (Tab. 1, Figs. 1, 2). Sampled material was preserved in vials with 96% alcohol in the field. Sampling time mostly varied between one and two hours at each locality. Sampling localities 1-6, 8 and 10 are restricted to the upper course of the Neretva River, with the most upstream locality at Krupac (Loc1) and the most downstream locality at Tajoraft (Loc10) at Glavatičevo village about 17 km southeast from Konjic. No samples were taken at intended localities 7 and 9. Additionally, samples were taken at Krupac stream (Kru), a right-bank tributary to the Neretva, about 500 meters upstream of the confluence with the Neretva at locality 1.

Table 1. Sampling localities for water beetles s.l. along the upper course of the Neretva River and its tributary Krupac stream.
Tabela 1. Lokacije vzorčenja vodnih hroščev s.l. vzdolž zgornjega toka reke Neretve in pritoka Krupac.
Tabela 1. Lokacije uzorkovanja vodenih kornjaša s.l. duž gornjeg toka rijeke Neretve i njene pritoke Krupac.

Locality name	Locality code	Latitude	Longitude	Altitude (m)	Date of sampling
Krupac (tributary to Neretva)	Kru	43.3309	18.4289	806	29.6.2022
Neretva (Krupac)	Loc1	43.3240	18.4305	790	29.6.2022
Neretva (Old Wooden Bridge)	Loc2	43.3652	18.3700	710	30.6.2022
Neretva (Cerova)	Loc3	43.3789	18.3562	695	30.6.2022
Neretva (Ulogski Buk)	Loc4	43.4053	18.3230	675	1.7.2022
Neretva (Swimming Beach)	Loc5	43.4241	18.3084	640	1.7.2022
Neretva (Nedavic)	Loc6	43.4580	18.3212	646	1.7.2022
Neretva (Brijestov Bridge)	Loc8	43.4823	18.2267	435	2.7.2022
Neretva (Tajoraft)	Loc10	43.5314	18.0817	346	3.7.2022

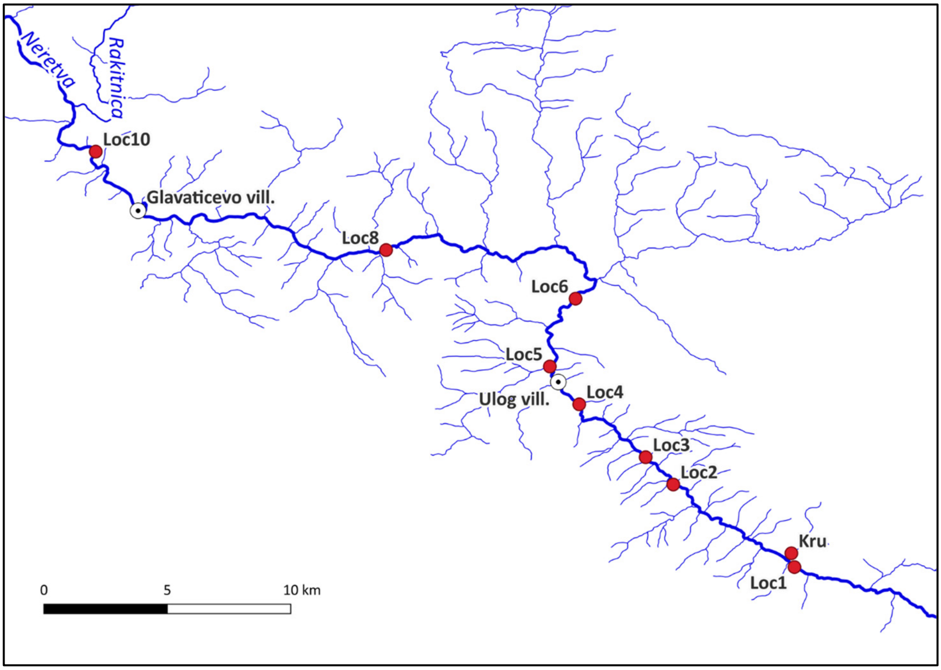

Figure 1. Map of the upper course of the Neretva River with sampling locations of water beetles s.l. marked with red dots. Locality codes as in Tab. 1. Abbreviations: vill. - village.
Slika 1. Zemljevid zgornjega toka reke Neretve, lokacije vzorčenja vodnih hroščev s. l. so označene z rdečimi pikami. Kode lokalitet kot v tabeli 1. Okrajšave: vill. – vas (village).
Slika 1. Karta gornjeg toka rijeke Neretve sa lokacijama uzorkovanja vodenih kornjaša s.l. označenim crvenim tačkama. Šifre lokaliteta su kao u Tab. 1. Skraćenice: vill. – selo (village).

Specimens were examined using a binocular Wild M-10 microscope and a light microscope Olympus BH-2. Macroscopic figures (Figs. 3, 4) are multilayer photographs generated by using a stereomicroscope (Leica MZ16) connected to a camera (DFC490) and were processed and edited with AutoMontage Pro and Adobe Photoshop 7.0. The Microscopic multilayer photograph (Fig. 5) was processed by using a microscope (Nikon Eclipse 80i) connected to a camera (Nikon DS-Fi1). The map (Fig. 1) was prepared using QGIS freeware (version 3.28.0-Firenze).

Various monographs, revisions and original descriptions were used for species identification, and the results were verified by comparison with specimens stored in the Coleoptera Collection of the Natural History Museum Vienna (NHMW). Voucher specimens of this survey are also stored in this collection. Additional material is stored in 96% alcohol and can be used for further molecular analysis. Helophoridae were determined by Robert Angus (Natural History Museum London), and Dytiscidae by Günther Wewalka and Helen Shaverdo (NHMW).

Distributional data was taken from Nilsson & Hájek (2022) (Dytiscidae), Hájek & Fery (2022) (Gyrinidae), Jäch & Skale (2015) (Hydraenidae), Przewoźny (2022) (Helophoridae, Georissidae, Hydrophilidae), Klausnitzer (2016) (Scirtidae), Jäch et al. (2016) (Elmidae), Kodada & Jäch (2016) (Dryopidae), Hernando & Ribera (2016) (Limnichidae) and Mascagni (2016) (Heteroceridae).

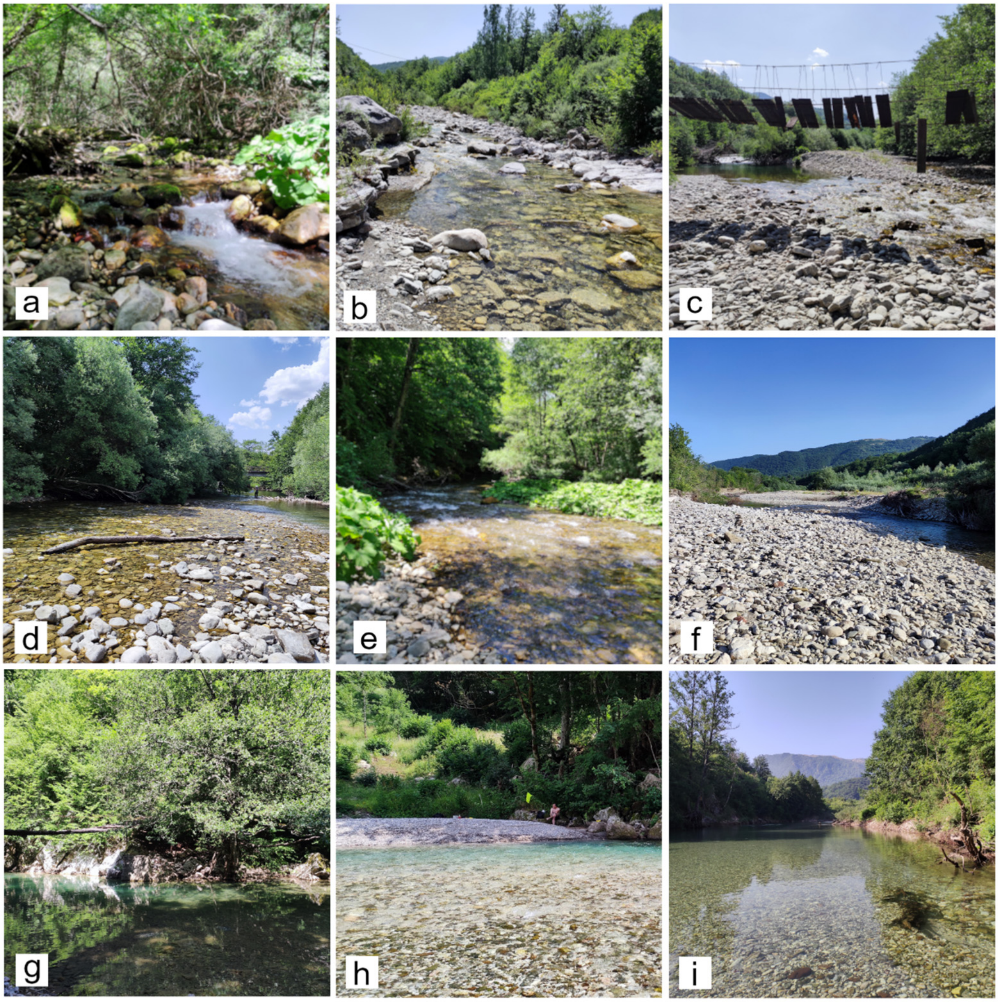

Figure 2. Photographs taken at sampling localities of water beetles s.l. at two rivers in BIH: at Krupac stream: a – trib.to Neretva (Kru) and Neretva River: b – Krupac (Loc1), c – Old Wooden Bridge (Loc2), d – Cerova (Loc3), e – Ulogski Buk (Loc4), f – Swimming Beach (Loc5), g – Nedavic (Loc6), h – Brijestov Bridge (Loc8), I – Tajoraft (Loc10).

Slika 2. Fotografije, posnete na lokacijah vzorčenja vodnih hroščev (s.lat.) ob dveh rekah v BIH: ob potoku Krupac: a – pritok Neretve (Kru) in ob reki Neretvi: b – Krupac (lok.), c – Stari leseni most (lok. 2), d – Cerova (lok. 3), e – Ulogski Buk (lok. 4), f – Plaža za kopanje (lok. 5), g – Nedavic (lok. 6), h – Brijestovski most (lok. 8), i – Tajoraft (lok. 10).

Slika 2. Fotografije snimljene na lokacijama uzorkovanja vodnenih tvrdokrilaca (s.lat.): a – prit. Neretve (Kru) i Neretvi: b – Krupac (Loc1), c – Stari drveni most (Loc2), d – Cerova (Loc3), e – Uloški Buk (Loc4), f – Plaža za plivanje (Loc5), g – Nedavić (Loc6), h – Most Brijestov (Loc8), i – Tajoraft (Loc10).

Results and discussion

Water beetle assemblage of the upper Neretva River

In total, approximately 1,000 adult specimens were examined from all sampling localities of the Neretva River and Krupac stream. Fifty-five species were identified, only two with caution due to the lack of male specimens or proper revisions (see below). Table 2 presents a list of the 57 recorded species, representing members of 11 families (Dytiscidae, Gyrinidae, Helophoridae, Georissidae, Hydrophilidae, Hydraenidae, Scirtidae, Elmidae, Dryopidae, Limnichidae, Heteroceridae).

With 18 species, Hydraenidae were the most species-rich group, followed by Elmidae with 12 species, and by Hydrophilidae (7), Dytiscidae (6) and Helophoridae (5), Gyrinidae, Georissidae and Scirtidae (2) and Dryopidae, Limnichidae and Heteroceridae had only one representative species. *Hydraena minutissima*, *H. morio*, *H. nigrita*, *H. subintegra*, *H. vedrasi*, *Esolus parallelepipedus*, *Limnius* cf. *intermedius* and *L. opacus* were collected at almost every locality. Other species were found along the localities more randomly or even only at specific conditions at a few or even at just a single locality.

Across the various localities, species numbers ranged from a low of 9 at Krupac stream to 38 at locality 2. The remaining localities had species numbers ranging between 19 and 25 species.

The rather low number of species in Krupac stream can be explained by the spring character of this locality. This was indicated by tufa deposits and a very low water temperature of - 8.1°C in comparison to temperatures between 13.2 and 18.3°C at remaining localities (del Campo et al., this issue). These conditions provide suitable habitat for only specialized species such as *Elmis bosnica*, a typical crenobiont species of Balkan rivers (Mičetić Stanković et la. 2021) and *Esolus angustatus*. Krupac stream had the most distinct water beetle species assemblage in comparison to all other localities.

Table 2 (next page). Species of water beetles s.l. collected at sampling localities along the upper Neretva River and Krupac stream (Kru). Column and row totals represent the number of species per locality and number of localities per species, respectively. Additional remarks identify a Balkan endemic species (BAE), a species endemic to Bosnia and Herzegovina (BHE), a new record for Bosnia and Herzegovina (NRBH). Locality codes as in Tab. 1.

Tabela 2 (naslednja stran). Vrste vodnih hroščev s.l., zbranih na vzorčiščih vzdolž zgornje Neretve in potoka Krupac (Kru). Vsota stolpcev in vrst ponazarja število vrst na lokaliteto oziroma število lokalitet na vrsto. Dodatne opombe označujejo balkanske endemite (BAE), endemite Bosne in Hercegovine (BHE), nov podatek za Bosno in Hercegovino (NRBH). Kode lokalitet kot v tabeli 1.

Tabela 2 (sljedeća stranica). Vrste vodenih tvrdokrilaca s.l. prikupljene na lokacijama uzorkovanja duž gornjeg toka Neretve i pritoke Krupac (Kru). Zbir u kolonama i redovima predstavljaju broj vrsta po lokalitetu i broj lokaliteta po vrsti. Dodatne napomene predstavljaju endemska vrsta za Balkan (BAE), endemska vrsta za Bosnu i Hercegovinu (BHE), novi podatak za Bosnu i Hercegovinu (NRBH). Šifre lokaliteta su iste kao u Tab. 1.

Species	Remarks	Kru	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc8	Loc10	Number of localities
DYTISCIDAE											
Bidessus delicatulus (Schaum, 1844)			●								1
Deronectes platynotus (Germar, 1834)			●	●						●	3
Hydroporus jonicus Miller, 1862				●							1
Hydroporus pubescens (Gyllenhal, 1808)				●	●						2
Nectoporus sanmarkii (Sahlberg, 1826)				●	●				●		3
Platambus maculatus (Linnaeus, 1758)				●			●				2
GYRINIDAE											
Gyrinus caspius Ménétriés, 1832				●							1
Orectochilus villosus (Müller, 1776)			●	●		●				●	4
HYDRAENIDAE											
Hydraena cf. *bosnica* Apfelbeck, 1909 (♀)	BHE	●						●			2
Hydraena egoni Jäch, 1986			●	●	●	●				●	5
Hydraena gracilis Germar, 1824										●	1
Hydraena minutissima Stephens, 1829			●	●	●	●	●	●	●	●	8
Hydraena morio Kiesenwetter, 1849		●	●	●	●	●	●	●	●	●	9
Hydraena nigrita Germar, 1824		●	●	●	●			●	●	●	7
Hydraena pygmaea Waterhouse, 1833		●		●	●	●		●	●		6
Hydraena subintegra Ganglbauer, 1901	BAE	●	●	●	●	●		●	●		7
Hydraena vedrasi Orchymont, 1931	BAE, NRBH		●	●	●	●	●	●	●	●	8
Limnebius fallaciosus Ganglbauer, 1904	BAE		●	●	●		●		●		5
Limnebius myrmidon Rey, 1883			●	●	●		●				4
Limnebius paganettii Ganglbauer, 1904	BAE, NRBH			●	●		●				3
Limnebius perparvulus Rey, 1884							●				1
Ochthebius dalmatinus Ganglbauer, 1904	BAE									●	1
Ochthebius melanescens Dalla Torre, 1877				●		●			●		3
Ochthebius metallescens Rosenhauer, 1847				●				●	●	●	4
Ochthebius montenegrinus Ganglbauer, 1901	BAE							●	●		2
Ochthebius sidanus Orchymont, 1942			●	●			●		●		4
HELOPHORIDAE											
Helophorus aquaticus (Linnaeus, 1758)		●		●					●		3
Helophorus discrepans Rey, 1885				●							1
Helophorus dorsalis (Marsham, 1802)				●							1
Helophorus montenegrinus Kuwert, 1885		●	●	●	●		●		●		6
Helophorus obscurus Mulsant, 1844									●		1

Species	Remarks	Kru	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc8	Loc10	Number of localities
GEORISSIDAE											
Georissus costatus Castelnau, 1840	NRBH						●		●		2
Georissus cf. *laesicollis* Germar, 1832	[NRBH]			●					●		2
HYDROPHILIDAE											
Cercyon ustulatus (Preyssler, 1790)	NRBH			●			●				2
Laccobius albipes Kuwert, 1890							●				1
Laccobius alternus Motschulsky, 1855			●	●			●		●		4
Laccobius bipunctatus Fabricius, 1775									●		1
Laccobius obscuratus Rottenberg, 1874			●	●	●						3
Laccobius simulatrix Orchymont, 1932									●		1
Laccobius striatulus Fabricius, 1801				●				●			2
SCIRTIDAE											
Hydrocyphon deflexicollis (Müller, 1821)							●				1
Hydrocyphon novaki Nyholm, 1867				●							1
ELMIDAE											
Elmis aenea (Müller, 1806)			●	●			●	●		●	5
Elmis bosnica (Zaitzev, 1908)		●	●	●							3
Elmis maugetii Latreille, 1802					●	●		●	●	●	5
Elmis rioloides (Müller, 1806)					●	●	●	●		●	5
Esolus angustatus (Müller, 1821)		●									1
Esolus parallelepipedus (Müller, 1806)			●	●	●	●	●	●	●	●	8
Limnius cf. *intermedius* Fairmaire, 1881			●	●	●	●	●	●	●	●	8
Limnius opacus Müller, 1806			●	●	●	●	●	●		●	7
Limnius volckmari (Panzer, 1793)					●	●	●	●		●	5
Riolus cupreus (Müller, 1806)			●					●			2
Riolus subviolaceus (Müller, 1817)			●	●	●	●		●	●	●	7
Stenelmis puberula Reitter, 1887							●				1
DRYOPIDAE											
Dryops lutulentus (Erichson, 1847)	NRBH			●							1
LIMNICHIDAE											
Limnichus incanus Kiesenwetter, 1851	NRBH									●	1
HETEROCERIDAE											
Augyles pruinosus (Kiesenwetter, 1851)				●			●		●		3
Total number of species		**9**	**22**	**38**	**21**	**15**	**23**	**19**	**25**	**19**	

While some species within this survey were mostly collected in the riffle zones and areas of higher water flow velocity (*Nectoporus sanmarkii*, *Hydraena vedrasi*, *H. subintegra, Esolus parallelepipedus*, *Limnius intermedius*, *L. opacus*, *L. volckmari*, *Stenelmis puberula*), other habitats preferred by different species included areas with lower water flow velocities (*Orectochilus villosus*, *Deronectes platynotus*, *Hydraena egoni*, *H. nigrita*, *H. morio*), mossy rocks and boulders (*Elmis aenea*, *E. bosnica*, *E. maugetii*, *E. rioloides*, *Riolus cupreus*, *R. subviolaceus*), pools with very low water flow velocity or even of a stagnant character (most Dytiscidae, *Gyrinus caspius*), partly submerged rocks in rather fast currents (*Ochthebius melanescens*, *O. montenegrinus*), rootlets (*Hydraena pygmaea*, *H. minutissima*), dead wood (*Limnichus incanus*), hygropetric habitats (*Ochthebius dalmatinus*, *O. metallescens*), rocks along the river bank (*Hydrocyphon* spp.), gravel banks (*Ochthebius sidanus*, *Limnebius* spp., *Dryops lutulentus*, *Bidessus delicatulus, Laccobius* spp.), moist sandy and muddy river banks (*Georissus* spp., *Augyles pruinosus*, *Laccobius* spp.) and erosion pools (*Helophorus* spp., *Laccobius* spp.). The availability of these various habitats reflect the number of species sampled within each locality. Furthermore, the presence of a variety of habitats along the course of the upper Neretva river, all of them being created by the natural flow of the river, inhabited by a specialized assemblage of aquatic Coleoptera, explains the high overall species richness found in this survey.

Elmidae are generally good indicators of longitudinal zonation along rivers and streams (Berthélemy 1964; Brojer et al. 2017). Small differences in species occurrence along the river's course, even if only represented by a relatively short section of the whole Neretva River, can be observed. While *Elmis bosnica* and *Esolus ang,ustatus* were found only in the uppermost localities, most other species of Elmidae were collected at almost all localities from Locality 1 down to Locality 10, except Krupac stream. *Elmis maugetii*, *E. rioloides* and *Limnius volckmari* were sampled further downstream, firstly at Locality 3.

A noteworthy finding is the occurrence of *Stenelmis puberula* (Fig. 3), a rarely collected species (Kodada et al. 2004) and, until today, reported from the Balkan region from only Bosnia and Herzegovina (Jäch et al. 2016) and recently from Bulgaria (Novaković et al. 2020). *Stenelmis* species are described as litho-rheophilic inhabitants of mountain streams with considerable water discharge, strong current and mainly stony substrates (Shapovalov et al. 2015). This agrees well with the habitat of Locality 5, where *Stenelmis puberula* was found within this survey.

Figure 3. Habitus photograph of *Stenelmis puberula*, collected at Loc5 (Swimming Beach) at the upper course of the Neretva River.
Slika 3. Fotografija habitusa *Stenelmis puberula*, z lokalitete 5 (Plaža za kopanje) v zgornjem toku reke Neretve.
Slika 3. Fotografija habitusa *Stenelmis puberula*, zabilježena na lokalitetu 5 (Plaža) na gornjem toku rijeke Neretve.

Balkan endemics

Hydraenidae are known to include numerous species with restricted distributions (Jäch & Balke 2008; Ribera et al. 2011). One-third of all hydraenid species collected in the course of this survey (*Hydraena subintegra*, *H. vedrasi*, *Limnebius fallaciosus*, *L. paganettii*, *Ochthebius dalmatinus*, *O. montenegrinus*) are Balkan endemics.

Unfortunately, one species of the *Hydraena*-lineage could not be identified with certainty, as only six female specimens were found. Based on external morphological features (elytral apices, tergit X and gonocoxites) it is probably *Hydraena bosnica*, a species especially noteworthy as it is endemic to Bosnia and Herzegovina and only known from the southeastern part of the country (Mičetić Stanković & Jäch 2012). This species was originally described from Krupa Valley at Pazarić village and Jablanica (Apfelbeck 1909), located in the Neretva basin. Specimens from Serbia and the Republic of Macedonia listed in the Palearctic Catalogue of Coleoptera (Jäch & Skale 2015) obviously belong to other species (M.A. Jäch, pers. comm.). The species has so far not been barcoded.

Counting two endemic species, known exclusively from Bosnia and Herzegovina, *Hydraena bosnica* and also *H. bimagua* (Jäch 1986), which is only known from the type locality close to Gacko village (Jäch 1986), further investigations, especially on mountain streams could reveal more species, as demonstrated by recent descriptions of new species with very restricted distribution ranges in the Dinaric Alps (Jäch & Díaz 2012; Mičetić Stanković & Jäch 2012; Freitag et al. 2021).

A comparison with studies carried out in Cetina River and Plitvice Lakes National Park (Mičetić Stanković et al. 2018, 2019) in Croatia, with the Neretva River basin, located in the European hydro-ecoregion of the Yugoslavian Karst (Wasson et al. 2007), shows that the water beetle assemblage of the upper course of the Neretva River is more closely linked to the Mediterranean region, especially due to the occurrence of a high number of Balkan endemics.

New records for the fauna of Bosnia and Herzegovina

By thoroughly washing sand from shorelines along the Neretva, specimens of *Georissus* Latreille, 1809 were collected at three localities (Loc2, Loc5 and Loc8).

Because of their somewhat cryptic lifestyle and tiny size, Georissidae are often overlooked. In Europe, they are mostly found on wet, muddy, or sandy shores of running and standing waters and sometimes near temporary pools. In the Catalogue of Palaearctic Coleoptera (Przewoźny 2022), no species of *Georissus* are listed for Bosnia and Herzegovina. This is surprising given the existence of historical specimens from Bosnia and Herzegovina (Ildiža, Sarajevo, Čelić), with some specimens dating back to the end of the 19th century (Apfelbeck 1895), in the collection of the Natural History Museum Vienna. Only *Georissus substriatus* (Heer, 1841) is imprecisely listed for the »Balkans«, without any precise data. *Georissus costatus* Castelnau, 1840 and *Georissus laesicollis* Germar, 1832 have not yet been recorded for Bosnia and Herzegovina.

The European members of *Georissus* have never been revised taxonomically. It has been assumed that the *G. laesicollis* group may include some undescribed species (Fikáček & Falamarzi 2010; Boukal et al. 2012). The specimens found at the Neretva can only be tentatively determined as *Georissus* cf. *laesicollis*. As *Georissus costatus* (Fig. 4a) was redescribed by Litovkin and Fikáček (2012) this species can be determined with certainty by aedeagal features (Fig 4b) and should be added to the species list of Bosnia and Herzegovina.

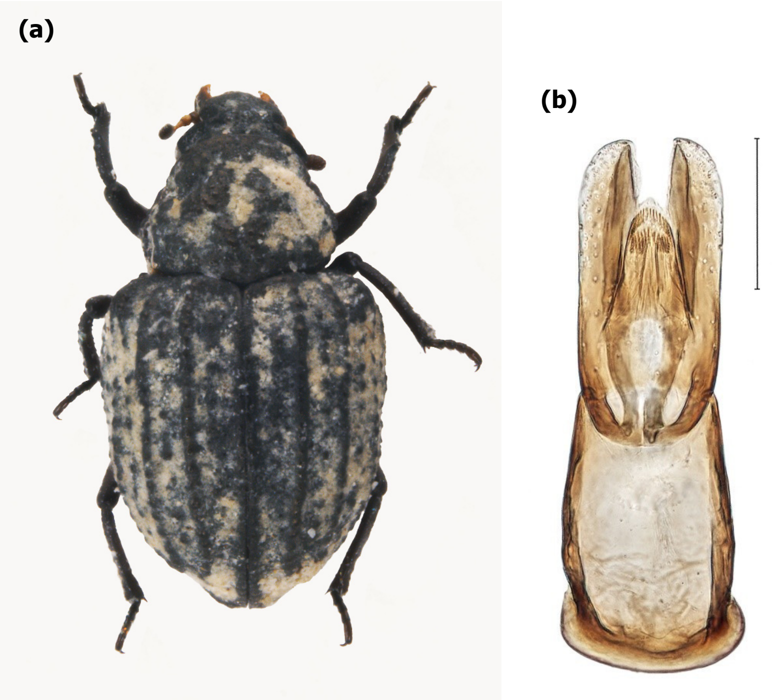

Figure 4. (a) Habitus photograph of *Georissus costatus*, collected at Locality 5 (Swimming Beach) and Locality 8 (Brijestov Bridge) at the upper course of the Neretva River. (b) Aedeagus of *Georissus costatus* (scale: 0.1 mm).
Slika 4. (a) Fotografija habitusa *Georissus costatus* z lokalitete 5 (Plaža za kopanje) in lokalitete 8 (Brijestovski most) v zgornjem toku reke Neretve. (b) Aedeagus *Georissus costatus* (merilo: 0,1 mm).
Slika 4. (a) Fotografija habitusa *Georissus costatus*, zabilježena na lokalitetu 5 (Plaža) i lokalitetu 8 (Brijestov most) na gornjem toku rijeke Neretve. (b) Aedeagus *Georissus costatus* (promjer: 0,1 mm).

The specimens of *Georissus* at the Neretva were found in sandy and muddy sediments along the riverbank – a habitat existing due to natural fluvial dynamics. Litovkin & Fikáček (2012) report their findings of *Georissus costatus* in Russia from undisturbed riverine habitats in unpolluted and remote areas. Hebauer & Klausnitzer (1998) point out their importance of being indicators of river pollution without further specification.

Surprisingly, *Hydraena vedrasi*, being one of the most common species at the upper Neretva, has not yet been reported in Bosnia and Herzegovina (Jäch & Skale 2015). Being well known, especially in southern Balkan countries, the locality in the Neretva seems to be the most northern one known to date (compare Trizzino et al. 2013).

An additional species collected within this survey and not found in respective catalogs listing Bosnian-Herzegovinian fauna is *Limnebius paganettii*. This species is known from several Balkan countries with its main occurrence in the south (Jäch & Skale 2015) and must be included in the species list of Bosnia and Herzegovina. Similarly, three additional riparian species, *Cercyon ustulatus*, *Dryops lutulentus,* and *Limnichus incanus* (Hernando & Ribera 2016; Kodada & Jäch

2016; Przewoźny 2022), should be included in the species list of Bosnia and Herzegovina. Notably, *Dryops lutulentus* was already reported for Bosnia and Herzegovina in Wanka (1908). Cases like *Dryops lutulentus* and the above mentioned *Georissus*, of which historical material was found in the collection of the Natural History Museum Vienna, suggest that historical material of these families housed in museum collections are still not appropriately considered.

Povzetek

V tednu »Neretva Science Week 2022« junija in julija 2022 smo kvalitativno vzorčili vodne hrošče sensu lato (Coleoptera) na devetih lokalitetah v zgornjem delu reke Neretve, vključno s pritokom Krupac. Glavni cilj študije je bil pridobiti vpogled v vrstno pestrost vodnih heroščev zgornjega toka reke Neretve.

Zgodovinsko so bile družine vodnih hroščev Elmidae in Hydraenidae v Bosni in Hercegovini, vključno z območjem Neretve, dobro raziskane. Vendar pa so novejše favnistične raziskave, še posebej vzdolž reke Neretve, redke, tudi zato, ker vodni hrošči (Coleoptera) niso vključeni v ocene biološke kakovosti vode in v raziskave o raznolikosti vodnih žuželk, opravljenih na spodnji Neretvi ali katerem koli njenem pritoku.

V tej študiji smo pregledali približno 1000 odraslih primerkov in določili 55 različnih vrst (le pri dveh z nekoliko zadržka zaradi pomanjkanja samcev ali ustreznih revizij), iz 11 družin (Dytiscidae, Gyrinidae, Helophoridae, Georissidae, Hydrophilidae, Hydraenidae, Scirtidae, Elmidae, Dryopidae, Limnichidae, Heteroceridae). Najbolj številčna družina z 18 vrstami so bili Hydraenidae, sledili so jim Elmidae. Vrste *Hydraena minutissima* Stephens, 1829, *H. morio* Kiesenwetter, 1849, *H. nigrita* Germar, 1824*, H. subintegra* Ganglbauer, 1901, *H. vedrasi* Orchymont, 1931, *Esolus parallelepipedus* (Müller, 1806), *Limnius cf. intermedius* Fairmaire, 1881 in *L. opacus* Müller, 1806 smo našli na skoraj vseh lokalitetah, druge vrste pa smo našli bolj ali manj naključno ali v nekaj primerih na nekaj ali celo zgolj na eni lokaciji. Število vrst na lokaliteto se je gibalo med 9 in 38. Celokupna vrstna pestrost je povezana z različnostjo habitatov, ki jih je ustvaril naravni tok reke; koliko the je na posamezni lokaliteti, se odraža v številu različlnih vrst, ki so bile tam najdene.

Sedem vrst Hydraenidae, najdenih med našo raziskavo, je endemičnih za Balkan. Med njimi je tudi *Hydraena* cf. *bosnica*, ki je endemična za Bosno in Hercegovino. Iz Bosne in Hercegovine prvič poročamo o več novih vrstah. Poleg *Hydraena vedrasi* Orchymont, 1931 so to večinoma vrste, specializirane za rečne bregove: *Georissus costatus* Castelnau, 1840, *G. cf. laesicollis* Germar, 1832, *Limnebius paganettii* Ganglbauer, 1904, *Cercyon ustulatus* Preyssler, 1790, *Dryops lutulentus* (Erichson, 1847) in *Limnichus incanus* Kiesenwetter, 1851. Še ena pomembna najdba je redko opažena vrsta *Stenelmis puberula*.

Sažetak

Tokom sedmice »Neretva Science Week 2022«, u junu i julu 2022. godine, kvalitativno smo uzorkovali vodene tvrdokrilce sensu lato (Coleoptera) na devet lokaliteta u gornjem toku rijeke Neretve, uključujući pritoku Krupac. Glavni cilj ovog istraživanja bio je da se dobije detaljan pregled sastava vrsta sa gornjeg toka rijeke Neretve.

Istorijski gledano porodice vodenih buba Elmidae i Hydraenidae bile su dobro istražene u Bosni i Hercegovini, uključujući i sliv Neretve. Međutim, nedostaju novija istraživanja faune, posebno duž rijeke Neretve, a posebno zato što vodeni tvrdokrilci (Coleoptera) nisu bili uključeni u biološku procjenu kvaliteta vode i istraživanja raznovrsnosti vodenih insekata koja se provode na donjem toku Neretve ili bilo kojoj od njenih pritoka.

U ovoj studiji, ukupno je pregledano oko 1.000 odraslih primjeraka, a identifikovano je 55 vrsta (samo dvije sa zadrškom zbog nedostatka muških primjeraka ili odgovarajućih revizija), koje predstavljaju 11 porodica (Dytiscidae, Gyrinidae, Helophoridae, Georissidae, Hydrophilidae, Hydraenidae, Scirtidae, Elmidae, Dryopidae, Limnichidae, Heteroceridae). Porodica Hydraenidae, sa 18 vrsta, bila je najbogatija porodica, a odmah iza nje je porodica Elmidae. Vrsta *Hydraena minutissima* Stephens, 1829, *H*. morio Kiesenwetter, 1849, *H. nigrita* Germar, 1824, *H. subintegra* Ganglbauer, 1901, *H. vedrasi* Orchymont, 1931, *Esolus parallelepipedus* (Müller, 1806), *Limnius cf. intermedius* Fairmaire, 1881 i *L. opacus* Müller, 1806 je pronađena na gotovo svakoj lokaciji a druge vrste su pronađene više nasumično ili samo u specifičnim uslovima na nekoliko ili čak samo na jednoj lokaciji. Broj vrsta na lokalitetima za uzorkovanje varirao je između 9 i 38. Ukupna raznovrsnost je rezultat različitih tipova staništa, stvorenih prirodnim tokom rijeke, a raspoloživost različitih staništa se odražava brojem vrsta na svakoj lokaciji.

Sedam vrsta Hydraenidae, sakupljenih tokom ovog istraživanja, su endemi Balkanskog poluostrva. To uključuje *Hydraena* cf. *bosnica*, koja je endem za Bosnu i Hercegovinu. Nekoliko vrsta je prvi put registrovano u Bosni i Hercegovini. Osim *Hydraena vedrasi* Orchymont, 1931, većina ovih vrsta su specijalizovane za riječne obale: *Georissus costatus* Castelnau, 1840, *G. cf. laesicollis* Germar, 1832, *Limnebius paganettii* Ganglbauer, 1904, *Cercyon ustulatus* Preyssler, 1790, *Dryops lutulentus* (Erichson, 1847) i *Limnichus incanus* Kiesenwetter, 1851. Još jedan vrijedan nalaz je rijetko zapažena vrsta *Stenelmis puberula*.

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Carabid beetles (Coleoptera: Carabidae) from the upper course of the Neretva River in Bosnia and Herzegovina

Wolfgang PAILL, Johanna GUNCZY

Universalmuseum Joanneum, Studienzentrum Naturkunde, Weinzöttlstraße 16, 8045 Graz, Austria;
E-mails: wolfgang.paill@museum-joanneum.at, johanna.gunczy@museum-joanneum.at

Abstract. During a four-day collection period, 89 ground beetle species were found in the upper course of the Neretva valley near Ulog. Many of them are classified as riparian floodplain specialists. A large proportion of these species, such as *Bembidion conforme*, have their main distribution in Central Europe, but are very rare in the Balkans. Consequently, these findings form important arguments revealing the upper Neretva to be of high importance in terms of nature conservation.

Key words: Carabidae, Bosnia and Herzegovina, Neretva, river, floodplain, nature conservation

Izvleček. Krešiči (Coleoptera: Carabidae) zgornjega toka reke Neretve v Bosni in Hercegovini – V štirih terenskih dneh smo v dolini zgornje Neretve, v okolici Uloga, našli 89 vrst krešičev. Mnogi od njih so ozko specializirani na obrežna poplavna območja. Veliko vrst, kot na primer *Bembidion conforme*, je pretežno razširjenih v srednji Evropi, na Balkanu pa so zelo redke. Posledično so te najdbe pomemben kazalec, da je zgornja Neretva naravovarstveno pomembno območje.

Ključne besede: Carabidae, Bosna in Hercegovina, Neretva, reka, poplavno območje, naravovarstvo

Apstrakt. Bauljari (Coleoptera: Carabidae) sa gornjeg toka rijeke Neretve u Bosni i Hercegovini – Tokom četverodnevnog terenskog rada, u gornjem toku Neretve kod Uloga pronađeno je 89 vrsta bauljara. Mnogi od njih su klasifikovani kao karakteristični za priobalne poplavne ravnice. Veliki dio ovih vrsta, kao što je *Bembidion conforme*, ima svoju glavnu rasprostranjenost u srednjoj Evropi, ali su vrlo rijetke na Balkanu. Posljedično, ovi nalazi predstavljaju važne argumente koji dokazuju da je gornji tok Neretve od velikog značaja u smislu očuvanja prirode.

Ključne riječi: Carabidae, Bosna i Hercegovina, Neretva, rijeka, poplavno područje, očuvanje prirode

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Introduction

The state of documentation of ground beetles from Bosnia and Herzegovina is moderately good. Although there is no annotated catalogue as for other Western Balkan countries (Ćurčić et al. 2007; Guéorguiev 2007a; Hristovski & Guéorguiev 2015), checklists from Drovenik & Peks (1999) and Lelo (2013) are available. A current overview of confirmed species is available from the Catalogue of Palaearctic Coleoptera (Löbl & Löbl 2017). In comparison, faunistic studies on the country's ground beetle fauna are rare. Bosnian carabid material is occasionally considered in the context of revisions (e. g. Jaeger 2007; Guéorguiev & Lohaj 2008; Bonavita & Vigna Taglianti 2010) and there are frequent descriptions of new species from caves (Ćurčić et al. 2012; Lohaj & Mlejnek 2012; Quéinnec & Ollivier 2021). Some papers also deal with the distribution of individual species within the genus *Carabus* (e. g. Jambrošić Vladić et al. 2018; Rapuzzi & Kleinfeld 2018; Kulijer et al. 2019). Beyond that, however, little is known about the composition of species communities from different regions or comparable habitats (e. g. Tabaković-Tošić 1996).

During the Neretva Science Week, which was organised and carried out by the Scientists for Balkan Rivers Network, there was an opportunity to work on ground beetles along the upper course of the Neretva River. Carabid beetles are known to inhabit floodplains along natural river systems in high species and specimen numbers. We focused on the question whether rare and sensitive species occur in the study area that would justify the protection of this near-natural river course.

Materials and methods

Study area

The study area is situated in the upper course of the Neretva River, in south-eastern Bosnia and Herzegovina, about 45 km east from Mostar. Five study sites were located along the river covering a distance of around 18 kilometres and stretching from Krupac, a small tributary that flows into the Neretva at 800 m a.s.l. to Ulog at an altitude of 640 m a.s.l. (Tab. 1).

Site 1b is located on the straight course of the Neretva and has developed narrow, coarse-grained gravel banks. At Sites 2 and 3, the Neretva shows a slightly meandering course with more or less extended, with high sun exposure, and mostly coarse-grained gravel banks. The Neretva is even wider and braided at Site 5, with fine-grained, sandy sediments deposited, in addition to gravel. Site 1a is a special location, a small shaded tributary stream with coarse-block banks and small spring brooks. A special location is also Site 4. It is located near the village of Ulog and is thus anthropogenically modified regarding riparian structures. Sampling was not carried out directly on the bank but rather in the area of an alluvial meadow, primarily by using light traps.

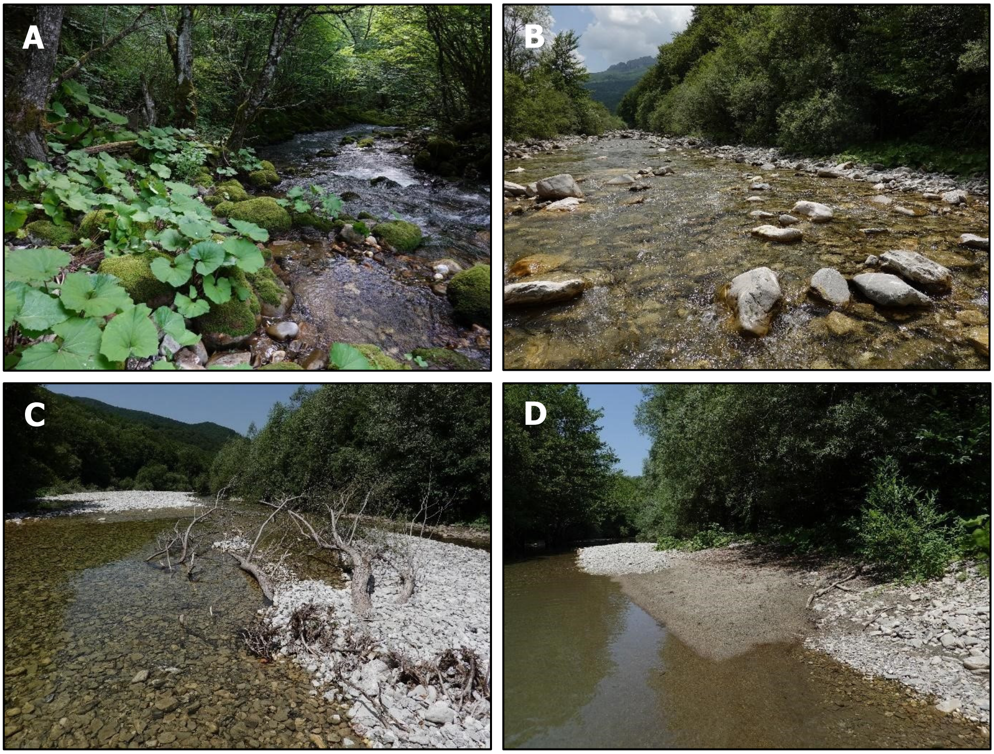

Figure 1. Views of the sample sites: a) Site 1a, b) Site 1b, c) Site 2 and d) Site 3 of the Neretva River (Bosnia and Herzegovina). Details in Tab. 1. (photos: W. Paill).
Slika 1. Pogledi na vzorčna mesta: a) lokaliteta 1a, b) lokaliteta 1b, c) lokaliteta 2 in d) okaliteta 3 na reki Neretvi (Bosna in Hercegovina). Podrobnosti v Tab. 1 (fotografije: W. Paill).
Slika 1. Pregled lokacija za uzorkovanje: a) lokalitet 1a, b) lokalitet 1b, c) lokalitet 2 i d) lokalitet 3 rijeke Neretve (Bosna i Hercegovina). Detajli u Tab. 1. (fotografije: W. Paill)

Table 1. Sampling sites and dates, when carabid beetles were sampled near Neretva River (Bosnia and Herzegovina).
Tabela 1. Lokacije in datumi, ko so bili vzorčeni krešiči ob reki Neretvi (Bosna in Hercegovina).
Tabela 1. Mjesta i datumi, kada su bili uzorkovani bauljari pored rijeke Neretve (Bosna i Hercegovina).

Site number	Coordinates	Elevation [m a.s.l.]	Method (Date)
Site 1a	43,331359 N 18,429952 E	812	Dry pitfall traps (28.–30.6.2022) Hand-collecting (28.6.2022)
Site 1b	43,329722 N 18,423889 E	783	Hand-collecting (30.6.2022)
Site 2	43,365278 N 18,369722 E	709	Hand-collecting (29.6.2022)
Site 3	43,379117 N 18,356051 E	696	Hand-collecting (29.6.2022)
Site 4	43,416944 N 18,312222 E	645	Hand-collecting (27.–30.6.2022) Light traps (27.–29.6.2022)
Site 5	43,425833 N 18,310278 E	638	Hand-collecting (28.6.2022) Light traps (30.6.2022)

Collection of beetles

The collection of carabid beetles was mostly restricted to the active floodplain channel of the river. The material was sampled during a 4-day period in 2022 (27.6. to 3.6.). Samples were collected mainly by hand, over a period of at least 3 hours at each site, either during the day or at night. Additionally, dry pitfall traps (at Site 1a, from 28.6. to 30.6.) and light traps (at Site 4, each night from 27.6. to 30.6.) were used. The field sampling was mostly done by Wolfgang Paill and Johanna Gunczy, some data were added by Wolfram Graf, Gernot Kunz and Rolf Niedringhaus.

Taxonomy and determination

Nomenclature of the taxa follows Löbl & Löbl (2017), considering one correction by Neri & Toledano (2021). The specimens were determined by Wolfgang Paill and Johanna Gunczy. All the material is stored in the Universalmuseum Joanneum (Studienzentrum Naturkunde).

Species traits

For the species, we collected information regarding habitat preference and chorotype. Concerning the habitat behaviour only riparian floodplain specialist and littoral species were selected following e. g. Apfelbeck (1904), GAC (2009), Kahlen (2010), Tallósi (2008), Teofilova et al. (2012), Paill et al. (2018), Hristovski (2017) and Hristovski et al. (2021). Chorotypes were attributed as defined by Vigna Taglianti et al. (1992) and Brandmayr et al. (2005), Stoch & Vigna Taglianti (2006) and Guéorguiev (2007b).

Results and discussion

List of species

In the study area, 89 carabid species were documented based on 763 specimens collected (Tab. 2). This corresponds to approximately 14% of the species known in Bosnia and Herzegovina.

Table 2. Annotated list of documented carabid species of the Neretva River (Bosnia and Herzegovina). Chorology: AFP = Afrotropical and Palearctic, ASE = Asiatic-European, BE = Balcan endemic, BSE = Balkan subendemic, CAE = Centralasiatic-European, CEM = Centralasiatic-Europeo-Mediterranean, CEU = Central European, EME = E-Mediterranean, EUM = Europeo-Mediterranean, EUR = European, OLA = Holarctic, PAL = Palearctic, SEU = S-European, SIE = Sibero-European, TEM = Turano-Europeo-Mediterranean, TUE = Turano-European, WBE = West Balcan endemic, WPA = W-Palearctic. Habitat (behaviour): lit = littoral species, rip = riparian floodplain specialist.

Tabela 2. Anotiran seznam zabeleženih vrst krešičev reke Neretve (Bosna in Hercegovina). Horologija: AFP = afrotropski in palearktični, ASE = azijsko-evropski, BE = balkanski endemit, BSE = balkanski subendemit, CAE = srednjeazijsko-evropski, CEM = centralnoazijsko-evropsko-mediteranski, CEU = srednjeevropski, EME = E-mediteranski, EUM = evropsko-mediteranski, EUR = evropski, OLA = holarktični, PAL = palearktični, SEU = južnoevropski, SIE = sibiro-evropski, TEM = tursko-evropsko-mediteranski, TUE = turansko-evropski, WBE = zahodno-balkanski endemit, WPA = zahodno-palearktični. Habitat (vedenje): lit = litoralna vrsta, rip = specialist obrežnih poplavnih ravnic.

Tabela 2. Popis sa komentarima dokumentovanih vrsta bauljara rijeke Neretve (Bosna i Hercegovina). Khorologija: AFP = afrotropski i palearktički, ASE = azijsko-evropski, BE = balkanski endem, BSE = balkanski subendem, CAE = centralnoazijski-evropski, CEM = centralnoazijski-evropsko-mediteranski, CEU = centralnoevropski, EME = e-mediteranski = evropsko-mediteranski, EUR = evropski, OLA = holarktik, PAL = palearktik, SEU = S-evropski, SIE = sibirsko-evropski, TEM = turano-evropsko-mediteranski, TUE = turano-evropski, WBE = zapadnobalkanski endem, WPA = W-palearktik. Stanište (ponašanje): lit = primorska vrsta, rip = specijalist za priobalne poplavne ravnice.

Species	Chorotype	Habitat	Site 1a	Site 1b	Site 2	Site 3	Site 4	Site 5	Sum
Abax carinatus (Duftschmid, 1812)	EUR						2	1	3
Abax ovalis (Duftschmid, 1812)	EUR		2				1		3
Acupalpus exiguus Dejean, 1829	SIE	lit					1		1
Agonum permoestum Puel, 1938	SEU	lit						1	1
Amara aulica (Panzer, 1796)	OLA						4		4
Amara bifrons (Gyllenhal, 1810)	CAE						3		3
Amara communis (Panzer, 1797)	ASE						1		1
Amara consularis (Duftschmid, 1812)	ASE						2		2
Amara montivaga Sturm, 1825	ASE						2		2
Amblystomus niger (Heer, 1841)	EUM	lit					4		4
Anchomenus dorsalis (Pontoppidan, 1763)	PAL						1		1
Aptinus bombarda (Illiger, 1800)	SEU							3	3
Badister peltatus (Panzer, 1796)	SIE	lit					6	2	8
Bembidion articulatum (Panzer, 1796)	ASE	lit			2	1		4	7
Bembidion azurescens Dalla Torre, 1877	EUR	rip			6			8	14
Bembidion brunnicorne Dejean, 1831	TUE	lit			1	14			15
Bembidion bualei Jacquelin du Val, 1852	PAL	rip			13	15	6	2	36
Bembidion concoeruleum Netolitzky, 1943	SEU	rip				2			2
Bembidion conforme Dejean, 1831	CEU	rip		1	38	60	3	9	111
Bembidion dalmatinum Dejean, 1831	SEU	lit	3	3		2	1		9
Bembidion decorum (Panzer, 1799)	CAE	rip		5	23	26	40	28	122
Bembidion deletum Audinet-Serville, 1821	EUR		2	1					3
Bembidion dentellum (Thunberg, 1787)	EUR	lit					1		1

Species	**Chorotype**	**Habitat**	**Site 1a**	**Site 1b**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Sum**
Bembidion femoratum Sturm, 1825	SIE				1				1
Bembidion guttula (Fabricius, 1792)	SIE	lit		1	1	9			11
Bembidion hypocrita illyricum Netolitzky, 1918	BE	rip				1			1
Bembidion inoptatum Schaum, 1857	SEU	lit	1						1
Bembidion minimum (Fabricius, 1792)	SIE	lit				1	1		2
Bembidion monticola Sturm, 1825	EUR	rip	2	6		18	1		27
Bembidion octomaculatum (Goeze, 1777)	PAL	lit					1		1
Bembidion punctulatum Drapiez, 1820	CEM	rip			2			4	6
Bembidion quadrimaculatum (Linnaeus, 1760)	OLA				1		1		2
Bembidion subcostatum vau Netolitzky, 1913	CEU	rip		2	2	4	1	1	10
Bembidion tetracolum Say, 1823	PAL	lit				1		14	15
Bembidion tibiale (Duftschmid, 1812)	EUR	rip	6	13	2	19			40
Bembidion varicolor (Fabricius, 1803)	EUR	rip		1	10	4	1	1	17
Bembidion vseteckai dissimile G. Müller, 1943	EME	lit				15	1	2	18
Blemus discus (Fabricius, 1792)	OLA						1		1
Brachinus crepitans (Linnaeus, 1758)	PAL						1	1	2
Brachinus explodens Duftschmid, 1812	ASE						1		1
Calathus fuscipes (Goeze, 1777)	EUM						2		2
Calathus melanocephalus (Linnaeus, 1758)	PAL						1		1
Carabus caelatus Fabricius, 1801	WBE						1		1
Carabus coriaceus excavatus Charpentier, 1825	WBE						2	1	3
Carabus gigas Creutzer, 1799	BSE		1						1
Carabus hortensis Linnaeus, 1758	EUR						1		1
Carabus intricatus Linnaeus, 1760	EUR		1					1	2
Carabus violaceus azurescens Dejean, 1826	BE						1		1
Chlaenius vestitus (Paykull, 1790)	EUM	lit						1	1
Cicindela monticola albanica Apfelbeck, 1909	BE	rip			1				1
Cylindera germanica (Linnaeus, 1758)	ASE						1		1
Drypta dentata (P. Rossi, 1790)	AFP						1		1
Dyschirius agnatus Motschulsky, 1844	TUE	rip						13	13
Elaphrus aureus P.W.J. Müller, 1821	EUR	rip			2			1	3
Harpalus affinis (Schrank, 1781)	ASE						1		1

Species	**Chorotype**	**Habitat**	**Site 1a**	**Site 1b**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Sum**
Harpalus dimidiatus (P. Rossi, 1790)	EUR						2		2
Harpalus rufipes (De Geer, 1774)	PAL							1	1
Harpalus serripes (Quensel, 1806)	PAL						4		4
Harpalus subcylindricus Dejean, 1829	SEU						1		1
Limodromus assimilis (Paykull, 1790)	SIE					1	1	2	4
Lionychus quadrillum (Duftschmid, 1812)	EUR	rip			12	2		1	15
Microlestes minutulus (Goeze, 1777)	OLA				1				1
Nebria picicornis (Fabricius, 1801)	EUR	rip					1		1
Omophron limbatum (Fabricius, 1777)	PAL	rip		1	4	2	2	3	12
Ophonus azureus (Fabricius, 1775)	CEM		1				1		2
Ophonus diffinis (Dejean, 1829)	EUR						4		4
Ophonus laticollis Mannerheim,1825	SIE						1		1
Ophonus melletii (Heer, 1837)	EUR						14	1	15
Ophonus rufibarbis (Fabricius, 1792)	TEM						2		2
Ophonus rupicola (Sturm, 1818)	EUR						1		1
Oxypselaphus obscurus (Herbst, 1784)	OLA	lit						1	1
Paranchus albipes (Fabricius, 1796)	EUM	rip		5	5	7	3	4	24
Parophonus maculicornis (Duftschmid, 1812)	SEU						1		1
Perileptus areolatus (Creutzer, 1799)	EUM	rip			5	1	4	4	14
Platynus scrobiculatus serbicus Csiki, 1904	BE	rip	4	1				1	6
Pterostichus melanarius (Illiger, 1798)	OLA				1				1
Pterostichus niger (Schaller, 1783)	ASE		2						2
Pterostichus nigrita (Paykull, 1790)	ASE	lit						8	8
Pterostichus strenuus (Panzer, 1796)	ASE			1					1
Pterostichus vernalis (Panzer, 1796)	PAL							1	1
Sinechostictus doderoi (Ganglbauer, 1891)	CEU	rip						1	1
Sinechostictus millerianus (Heyden, 1883)	CEU	rip			8	11		2	21
Sinechostictus tarsicus (Peyron, 1858)	SEU	rip				2			2
Stenolophus mixtus (Herbst, 1784)	PAL	lit					3	1	4
Tachys bistriatus (Duftschmid, 1812)	WPA	lit					1		1
Tachys micros (Fischer von Waldheim, 1828)	EUM	rip			6			1	7
Tachyura quadrisignata (Duftschmid, 1812)	EUM	rip			14			4	18
Tachyura sexstriata (Duftschmid, 1812)	EUR	rip			3	1	6	25	35
Trechus subnotatus Dejean, 1831	BE	lit	4						4

Comparison of the sites

The number of detected species corresponds to the structural richness of the habitats of the river course. 12-13 species were found in the stretched, partially shaded section (Sites 1a and 1b), 24-25 species in the slightly meandering course (Sites 2 and 3), and 37 species in the wider section (Site 5). The share of riparian floodplain specialists is very similar at Sites 1b, 2, 3 and is between 67% and 72% (Fig. 2). Deviating values were determined for Site 1a (25%), where forest species increasingly reach the linear floodplain zone, at Site 5 (51%), where further open land species complete the local ground beetle community, and at Site 4 (21%), where species from outside the floodplain zone were additionally caught by the light trap. A similar picture emerges if the littoral species are considered in addition to the riparian species. At Site 3, almost all species (96%) belong to this group, which may be explained by the extended alluvial forest on this sample site.

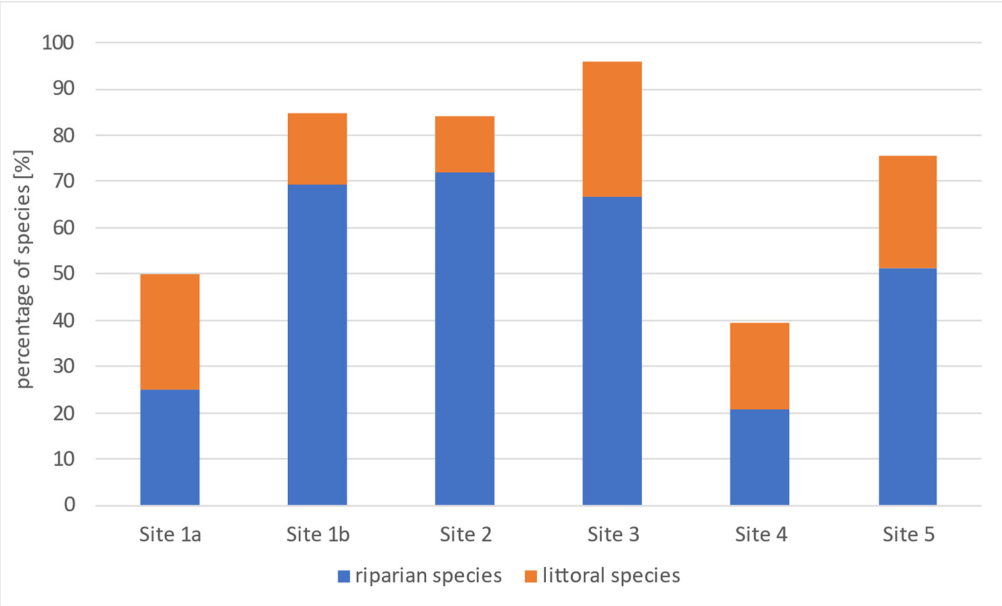

Figure 2. Comparison of the proportion of riparian and littoral carabid species from the upper course of the Neretva River (Bosnia and Herzegovina).
Slika 2. Primerjava deleža obrežnih in litoralnih vrst krešičev iz zgornjega toka reke Neretve (Bosna in Hercegovina).
Slika 2. Poređenje udjela priobalnih i litoralnih bauljara iz gornjeg toka rijeke Neretve (Bosna i Hercegovina).

Chorological data

The collected species can be assigned to 18 zoogeographical chorotypes (Fig. 3). For a better overview, they are classified into five groups: I) Subcosmopolitan species (chorotype AFP); II) widely distributed species in the Holarctic region (ASE, CAE, CEM, EUM, OLA, PAL, SIE, TEM, TUE, WPA); III) species in the European area (CEU, EUR, SEU); IV) species in the Mediterranean area (EME) and V) Balkan endemics (BE, BSE, WBE). A large proportion of widespread Holarctic (55% of the species) as well as European species (34%) can be seen. On the other hand, the proportion of Mediterranean species is low. Including the Balkan endemics, this group only comprises 10% of the recorded diversity.

When focusing on riparian floodplain specialists, the ratio between Holarctic (35%) and European (54%) species is reversed. The dominance of European species is also reflected in the captured individuals of these species. For example, the Central European *Bembidion conforme* is the second most common representative of the riparian floodplain fauna, and *Bembidion monticola*, *Bembidion tibiale* and *Sinechostictus millerianus* are also quite abundant. Findings of *Nebria picicornis*, *Elaphrus aureus*, *Bembidion azurescens*, *Bembidion dentellum*, *Lionychus quadrillum*, and *Tachyura sexstriata* complete the set of alluvial specialists with a (Central) European distribution focus.

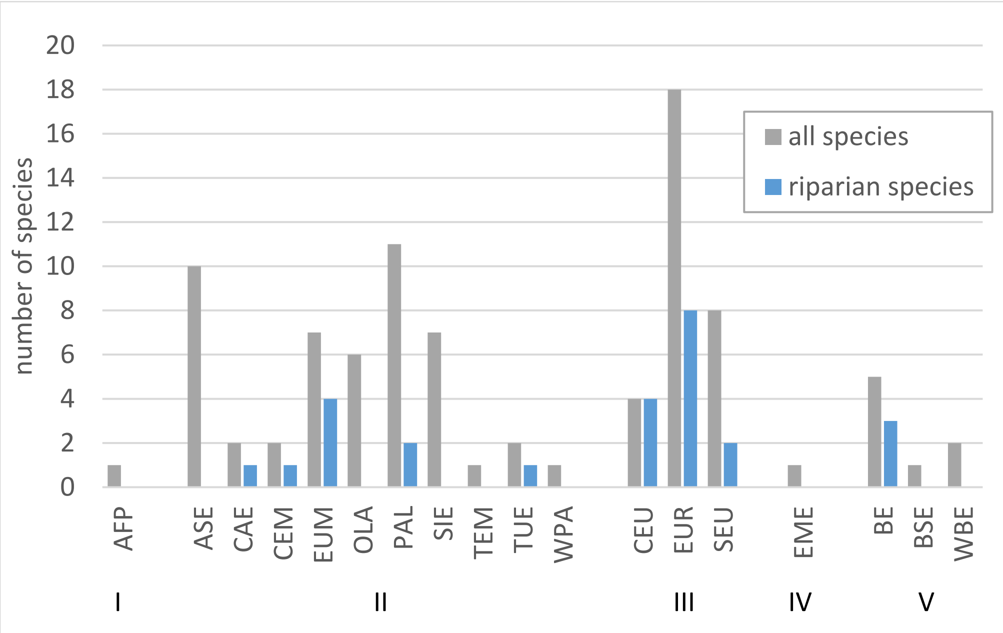

Figure 3. Chorological spectrum of the carabid beetles collected in the study area at the Neretva River (Bosnia and Herzegovina). See the text for abbreviations.

Slika 3. Horološki spekter krešičev, najdenih na raziskovanem območju ob reki Neretvi (Bosna in Hercegovina). Za okrajšave glej besedilo.

Slika 3. Horološki spektar bauljara sakupljenih na istraživanom području na rijeci Neretvi (Bosna i Hercegovina). Za skraćenice vidi tekst.

Comments on remarkable species

Little is known about the distribution and frequency of ground beetles within Bosnia and Herzegovina (see above). Based on a comparison with the better documented fauna of Croatia, Serbia, North Macedonia and Albania, an approximate assessment of the frequency or rarity of selected species can nevertheless be made. Accordingly, the following commented species are of particular faunistic significance.

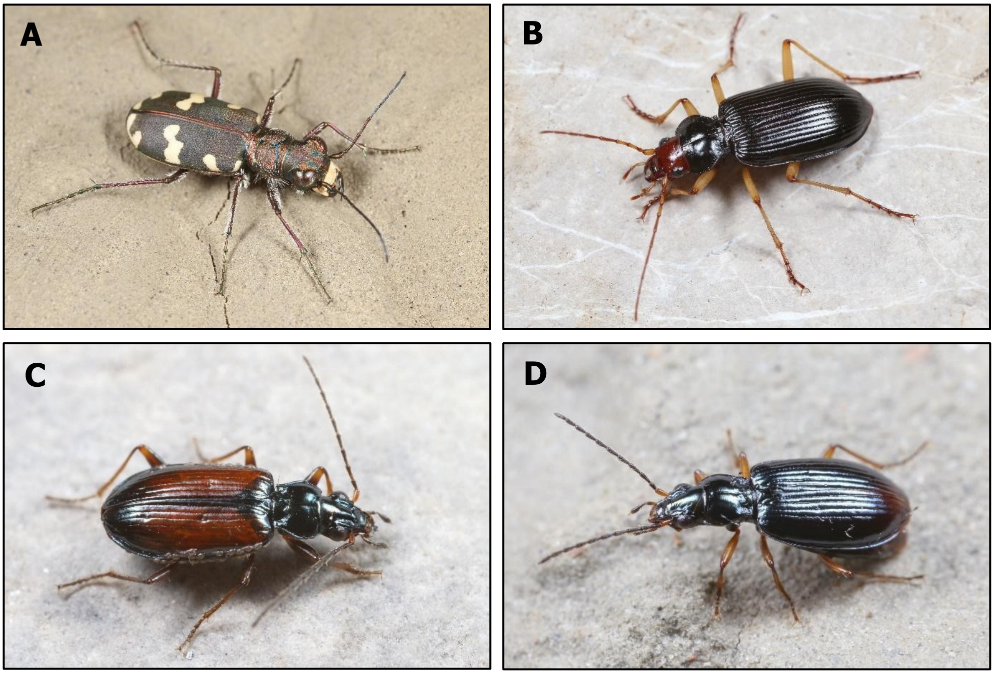

Figure 4. a) *Cicindela monticola albanica* (body size 11-17 mm), b) *Nebria picicornis* (body size 10-15 mm), c) *Bembidion conforme* (body size 4.5-6 mm), d) *Bembidion monticola* (body size 4.5-5.5 mm). The pictures were not taken from Bosnian material (photos: W. Paill).

Slika 4. a) *Cicindela monticola albanica* (velikost telesa 11-17 mm), b) *Nebria picicornis* (velikost telesa 10-15 mm), c) *Bembidion conforme* (velikost telesa 4,5-6 mm), d) *Bembidion monticola* (velikost telesa 4,5-5,5 mm). Slikani primerki niso bili nabrani v BiH (fotografije: W. Paill).

Slika 4. a) *Cicindela monticola albanica* (veličina tijela 11-17 mm), b) *Nebria picicornis* (veličina tijela 10-15 mm), c) *Bembidion conforme* (veličina tijela 4,5-6 mm), d) *Bembidion monticola* (veličina tijela 4,5-5,5 mm). Fotografisani primjerci nisu prikupljeni u BiH (fotografije: W. Paill).

***Cicindela monticola albanica* Apfelbeck, 1909**

This Cicindelini species is endemic to the Balkans and occurs infrequently from Croatia over Bulgaria, Serbia, North Macedonia, Montenegro, Albania and Greece (e. g. Jaskuła et al. 2005; Jaskuła 2007). From Bosnia and Herzegovina, only three detailed findings are published (Gebert 1995). We found a single specimen of this species which prefers broad riverbanks with a mixture of fine-grained and coarse-grained sediments.

***Nebria picicornis* (Fabricius, 1801)**

In the Balkans, this European species becomes very rare towards the south. This is indicated by its absence in Albania and Greece (e. g. Huber 2017) and the first individual findings from Croatia and Kosovo published only a few years ago (Guéorguiev 2011), as well as the restricted occurrence in North Macedonia (Hristovski & Guéorguiev 2015). We found one single specimen of this inhabitant of coarse-grained gravel banks.

***Dyschirius agnatus* Motschulsky, 1844**

This species was not listed by Apfelbeck (1904), Drovenik & Peks (1999) or Lelo (2013) for Bosnia and Herzegovina, although it was published by Müller (1922) and by Hieke & Wrase (1988), who listed it as *Dyschirius lucidus* Putzeys, 1867. Further finds were mentioned by Bulirsch & Pavićević (2008) and Guéorguiev (2011). We found a good population at Site 5.

***Bembidion azurescens* Dalla Torre, 1877**

This species was not listed by Apfelbeck (1904), Drovenik & Peks (1999) or Lelo (2013) for Bosnia and Herzegovina. The first unambiguous reports were published by Tabaković-Tošić (1991) and Guéorguiev (2011), but only included historical collection material. We found 14 specimens at Site 2 and Site 5 of the study area.

***Bembidion conforme* Dejean, 1831**

This Central European species is mentioned in all lists and catalogues for Bosnia and Herzegovina (Apfelbeck 1904; Drovenik & Peks 1999; Lelo 2013). However, *Bembidion conforme* is likely to be rare in the Balkans. There are only isolated findings from Croatia (Guéorguiev 2011), Montenegro (Apfelbeck 1904) and Albania (Guéorguiev 2007a), whereas in North Macedonia and most likely Serbia (Ćurčić et al. 2007; Hristovski & Guéorguiev 2015) the species is not yet known. In our study area, *Bembidion conforme* together with *Bembidion decorum* was the most common inhabitant of coarse-grained gravel banks.

***Bembidion dentellum* (Thunberg, 1787)**

In the Balkans, this European species becomes rare towards the south and is missing in North Macedonia, Albania and Greece (e. g. Marggi et al. 2017). As a typical inhabitant of the banks of floodplain waters, their main distribution point along the Neretva is likely to be further downstream. We found only one single specimen at Site 4.

***Bembidion femoratum* Sturm, 1825**

This Sibero-European species was not listed by Apfelbeck (1904), Drovenik & Peks (1999) or Lelo (2013) for Bosnia and Herzegovina. The first confirmed findings were published by Guéorguiev (2011), but only concern two single historical specimens. We found one single exemplar at Site 2.

Bembidion hypocrita illyricum **Netolitzky, 1918**

The distribution of this Balkan endemic subspecies extends from Slovenia to the Peloponnese (Drovenik & Peks 1999; Bonavita & Vigna Taglianti 2005; Marggi et al. 2017), with single, widely disjunct locations. For example, the species was only recently definitely reported from Bulgaria for the first time with only a single specimen by Guéorguiev (2011). In addition, from North Macedonia only two historic specimens and a single recent finding are documented (Hristovski et al. 2010; Hristovski & Guéorguiev 2015). Only one historical find is also documented from Bosnia and Herzegovina (Apfelbeck 1904). We found one individual at Site 3.

Bembidion monticola **Sturm, 1825**

According to Apfelbeck (1904), this European species is rare in the Balkans. The species is missing in Albania, and only a few specimens are known from Serbia, Montenegro and Greece (Ćurčić et al. 2007; Guéorguiev 2011; Marggi 2011; Hristovski & Guéorguiev 2015). The historic Bosnian findings from Apfelbeck (1904) are now confirmed by recent evidence from the upper Neretva.

Sinechostictus doderoi **(Ganglbauer, 1891)**

This Central European species was not listed by Apfelbeck (1904), Drovenik & Peks (1999) or Lelo (2013) for Bosnia and Herzegovina. The first unambiguous cases were published by Guéorguiev (2011), but only concerned historical collection material. We found one single specimen.

Sinechostictus millerianus **(Heyden, 1883)**

The distribution of this Central European species on the Balkans is rather scattered. This is indicated, for example, by the data from Croatia, Serbia, North Macedonia and Albania (Ćurčić et al. 2007; Guéorguiev 2007a; Guéorguiev 2008; Guéorguiev 2011; Hristovski & Guéorguiev 2015; Paill & Gunczy unpubl.). In the study area, *Sinechostictus millerianus* was quite commonly found to inhabit coarse-grained gravel banks.

Tachyura quadrisignata **(Duftschmid, 1812)**

This Europeo-Mediterranean species seems to be rare in the Balkans, as it was only recently documented for the first time for Croatia (Tallósi 2008; Guéorguiev 2011) and North Macedonia (Hristovski & Guéorguiev 2015) and is not known from Albania (Guéorguiev 2007a). We found several specimens at sampling Sites 1b and 5.

Povzetek

Študija je pomemben prispevek k poznavanju do sedaj le malo raziskovanih talnih hroščev v Bosni in Hercegovini. Na petih lokacijah vzdolž zgornjega toka reke Neretve smo v habitatih, povezanih z reko (poplavne ravnice, prodišča, obrežna vegetacija), zabeležili 89 vrst krešičev, s skupno 763 osebki. Osredotočili smo se na dokumentiranje redkih in občutljivih vrst, katerih najdbe bi bile naravovarstveno pomembne za zaščito te skoraj naravne reke. Vzorce smo nabirali ročno (vsaj 3 ure na vsaki lokaciji), s suhimi in svetlobnimi pastmi. Število odkritih vrst je ustrezalo strukturni pestrosti habitatov na petih lokacijah. V raztegnjenem, delno osenčenem delu je bilo najdenih 12-13 vrst, v rahlo vijugastem delu 24-25 vrst, v širšem rečnem delu pa 37 vrst. Delež specialistov obrežnih poplavnih ravnic je bil visok, razen na območjih, kjer so gozdne vrste zlahka dosegle poplavno ravnico ali pa so lokalne vrste hroščev dopolnjevale kopenske vrste odprtih habitatov. Vrste zunaj poplavnega območja smo ulovili tudi s svetlobnimi pastmi. Večina najdenih hroščev je holarktično razširjenih (55 % vrst). Delež evropskih in sredozemskih vrst (vključno z balkanskimi endemiti) je bil 34- oziroma 10-odstoten. Znotraj specialistov obrežnih poplavnih ravnic je razmerje med holarktičnimi (35 %) in evropskimi (54 %) vrstami obrnjeno.

Na podlagi primerjave z bolje dokumentirano favno v državah, ki mejijo na Bosno in Hercegovino, je mogoče narediti približno oceno redkosti različnih vrst. Naslednje vrste so posebnega favnističnega pomena: *Cicindela monticola albanica* Apfelbeck, 1909 (balkanski endemit, ki ima najraje široka rečna prodišča); *Nebria picicornis* (Fabricius, 1801) (evropska vrsta, ki je redkejša južneje vzdolž Balkanskega polotoka in naseljuje prodnate brežine); *Dyschirius agnatus* Motschulsky, 1844; *Bembidion azurescens* Dalla Torre, 1877; *Bembidion conforme* Dejean, 1831 (srednjeevropska vrsta, redka na Balkanu, običajen prebivalec grobozrnatih prodišč); *Bembidion dentellum* (Thunberg, 1787) (evropska vrsta, redkejša južneje vzdolž Balkanskega polotoka in naseljuje bregove vodnih teles poplavnih nižin); *Bembidion femoratum* Sturm, 1825 (sibiroevropska vrsta); *Bembidion hypocrita illyricum* Netolitzky, 1918 (balkanska endemična podvrsta z eno samo, močno ločeno populacijo); *Bembidion monticola* Sturm, 1825 (evropska vrsta, redka na Balkanu in v BiH znana samo iz zgodovinskih zapisov); *Sinechostictus doderoi* (Ganglbauer, 1891) (srednjeevropska vrsta); *Sinechostictus millerianus* (Heyden, 1883) (srednjeevropska vrsta s precej razpršeno razširjenostjo na Balkanskem polotoku, ki naseljuje grobozrnata prodišča); *Tachyura quadrisignata* (Duftschmid, 1812) (evropsko-mediteranska vrsta, redka na Balkanu).

Sažetak

Ova studija daje značajan doprinos relativno slabo istraženim bauljarima u Bosni i Hercegovini. Na pet lokacija duž gornjeg toka Neretve evidentirano je 89 vrsta, sa ukupno 763 jedinke, u staništima vezanim za rijeku (plavne ravnice, sprudovi, obalna vegetacija). Fokusirali smo se na dokumentovanje rijetkih i osjetljivih vrsta, čiji bi nalazi bili od značaja za zaštitu ove gotovo netaknute rijeke. Uzorci su prikupljani: ručno tokom najmanje 3 sata na svakoj lokaciji, suhim zamkama i svjetlosnim zamkama. Broj otkrivenih vrsta odgovara strukturnom bogatstvu staništa na pet lokaliteta. 12-13 vrsta pronađeno je u izduženom, djelomično zasjenjenom dijelu, 24-25 vrsta u blago krivudavom dijelu, a 37 vrsta u širem dijelu rijeke. Udio vrsta za obalne poplavne ravnice bio je visok osim na mjestima gdje su šumske vrste lako mogle doći do poplavne ravnice ili su dodatne vrste otvorenog zemljišta dopunjavale lokalnu zajednicu bauljara. Također,svjetlosnim zamkama su uhvaćene vrste izvan poplavne zone. Većina pronađenih bauljara ima holarktičnu rasprostranjenost (55% vrsta). Udio evropskih i mediteranskih vrsta (uključujući balkanske endeme) iznosio je 34%, odnosno 10%. Unutar vrsta za obalne poplavne ravnice, odnos holarktičkih (35%) prema evropskim (54%) vrsta je obrnut.

Na osnovu poređenja sa bolje dokumentovanom faunom zemalja u susjedstvu Bosne i Hercegovine, može se napraviti približna procjena rijetkosti različitih vrsta. Prema tome, sljedeće vrste su od posebnog faunističkog značaja: *Cicindela monticola albanica* Apfelbeck, 1909 (balkanski endem koji preferira široke šljunkovite obale rijeka); *Nebria picicornis* (Fabricius, 1801) (evropska vrsta koja postaje rijetka južnije duž Balkanskog poluostrva i naseljava šljunkovite obale); *Dyschirius agnatus* Motschulsky, 1844; *Bembidion azurescens* Dalla Torre, 1877; *Bembidion conforme* Dejean, 1831 (srednjoevropska vrsta rijetka na Balkanu, pronađena kao običan stanovnik krupnozrnih šljunčanih obala); *Bembidion dentellum* (Thunberg, 1787) (evropska vrsta koja postaje rijetka južnije duž Balkanskog poluostrva i naseljava obale poplavnih vodnih područja); *Bembidion femoratum* Sturm, 1825 (sibero-evropska vrsta); *Bembidion hipocrita illyricum* Netolitzky, 1918 (balkanska endemska podvrsta sa pojedinačnim, široko odvojenim populacijama); *Bembidion monticola* Sturm, 1825 (evropska vrsta rijetka na Balkanu i istorijski opisana samo za Bosnu i Hercegovinu); *Sinechostictus doderoi* (Ganglbauer, 1891) (srednjoevropska vrsta); *Sinechostictus millerianus* (Heyden, 1883) (srednjoevropska vrsta sa prilično raštrkanom rasprostranjenošću na Balkanskom poluostrvu, koja naseljava krupne šljunkovite obale); *Tachyura quadrisignata* (Duftschmid, 1812) (evropsko-mediteranske vrste rijetka na Balkanu).

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The impacts of the Ulog dam construction become impressively clear when flying a drone. Photos: Vladimir Tadić

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New records of *Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 (Neuroptera: Nevrorthidae) from Bosnia and Herzegovina

Susanne RANDOLF[1*], Stefan ANDJUS[2], Bojana TUBIĆ[2], Harald BRUCKNER[1], Michaela BROJER[1], Maja ZAGMAJSTER[3]

[1]Natural History Museum Vienna, 2nd Zoological department, Burgring 7, 1010 Vienna, Austria
[2]University of Belgrade, Department for Hydroecology and Water protection, Institute for Biological Research »Siniša Stanković«, National Institute of the Republic of Serbia, Bulevar despota Stefana 142, 11060 Belgrade, Serbia
[3]University of Ljubljana, Biotechnical Faculty, Department of Biology, SubBioLab, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[*]Corresponding author. E-mail: susanne.randolf@nhm-wien.ac.at

Abstract. During the »Neretva Science Week« in summer 2022, two larvae of *Nevrorthus apatelios* were found in Eastern Bosnia and Herzegovina. These are the first confirmations of the species in the country since a single observation of one adult from Sarajevo almost 100 years ago. New records come from two sites of the upper course of the Neretva River, near Ulog village: one larva was found in a benthic fauna sample collected at the river bottom near Rastovac, while the second was pumped from the river interstitial at depths 30-60 cm, in the area near Cerova. Based on these findings we provide a description of the larva. *Nevrorthus apatelios* lives in highly oxygenated waters, and is a good indicator of clean waters. We discuss the potential reasons for rare observations of the species in the country, and conservation implications in light of ongoing environmental pressures in the currently pristine part of the upper course of Neretva River.

Key words: Nevrorthidae, new locality, Neretva River, conservation

Izvleček. Nova opažanja vrste *Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 (Neuroptera: Nevrorthidae) v Bosni in Hercegovini – Med »Tednom znanosti na Neretvi« poleti 2022 sta bili v vzhodni Bosni in Hercegovini najdeni dve ličinki vrste *Nevrorthus apatelios*. To je prva potrditev pojavljanja vrste v državi, vse zabeležbe enega odraslega osebka v Sarajevu pred skoraj 100 leti. Vrsta je bila tokrat opažena na dveh lokacijah v zgornjem toku reke Neretve pri vasi Ulog: ena ličinka je bila najdena v vzorcu bentoške favne, zbranem na dnu reke pri Rastovcu, medtem ko je bila druga najdena v vzorcu vode, načrpane iz rečnega intersticija na globinah 30-60 cm na območju pri Cerovi. Na podlagi najdb podajamo opis ličinke. *Nevrorthus apatelios* živi v vodah z visoko vsebnostjo kisika in je dober kazalec čistih voda. V nadaljevanju razpravljamo o morebitnih razlogih za redke najdbe te vrste v državi in o varstvenih posledicah, v luči nenehnih pritiskov na okolje v tem za zdaj še neokrnjenem delu zgornjega toka reke Neretve.

Ključne besede: Nevrorthidae, nova lokaliteta, reka Neretva, varstvo

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Apstrakt. Novi nalaz vrste *Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 (Neuroptera: Nevrorthidae) u Bosni i Hercegovini – Tokom »Sedmice nauke na Neretvi« u ljeto 2022. godine u istočnoj Bosni i Hercegovini pronađene su dvije larve *Nevrorthus apatelios*. Ovo su prva zapažanja vrste u zemlji od prije skoro 100 godina od poslednjeg opažanja. Novi podaci dolaze sa dva lokaliteta gornjeg toka rijeke Neretve: u blizini sela Ulog jedna larva je pronađena u uzorku bentoske faune sakupljenog na dnu rijeke kod Rastovca, dok je druga ispumpana iz riječnog segmentaa na dubinama 30-60 cm, u okolini Cerove. Na osnovu ovih nalaza dajemo opis larve *Nevrorthus apatelios* koja živi u vodama bogatim kiseonikom i dobar je pokazatelj čistih voda. Razgovaramo o potencijalnim razlozima rijetkih opažanja vrsta u zemlji, te implikacijama na očuvanje u svjetlu trenutnih pritisaka na životnu sredinu u trenutno netaknutom dijelu gornjeg toka rijeke Neretve.

Ključne riječi: Nevrorthidae, novi lokalitet, rijeka Neretva, očuvanje

Introduction

The Nevrorthidae are a small neuropteran family that currently contains 19 described extant species assigned to four genera: *Nevrorthus* Costa, 1863, with five species distributed in the Mediterranean, *Nipponeurorthus* Nakahara, 1958, with 11 species on the Japanese islands, mainland China and Taiwan, *Austroneurorthus* Nakahara, 1958 with two species in eastern Australia, and *Sinoneurorthus* Liu et al., 2012, with one species in mainland China (Aspöck et al. 2017). The disjunct distribution of nevrorthid genera indicates an ancient, wide Pangaean distribution, which was subsequently fragmented by continental drift. Because of the great morphological similarity with fossil Nevrorthidae from Baltic amber, the extant species have even been considered »living fossils« (Aspöck & Aspöck 1994; Wichard et al. 2010).

The life cycle of Nevrorthidae includes aquatic and terrestrial life forms. From the first larval instar to the pupal stage they are bound to aquatic habitats, which is unique for Neuroptera. There are only two more families, Osmylidae and Sisyridae, having partially amphibious larval behavior or having aquatic larvae going ashore to pupate, respectively (Weißmair 1999; Wichard et al. 2009).

Larvae of Nevrorthidae are slender and elongated. They rapidly wriggle forward and backward through the gaps between gravel and coarse sand in fast-flowing streams (Malicky 1984). They are equipped with a complex joint between the head and the elongated anterior part of the pronotum, that allows them to move their head up quickly, but they have no sideways motions of the head. The function of this fast head movement in the life of the larva is not known (Zwick 1967). The conspicuous sucking stylets, a synapomorphy of neuropteran larvae, are curved at the tip and can move against each other. The association of the stylets with a venom gland suggests that they are carnivorous (Gaumont 1976), yet nothing is known about the type of prey (Malicky 1984). Gills are absent and the tracheal system is reduced. The abundance of oxygen in flowing water is apparently sufficient to be absorbed by the cuticle (Zwick 1967). The last instar larva spins a two-layered cocoon on the underside of stones in which air can be stored, covering the pupal body and thus enabling respiration (Wichard et al. 2010). The pupal stage probably only lasts a few days (Malicky 1984). The total length of development has not been appropriately investigated, Malicky (1984) and Popov (2005) estimate a duration of one year with the larva overwintering. The number of eggs and site for egg deposition are unknown (New 1978; Aspöck et al. 2017).

Adults are found on overhanging branches of deciduous trees, in bushes and low vegetation close to the water (Aspöck et al. 2017). Malicky (1984) often found adults on the leaves of alder species sticky with aphid exudates and thought it possible that they were feeding on honeydew. Additionally, the significant number of fungal spores that Monserrat (2005) reported in their digestive tract suggests a glycophagous diet, as some fungi develop in honeydew (Magyar et al. 2005). Furthermore, there is a special formation of the mouthparts, namely a secondary prolongation of the salivary system, which indicates that even dried honeydew can be liquefied and ingested (Randolf et al. 2014).

The genus *Nevrorthus*, as the family Nevrorthidae, has a fragmented and relict distribution, with five species living isolated from each other in different parts of the Mediterranean regions: *Nevrorthus fallax* (Rambur, 1842) is endemic to Sardinia and Corsica, *Nevrorthus iridipennis* Costa, 1863 occurs in the south of the Italian Peninsula and Sicily, *Nevrorthus hannibal* U. Aspöck & H. Aspöck, 1983 in Tunisia and Algeria, and *Nevrorthus reconditus* Monserrat & Gavira, 2014 in the south of the Iberian Peninsula. Amongst the five species of the genus, *Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 is the one with the widest distribution: it extends from Northeast of Italy, via Slovenia, Bosnia and Herzegovina, Montenegro, Serbia, Kosovo, Albania, North Macedonia, Greece, Bulgaria to the Cernei mountains in Romania (e. g. Zelený 1964 [as *N. iridipennis*]; Aspöck et al. 1977; Aspöck & Aspöck 1983; Popov 1992, 2002, 2005; Devetak 1992; Bernardi Iori et al. 1995; Aspöck et al. 2001; Devetak & Jakšić 2003; Letardi et al. 2005; Sziráki 2008; Jones & Devetak 2009; Nicoli Aldini et al. 2012; Monserrat & Gavira 2014; Marković et al. 2016; Devetak 2021).

One of the countries where *Nevrorthus apatelios* had been reported, although only by a single observation, was Bosnia and Herzegovina. The specimen was an adult male, collected in 1929 from Sarajevo (Aspöck et al. 1977). Here we present new findings of the species in the upper Neretva River, during the studies conducted within the »Neretva Science Week« in summer 2022. They are confirming its presence in Bosnia and Herzegovina (BIH) after nearly 100 years since the first report, being the first observations of the larval stages for the country. We discuss the possible reasons for such rare observations of the species in its whole distribution area and conservation implications.

Materials and methods

From the 28.6. to 5.7. 2022, samples of benthic and interstitial macroinvertebrates were collected in the upper Neretva River, Eastern BIH, in the stretch of river between the confluence with Krupac River and the city of Konjic (see respective contributions of this special issue for details).

The invertebrates of the river bottom were sampled using the Kick and Sweep (K&S) method, from all microhabitat types using the standardised approach of handnet sampling of aquatic macroinvertebrates (EN 27828:1994). The biological material was transferred to sample containers (size 250, 500 ml) and preserved with 96 or 70% ethanol. Invertebrates were sampled with this method at nine different locations.

The river interstitial was sampled using Bou-Rouch pumping, conducted at multiple points of each gravel bar, and at two different depths to which the metal pole with holes was dug in: 30-60 cm and 60-90 cm. At each point and depth, at least 30 l of water with sediments and organisms was pumped out and filtered through a net of 0.5 and 0.1 mm mesh size. The remaining material was stored in 96% ethanol, keeping the information on each sampling point and depth. All together, five gravel bars of Neretva were sampled with Bou-Rouch pumping for a total of 30 taken samples (see respective contribution of this special issue for details).

All the samples were checked in the laboratories, using the stereomicroscope, and organisms sorted according to taxonomic levels. Identification of the aquatic macroinvertebrates was done based on morphological characteristics to the lowest possible systematic level using appropriate identification keys., ZEISS Stemi 2000C (x50) and Nikon SMZ 800N (x75) binocular stereoscopic magnifiers, ZEISS Axio Lab. A1 (x630) microscope.

Photographs of the specimen were taken with a Leica DFC490 camera attached to a Leica Z16APO optics carrier, using Leica Application Suite V3.8, and were subsequently stacked with ZereneStacker 64-bit, and processed with Adobe Photoshop 7.0.

Results and discussion

Localities of *N. apatelios* in BIH

The only previous locality of the specimen in BIH was reported from »Sarajevo«, with no more details on the labels (Tab. 1; Fig.1). Considering the habitat requirements and behaviour of *N. apatelios*, this adult male was probably collected along a mountain stream in the vicinity of the city. Two new specimens of *N. apatelios* in BIH were found at two localities of the upper Neretva River (Tab. 1; Fig. 1), about 55 km south from Sarajevo. While the first one was found in the water pumped from the 60-90 cm depth of the river interstitial, the second one was found in the sample taken from the bottom of the Neretva River.

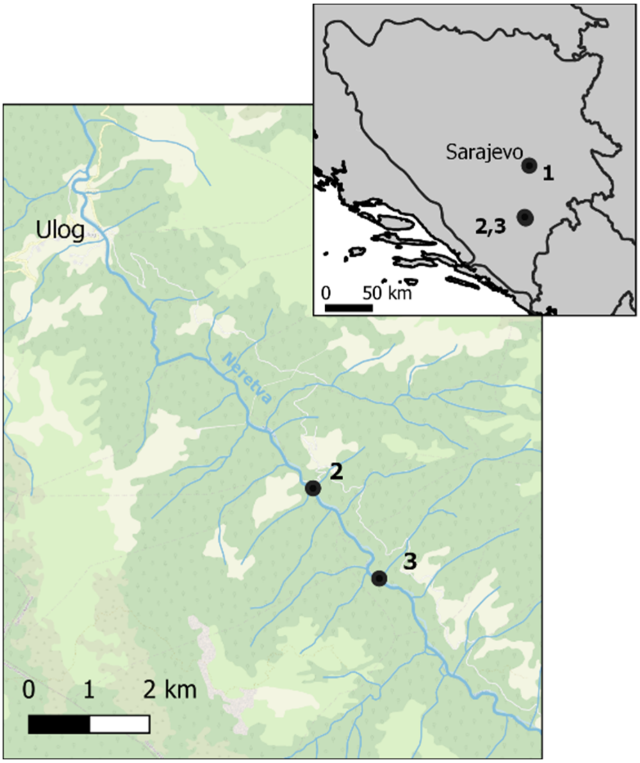

Figure 1. All known localities of *N. apatelios* in BIH, with two summer 2022 findings at upper Neretva. Numbers refer to descriptions of localities in Tab. 1.

Slika 1. Vsa znana nahajališča *N. apatelios* v BiH, z dvema najdbama poleti 2022 v zgornji Neretvi. Številke se nanašajo na opise lokalitet v Tab. 1.

Slika 1. Svi poznati lokaliteti *N. apatelios* u BiH, sa dva nalaza u ljeto 2022. na gornjoj Neretvi. Brojevi se odnose na opise lokaliteta u Tab. 1.

Description of the *N. apatelios* larva

Here we describe the morphology of the larva from the Neretva gravel bar at Cerova, since the two larvae do not differ in their morphological characteristics. The larva is slender and about 9 mm long, with thin bristles all over the body, which are up to 2 mm long on the last abdominal segment (Fig. 2). The head is dorsoventrally flattened, the sucking stylets are longer than the head capsule. Head, neck and pronotum are heavily sclerotised, while on the meso- and metanotum only one oval spot is sclerotised on each side. The thoracic sterna are completely unsclerotised. On both sides of the thoracic segments two small pleural sclerites form the articulation sites for the coxae. The abdominal segments, except for the last one, are unsclerotised. They are white, dorsally with a pattern of brownish pigmentation with one light transverse and two light lateral longitudinal stripes.

Table 1. All *N. apatelios* localities in BIH, with two recent ones from summer 2022 sampling at upper Neretva River. Details on sampling points, dates, legators (Leg.) and determinators (Det.) are given. Acronyms refer to: BR – Behare Rexhepi, BT – Bojana Tubić, EP - Ester Premate, HA – Horst Aspöck, HH – Herbert Hölzel, MZ – Maja Zagmajster, SA – Stefan Andjus, SR – Susanne Randolf, ŠB – Špela Borko, UA – Ulrike Aspöck, VM – Vojo Milanović, Z – Zerny, NHMW – Natural History Museum Vienna, IBISS – Institute for Biological Research »Siniša Stanković«, Belgrade.

Tabela 1. Vsa nahajališča *N. apatelios* v BiH, vključno z najdbama poleti 2022 v zgornjem delu reke Neretve. Podane so podrobnosti o vzorčiščih, datumih, legatorjih (Leg.) in determinatorjih (Det.). Kratice se nanašajo na: BR – Behare Rexhepi, BT – Bojana Tubić, EP – Ester Premate, HA – Horst Aspöck, HH – Herbert Hölzel, MZ – Maja Zagmajster, SA – Stefan Andjus, SR – Susanne Randolf, ŠB – Špela Borko, UA – Ulrike Aspöck, VM – Vojo Milanović, Z – Zerny, NHMW – Naravoslovni muzej Dunaj, IBISS – Inštitut za biološke raziskave »Siniša Stanković«, Beograd.

Tabela 1. Svi lokaliteti *N. apatelios* u BiH, sa dva nedavna uzorkovanja iz ljeta 2022. godine u gornjem dijelu rijeke Neretve. Dati su detalji o tačkama uzorkovanja, datumima, legatorima (Leg.) i determinatorima (Det.). Akronimi se odnose na: BR – Behare Rexhepi, BT – Bojana Tubić, EP – Ester Premate, HA – Horst Aspöck, HH – Herbert Hölzel, MZ – Maja Zagmajster, SA – Stefan Anđus, SR – Susanne Randolf, ŠB – Špela Borko, UA – Ulrike Aspöck, VM – Vojo Milanović, Z – Zerny, NHMW – Prirodnjački muzej Beč, IBISS – Institut za biološka istraživanja »Siniša Stanković«, Beograd.

No.	Locality	Coordinates	Description	Date	Leg.	Det.	Depository
1	Sarajevo, BIH	18.416667, 43.866667	1 adult male	16.7.1929	Z	HA, UA, HH	NHMW
2	Neretva gravel bar at Cerova, Ulog, Kalinovik, BIH	18.356472, 43.378611	Bou-Rouch pump at the river bank, depth 60-90 cm (Point 5); 1 larva	1.7.2022	BR, EP, MZ, ŠB, VM	SR	NHMW
3	Neretva River above the old wooden bridge, Rrastovac Ulog, Kalinovik, BIH	18.369990, 43.365230	hand-net benthic sample in about 40 cm depth; 1 larva	30.6.2022	SA	BT	IBISS

Figure 2. *Nevrorthus apatelios*, larva from the Neretva gravel bar at Cerova, dorsal view (photo: H. Bruckner).
Slika 2. *Nevrorthus apatelios*, ličinka iz prodišča na Neretvi pri Cerovi, dorzalni pogled (foto: H. Bruckner).
Slika 2. *Nevrorthus apatelios*, larva iz neretvanskog šljunka na Cerovi, pogled s leđne strane (foto: H. Bruckner).

Comments of the new findings

The habitat where Nevrorthidae larvae are found is limited to perennial, oxygen rich and clean water systems with gravel in various forms, larger blocks and coarse sand in non-urbanised areas with intact natural vegetation such as maquis, montane forests, or deciduous forests, usually in coastal mountain ranges (Zwick 1967; New 1978; Aspöck & Aspöck 1983; Malicky 1984; Letardi et al. 2005; Jones & Devetak 2009; Gavira et al. 2012; Monserrat & Gavira 2014). The two locations on the Neretva River fit this description – both are situated in the natural river course, surrounded by the well-preserved natural riparian and deciduous forests, with almost no human settlements (Fig. 3). The Nevrorthidae are tolerant towards wide ranges of water temperatures, but sensitive to pollution, so they can be regarded as indicators of clean water (Malicky 1984). Their presence in the upper Neretva part supports the pristine and non-polluted nature of this part of the river.

Figure 3. One new locality of *N. apatelios* on the Neretva River at Cerova (Loc. 2, Fig, 1, Tab. 1), with gravel bars and surrounding forests (photo: M. Zagmajster).

Slika 3. Pogled na novo nahajališče vrste *N. apatelios* na reki Neretvi pri Cerovi (Loc. 2, Sl. 1, Tab. 1), s prodišči in okoliškimi gozdovi (foto: M. Zagmajster).

Slika 3. Pogled na novo nalazište *N. apatelios* na rijeci Neretvi kod Cerove (Loc. 2, Sl. 1, Tab. 1), sa šljunkom i okolnim šumama (foto: M. Zagmajster).

Although a high abundance has been reported for some species of Nevrorthidae at certain localities (e.g. *N. fallax* in Corsica and Sardinia; Mosely 1932; Zwick 1967; Malicky 1984), this is not likely to be the general case. Nevrorthidae are rarely collected even in targeted searches and appear to be very locally distributed (Popov 2002; Jones & Devetak 2009; Gavira et al. 2012; Monserrat & Gavira 2014). This is especially true for *N. apatelios*, having only a few confirmed records for most countries within its range. Even though in some countries of its range, ecological river monitoring is conducted also by standardised monitoring of benthic communities, this is not reflected in more common findings of the species. But, it is most likely that a lack of studies of river invertebrates within Bosnia and Herzegovina is the main reason for the paucity of records and the rediscovery of the species after almost 100 years.

The finding of the larva in a deep interstitial zone indicates that in this developmental stage the species occurs in deeper interstitial habitats, too, which are rarely sampled by regular river monitoring practices. Even if the specimen was pumped in from the surrounding area of the Bou-Rouch pole at 60-90 cm depths, this would still present a subterranean interstitial layer. The slender body of the larvae and known ecology of living between the pieces of sand and gravel support the possibility of the species actively entering this kind of habitat. On the other hand, it is not only the larvae that are rarely found, but also the adults (Letardi et al. 2005, Jones & Devetak 2009). So, if larvae in deep interstitial zones had been overlooked on a large scale, one would expect to find numerous adults – which evidently does not seem to be the case.

General paucity of Nevrorthidae records and their sensitivity and dependence on clean waters (Zwick 1967; New 1978; Aspöck & Aspöck 1983; Malicky 1984; Letardi et al. 2005; Jones & Devetak 2009; Gavira et al. 2012; Monserrat & Gavira 2014) may also indicate the possibility of their disappearance due to human activities, as assumed by Aspöck & Aspöck (2010). This makes the two findings within the upper course of the Neretva River even more important, and supports protection of the pristine and unaltered nature of the river and its wider area.

To our knowledge, *N. apatelios* has no conservation status within Bosnia and Herzegovina, nor within the countries in other parts of its range. A first attempt to protect this species was made in Bulgaria, where it is listed as »vulnerable« in the Red Data Book of the Republic of Bulgaria with B2ab(ii) conservation status according to IUCN criteria (Golemanski et al. 2011; Marković et al. 2016). Due to its scattered distribution, rarity of individuals found at the site, and assumed dependence on clean non-polluted habitats, *N. apatelios* would have to be put on the IUCN Red List of Threatened Species. However, more targeted studies, directed toward sampling larvae as well as adult forms, would be needed to achieve a better understanding of species distribution and ecology.

Povzetek

Edini zapis o vrsti *Nevrorthus apatelios* v Bosni in Hercegovini, odraslem samcu, sega skoraj sto let v preteklost. V Tednu znanosti na Neretvi 2022 sta bili med vodnimi makroskopskimi nevretenčarji najdeni tudi dve ličinki vrste *N. apatelios*. To je izjemno odkritje, saj gre za redko vrsto, ki se pojavlja le zelo lokalno. Njena redkost in odvisnost ličink od čiste, neonesnažene vode poudarjata pomen varovanja neokrnjene narave te reke in njene okolice.

Sažetak

Jedini zapis o *Nevrorthus apatelios* u Bosni i Hercegovini, odraslom mužjaku, datira skoro stotinu godina unazad. Tokom »Neretvanske Sedmice nauke« 2022. godine među vodenim makrobeskičmenjacima prikupljene su i dvije larve *N. apatelios*. Ovo je izvanredno jer je rijetka vrsta koja se javlja samo vrlo lokalno. Njena rijetkost i ovisnost larvi o čistoj, nezagađenoj vodi naglašavaju važnost zaštite netaknute prirode rijeke Neretve i njene okoline.

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A collage of various scientists during the Neretva Science Week looking for macrozoobenthic invertebrates in the river. Photos: Vladimir Tadić

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Study of subterranean biodiversity of the upper Neretva River catchment in Bosnia and Herzegovina

Maja ZAGMAJSTER[1*], Ester PREMATE[1], Špela BORKO[1], Behare REXHEPI[1], Vojo MILANOVIĆ[2], Anton BRANCELJ[3], Michaela BROJER[4], Christophe DOUADY[5], Josef GREGO[1], Milica JOVANOVIĆ[6], Florian MALARD[5], Nataša MORI[3], Vladimir PEŠIĆ[6]

[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, SubBioLab, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; E-mails: maja.zagmajster@bf.uni-lj.si, ester.premate@bf.uni-lj.si, borko.spela@gmail.com, behare.rexhepi@bf.uni-lj.si, jozef.grego@gmail.com
[2]Centre for Karst and Speleology, Branilaca Sarajeva 30, 71 000 Sarajevo, Bosnia and Herzegovina; E-mail: milanovic.vojo9@gmail.com
[3]National Institute of Biology, Department of Organisms and Ecosystems Research, Večna pot 121, SI-1000 Ljubljana, Slovenia; E-mails: anton.brancelj@nib.si, natasa.mori@nib.si
[4]Natural History Museum of Vienna, Burgring 7, 1010 Vienna, Austria; E-mail: michaela.brojer@nhm-wien.ac.at
[5]Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 Lehna, Villeurbanne, France; E-mails: christophe.douady@univ-lyon1.fr, florian.malard@univ-lyon1.fr
[6]Department of Biology, University of Montenegro, Cetinjski put bb, 81000 Podgorica, Montenegro; E-mails: milicajovanovic93@yahoo.com, vladimirp@ac.me
[*]Corresponding author

Abstract. The Dinarides in the Western Balkans host a globally exceptional subterranean biodiversity, but still areas remain with little or no data on subterranean species. In this work, we present the study of subterranean fauna in the upper Neretva River catchment (Eastern Bosnia and Herzegovina), which included the first systematic exploration of the interstitial habitats in the country. During the five-day »Neretva Science Week« in the summer of 2022, we sampled six gravel bars, six springs and one cave. We gathered 268 records (taxon-locality-date) of 116 taxa, with 35 records referring to 27 terrestrial taxa. Nine terrestrial taxa were sampled in a cave, while 19 were found in aquatic samples, mostly from springs. 27 taxa were identified as obligate subterranean (troglobiotic) species, one terrestrial beetle from the cave, and 26 aquatic species, belonging to water mites, snails, and crustaceans. Eight of the aquatic troglobionts present the potential for new species to science. Of the latter, four species were distinguished based on morphology: two species of snails from the families Hydrobiidae and Moitessieriidae, one representative of Ostracoda and one Copepoda. Individuals of the aquatic troglobiotic isopods and amphipods were analysed molecularly, and based on molecular differences, two new species within the genus *Proasellus* and two within the genus *Niphargus* were proposed. Further work on all these taxa is needed for the formal descriptions of the new species to science. Despite the short study period, we have shown that the area is rich in endemic groundwater fauna. The hydroelectric power plant constructions planned in the region threaten groundwater communities by altering subterranean habitats and their connectivity. Further studies are needed to properly highlight the great diversity of the subterranean habitats of the Neretva River catchment and its wider region, and to include this knowledge in conservation strategies.

Key words: troglobiont, *Proasellus*, *Niphargus*, *Kerkia, Paladilhiopsis, Typhlocypris*, *Bryocamptus*, water mites, conservation, hyporheic

Izvleček. Raziskava podzemne biodiverzitete zgornjega porečja reke Neretve v Bosni in Hercegovini – Čeprav je bogastvo podzemne biodiverzitete Dinaridov na zahodnem Balkanu izjemno v svetovnem merilu, tu še vedno najdemo območja z malo ali nič podatki o podzemnih vrstah. V tem delu predstavljamo študijo podzemne favne v zgornjem porečju reke Neretve (vzhodna Bosna in Hercegovina), ki je tudi prva sistematična raziskava intersticialnih habitatov v tej državi. V okviru dogodka »Neretva Science Week« poleti 2022 smo v petih dneh vzorčili šest prodišč, šest izvirov in eno jamo. Zbrali smo 268 podatkov (takson-lokacija-datum) o 116 taksonih, od tega 35 podatkov o 27 kopenskih taksonih. Devet podzemnih taksonov je bilo najdenih v jami, 19 pa v vodnih vzorcih, večinoma iz izvirov. Identificiranih je bilo 27 izključno podzemnih (troglobiotskih) vrst, od tega en kopenski hrošč iz jame in 26 vodnih vrst, ki pripadajo pršicam, polžem in rakom. Osem vodnih troglobiontov predstavlja potencialno nove vrste za znanost. Od slednjih smo morfološko razločili štiri: dve vrsti polžev iz družin Hydrobiidae in Moitessieriidae, eno vrsto dvoklopnikov (Ostracoda) in eno vrsto ceponožcev (Copepoda). Molekularno smo analizirali osebke vodnih troglobiotskih enakonožcev in postranic ter na podlagi molekularnih razlik identificirali dve novi vrsti v rodu *Proasellus* in dve v rodu *Niphargus.* Do opisov novih vrst za znanost bodo potrebne dodatne raziskave teh taksonov. Kljub kratkemu obdobju raziskave so smo pokazali, da je območje bogato z endemično podzemno vodno favno. Gradnje hidroelektrarn, ki so načrtovane v regiji, ogrožajo živalstvo podzemnih voda, tako s spreminjanjem podzemnih habitatov kot tudi njihove povezljivosti. Priporočamo nadaljnje študije, ki bodo lahko dodatno potrdile visoko vrstno pestrost podzemnih habitatov v porečju reke Neretve in širši regiji, to znanje pa je treba vključili v varstvene strategije.

Ključne besede: troglobiont, *Proasellus*, *Niphargus*, *Kerkia, Paladilhiopsis, Typhlocypris*, *Bryocamptus*, vodne pršice, varstvo, hiporeik

Apstrakt. Studija podzemnog biodiverziteta gornjeg toka rijeke Neretve u Bosni i Hercegovini – Iako je bogatstvo podzemnog biodiverziteta Dinarida na zapadnom Balkanu izuzetno na globalnom nivou, još uvijek postoje područja sa malo ili nimalo podataka o podzemnim vrstama. U ovom radu predstavljamo proučavanje podzemne faune u slivu rijeke Neretve (istočni dio Bosne i Hercegovine), koje je uključivalo prvo sistematsko istraživanje intersticijalnih staništa u zemlji. Tokom petodnevne »Sedmice nauke na Neretvi« u ljeto 2022. godine uzorkovali smo: šest lokacija sa šljunkom, šest izvora i jednu pećinu. Prikupili smo 268 zapisa (takson-lokalitet-datum) od 116 taksona, sa 35 zapisa koji se odnose na 27 kopnenih taksona. Devet kopnenih taksona uzorkovano je u pećini, dok je 19 pronađeno u vodenim uzorcima, uglavnom iz izvora. Identificirano je 27 taksona kao obavezne podzemne (troglobiotske) vrste, jedan tvrdokrilac iz pećine i 26 vodenih vrsta, koje pripadaju vodenim grinjama, puževima i rakovima. Osam vodenih troglobionta predstavljaju potencijalne nove vrste za nauku, od kojih, četiri vrste su izdvojene na osnovu morfologije: dvije vrste puževa iz porodica Hydrobiidae i Moitessieriidae, jedan predstavnik Ostracoda i jedan Copepoda. Molekularno su analizirane jedinke vodenih troglobiotičkih izopoda i amfipoda, a na osnovu molekularnih razlika predložene su dvije nove vrste u okviru roda *Proasellus* i dvije unutar roda *Niphargus*. Potreban je dalji rad na svim ovim taksonima do formalnih opisa novih vrsta za nauku. Unatoč kratkom periodu istraživanja, pokazali smo da je područje bogato endemskom faunom podzemnih voda. Planirana izgradnja hidroelektrana u regionu ugrožavaju zajednice podzemnih voda mijenjajući podzemna staništa i njihovu povezanost. Potrebna su dalja istraživanja, kako bi se na pravi način istakla velika raznovrsnost podzemnih staništa sliva rijeke Neretve i šireg regiona te da bi se ovo znanje uključilo u strategije očuvanja.

Ključne riječi: troglobiont, *Proasellus*, *Niphargus*, *Kerkia*, *Paladilhiopsis*, *Typhlocypris*, *Bryocamptus*, vodene grinje, očuvanje, hiporeično stanište

Introduction

The Dinarides are a mountainous karst ridge in the Western Balkans, with globally exceptional subterranean biodiversity (Sket 2012). Besides being a global hotspot in the number of subterranean species, it is also the home to some of the world's unique subterranean taxa, e.g. cave tube worm (*Marifugia cavatica* Absolon & Hrabe, 1930), cave hydrozoan (*Velkovrhia enigmatica* Matjašič & Sket, 1971), cave mussels (*Congeria* spp.), and the world's largest subterranean amphibian, the olm (*Proteus anguinus* Laurenti, 1768) (Sket 2012). Previous studies have identified regional hotspots of subterranean biodiversity but also knowledge gaps in the distribution of subterranean species (Zagmajster et al. 2008; Bregović et al. 2019; Borko et al. 2022). Both are characteristics of Bosnia and Herzegovina (BIH), extending over the middle and southeastern parts of the Dinarides. Whilst its southern parts have long been recognised as subterranean biodiversity hotspots (Culver et al. 2009; Zagmajster et al. 2014), there are parts of the country with few or even no data on subterranean species. The upper part of the Neretva River in eastern BIH, the focus of the studies conducted during the »Neretva Science Week« in the summer 2022, was also considered an overlooked area.

Studies of subterranean fauna typically focus on the sampling of caves and to a lesser extent on other subterranean habitats (Culver & Pipan 2014, 2019). Yet, subterranean species are not limited to caves, nor karst areas, but also occur outside consolidated geological substrata (Culver & Pipan 2014). An example of such are interstitial habitats, defined as a complex of dry or water-filled voids between sediment particles of different sizes (Culver & Pipan 2014). Even though many subterranean species have been described from the aquatic interstitial habitats of the Western Balkans, they received far less research attention than karst habitats, even within the Dinarides (SubBioDB 2023).

Prior to the present study in summer 2022, there were only a few records of subterranean species from the upper Neretva River (SubBioDB 2023). Most of the records refer to terrestrial species, especially Coleoptera. For example, the cave Velika Đeverđela near Ulog is a type locality of the subterranean beetle subspecies, *Anthroherpon ganglbaueri distinguendum* (Müller, 1913) (Giachino & Vailati 2005). Data on aquatic subterranean species were scarce (SubBioDB 2023), with two localities of *Niphargus borkanus* (Karaman S., 1960) described from a spring near Boračko jezero (Karaman 2014) and two endemic snail species, *Plagigeyeria konjicensis* Grego, 2020 and *P. ljutaensis* Grego, 2020, described from springs in Ljuta canyon close to Konjic (Grego 2020). The upper part of the Neretva River presents an interesting area for subterranean species, due to its pristine nature, presence of many gravel bars and karst hills surrounding the valley. Improved knowledge of the subterranean fauna of this area is especially important as there are many hydropower plants planned for the upper part of Neretva River, which will greatly affect the currently well-preserved area. Negative effects must also be expected for the down- stream sections, as they disturb the natural seasonal flow regime, and, in the worst case, create unexpected flow peaks (hydro-peaking). Hydropower constructions will affect the subterranean realm, by changing the natural subterranean water courses, their connections and water table levels, and will consequently negatively affect subterranean habitats (Fišer et al. 2022).

In this contribution, we present the results of the survey on the subterranean fauna conducted during the »Neretva Science Week« in the summer of 2022. We sampled various subterranean habitats, but specifically focused on the interstitial waters of the upper Neretva River, investigated here for the first time.

Materials and methods

Sampling area and sampling sites

The study was conducted in the upper Neretva River catchment, in the area extending from Krupac to Konjic (Fig. 1). We sampled river interstitial habitats on the accessible gravel bars near the river, but also springs, when found, and one cave. We determined the geographical position of each sampled locality using a Garmin handheld GPS or areal maps accessed via Google Maps web map service (http:\\maps.google.com).

The selection of sampling localities was carried out differently for the different habitat types. We selected gravel bars based on the »main« or »additional« sampling sites suggested by the organisers of the Neretva Science Week. We checked these places using areal images, as provided by the aforementioned web map service, and searched for accessible gravel bars (those being large enough to permit sampling; and being easily accessed by vehicles). Thereby, we identified five localities distributed along the upper stretch of the Neretva River for our work (Fig. 1). In addition, we searched for springs in the upper-most part of the focal area (following the upstream path along the Krupac tributary) but also sampled any spring we discovered during the fieldwork. We visited a cave thanks to the help of local inhabitants of the town of Ulog who guided us to the cave entrance.

Fieldwork

River interstitial habitats

We used the Bou-Rouch method (Bou & Rouch 1967) to sample interstitial waters at all gravel bars except one (Tab. 1). This sampling device consists of a hollow metal pipe with a pointed end, with holes of about 0.5 cm diameter distributed within about 30 cm length at its lower end. The pipe was pushed into the gravel to the desired depth by using a mallet, after which a hand pump was set on its upper part (Fig. 2A). Pumping creates a negative pressure pulls the interstitial water along with sediments and animals, into to the pipe and through the pump into a collection bucket. We sampled at multiple points at each gravel bar, to account for the potential variation amongst sampling points within a single gravel bar. At each locality, we identified at least three sampling points at the river bank with a distance of approx. 5 – 10 m between neighbouring points. We positioned the Bou-Rouch pipe at two depths – so that the section with holes reached depths between 30 – 60 cm, and between 60 – 90 cm. From each depth, we sampled at least 30 litres of water with sediment and organisms and then filtered these through hand water nets of two different mesh sizes – 0.5 mm for the coarse fraction, and 0.1 mm for the fine fraction. We sampled from two depths at three points along each gravel bar whenever possible. This protocol could not be completed at two gravel bars, due to equipment and weather problems (Tab. 1). At two localities, we sampled at more points on the gravel bar to collect more individuals from microcrustacean groups. We report cumulative findings of different taxa per gravel bar, without considering their abundance or differences in number of sampled points per locality.

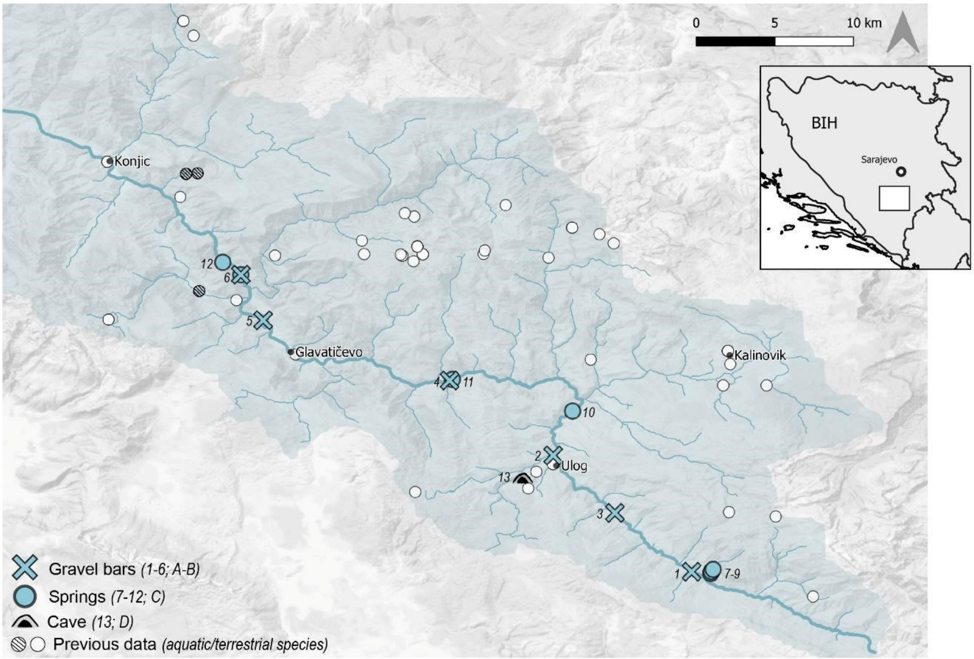

Figure 1. Map of studied subterranean localities in the upper catchment of Neretva River, combining those surveyed during »Neretva Science Week 2022« and those for which data existed before this study (SubBioDB 2023). The numbers on the map correspond to those in Tab. 1. The blue shaded area on the map represents the upper Neretva River catchment (HydroBASINS 1.0, level 08; Linke et al. 2019). The embedded map shows the position of the study area within Bosnia and Herzegovina. Photographs below show the sampling methods used during the study: A: Bou-Rouch method, B: Karaman-Chappuis method, C: sampling of a spring using a hand water net, D: sampling of invertebrates using visual inspection and direct collection. Photo: M. Zagmajster (A, B), E. Premate (C, D).

Slika 1. Zemljevid z lokacijami raziskanih podzemnih habitatov v zgornjem porečju reke Neretve, kjer so združeno prikazane lokalitete, raziskane med tednom »Neretva Science Week 2022«, in tiste, za katere so bili podatki zbrani že pred to študijo (SubBioDB 2023). Številke na karti se ujemajo s tistimi v tab. 1. Modro obarvano območje ponazarja porečje gornje Neretve (HydroBASINS 1.0, nivo 08; Linke et al. 2019). Vstavljena karta prikazuje položaj območja raziskave znotraj Bosne in Hercegovine. Na fotografijah spodaj so prikazane metode vzorčenja med raziskavo: A – Bou-Rouch-metoda; B – Karaman-Chappuis-metoda; C – vzorčenje izvira z vodno mrežo; D – vzorčenje nevretenčarjev z opazovanjem in neposrednim pobiranjem. Foto: M. Zagmajster (A, B), E. Premate (C, D).

Slika 1. Karta proučavanih podzemnih lokaliteta u gornjem slivu rijeke Neretve, kombinujući one istražene tokom »Sedmice nauke na Neretvi 2022.« i one za koje su postojali podaci prije ove studije (SubBioDB, 2023). Brojevi na karti odgovaraju onima u Tab. 1. Plavo osjenčano područje na karti predstavlja sliv gornje Neretve (HydroBASINS 1.0, nivo 08; Linke et al. 2019). Ugrađena mapa pokazuje položaj istraživanog područja unutar Bosne i Hercegovine. Fotografije ispod prikazuju metode uzorkovanja koje su korištene tokom istraživanja: A: Bou-Rouch metoda, B: Karaman-Chappuis metoda, C: uzorkovanje izvora korištenjem ručne mreže za vodu, D: uzorkovanje beskičmenjaka vizualnim pregledom i direktnim prikupljanjem. Foto: M. Zagmajster (A, B), E. Premate (C, D).

Figure 2. Presentation of sampling methods used during the study of subterranean fauna in the upper catchment of Neretva River. A: Bou-Rouch method, B: Karaman-Chappuis method, C: sampling of a spring using a hand water net, D: sampling of invertebrates using visual inspection and direct collection. Photo: M. Zagmajster (A, B), E. Premate (C, D).

Slika 2. Prikaz metod vzorčenja med raziskavo podzemne fauna v zgornjem porečju reke Neretve: A – Bou-Rouch-metoda; B – Karaman-Chappuis-metoda; C – vzorčenje izvira z vodno mrežo; D – vzorčenje nevretenčarjev z opazovanjem in neposrednim pobiranjem. Foto: M. Zagmajster (A, B), E. Premate (C,D).

Slika 2. Prikaz metoda uzorkovanja podzemne faune u gornjem slivu rijeke Neretve, koje su korištene tokom istraživanja: A: Bou-Rouch metoda, B: Karaman-Chappuis metoda, C: uzorkovanje izvora korištenjem ručne mreže za vodu, D: uzorkovanje beskičmenjaka vizualnim pregledom i direktnim prikupljanjem. Foto: M. Zagmajster (A, B), E. Premate (C,D).

At one gravel bar, we used the Karaman-Chappuis method to sample interstitial fauna (Fig. 2B; Karaman 1935; Chappuis 1942). We dug a hole in the gravel with a diameter of about one metre and a depth of about half a metre. Once the hole became filled with water, we sampled it using a hand water net with mesh size 0.5 mm, catching the animals that were flushed into the hole by the flow of water from the surrounding interstitial habitats. Smaller invertebrates were likely overlooked due to the mesh size.

Whilst in the field, whenever bigger animals were noticed by sight in the collected samples, they were directly collected with forceps and then stored in 96% ethanol. The remaining material was stored in large containers and also preserved with 96% ethanol.

Springs and caves

We sampled the springs using a kick-sampling method and collected the animals using hand water nets with 0.5 mm mesh size, again being aware of the possible loss of smaller invertebrates due to mesh size (Fig. 2C). The sampled springs were rheocrenes: places of groundwater discharge in the form of running water forming a channel or a spring brook. We visually checked the under sides of stones, and in some cases, collected samples of sand for later inspection.

To explore the cave, we used standard speleological equipment including caving harnesses and ropes. We only sampled the animals in the cave by visual inspection of different microhabitats: cave walls, ceiling, floor, puddles, beneath the stones, and near organic remains (Fig. 2D). Whenever an animal was found, it was carefully collected and stored in a vial with 96% ethanol.

Abiotic parameters

At each locality, we measured the abiotic parameters in the surface river water, and in 10 litres of each sampling point immediately after pumping, using the Eutech multimeter instrument CyberScan CD 650 (Eutech Instruments Pte Ltd.). We measured water temperature (°C), dissolved oxygen concentration (mg/l) and saturation (%), pH and conductivity (μS/cm). Measuring dissolved oxygen in water withdrawn with the Bou-Rouch pump might have slightly overestimated the oxygen content of sediment because of oxygenation introduced by the pump itself. Our previous experiences and tests indicated small deviations between the two when water was gently pumped to minimise mixing with the air. Measurements were carried out immediately after pumping and at the bottom of the bucket. Abiotic parameters were not measured in springs and caves, but at some springs we measured water temperature using a hand thermometer.

Sample processing

We sorted the samples in the laboratory using a stereomicroscope (10 – 40 x magnifications) and classified the animals into higher taxonomic groups. When the samples contained a lot of sediment with particles, we only analysed a subsample (of about 1/5 to 1/3 of the whole sample).

We sent specimens of various taxonomic groups to the relevant experts, but due to a large amount of material, identifications of some groups were not finished in time for the preparation of this publication. Most specimens were identified based on morphological characters; which have certain limitations in species identification. This approach enabled the identification of potential new species, but for the final description, further work, including molecular analyses, is needed. The coauthors of this contribution checked the following taxa: JG – Mollusca, MJ & VP – Acarina, NM – Ostracoda, AB – Copepoda, FM & CD – aquatic Isopoda, EP, BR& ŠB – Amphipoda, MB – aquatic Coleoptera. The remaining groups were identified by Miloš Vittori (terrestrial Isopoda), Peter Trontelj (Hirudinea) and Cene Fišer (Amphipoda). The identification of Neuroptera was carried out by Susanne Randolf, respective results are presented separately (see Randolf et al., this issue).

Two taxa, for which molecular analyses were conducted due to limitations in morphological identifications, were subterranean macrocrustaceans from the orders Amphipoda and Isopoda. In these cases, the individuals were identified using molecular markers. For DNA-isolation and COI-amplification in amphipods, we followed the protocol described in Borko et al. (2022). We used the COI LCO-HCO barcoding marker, following the procedure in Delić et al. (2017). We compared the COI sequences to those available in GenBank and our own DNA sequence collection (SubBioDB 2023) according to Borko et al. (2022). We calculated uncorrected p-distance and inferred the maximum likelihood phylogeny with IQ-TREE (Minh et al. 2020). Molecular analyses of asellid isopods (Isopoda, Asellidae) followed the standard procedure described in Morvan et al. (2013). Specimens were identified as belonging to new species based on their genetic divergence with other species, as evaluated from the COI gene. Following Lefébure et al. (2006), two clades diverging by more than 0.16 substitution per site, as measured by patristic distances, have a strong probability (ca. 0.99) of belonging to different species.

In the overview of taxa, we indicated the taxa that presumably live their whole life cycle in subterranean habitats as obligate subterranean (troglobiotic) taxa.

Results

We surveyed six gravel bars, six springs, and one cave (Tab. 1). The entire sampling protocol using the Bou-Rouch method, including sample collection at two depths and at three spots, was only carried out at three of the five gravel bars. At all the gravel bars, physical-chemical parameters of the interstitial water differed from those of the surface water, i.e. temperatures were slightly higher, pH was slightly lower, and dissolved oxygen concentrations were lower in the interstitial water (Tab. 2). Electric conductivity was highest just below Glavatičevo village (Loc. 5, Tab. 1).

Table 1. The list of localities sampled in the upper Neretva River catchment in summer 2022. Names in brackets and italic font refer to pre-determined localities by the »Neretva Science Week« team, coordinates are in decimal degrees (WGS84). Abbreviations refer to: S - shallow (30-60 cm) and D - deep (60-90 cm) interstitial layers.
Tabela 1. Seznam lokalitet na območju zgornjega porečja reke Neretve, kjer smo vzorčili poleti 2022. Imena v oklepajih in poševnem tisku se nanašajo na predhodno določene lokalitete s strani ekipe dogodka »Neretva Science Week«, koordinate so v decimalnih stopinjah (WGS84). Okrajšave pomenijo: S – plitki (30-60 cm) in D – globoki (60-90 cm) sloji intersticiala.
Tabela 1. Spisak uzorkovanih lokaliteta u slivu gornjeg toka Neretve u ljeto 2022. godine. Imena u zagradama odnose se na unaprijed određene lokalitete od strane tima »Sedmice nauke na Neretvi«, koordinate su u decimalnim stepenima (WGS84). Skraćenice se odnose na: S - plitki (30-60 cm) i D - duboki (60-90 cm) međuprostorni slojevi.

No.	Locality	Coordinates	Date	Methods	Number of samples
1	Neretva gravel bar, near the farm downstream from confluence Krupac-Neretva (*Krupac Confluence (1), main locality*)	18.41654, 43.33251	29.6.2022	Bou-Rouch method	3 x S 5 x D
2	Neretva gravel bar, at the swimming beach downstream from Ulog (*Swimming beach (5), main locality*)	18.308137, 43.423636	30.6.2022	Bou-Rouch method	3 x S 3 x D
3	Neretva gravel bar at Cerova (*Cerova (3), main locality*)	18.35621, 43.37887	1.7.2022	Bou-Rouch method	3 x S 5 x D
4	Neretva gravel bar upstream from Brijestov bridge (*Brijestov bridge (8), main locality*)	18.227032, 43.482262	2.7.2022	Bou-Rouch method	3 x S 2 x D
5	Neretva gravel bar below Tajoraft (*Tajoraft (10), additional locality*)	18.081052, 43.529472	3.7.2022	Bou-Rouch method	2 x S 1 x D
6	Spring in the gravel on the right bank of Neretva (rafting lunch location)	18.063583, 43.565139	3.7.2022	Karaman – Chappuis method	1
7	Spring 1 above Krupac confluence	18.43152, 43.33118	29.6.2022	Water net, direct collecting	1
8	Spring 2 above Krupac confluence	18.43196, 43.33272	29.6.2022	Water net, direct collecting	1
9	Spring 3 above Krupac confluence	18.43337, 43.33436	29.6.2022	Water net, direct collecting	1
10	Spring near the dirt road to Neretva at Nedavić	18.323332, 43.458369	2.7.2022	Direct collecting	1
11	Neretva tributary springs, left bank of Neretva, 200 m upstream from Brijestov most	18.22869, 43.482222	2.7.2022	Water net, direct collecting	1
12	Spring in the Neretva canyon, left bank (rafting)	18.049440, 43.574848 (+/-200m)	3.7.2022	Water net	1
13	Velika Đeverđela	18.284287 43.405874	30.6.2022	Direct collecting	1

Table 2. The abiotic parameters and number of taxa collected at each locality (Loc. no. refers to Tab. 1). Average value, standard deviation and number of measurements (N = number of samples; see Tab. 1) are given for each abiotic parameter. Measurements of the surface river water samples are given for the first five localities (R), while S indicates the groundwater measurements. At some springs, abiotic parameters are not reported, as they were not measured. The last four columns contain: all - the number of all taxa, aq – the number of aquatic taxa, tgb – the number of troglobiotic taxa, nsp - the number of potentially new species for science. See Tab. 3 for details on taxa.

Tabela 2. Abiotski parametri in število taksonov, nabranih na vsaki lokaliteti (Loc. no. se nanaša na oznake v tab. 1). Za vsak abiotski parameter so podane povprečne vrednosti, standardna deviacija in število meritev (N = število vzorcev; glej tab. 1). Meritve površinske vode reke (R) so podane za prvih pet lokalitet, medtem ko S označuje meritve podzemnih voda. Na nekaj izvirih nismo merili abiotskih parametrov, zato jih v tabeli ni. Zadnji štirje stolpci podajajo: all – število vseh taksonov, aq b- število vodnih taksonov, tgb – število troglobiotskih taksonov, nsp – število potencialnih novih vrst za znanost. Glej tab. 3 za podrobnost o taksonih.

Tabela 2. Abiotički parametri i broj taksona prikupljenih na svakom lokalitetu (lok. br. se odnosi na tab. 1). Prosječna vrijednost, standardna devijacija i broj mjerenja (N = broj uzoraka; vidjeti tab. 1) dati su za svaki abiotički parametar. Mjerenja površinske riječne vode data su na prvih pet lokaliteta (R), dok S označava mjerenja podzemnih voda. Na nekim izvorima abiotički parametri nisu prijavljeni, jer nisu mjereni. Posljednje četiri kolone sadrže: all - broj svih taksona, aq - broj vodenih taksona, tgb - broj troglobiotičkih svojti, nsp - broj potencijalno novih vrsta za nauku. Vidi tab. 3. za detalje o taksonima.

Loc. No.	Depth	N	T (°C)	pH	O_2 (%)	O_2 (mg/L)	Cond. (µS)	all	aq	tgb	nsp
1	S	8	17.1 (± 0.8)	8.2 (±0.1)	84.7 (± 5.3)	7.4 (±0.4)	253.6 (± 6.6)	34	33	8	3
	R	1	16.9	8.34	102.2	9.18	246.4				
2	S	6	18.8 (± 0.9)	7.7 (±0.2)	68.0 (±10.1)	5.8 (±1.0)	308,2 (± 5.8)	43	39	14	4
	R	1	17.8	7.8	86.9	7.97	298				
3	S	8	19.2 (±1.2)	7.8 (±0.1)	80.5 (± 8.6)	6.8 (±0.6)	287.3 (±3.4)	33	31	10	3
	R	1	16.8	7 8.	94.1	8.59	286.9				
4	S	5	17.2 (±1.7)	7.9 (±0.0)	82.7 (±8.7)	7.8 (±0.8)	276.5 (±3.1)	34	32	13	4
	R	1	15.4	8.28	105.3	10.27	277.5				
5	S	3	16.8 (± 0.6)	7.9 (±0.3)	59.0 (±10.2)	5.7 (± 0.9)	371.2 (± 31.6)	23	22	4	1
	R	1	12.5	8.03	91.7	10.38	322.9				
6	S	1	/	/	/	/	/	15	13	2	1
7	S	1	7.5	7.3	48.1	4.52	437.7	1	1	0	0
8	S	1	7.8	7.49	82	8.78	409.6	7	7	0	0
9	S	1	7	7.73	92.7	9.97	241.9	29	16	1	0
10	S	1	/	/	/	/	/	2	2	1	0
11	S	1	/	/	/	/	/	19	19	2	0
12	S	1	/	/	/	/	/	19	19	2	0
13	/	1	/	/	/	/	/	9	0	1	0

Altogether, 268 records (taxon-locality-date) of 116 taxa were recorded from the 13 localities (Tab. 3), with 35 records referring to 27 terrestrial taxa. While we sampled terrestrial taxa only in the cave, 19 were found in aquatic samples, mostly from springs. Overall, 27 obligate subterranean (troglobiotic) species were identified, one terrestrial beetle from the cave, and 26 aquatic species, belonging to water mites, snails and crustaceans (Tab. 3).

The highest richness of aquatic taxa was recorded in the Neretva gravel bar at the swimming beach near Ulog (43 taxa, Loc. 2, Tab. 1), followed by the gravel bar downstream of the Krupac-Neretva confluence (34 taxa, Loc. 1, Tab. 2) and the gravel bar upstream of Brijestov bridge (34 taxa, Loc. 4, Tab. 1) as well as the gravel bar near Cerova (33 taxa, Loc. 3, Tab. 2). The gravel bars at Ulog and Brijestov bridge harboured the highest number of troglobionts, followed by the other two aforementioned gravel bars (Tab. 2). There were up to four potentially new species per gravel bar – with at least one present at each of them.

We recorded much lower species diversity at springs. Some localities had many terrestrial taxa that were accidentally present in the aquatic samples (Tabs. 2,3). Troglobiotic taxa were found at four springs (Tab. 2).

Table 3. The list of taxa found in subterranean habitats of the upper Neretva River catchment between Krupac and Konjic in the summer of 2022. Acronyms refer to: T – troglobiont, L – larval state (only in insects), N.SP. – potentially new species for science, ter – terrestrial taxon, B – bone remains. The numbers of localities, given in the last three columns, refer to Tab. 1.

Tabela 3. Seznam taksonov, najdenih v podzemnih habitatih porečja zgornje rege Neretve med krajema Krupac in Konjic poleti 2022. Okrajšave pomenijo: T – troglobiont, L – ličinka (le pri žuželkah), N. SP. – potencialno nova vrsta za znanost, ter – kopenski takson, B – kostni ostanki. Številke lokalitet, podane v zadnjih treh stolpcih, se nanašajo na Tab. 1.

Tabela 3. Spisak taksona pronađenih u podzemnim staništima gornjeg sliva rijeke Neretve između Krupca i Konjica u ljeto 2022. godine. Akronimi se odnose na: T – troglobiont, L – stanje larve (samo kod insekata), N.SP. – potencijalno nova vrsta za nauku, ter – kopneni takson, B – ostaci kostiju. Brojevi lokaliteta, dati u posljednje tri kolone, odnose se na Tab. 1.

Group	**Family**	**Taxon**	**Gravel bars**	**Springs**	**Cave**
Nematoda		Nematoda	1, 2, 3, 4, 5, 6	9, 11, 12	
Turbellaria		Turbellaria		9	
Oligochaeta		Oligochaeta	1, 2, 3, 4, 5, 6	11, 12	
Hirudinea	Erpobdellidae	*Dina* cf. *dinarica*		8	
Gastropoda	Hydrobiidae	*Belgrandiella* sp. T	1, 3, 5	9, 11, 12	
		Bythinella sp.	2, 3, 4, 5	9, 10	
		Islamia sp. T	4	11, 12	
		Kerkia sp. T	2, 4		
		Kerkia sp.nov. T - N.SP.	4		
	Moitessieriidae	*Paladilhiopsis* sp.nov. T - N.SP.	2, 3, 4, 5		
		Paladilhiopsis sp. T	5, 6	10	
	Planorbidae	*Ancylus fluviatilis* O. F. Müller, 1774	5, 6	11, 12	
		Ancylus sp.	5	11	
	Lymnaeidae	*Galba truncatula* (O. F. Müller, 1774)	5		
		Radix sp.		12	
	Valloniidae	*Vallonia* sp. ter	2		
	Ellobiidae	*Carychium tridentatum* (Risso, 1826) ter	2		
		Carychium sp. ter	2, 4	9	
	Pristilomatidae	*Vitrea* sp. ter	5, 6	9	
	Zonitidae	*Aegopis* sp. ter		9	
	Punctidae	*Punctum pygmaeum* (Draparnaud, 1801) ter		9	

Group	**Family**	**Taxon**	**Gravel bars**	**Springs**	**Cave**
	Gastrodontidae	*Aegopinella* sp.	5		
		Zonitoides nitidus (O. F. Müller, 1774) [ter]		9	
	Discidae	*Discus perspectivus* (Megerle von Mühlfeld, 1816) [ter]	6		
	Cochlostomatidae	*Cochlostoma* sp. [ter]		9	
	Pupillidae	*Pupilla* sp. [ter]	3		
	Hygromiidae	*Monacha* sp. [ter]	3		
	Oxychilidae	*Oxychilus* sp. [ter]	4		
Bivalvia	Sphaeriidae	*Pisidium* sp.	1, 5		
Diplopoda		Diplopoda [ter]		9	
Symphyla		Symphyla [ter]			13
Acarina	Aturidae	*Aturus crinitus* Thor, 1902	3		
		Erebaxonopsis brevipes Motas & Tanasachi, 1947 [T]	2		
		Paraxonopsis inferorum Motas & Tanasachi, 1947 [T]	1, 2		
		Paraxonopsis vietsi (Motas & Tanasachi, 1947)[T]	2		
	Frontipodopsidae	*Frontipodopsis reticulatifrons* Szalay, 1945 [T]	2, 3, 4		
	Halacaridae	*Parasoldanellonyx typhlops* Viets, 1933 [T]	4		
		Parasoldanellonyx sp.	4		
	Hungarohydracari-dae	*Hungarohydracarus subterraneus* Szalay, 1943 [T]	2, 3		
	Hydryphantidae	*Partnunia* sp.		8	
	Hygrobatidae	*Atractides latipes* Szalay, 1935	3		
		Atractides pumilus Szalay, 1946 [T]	1, 2, 3, 4		
		Atractides sp.	1	9	
	Lethaxonidae	*Lethaxona pygmaea* Viets, 1932[T]	2		
	Momoniidae	*Stygomomonia latipes* Szalay, 1934 [T]	4		
	Sperchontidae	*Sperchon glandulosus* Koenike, 1886		9	
	Torrenticolidae	*Torrenticola anomala* Koch, 1837	2, 3		
		Torrenticola jeanneli Motas & Tanasachi, 1947 [T]	3		
		Torrenticola tenuirostris Viets, 1936	3		
		Torrenticola sp.	1, 2		
	Oribatida fam.	Oribatida [ter]	1, 2	9	
	Acarina fam.	Acarina g.sp.	1, 2, 3, 4		
		Acarina g.sp. [ter]			13
Aranea	Aranea fam.	Aranea g.sp. [ter]			13
Opiliones	Phalangiidae	Phalangiidae g.sp. [ter]			13

Group	Family	Taxon	Gravel bars	Springs	Cave
Ostracoda	Cyprididae	Cyprididae	3		
		Cavernocypris subterranean (Wolf, 1920)	1		
		Cypridopsis sp.	4		
		Potamocypris sp.	4, 5		
	Candonidae	Candoninae	2, 3, 4, 5, 6		
		Candona candida (O.F.Müller, 1776)	4		
		Neglecandona sp.	1, 2, 4	8, 9	
		Pseudocandona albicans (Brady, 1864)	2, 4		
		Typhlocypris sp. [T] - N.SP.	4		
Copepoda	Cyclopoida fam.	Cyclopoida	1		
	Cyclopidae	*Diacyclops antrincola* Kiefer, 1968 [T]	4		
		Diacyclops cf. *antrincola* [T]	4		
		Diacyclops clandestinus (Yeatman, 1964) [T]	1, 2, 3, 4, 5		
		Eucyclops serrulatus (Fischer, 1851)	2		
		Megacyclops viridis (Jurine, 1820)	1, 2, 3, 4	11, 12	
	Harpacticoida fam.	Harpacticoida	5		
	Canthocamptidae	*Attheyella crassa* (G.O. Sars, 1863)	2, 3		
		Attheyella wierzejskii (Mrazek, 1893)	1, 2, 4	9	
		Bryocamptus cf. *macedonicus* [T] - N.SP.	1, 3		
		Bryocamptus pygmaeus (Sars G. O., 1863)	1		
		Bryocamptus typhlops (Mrazek, 1893) [T]	1, 2, 3		
		Bryocamptus zschokkei (Schmeil, 1893)	2		
		Bryocamptus sp.	1, 3		
		Elaphoidella elaphoides (Chappuis, 1923)	4		
		Moraria poppei (Mrazek, 1893)	1, 3		
Amphipoda	Niphargidae	*Niphargus* sp. [T] – MOTU 1 - N.SP.	1, 2, 4		
		Niphargus sp. [T] – MOTU 2 - N.SP.	2		
	Crangonyctidae	*Synurella* sp.	1, 2, 4, 5		
	Gammaridae	*Gammarus* sp.		7, 8, 9, 11, 12	
Isopoda	Asellidae	*Proasellus* sp. [T]	2	11, 12	
		Proasellus n.sp. *- MOTU 1* [T] *(P. anophthalmus clade)* - N.SP.	1, 2, 3		

Group	**Family**	**Taxon**	**Gravel bars**	**Springs**	**Cave**
	Asellidae	*Proasellus* n.sp. - *MOTU 2* [T] *(P. anophthalmus clade)* - N.SP.	6		
	Ligiidae	*Ligidium germanicum* Verhoeff, 1901 [ter]		9	
	Trichoniscidae	*Trichoniscus* sp. [ter]		9	
		Hyloniscus sp. [ter]		9	
		Trichoniscidae g.sp. [ter]			13
Collembola		Collembola [ter]		9	13
Diptera		Diptera (L)	1, 2, 3, 4, 5, 6		
	Chironomidae	Chironomidae (L)	1, 2, 3, 4, 5, 6	9, 11, 12	
Plecoptera		Plecoptera (L)	1, 2, 3, 4, 5, 6	9, 11, 12	
Ephemero-ptera		Ephemeroptera (L)	1, 2, 3, 4, 5, 6	8, 9, 11, 12	
Trichoptera		Trichoptera (L)	1, 2, 3, 5, 6	8, 9, 12	
Neuroptera	Nevrorthidae	*Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 (L)	3		
Coleoptera	Coleoptera	Coleoptera (L)	1		
	Scirtidae	*Hydrocyphon* sp. (L)	1, 2		
		Elodes sp. (L)		8	
	Hydraenidae	*Hydraena* cf. *bosnica*		9	
	Elmidae	*Elmis bosnica* (Zaitzev, 1908)		9, 11, 12	
		Elmis sp. (L)		9, 11, 12	
		Esolus cf. *angustatus* (L)	6	11, 12	
		Esolus cf. *parallelepipedus* (L)		11, 12	
		Esolus sp. (L)	1, 2, 3, 5, 6		
		Limnius sp. (L)	1, 2		
		Limnius sp. 1 (L)		11, 12	
		Limnius sp. 2 (L)		11, 12	
		Stenelmis cf. *puberula* (L)	1, 2		
	Staphylinidae	Staphylinidae [ter]		9	
	Leiodidae	*Anthroherpon ganglaueri distinguendum* (Müller, 1913) [ter, T]			13
	Carabidae	cf. *Laemostenus* sp.			13
Pisces		Pisces		11	
Mammalia	Rhinolophidae	*Rhinolophus hipposideros* Bechstein, 1800 (B)			13

Molecular identifications of *Niphargus* sp. and *Proasellus* sp.

Representatives of the genus *Niphargus* sp. were found at three gravel bars (Tab. 1). Individuals from all localities were molecularly analysed, which revealed two different MOTUs (molecular operational taxonomic units). At three localities (Locs. 1, 2, and 4) we found a total of four individuals (voucher codes NG679, NG680, NG681 and NG682, SubBioDB 2023) that belong to the same MOTU 1 (Tab. 3). According to the COI marker, they form a closely related clade with less than 2% uncorrected p-distance, and with 9% uncorrected p- distance to the closest sister clade. In addition, we found one individual of a different MOTU (MOTU 2) at locality 2, which also has the potential to be a new species (voucher code: NG678, SubBioDB 2023). According to the COI results, it is highly divergent from all other *Niphargus* species, with more than a 15% uncorrected p-distance.

Representatives of the genus *Proasellus* sp. were found at three gravel bars (Locs. 1, 2 and 3) and at one gravel bar with the spring (Loc. 6). All specimens collected at the three gravel bars belonged to the same MOTU 1, which is most likely a species new to science. One spring located in the gravel on the right bank of Neretva (Loc. 6, rafting lunch location) contained different MOTU 2, which was also a species new to science (Tab. 3). According to the COI gene, the two MOTUs were highly divergent from all other known species of *Proasellus*, with divergence higher than 16%, as measured with patristic distances.

Discussion

In this study, we conducted the first systematic survey of subterranean fauna in the upper Neretva River. Despite the limited time frame, we gathered valuable new information on subterranean species in the upper Neretva River. Our efforts not only expanded the knowledge on the distribution of known subterranean species but also led to the discovery of species previously unknown to science.

To confirm the existence of these new species and prepare their formal descriptions, further in-depth studies combining molecular, morphological, and distribution data are needed. Molecular analyses conducted in the genera *Proasellus* and *Niphargus* revealed strong indication of new species to science. The comparative analyses of distances to other *Niphargus* species (data taken from Borko et al. 2022) have shown that the MOTUs found during this study are at 9% to 15% uncorrected p-distances to other known species. The MOTU 1, found at three localities, belong to the same MOTU as the MOTU we sampled in a spring near Gacko, south of this study's localities, in March 2021 (SubBioDB 2023, unpublished data). According to the COI marker, they form closely related clades with less than 2% uncorrected p-distance. The distance to other analysed *Niphargus* species is > 9%. Although we lack molecular data for a few species from BIH, they differed in morphology from the ones we sampled. Therefore, we can conclude that these four individuals from Neretva, as well as an individual from a locality near Gacko, belong to one new species of *Niphargus*. It is nested within the south Dinaric clade, together with *N. brevicuspis* and *N. factor* (Borko et al. 2022). The second MOTU and potentially new species of *Niphargus* was collected at the gravel bar near Ulog, in the same area as the previous one. According to the COI analysis, it is highly divergent from all other *Niphargus* species, with even more than a 15% uncorrected p-distance, and falls into the Pontic clade of primarily interstitial species (Borko et al. 2022).

Improving the knowledge on the subterranean fauna of this area is not only an interesting research question per se but is also important from a conservation point of view. Typically, subterranean species are highly endemic, with small distribution ranges, many being known from only single locality (Bregović et al. 2019). Groundwater biota of interstitial and surrounding aquatic habitats are an important part of the river ecosystem, being involved in both nutrient cycling and bioturbation (Hose et al. 2022). Groundwater species also significantly contribute to the overall biodiversity of freshwater ecosystems (Boulton et al. 2008), especially in subterranean diversity hotspots such as the Dinarides.

When limited knowledge on the general biodiversity is confronted with high developmental and economic interests, this makes the Neretva River region very vulnerable to destruction. Due to the lack of data on the existence of endemic species, it is essential to conduct baseline studies gathering both fundamental distribution and ecological data within the upper Neretva River. This is also important for subterranean habitats, which can be heavily affected by the planned hydropower plants in multiple ways (Fišer et al. 2022). The hydropower plant construction can irreversibly destroy the interstitial habitats during its construction. Additionally, following the formation of the reservoir behind the dam, groundwater habitats can be affected by colmation (i. e. filling the voids between gravel with fine sediments) and fragmentation of the previously connected habitats along the river valley. Dams can have a negative impact on the invertebrate communities living beneath them by causing disturbances in the water flow and temperature, leading to a decreased quantity and diversity of invertebrates (Dolédec et al. 2021). This latter study also showed that overall, there are fewer and less diverse invertebrate communities below the dam, favouring species with higher resistance and lower food specialisation and sensitivity to pollution.

The upper parts of the Neretva River are currently preserved in an almost intact state, surrounded by vast forests, and without large dams on the river. The similarity in taxonomic richness of interstitial communities along the studied part of the Neretva River indicates the intact nature of the river, which indicates the longitudinal connectivity of the interstitial habitats and connectivity with surrounding karstic subterranean habitats. It has been shown that karstic subterranean environments are connected through hydrological pathways (Bonacci et al. 2009), and that interstitial habitats are a highway or a migration corridor for subterranean fauna (Ward & Palmer 1994). The upper Neretva still provides such connectivity and migration possibilities, which could be severely affected by damming and hydropower plant construction.

These first results originating from a single field sampling campaign indicate that the upper Neretva area needs further, more extensive research. However, groundwater fauna is still often neglected in freshwater biodiversity studies. We encourage the inclusion of groundwater components in future studies of river biodiversity, especially in understudied areas that are at the same time threatened by destructive hydropower and other construction projects.

Povzetek

Dinaridi so kraško območje na Zahodnem Balkanu z globalno izjemno podzemno biotsko raznovrstnostjo (Sket 2012). A nekatera območja so slabo raziskana (Zagmajster et al. 2008; Bregović et al. 2019; Borko et al. 2022) in med taka spada tudi zgornji del porečja reke Neretve v vzhodni Bosni in Hercegovini. To območje je še zelo dobro ohranjeno, tu je veliko prodišč ob reki Neretvi in njenih pritokih ter kraških gričev, ki obkrožajo dolino. Pred našo študijo je bilo od tod le nekaj podatkov o podzemnih vrstah (SubBioDB 2023).

Raziskava je potekala na območju med krajema Krupac in Konjic (Sl. 1). Vzorčili smo rečni intersticij na dostopnih prodiščih ob reki, a tudi izvire in eno jamo. Z metodo Bou-Rouch (Bou & Rouch 1967) smo vzorčili intersticijske vode na vseh razen na enem prodišču (Tab. 1), kjer smo uporabili metodo Karaman-Chappuis (Karaman 1935; Chappuis 1942). Izvire smo vzorčili z metodo »kick-sampling« in živali zbirali z ročnimi vodnimi mrežami. V jami smo živali iskali s pregledovanjem različnih mikrohabitatov. Abiotske parametre smo merili v površinski rečni vodi in v 10 litrih načrpane podzemne vode na vseh prodiščih razen enem. Na nekaj izvirih smo izmerili temperaturo vode z ročnim termometrom.

Skupaj smo pregledali šest prodišč, šest izvirov in eno jamo. Zbrali smo 268 podatkov (takson-lokacija-datum) o 116 taksonih, od tega 35 podatkov za 27 kopenskih (Tab. 3). Medtem ko smo kopenske taksone vzorčili samo v jami, smo jih 19 našli v vodnih vzorcih, večinoma iz izvirov. Skupno smo zabeležili 27 izključno podzemnih (troglobiotskih) vrst, enega kopenskega hrošča v jami in 26 vodnih vrst, ki pripadajo pršicam, polžem in rakom (Tab. 3).

Največje bogastvo vodnih taksonov je bilo zabeleženo na prodišču Neretve na kopališču pri Ulogu (43 taksonov), sledita prodišče dolvodno od sotočja Krupac-Neretva (34 taksonov) in prodišče gorvodno od Brijestovega mostu (34 taksonov) ter prodišče pri Cerovi (33 taksonov). Največ troglobiontov je bilo v prodiščih pri Ulogu in Brijestovem mostu.

Osem vodnih troglobiontov kaže na potencialne nove vrste za znanost. Od slednjih smo morfološko ločili štiri vrste: dve vrsti polžev iz družin Hydrobiidae in Moitessieriidae, enega predstavnika Ostracoda in enega Copepoda. Na podlagi molekularnih razlik smo identificirali po dve novi vrsti v rodu *Proasellus* (Isopoda) in rodu *Niphargus* (Amphipoda).

Podzemne vrste so zelo endemične, imajo majhna območja razširjenostmi, mnoge so znane le iz po ene lokalitete, kar jih dela zelo ranljive (Bregović et al. 2019). Dokazano je, da so kraška podzemna okolja povezana s hidrološkimi povezavami (Bonacci et al. 2009), intersticialni habitati pa predstavljajo selitvene koridorje za podzemno favno (Ward & Palmer 1994). Zgornja Neretva še vedno omogoča takšno povezljivost in možnosti selitev, ki pa bi jih lahko ogrozile načrtovane zajezitve zaradi gradenj hidroelektrarn. Problematika podzemnih voda bi morala biti del študij biotske raznovrstnosti rek, zlasti na zelo ohranjenih, a premalo raziskanih območjih, ki jih ogrožajo načrtovane hidroelektrarne in druge večje konstrukcije.

Sažetak

Dinaridi su kraško područje na zapadnom Balkanu sa globalno izuzetnim podzemnim biodiverzitetom (Sket 2012). Međutim, neka područja su slabo istražena (Zagmajster et al. 2008; Bregović et al. 2019; Borko et al. 2022), a među njima je i gornji dio sliva rijeke Neretve u istočnom dijelu Bosne i Hercegovine. Ovo područje je još uvijek vrlo dobro očuvano, ima mnogo pješčanih sprudova duž rijeke Neretve i njenih pritoka i kraških brda koja okružuju dolinu. Prije našeg istraživanja, postojalo je samo nekoliko podataka o podzemnim vrstama sa ovog terena (SubBioDB 2023).

Istraživanja su obavljena na području između Krupca i Konjica (Sl. 1). Uzorkovali smo riječni intersticij na pristupačnim dijelovima riječnog korita, izvore i jednu pećinu. Intersticijalne vode uzorkovane su Bou-Rouch metodom (Bou & Rouch 1967) u svim šljunčanim sprudovima osim u jednom (Tab. 1), gdje je korištena Karaman-Chappuis metoda (Karaman 1935; Chappuis 1942). Izvori su uzorkovani metodom »kick-sampling«, a životinje su sakupljene ručnim mrežama za vodu. Tražili smo životinje u pećini ispitujući različita mikrostaništa. Abiotički parametri su izmjereni u površinskoj riječnoj vodi i u 10 litara ispumpane podzemne vode na svim sprudovima osim u jednoj. Na nekoliko izvora mjerili smo temperaturu vode ručnim termometrom.

Ukupno smo ispitali: šest šljunčanih sprudova, šest izvora i jednu pećinu. Prikupili smo 268 podataka (takson-lokacija-datum) za 116 taksona, od čega 35 podataka za 27 kopnenih (Tab. 3). Kopneni taksoni uzorkovani su samo u pećini, 19 ih je pronađeno u uzorcima vode, uglavnom iz izvora. Ukupno smo evidentirali: 27 isključivo podzemnih (troglobiotičkih) vrsta, jednog tvrdokrilca u pećini i 26 vodenih vrsta grinja, puževa i rakova (Tab. 3).

Najveće bogatstvo vodenih taksona zabilježeno je na ušću Neretve i plaži kod Uloga (43 taksona), zatim ušće nizvodno od ušća Krupac u Neretvu (34 taksona), ušće uzvodno od Brijestovog mosta (34 taksona) i ušće kod Cerove (33 taksona). Najviše troglobionta bilo je u šljunčanim sprudovima kod Uloga i Brijestovog mosta.

Osam vodenih troglobionta predstavljaju potencijalne nove vrste za nauku, od kojih su morfološki izdvojene četiri vrste: dvije vrste puževa iz porodica Hydrobiidae i Moitessieriidae, jedan predstavnik Ostracoda i jedan predstavnik Copepoda. Na osnovu molekularnih razlika identifikovali smo po dvije nove vrste u rodu *Proasellus* (Isopoda) i rodu *Niphargus* (Amphipoda).

Podzemne vrste su visokoendemične, imaju mala područja rasprostranjenja, mnoge su poznate samo sa jednog lokaliteta, što ih čini veoma ranjivim (Bregović et al. 2019). Pokazalo se, da su podzemne sredine u kršu povezane hidrološkim vezama (Bonaci et al. 2009), a intersticijska staništa predstavljaju migracione koridore za podzemnu faunu (Ward & Palmer 1994). Gornja Neretva još uvijek pruža takvu povezanost i mogućnosti migracije, koje bi mogle biti ugrožene planiranim branama zbog izgradnje hidroelektrana. Problem podzemnih voda trebao bi biti dio proučavanja biodiverziteta rijeka, posebno u dobro očuvanim, ali nedovoljno istraženim područjima ugroženim planiranim hidroelektranama i drugim velikim projektima.

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The upper Neretva River discontinuum: gradients of taxonomic and functional diversity of benthic invertebrates in a wild Balkan river

Edurne ESTÉVEZ[1*], Davina DIETRICH[1], Simone SAHLER[1], Fabian VASSANELLI[1], Sara WIDERA[1], Jan MARTINI[1,2], Wolfgang WANEK[3], Gabriel SINGER[1]

[1]Department of Ecology, Fluvial Ecosystem Ecology, University of Innsbruck, Austria
[2]Eurac Research, Institute for Alpine Environment, Viale Durso 1, 39100 Bolzano/Bozen, Italy
[3]Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, University of Vienna, Austria
[*]Corresponding author. E-mail: edurne.estevez-cano@uibk.ac.at

Abstract. Free-flowing rivers are highly valuable to understand the taxonomic and functional community structure of organisms that inhabit fluvial ecosystems. We investigated patterns of macroinvertebrate community composition and food web structure using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), including potential environmental drivers, over a free-flowing river continuum in the upper course of the Neretva River in Bosnia and Herzegovina. Results showed a high taxonomic diversity, a high percentage of EPT taxa (Ephemeroptera, Plecoptera, Trichoptera), and a slightly heterotrophic ecosystem (average allochthonous assimilation = 54%). The taxonomic composition differed prominently between a tributary located upstream and the mainstem. However, we also found notable species turnover between the upper (headwater and midsection) reaches and the downstream reaches of the mainstem. Macroinvertebrate abundance and the percentage of EPT peaked downstream while midsection reaches showed the highest taxonomic richness and diversity and allochthonous assimilation. Most of the functional metrics (maximum and average trophic position and isotopic richness and evenness) showed pronounced discontinuities in their spatial patterns, which did not follow the predictions of the River Continuum Concept. These results highlight the uniqueness of this section of the Neretva, where natural discontinuities structure macroinvertebrate communities in ways and by mechanisms not captured by smooth ecological concepts.

Key words: allochthonous assimilation, trophic position, stable isotopes, EPT taxa, food web

Izvleček. Diskontinuum Gornje Neretve: gradienti taksonomske in funkcionalne raznolikosti bentoških nevretenčarjev v divji balkanski reki – Prosto tekoče reke so pomembne za razumevanje taksonomske in funkcionalne strukture združb organizmov, ki naseljujejo rečne ekosisteme. Raziskovali smo vzorce sestave združb makroskopskih nevretenčarjev in strukturo prehranjevalnih spletov z uporabo stabilnih izotopov (δ13C in δ15N), ter potencialne okoljske dejavnike v prosto tekočem rečnem kontinuumu v zgornjem toku reke Neretve v Bosni in Hercegovini. Rezultati so pokazali visoko taksonomsko raznolikost, visok odstotek taksonov EPT (Ephemeroptera, Plecoptera, Trichoptera) in rahlo heterotrofen ekosistem (povprečna alohtona asimilacija = 54 %). Taksonomska sestava se je vidno razlikovala med gorvodnim pritokom in glavnim dolvodnim segmentom reke. Pokazali smo izrazito zamenjevanje vrst med zgornjim delom (povirni in srednji del) in spodnjim delom glavnega rečnega segmenta. Številčnost makroskopskih nevretenčarjev in odstotek EPT sta dosegla najvišjo vrednost dolvodno, medtem ko so srednji odseki pokazali največji taksonomsko pestrost in raznolikost ter alohtono asimilacijo. Večina funkcionalnih meritev (največji in povprečni trofični položaj ter izotopska pestrost in enakomernost) je pokazala izrazite diskontinuitete v prostoru, ki niso v skladu z napovedmi koncepta rečnega kontinuuma. Ti rezultati poudarjajo edinstvenost tega odseka Neretve, kjer naravne diskontinuitete strukturirajo združbe makroskopskih nevretenčarjev na načine in z mehanizmi, ki jih enostavni ekološki koncepti ne zajemajo.

Ključne besede: alohtona asimilacija, trofični položaj, stabilni izotopi, taksoni EPT, prehranjevalni splet

Apstrakt. Diskontinum gornje Neretve: Gradijent taksonomske i funkcionalne raznovrsnosti bentoskih beskičmenjaka u divljoj balkanskoj rijeci – Rijeke slobodnog toka su veoma vrijedne za razumijevanje taksonomske i funkcionalne strukture zajednica organizama koji naseljavaju riječne ekosisteme. Istraživali smo obrasce sastava zajednice makrobeskičmenjaka i strukture trofičke mreže koristeći stabilne izotope (δ13C i δ15N), uključujući potencijalne ekološke pokretače, preko slobodnog riječnog kontinuma u gornjem toku rijeke Neretve u Bosni i Hercegovini. Rezultati su pokazali visoku taksonomsku raznovrsnost, visok postotak EPT taksona (Ephemeroptera, Plecoptera, Trichoptera) i blago heterotrofni ekosistem (prosječna alohtona asimilacija = 54 %). Taksonomski sastav se značajno razlikovao između pritoke koja se nalazi uzvodno i glavnog toka. Takođe smo otkrili značajnu smjenu vrsta između gornjih (izvorište i srednji dio) tokova i nizvodnih tokova glavnog vodotoka. Obilje makrobeskičmenjaka i postotak EPT-a dostigli su vrhunac nizvodno, dok su dosezi srednjeg dijela pokazali najveće taksonomsko bogatstvo, raznovrsnost i alohtonu asimilaciju. Većina funkcionalnih metrika (maksimalna i prosječna trofička pozicija te izotopsko bogatstvo i ravnomjernost) pokazala je izražene diskontinuitete u svojim prostornim obrascima, koji nisu slijedili predviđanja koncepta riječnog kontinuma. Ovi rezultati naglašavaju jedinstvenost ovog dijela Neretve, gdje prirodni diskontinuiteti kreiraju zajednice makrobeskičmenjaka na načine i mehanizme koji nisu obuhvaćeni jednostavnim ekološkim konceptima.

Ključne riječi: alohtona asimilacija, trofički položaj, stabilni izotopi, EPT taksoni, trofička mreža

Introduction

Free-flowing rivers are diverse and dynamic ecosystems that host a high biodiversity and provide important ecosystem services for society (Daily 2012). In Europe, only a few sections of rivers remain free-flowing, mostly located in Alpine spaces, such as the Tagliamento in Italy or the upper Lech in Austria (Belletti et al. 2020; Tockner et al. 2022). Notably, many rivers in the mountainous areas of the Balkans are still in natural conditions at larger spatial scales, such as the Albanian Vjosa River (Schiemer et al. 2020) and the upper Neretva River (Grill et al. 2019). Unfortunately, these free-flowing rivers are threatened by the planned construction of numerous dams for hydropower generation (Reid et al. 2019). The protection of these last European natural rivers is of utmost importance to ensure the conservation of continuously diminishing freshwater biodiversity (Albert et al. 2021) and the continued existence of naturally functioning ecosystems capable of providing important services. Since such undisturbed ecosystems are becoming increasingly rare, they are highly valuable to understand patterns of biodiversity and the mechanisms that generate and maintain this biodiversity and thus ensure natural ecosystem functioning. Indeed, it has been argued that river systems which are hydromorphologically intact at larger spatial scales, such as the Vjosa river network, may be important reference systems that are capable of guiding urgently needed restoration activities of degraded European rivers (Schiemer et al. 2020). Here, a notable feature of such free-flowing rivers is their ability to support natural ecological mechanisms acting across several spatial scales. They also showcase the effect of natural discontinuities, which structure ecosystems and communities at the larger spatial scale often stronger than anticipated from the textbook-derived conceptual models geared towards smooth ecological gradients.

Smooth gradients from source to sea have long been postulated behind the distribution of certain taxa along riverine continua based on their reaction to dominant environmental gradients (Illies & Botosaneanu 1963). Lately, besides this deterministic niche-based mechanism, neutral mechanisms related to extinction, recolonisation and thus dispersal across the complex spatial domain of river networks have been additionally recognised as structuring patterns of community composition and taxonomic diversity (Brown et al. 2011; Heino et al. 2015). The most pivotal conceptual model in river ecology describing a smooth monotonic downstream variation in abiotic and biotic parameters is probably the River Continuum Concept (RCC; Vannote et al. 1980). Instead of relying on species-specific reaction norms, this concept proposes that the relative availability of major food resources creates distinct zones with functionally different macroinvertebrate communities along a riverine continuum. For example, headwaters are expected to be areas where low light availability and a high influx of coarse detritus (leaves) cause a dominance of allochthonous food resources, and, consequently, lead to macroinvertebrate communities dominated by shredders and detritivores. Moving downstream, where the river widens and light availability increases, benthic primary production, and hence, autochthonous organic matter, may become the main source of energy for a macroinvertebrate community dominated by grazers. Fine particulate organic matter, may be present from headwaters to far downstream, yet originating from different sources along the riverine continuum: direct input from terrestrial systems in headwaters, and breakdown of larger sized leaf material or sloughed off algal biomass anywhere below the respective source materials exist. This resource feeds collector-gatherer and filter feeders, which become the dominant functional group in far downstream sections where the river width limits terrestrial input and turbid water does not allow much primary production.

This stereotypical zonation predicted by the RCC can be broken by natural environmental discontinuities such as changes in river morphology, the presence of large tributaries contributing water and sediment, geomorphology-driven flow intermittency, and massive groundwater inputs with very different physicochemical properties as occurring in karstic systems. Additionally, changes in the terrestrial vegetation, which are often related to the presence of agricultural fields and grasslands or open vegetation at higher elevations, can produce discontinuities of allochthonous input with implications for the invertebrate communities (see Doretto et al. 2020 for a review on natural discontinuities). Notably, the fact that rivers are dendritically shaped hierarchical networks with unidirectional flow of resource suggests frequent discontinuities at confluences, which in fact shape the creation of gradients in functional feeding group composition along a river continuum (Jacquet et al. 2022).

The RCC only foresees the turnover in the composition of functional feeding groups, but the underlying variations in food resources must in fact be reflected in alterations of the structure and functioning of the food web. For example, longer food webs are expected with increasing resource availability, because more energy will be available to sustain higher trophic levels (Post 2002). Also, a greater variety of resources should drive higher functional trophic diversity by providing a larger resource niche space for consumers. Extending a functional assessment from functional feeding groups to the reality of a food web seems to be a timely scientific endeavour to be done at the larger spatial scale of a river continuum, if not river network. Such a larger scale view should also respect naturally occurring discontinuities instead of forcing synthetic continua, i.e. selecting (certainly existing) data dimensions that correspond to a growth in river size as the master variable. Although some studies have investigated the effects of environmental discontinuities on macroinvertebrate communities (Bruns et al. 1984; Torgersen et al. 2008; Fenoglio et al. 2014; Datry et al. 2016), the consequences for food web structure and trophic functionality have so far been poorly investigated. The Neretva river represents an exceptional place to carry out such investigations given the rather long river continuum that flows undammed and the multiple discontinuities the river experiences (groundwater springs, tributaries, geomorphological and vegetation changes; see »Study area and sampling design« section for a detailed description).

In this study we aimed to (i) describe patterns of macroinvertebrate community composition and taxonomic as well as functional diversity along the river continuum of the upper course of the Neretva River, and (ii) investigate their main environmental drivers, given the environmental discontinuities that characterise this ecosystem. To adequately characterise the community functionally, we specifically studied food web structure and trophic functionality besides functional feeding group composition. We expected macroinvertebrate communities to vary following the available food resources and environmental characteristics. Moreover, we predicted taxonomic and functional diversity to show similar patterns as different taxa can accomplish different functions in the ecosystem.

Materials and methods

Study area and sampling design

The study was performed along the upper course of the Neretva River in Bosnia and Herzegovina at the end of June and beginning of July 2022. The Neretva River originates in the Dinaric Alps and flows through a karstic area that is distinguished by an extremely high groundwater exchange (Operta & Pamuk 2015). Downstream of Konjic, starting with the Jablanica reservoir, the Neretva River is impacted by a series of larger dams until it drains into the Adriatic Sea in Croatia. For this study, we sampled in the upper and still entirely free-flowing river section (approx. 70 km) between the source and the town of Konjic. In this section, a few kilometres downstream the city of Ulog, a new reservoir is starting to be constructed. At the time of sampling, 200 m of river flowed through a large pipe without the river connectivity being affected. Along the studied section, the river experiences multiple discontinuities generated by high-volume water inputs from karstic springs, the presence of tributaries (e.g., Ljuta, Krupac), the sudden changes in geomorphology between sediment-rich and calmer flowing alluvial reaches and steep and narrow canyons, and the variation of vegetation in the riparian areas. This creates discontinuities in the environmental conditions of the mainstem continuum that can be reflected in the biota that inhabit this river section. Here, we sampled nine sites (Fig. 1). Site -1, was located in the Krupac stream, a small spring-fed tributary in the headwaters of the Neretva River that has low temperature (daily average = 8.9 °C) and low Na and K but higher nitrogen concentrations (DN, NO_3N) compared to closely located sites (Tab. 1). This site was characterised by a dense arboreous riparian vegetation and the dominance of moss in the river bed and scarce development of biofilm and algae. The remaining sites were located along the mainstem of the upper Neretva. Site 0 was in the river headwaters and characterised by little arboreous riparian vegetation and large sediments. Sites 3 to 6 were located in the midsection reaches whereas Sites 7, 8 and 10 were the most downstream sites, located downstream of a major karst spring tributary, Ljuta.

In these sites, we characterised the macroinvertebrate community taxonomically and functionally by studying food web features based on stable isotope analysis. We measured several environmental variables to explain observed biotic patterns.

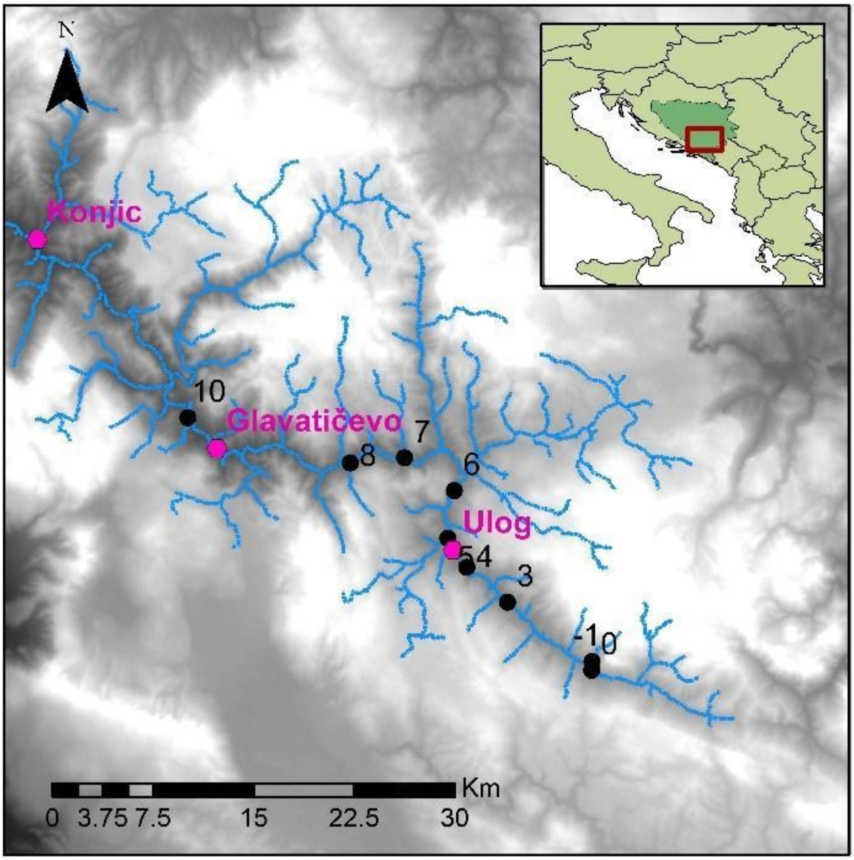

Figure 1. Location of the nine sampling sites (black) in the upper course of the Neretva River in Bosnia and Herzegovina. Site -1 is located in the Krupac stream, a small tributary in the headwaters of the Neretva River. Cities and villages are shown in pink.

Slika 1. Lokacija devetih vzorčišč (črno) v zgornjem toku reke Neretve v Bosni in Hercegovini. Lokacija -1 je v potoku Krupac, majhnem pritoku v povirju reke Neretve. Mesta in vasi so označeni rožnato.

Slika 1. Lokacije devet mjesta za uzorkovanje (crna) u gornjem toku rijeke Neretve u Bosni i Hercegovini. Lokacija -1 nalazi se na potoku Krupac, maloj pritoci pri izvorištu rijeke Neretve. Gradovi i sela prikazani su ružičastom bojom.

Environmental parameters

At each site, we calculated the catchment area and measured canopy cover, discharge, water temperature, and average sediment grain size. We also collected water samples for an analysis of major water chemical variables (dissolved organic carbon (DOC), dissolved nitrogen (DN), Cl, NO_3N, SO_4, Na, K and Mg; Tab. 1).

We derived catchment area from a digital river network built from a digital elevation model (Copernicus 2022) with a resolution of 30 metres using watershed R package (version 0.4.9, https://github.com/flee-group/watershed). To estimate the canopy cover, a proxy for light availability, we took a hemispherical sky photograph with a fisheye lens at three different spots at each site. The camera was levelled and horizontally positioned. Canopy cover from hemispherical photographs was analysed with the Gap Light Analyzer software (Frazer et al. 1999). We calculated discharge for each site as the product of the river width, average depth and average flow velocity at 40% of the depth. For this, we measured depth and velocity at 10-15 spots along a transect perpendicular to the shore. For each site, we computed average water temperature from a temperature time series recorded by oxygen loggers (MiniDOT, PME) at ten-minute intervals over at least seven days. We computed the average grain size from a pebble count of at least 90 stones randomly picked along a zig-zag walking course through a representative reach at least 15 times longer than the average river width. At each site, we filtered a water sample through a pre-rinsed 0.2 µm membrane filter (Sartorius) and stored samples in the cold and dark until laboratory analysis. We analysed nutrients (Cl, NO_3N, SO_4, Na, K, Mg) by ion chromatography. DOC and DN concentrations were determined with a TOC Analyzer (Shimadzu). To summarise water chemical information, we performed a principal component analysis. The resulting principal component PC1 explained 47.4% of the water chemistry dataset's total variability and was negatively related to Na and K, and positively to DN and NO_3N; further PCA-axes had much lower explanatory power. PC1 was then included as a »water chemistry« variable in further analyses, likely indicating mostly nitrogen availability.

Table 1. Environmental characteristics of the sampled sites. Concentrations of major water chemical constituents are all reported in mg/L. Catch. Area = Catchment area, DOC = Dissolved organic carbon, DN = Dissolved nitrogen.
Tabela 1. Okoljske značilnosti na mestih vzorčenja. Vse koncentracije glavnih kemičnih sestavin vode so navedene v mg/L. Catch. Area = povodje, DOC = raztopljeni organski ogljik, DN = raztopljeni dušik.
Tabela 1. Karakteristike sredine uzorkovanih lokacija. Koncentracije glavnih hemijskih sastojaka vode iskazane su u mg/L. Catch. area = sliv, DOC = rastvoreni organski ugljenik, DN = rastvoreni azot.

Site code	Catch. Area (km^2)	Canopy cover (%)	Discharge ($m^3.s^{-1}$)	Avg. Tª(°C)	Sediment size (mm)	Na	K	Mg	Cl	SO_4	NO_3-N	DOC	DN
-1	10.2	61.0	0.42	8.9	207	0.66	0.19	2.59	0.83	2.63	0.15	0.36	0.17
0	95.5	28.7	0.50	14.9	195	1.74	0.39	3.29	1.12	5.71	0.02	0.46	0.05
3	169.1	53.1	0.81	16.7	74	1.86	0.43	3.43	1.11	5.39	0.03	0.45	0.06
4	203.7	53.0	1.04	16.3	184	1.95	0.49	3.49	1.12	5.31	0.04	0.52	0.08
5	289.6	7.6	0.79	19.1	77	1.98	0.55	3.61	1.16	5.38	0.03	0.51	0.07
6	317.2	53.1	1.04	16.9	210	2.11	0.56	4.05	1.22	5.92	0.06	0.52	0.10
7	666.2	26.1	5.92	12.9	337	1.16	0.37	4.06	1.2	4.13	0.20	1.26	0.20
8	703.5	24.3	6.06	14.7	89	1.2	0.37	4.38	1.27	4.45	0.19	0.32	0.20
10	921.64	21.3	12.27	16.9	55	1.32	0.37	8.76	1.65	6.35	0.25	0.33	0.24

Macroinvertebrate characterisation

At each site, following a multi-habitat sampling design (Consortium et al. 2002), we collected a composite sample of 20 single kicks distributed over all dominant habitat types according to their estimated coverage in a 100 m reach. The used hand-net had a 25 × 25 cm frame and 500 µm mesh size. We further processed half of each homogenised composite sample for taxonomic identification and stable isotope analyses (see below), except for Site 6, from where the entire sample was processed as it became apparent fewer individuals were present than in the remaining sites.

We identified and counted all macroinvertebrates except Acari (and 14 Diptera at site 5) at least to family level (some were identified to genus and others to species level). We calculated the abundance (ind. m^{-2}) of all taxa for each site. We discarded empty shells, eggs or terrestrial forms, and when fragmented animals appeared, we only counted the head. Moreover, we assigned invertebrates to one of the following six feeding groups: predator, shredder, grazer, collector/gatherer, filter feeder, grazer & collector/gatherer reflecting their dominant feeding behaviour (Schmidt-Kloiber & Hering 2015). We separated organic matter and algae from the invertebrates, divided into allochthonous (leaves) and autochthonous (algae) organic matter and froze them at – 20 °C until analysis. We kept specimens of each macroinvertebrate taxon in an individual vial with water for a few hours to allow gut clearance and then froze them at – 20 °C until analysis. We extracted the gut only for very large specimens and not for small and abundant taxa, for which a slight contamination of stable isotopic signatures by food resource or prey organisms was deemed acceptable.

Stable isotope analysis

We measured $\delta^{13}C$ and $\delta^{15}N$ isotopic signals of macroinvertebrate tissue and of autochthonous and allochthonous resources. We thawed the samples and dried them for 72 hours at 50 °C in individual Eppendorf tubes. We milled and ground the dried samples into fine powder and then fumigated them in 10 M 37% HCl for approximately eight hours to remove inorganic carbon as outgassing CO_2. We encapsulated between 0.2 - 0.3 mg of the decalcified powdered samples in tin capsules and analysed in an elemental analyzer (EA Isolink, Thermo Scientific) coupled by a ConFlo IV interface (Thermo Scientific) to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific). We measured the samples against calibrated reference gases (high purity N2 and CO2, Air Liquide) and laboratory standards, which were referenced against international certified reference materials (IAEA-N-1, USGS-65 and USGS-66). We expressed isotope abundances in delta notation ($\delta^{13}C$ and $\delta^{15}N$ values) relative to Vienna PeeDee belemnite and atmospheric nitrogen.

Data analysis

Taxonomic composition and diversity

We computed taxa richness and the percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) for each site. We also computed total abundance as an indicator of the overall productivity of a site, the relative abundance of each of the defined feeding groups, the Shannon diversity index and Pielou's evenness, by dividing the Shannon diversity index by the natural logarithm of the taxa richness.

We performed Spearman correlations between catchment area and taxa richness and evenness, the Shannon diversity index and the percentage of EPT and total macroinvertebrate abundance to understand how the macroinvertebrate communities changed along the river continuum. Additionally, we performed a Principal Coordinate Analysis (PCoA) based on a Bray–Curtis dissimilarity matrix computed from absolute abundances. This allowed a graphical investigation of turnover of community composition along the river continuum. To further test if environmental variables explained variability in community composition, we correlated the measured environmental variables with the first two components of the PCoA. Moreover, to test if community taxonomic metrics were associated with variability in community composition, we correlated them with the first two components of the PCoA.

Food web structure and functional diversity

For each site, we computed average allochthonous assimilation, average trophic position and maximum trophic position from stable isotope data.

The allochthonous assimilation of each taxon (*Alloc*) was calculated as:

$$Alloc = \frac{\delta^{13}C_{Consumer} - \delta^{13}C_{min}}{\delta^{13}C_{max} - \delta^{13}C_{min}} * 100$$

Here, $\delta^{13}C_{Consumer}$ is the $\delta^{13}C$ signal of a given taxon, and $\delta^{13}C_{min}$ and $\delta^{13}C_{max}$ are the minimum and maximum $\delta^{13}C$ invertebrate values of each site, which we used to represent signatures of autochthonous and allochthonous organic matter. This approach is justified by the consistent and clearly distinct position of grazers and shredders close to observed $\delta^{13}C_{min}$and $\delta^{13}C_{max}$ in stable isotope plots (Fig. 2A). $\delta^{13}C_{max}$ was mostly very close to -28‰, the often assumed signature of terrestrial leaf litter. Benthic periphytic biofilms tend to have a very variable and microhabitat-specific carbon signature depending on flow velocity (Singer et al. 2005), suggesting that local integration by exclusive grazers may provide the most reliable signature for autochthonous organic matter of a given site. $\delta^{13}C$ signals of collected autochthonous and allochthonous organic matter resources were unfortunately often very similar and could not be used in the analyses. For each site, we computed the average allochthonous assimilation as the average of the allochthonous assimilation of all the taxa.

Trophic position (tp) for each taxon was calculated as:

$$tp_{Consumer} = 2 + \frac{\delta^{15}N_{Consumer} - \delta^{15}N_{Base}}{3.4}$$

Here, $\delta^{15}N_{Consumer}$ is the $\delta^{15}N$ signal for a taxon, $\delta^{15}N_{Base}$ is the minimum $\delta^{15}N$ value of a primary consumer at each site, and 3.4‰ is the assumed enrichment in $\delta^{15}N$ per trophic level (Post 2002). For each site, we computed the average trophic position as the average of the trophic positions of all the taxa. The highest trophic position at each site was the maximum trophic position.

We further use isotopic richness and isotopic evenness as indicators of trophic diversity, adapting the computations from (Cucherousset & Villéger 2015). Both measures integrate (horizontal) variation at a food web base and (vertical) extension across trophic levels. Isotopic richness is the amount of isotopic space filled by the invertebrate community at a site and computed as the convex hull area encompassed by all organisms of each site in the % allochthonous assimilation-trophic position bi-plot space (see Fig. 2B). We used the % allochthonous assimilation-trophic position bi-plot space instead of the isotopic space ($\delta^{13}C$ – $\delta^{15}N$ bi-plot space) for standardisation processes, to be able to compare amongst sites with very different isotopic signals due to the differences in the food resource signals.

Isotopic evenness is the evenness in the distribution of taxa along the shortest tree that links all the points (e.g., taxa) in the % allochthonous assimilation-trophic position bi-plot space. Isotopic evenness (*IEve*) is computed as:

$$IEve = \frac{\sum_{l-1}^{N-1} min\left(\frac{E_l}{\sum_{l-1}^{N-1} E_l}, \frac{1}{N-1}\right) - \frac{1}{N-1}}{1 - \frac{1}{N-1}}$$

With

$$E_{l(i,j)} = \sqrt{\sum_{k=1}^{Sl} \left(k_i - k_j\right)^2}$$

where *N* is the total number of taxa, *l* is the branch of the minimum spanning tree linking organisms in the % allochthonous assimilation-trophic position bi-plot space and *E* the evenness. *S* is each segment of the spanning tree, k_i is the position of taxon *i* in the % allochthonous assimilation-trophic position bi-plot space and k_j is the position of taxon *j*.

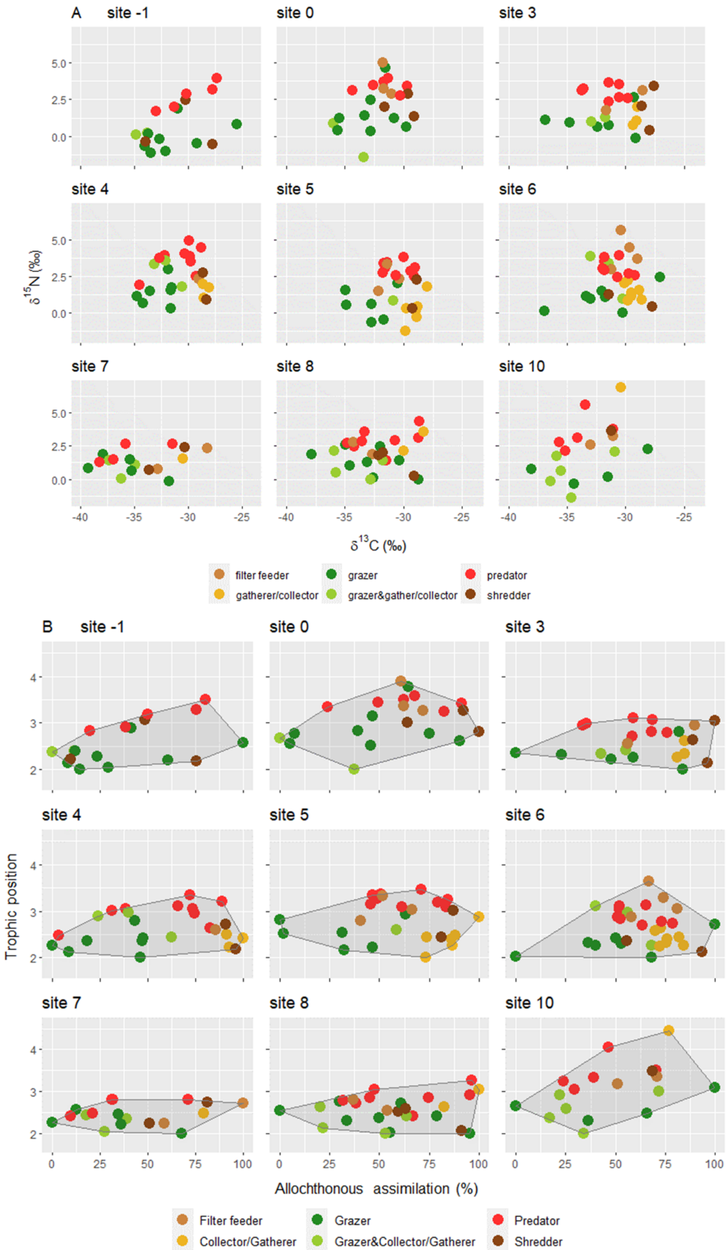

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ signals of the macroinvertebrate taxa analyszed at each site (A). The $\delta^{13}C$ and $\delta^{15}N$ were used to compute the % allochthonous assimilation and the trophic position of each of the analysed taxa (B).

Slika 2. Signali $\delta^{13}C$ in $\delta^{15}N$ preučevanih taksonov makroskopskih nevretenčarjev, na vsakem mestu vzorčenja (A). $\delta^{13}C$ in $\delta^{15}N$ sta bila uporabljena za izračun % alohtone asimilacije in trofične lege vsakega izmed preučevanih taksonov (B).

Slika 2. Signali $\delta^{13}C$ i $\delta^{15}N$ taksona makrobeskičmenjaka analiziranih na svakoj lokaciji (A). $\delta^{13}C$ i $\delta^{15}N$ korišteni su za izračunavanje % alohtone asimilacije i trofičke pozicije svake od analiziranih taksona (B).

We computed Spearman correlations between catchment area and average allochthonous assimilation, average trophic position, maximum trophic position, isotopic richness and isotopic evenness to understand how food web structure and functional diversity changed along the river continuum. Moreover, to test if food web and functional diversity metrics were associated with variability in community composition, we correlated them with the first two components of the PCoA. Finally, to check for relationships between taxonomic and functional variables, we performed Spearman correlations between taxonomic and food web structure and functional diversity metrics.

We performed the data analysis in RStudio (Version 2022.02.2+485, RStudio Team 2022) using GGPLOT2 (Wickham 2016) and VEGAN packages (Oksanen et al. 2022).

Results

Taxonomic composition

We collected a total of 92 macroinvertebrate taxa at the nine sites sampled along the upper course of the Neretva River (Tab. S1). The average taxon richness per site was 36, ranging between 48 at Site 6 and 26 at Site -1. On average, 65.2% of all taxa belonged to EPT. The highest fraction of EPT was found at Site 7 (77.9%) and the lowest at Site 0 (53.5%). Similarly, Shannon diversity was highest at Site 5 (3.01) and lowest at Site 1 (2.07) and on average it was 2.60, while the average taxon evenness was 0.72, ranging between 0.78 at Site 5 and 0.64 at Site -1. Average abundance was 1645 ind. m^{-2}, ranging between 2675 ind. m^{-2} at Site 8 and 848 ind. m^{-2} at Site 7. Regarding the feeding groups (Tabs. S1, S2), grazers represented an average of 28.3% (max 53.7% at Site 4 and min 8.5% at Site 8), grazers & collector/gatherers 25.3% (max 51.1% at Site 7 and min 10.6% at Site 0) of the macroinvertebrate abundance, collector/gatherers 14.2% (max 28.0% at Site 6 and min 0.2% at Site -1), filter feeders 13.0% (max 26.6% at Site 0 and min 0% at Site 4, not present at Site -1), shredders 11.0% (max 54.8% at Site -1 and min 1.9% at Site 4) and predators 8.0% (max 13.7% at Site 6 and min 2.7% at Site 10; Fig. 3, Tab. S2). None of the metrics (richness, percentage of EPT, Shannon diversity, evenness, abundance; Tab. S3) showed a significant correlation with catchment area ($p = 0.75$, $p = 0.15$, $p = 0.61$, $p = 0.74$ and $p = 0.74$, respectively). Amongst the different feeding groups, only the percentage of grazers & collector/gatherers significantly increased with catchment area (cor. = 0.87 $p < 0.01$). Neither grazers nor collector/gatherers, filter feeders, shredders or predators showed a significant correlation with catchment area ($p = 0.08$, $p = 0.25$, $p = 0.29$, $p = 0.64$, $p = 0.98$, respectively).

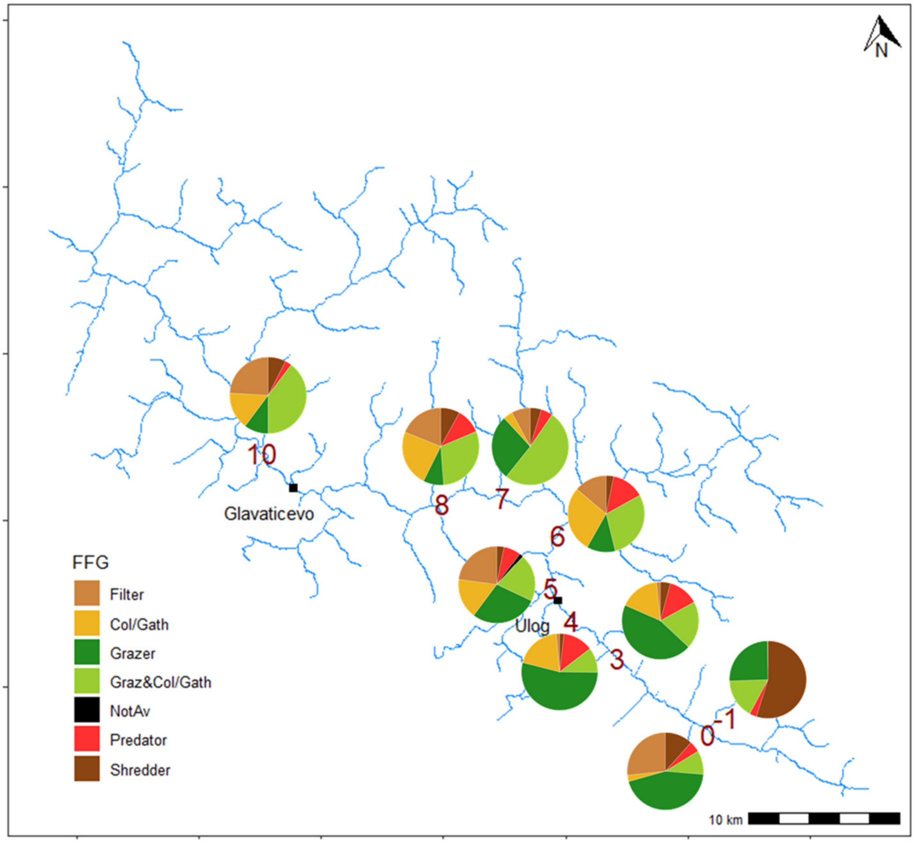

Figure 3. Relative abundance of feeding groups along the upper course of the Neretva River. Site codes are shown in dark red. FFG = functional feeding group, Col/Gath = Collector/gatherers, Graz&Col/Gath = Grazer & collector/gatherers, NotAv = Not Available (14 Diptera taxa only identified to order).

Slika 3. Relativna številčnost prehranjevalnih skupin vzdolž zgornjega toka reke Neretve. Kode vzorčnih mest so temno rdeče. FFG = funkcionalna prehranjevalna skupina, Col/Gath = zbiralci/nabiralci, Graz&Col/Gath = strgalci in zbiralci/nabiralci, NotAv = Ni na voljo (14 taksonov Diptera identificiranih samo do redu).

Slika 3. Relativna brojnost trofičkih grupa duž gornjeg toka rijeke Neretve. Kodovi mjesta prikazani su tamno crvenom bojom. FFG = funkcionalna trofička grupa, Col/Gath = Kolektori/sakupljači, Graz&Col/Gath = Grazer i kolektori/sakupljači, NotAv = Nije dostupno (14 taksona Diptera identifikovano samo do nivoa »ordo«).

Despite the lack of correlations with catchment area, diversity and richness were lower at the most upstream sites (Site 0 and -1) and downstream sites (Sites 7, 8 and 10) but highest at midsection sites (Sites 5 and 6; Fig. 4A, Tab. S3). In contrast, macroinvertebrate abundance and the percentage of EPT were highest at the downstream sites (except for abundance at Site 7, which was the lowest amongst sites) and lower at the upstream and midsection sites of the mainstem (except for the percentage of EPT at Site 4, which was quite high; Fig. 4B and 4C, Tab. S3). Grazers represented a high percentage of the community in headwaters and midsection sites until Site 4, after which the percentage was quite low (except for Site 7; Fig. 3, Tab. S2). Moreover, collector/gatherers were very little abundant in the smallest sites (headwater Site 0 and Krupac tributary Site -1; Fig. 3, Tab. S2) and Site 7.

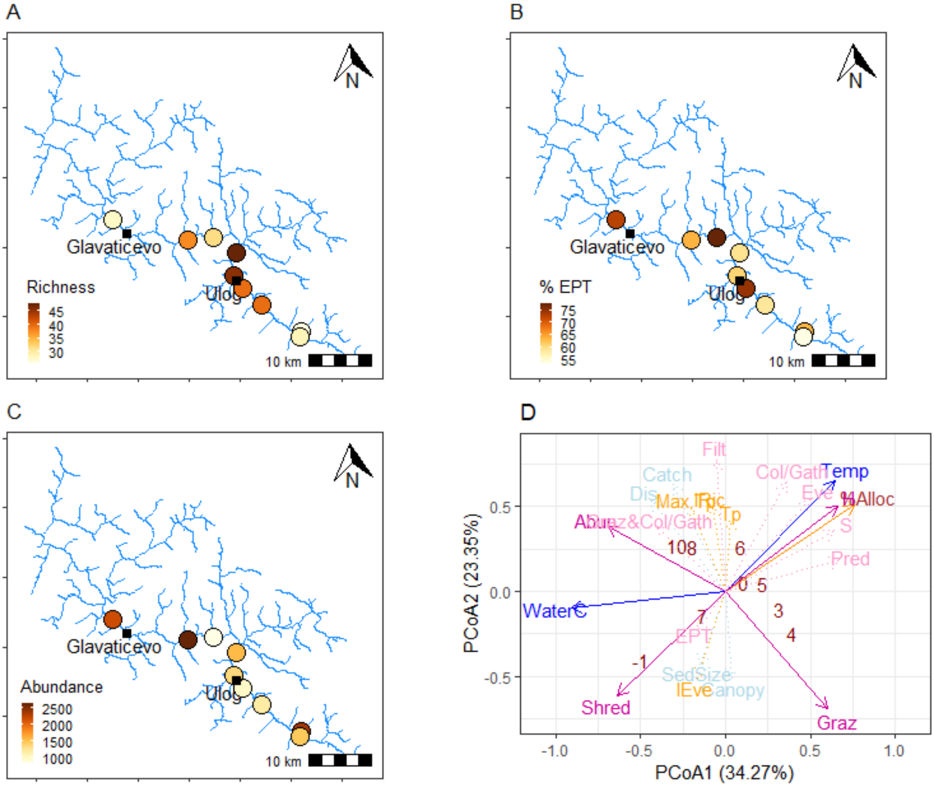

Figure 4. Macroinvertebrate richness (A), percentage of EPT (B) and total macroinvertebrate abundance (C) along the upper course of the Neretva River. (D) Variation in the composition of the macroinvertebrate community of the upper course of the Neretva River were assessed by Principal Coordinate analysis (PCoA) based on Bray–Curtis dissimilarities computed from abundances. Turnover of community composition is related to changes in water chemistry, temperature, the percentage of allochthonous assimilation of the community and the Shannon diversity, the percentage of shredders and grazers, and total macroinvertebrate abundance as an indicator of a site's overall productivity. Site codes are shown in dark red. Environmental variables are shown in blue, taxonomic diversity metrics in pink and food web metrics in orange. Dark colours and solid lines show variables that are significantly correlated with the PCoA ordination whereas light colours and dotted lines show variables that are not correlated. Temp = Temperature, Catch = Catchment area, Dis = Discharge, Canopy = Canopy cover, SedSize = Sediment size, WaterC = Water chemistry (is the PC1 of PCA performed to summarise the water chemistry), Max.Tp = Maximum trophic position, %Alloc = Percentage of allochthonous assimilation, Tp = Average trophic position, IRic = Isotopic richness, IEve = Isotopic evenness, H = Diversity, S = Richness, Eve = Evenness, Abun = Abundance, EPT = Percentage of EPT, Filt = Percentage of filter feeders, Graz&Col/Gath = Percentage of grazer & collector/gatherers, Pred = Percentage of predators, Graz = Percentage of grazers, Shred = Percentage of shredders, Col/Gath = Percentage of collector/gatherers.

Slika 4. Bogastvo makroskopskih nevretenčarjev (A), odstotek EPT (B) in skupna številčnost makroskopskih nevretenčarjev (C) vzdolž zgornjega toka reke Neretve. (D) Spremembe v sestavi združb makroskopskih nevretenčarjev v zgornjem toku reke Neretve, ocenjene z analizo glavnih koordinat (PCoA), z Bray–Curtisovim različnostnim indeksom, izračunanim iz številčnosti. Sprememba sestave združbe je povezana s spremembami v kemiji vode, temperaturo, odstotkom alohtone asimilacije združbe in raznovrstnosti (Shannonov indeks), odstotkom drobilcev in strgalcev ter skupno številčnostjo makroskopskih nevretenčarjev kot indikatorjem splošne produktivnosti območja. Kode vzorčnih mest so temno rdeče. Okoljske spremenljivke so modre, metrike taksonomske raznolikosti rožnate, metrike prehranjevalnih spletov pa oranžne barve. Temne barve in polne črte prikazujejo spremenljivke, ki statistično značilno korelirajo z ordinacijo PCoA, medtem ko svetle barve in pikčaste črte prikazujejo spremenljivke, ki niso korelirane. Temp = Temperatura, Catch = Povodje, Dis = Pretok, Canopy = Krošnja, SedSize = Velikost sedimenta, WaterC = Vodna kemija (je PC1 os PCA, izračunana za rezimiranje kemijskih parametrov vode), Max.Tp = Največji trofični položaj, % Alloc = odstotek alohtone asimilacije, Tp = povprečni trofični položaj, IRic = izotopska pestrost, IEve = izotopska enakomernost, H = raznolikost, S = pestrost, Eve = enakomernost, Abun = številčnost, EPT = odstotek EPT, Filt = odstotek filtratorjev, Graz&Col/Gath = Odstotek strgalcev in zbiralcev/nabiralcev, Pred = Odstotek plenilcev, Graz = Odstotek strgalcev, Shred = Odstotek drobilcev, Col/Gath = Odstotek zbiralcev/nabiralcev.

Slika 4. Bogatstvo makrobeskičmenjaka (A), postotak EPT (B) i ukupna brojnost makrobeskičmenjaka (C) duž gornjeg toka rijeke Neretve. (D) Varijacije u sastavu zajednice makrobeskičmenjaka gornjeg toka rijeke Neretve procijenjene analizom glavnih koordinata (PCoA) na osnovu Bray-Curtisovih razlika izračunatih iz brojnosti. Promet sastava zajednice povezan je sa promjenama: u hemiji vode, temperaturi, postotku alohtone asimilacije zajednice i raznovrsnosti (Shannon metoda), postotku drobilica i sjekača, te ukupnom obilju makrobeskičmenjaka kao pokazatelju ukupne produktivnosti lokacije. Kodovi lokacija prikazani su tamno crvenom bojom. Varijable okoline su prikazane plavom bojom, metrika taksonomske raznolikosti ružičastom, a metrika mreže hrane narandžastom. Tamne boje i pune linije pokazuju varijable koje su u značajnoj korelaciji s PCoA ordinacijom, dok svijetle boje i isprekidane linije pokazuju varijable koje nisu u korelaciji. Temp = Temperatura, Catch = Područje sliva, Dis = Ispuštanje, Canopy = Krošnja, SedSize = Veličina sedimenta, WaterC = Hemija vode (da li je PC1 PCA izveden za sumiranje hemije vode), Max.Tp = Maksimalna trofička pozicija, % Alloc = Postotak alohtone asimilacije, Tp = Prosječna trofička pozicija, IRic = Izotopsko bogatstvo, IEve = Izotopska ujednačenost, H = Raznovrsnost, S = Bogatstvo, Eve = Ravnomjernost, Abun = Obilje, EPT = Postotak EPT, Filt = Postotak filtera hranilice, Graz&Col/Gath = Postotak strugača i berača/sakupljača, Pred = Postotak predatora, Graz = Postotak drobilica, Shred = Postotak sjekača, Col/Gath = Postotak sakupljača.

We summarised the macroinvertebrate community composition in a PCoA, in which the first and second axis explained 34.27% and 23.35%, respectively (Fig. 4D). The Krupac tributary (Site -1) had the most distinct invertebrate community, characterised by the dominance of shredders, being particularly abundant *Gammarus* spp. (Amphipoda) and *Protonemura* spp. (Plecoptera) which together represented more than 50% of the community, and grazers compared to the mainstem. This site was also characterised by dominance of the stonefly *Isoperla* spp. (Plecoptera), the mayfly family Heptageniidae (*Rhithrogena* spp., *Ecdyonurus* spp., *Epeorus* spp.), *Baetis* spp. (Emphemeroptera), and *Elmis* spp*.* (Coleoptera) and the site-specific occurrence of *Diamesa* spp. (Diptera), *Chaetopteryx* spp. (Trichoptera), *Ancylus* spp. and *Bythinella* spp (Mollusca)*.* The Krupac tributary was associated with clearly lower water temperature and a lower concentration of Na and K but higher DN and NO_3N than any site on the mainstem. Amongst the mainstem sites, the downstream sites (8, 10) were also distinct in the PCoA ordination space from the upstream and midsection sites (0, 3, 4, 5, 6). This indicates a change in the macroinvertebrate community composition in the river continuum which was associated with a higher abundance of macroinvertebrates but a lower proportion of grazers downstream (see also Fig.3). The downstream sites were dominated by *Leuctra* spp. (Plecoptera) and characterised by a higher abundance of Blephariceridae (Diptera). Moreover, Dytiscidae (Coleoptera) and Tanytarsini (Diptera) were more abundant at Site 8 than at other sites, while *Riolus* spp. (Coleoptera) only appeared at Site 10. In contrast, the upstream and midsection sites were characterised by a higher abundance of *Micrasema* spp. (Trichoptera), Polycentropodidae (Trichoptera) and Psychodidae (Diptera) than the downstream sites. Additionally, Corixidae (Hemiptera) and Leptophlebiidae (Ephemeroptera) were highly abundant at the midsection sites but did not appear at Site 0, the headwater site. Amongst the mainstem sites, upstream and midsection sites exhibited slightly higher temperature, a higher concentration of Na and K but lower DN and NO_3N than downstream sites. Site 7 was located in the PCoA ordination between the Krupac tributary and the mainstem sites. This site was dominated by *Acentrella* spp. and, similarly to the Krupac tributary, *Baetis* spp. (Ephemeroptera), while it showed very low abundances of taxa that were abundant at the downstream sites (e.g., *Leuctra* spp., Simuliidae, *Ephemerella* spp.). Moreover, the taxa *Rhyacophila laevis*, *Rhyacophila fasciata* and *Psychomia* spp. (Trichoptera) only occurred at this site.

Food web structure and functional diversity

$\delta^{13}C$ of invertebrates ranged from -39.29 to -25.46‰ across all sites, expanding on average over a range of 8.86‰ at individual sites (Fig. 2A). Grazer macroinvertebrates consistently showed the most negative $\delta^{13}C$ values, while shredders (predominantly), filter feeders and collector/gatherers the most positive ones, which were also close to -28‰, the frequently measured signature of terrestrial leaf litter. Overall, the studied sites showed an average allochthonous assimilation of 54.2%, which suggests a slightly heterotrophic system. Sites -1, 7 and 10 were mostly autochthonous compared to the remaining sites, which showed a predominantly allochthonous assimilation (Fig. 2B and 5A; Tab. S3). Site 3 showed the highest average allochthonous assimilation (64.1%), whereas Site -1 the lowest one (38.9%). The average allochthonous assimilation did not correlate with catchment area ($p = 0.91$).

Across all sites, $\delta^{15}N$ values ranged from -1.44‰ (Site 0) to 6.92 ‰ (Site 10), expanding on average over a range of 5.11‰ per site (Fig. 2A). Grazers showed the lowest $\delta^{15}N$ values, while predators had the highest ones. Average trophic position ranged between 3.1 at Site 10 and 2.5 at Site 7 (average = 2.7; Fig. 2B and 5B; Tab. S3). The maximum trophic position ranged between 4.5 at Site 10 and 2.8 at Site 7 (average = 3.5). Both extremes corresponded to taxa from the order Plecoptera, *Leuctra* spp. at Site 10 (collector/gatherer) and *Dinocras* spp. (predator) at Site 7. At the remaining sites, mostly predators such as Limoniidae, *Tabanus* spp. (Diptera) or Dytiscidae (Coleoptera) held the highest trophic position. Neither average trophic position nor maximum trophic position varied significantly with catchment area ($p = 0.74$ and $p = 0.95$, respectively).

Isotopic richness ranged between 127.0 at Site 8 and 56.5 at Site 6 (average = 91.2) while isotopic evenness, with an average of 0.61, showed little variation among sites (0.68 at Site 4 and 0.50 at Site 6; Tab. S3). Neither isotopic richness nor isotopic evenness varied significantly with catchment area ($p = 1$ and $p = 0.64$, respectively; Fig. 5C and 5D).

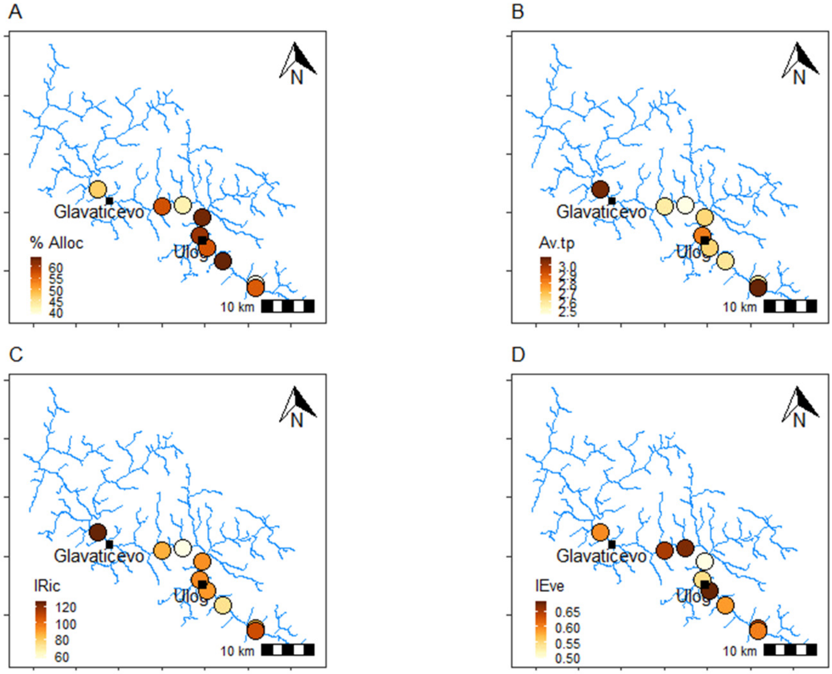

Figure 5. Macroinvertebrate average allochthonous assimilation (A), average trophic position (B), isotopic richness (C) and isotopic evenness (D) along the upper course of the Neretva River.

Slika 5. Povprečna alohtona asimilacija makroskopskih nevretenčarjev (A), povprečna trofična lega (B), izotopska pestrost (C) in izotopska enakomernost (D) vzdolž zgornjega toka reke Neretve.

Slika 5. Prosječna alohtona asimilacija makrobeskičmenjaka (A), prosječna trofička pozicija (B), izotopsko bogatstvo (C) i izotopska ravnomjernost (D) duž gornjeg toka rijeke Neretve.

Relationships between taxonomic diversity and food web structure and functional diversity

Amongst the relationships between taxonomic and functional metrics, only the average allochthonous assimilation was positively correlated with Shannon diversity and richness (Fig. 6). Additionally, both the average allochthonous assimilation and Shannon diversity were associated to the PCoA ordination (Fig. 4D), showing that the midsection sites of the mainstem were the most diverse and had the highest allochthonous assimilation.

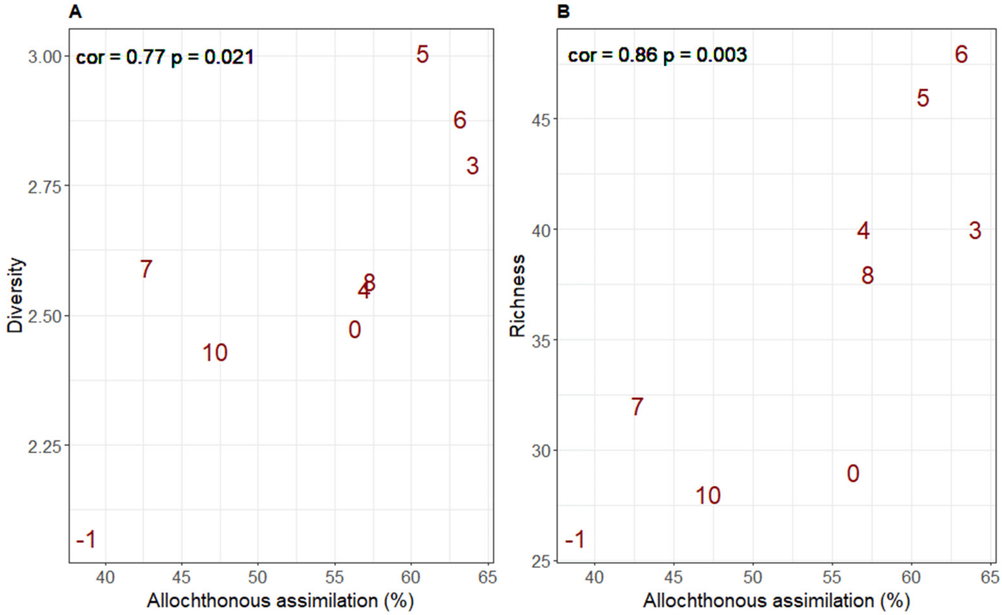

Figure 6. Relationship between the percentage of allochthonous assimilation with Shannon diversity (A) and taxa richness (B). Site codes are shown in dark red.

Slika 6. Razmerje med odstotkom alohtone asimilacije in Shannonovim indeksom pestrosti (A) ter taksonomsko pestrostjo (B). Kode vzorčnih mest so temno rdeče.

Slika 6. Odnos između procenta alohtone asimilacije sa Šenonovom raznovrsnošću (A) i bogatstvom taksona (B). Kodovi mjesta prikazani su tamno crvenom bojom.

Discussion

Overall, the studied section of the Neretva River showed a high macroinvertebrate taxonomic richness and diversity, comparable to other reports of karstic rivers of the Dinaric Alps (Previšić et al. 2009), and a high percentage of EPT, which evidenced its good conservation status (Bakrac et al. 2021). Macroinvertebrates are essential components of river ecosystems, playing a vital role in fluvial food webs by connecting organic material to higher trophic levels (Wallace & Webster 1996). The results from isotope analyses shows that the upper course of the Neretva is a slightly heterotrophic ecosystem, in which most taxa are sustained by allochthonous resources. Although without considering organism biomass we cannot fully estimate the role of autochthonous versus allochthonous resources in the food webs, the fact that allochthonous energy pathways dominated most of the studied sites during the most productive period of the year (i.e., early summer), supports the idea that food webs are likely sustained by allochthonous food resources throughout the year. This highlights the importance of preserving the terrestrial vegetation and the aquatic-terrestrial linkage to maintain a healthy fluvial ecosystem.

Regarding patterns of macroinvertebrate community composition, the Krupac tributary (Site -1) showed a particularly different macroinvertebrate community compared to the mainstem. This was related to the environmental characteristics of this tributary (i.e., low temperature, low Na and K but high DN and NO_3N). Moreover, spring-fed streams are characterised by their stable conditions throughout the year, including low temperature variability, low flow variability and a high sediment stability (e.g., Stevens et al. 2022). These properties support an observably high abundance of moss in the river, which could contribute to the dominance of the autochthonous energy pathway in this stream, despite the high canopy cover (61%). Interestingly, the scarce biofilm and algae development in spite of the obviously coarse and stable substrate, which we attributed to an intensive grazing, not necessarily to a low primary productivity (gross primary production = 0.27 g $O_2.m^{-2}.d^{-1}$; Del Campo et al., this issue). In fact, the macroinvertebrate community in this tributary was dominated by the shredders *Protonemura* spp. or *Gammarus* spp, which together accounted for more than 50% of the total macroinvertebrate abundance, but showed very low allochthonous assimilation (*Protonemura* spp. = 9.8%, *Gammarus* spp. = 49.0%). The following most abundant taxa were grazers (whose abundance was relatively high compared to other sites) and grazer & collector/gatherers (*Rhithrogena* spp., *Baetis* spp., *Ecdyonurus* spp.), which also showed a very low allochthonous assimilation (12.26%, 0% and 11.64%, respectively). This finding resembles previous studies, which have shown that despite autochthonous resources (particularly algae) not being dominant biomass-wise, the autochthonous energy pathways can still be the main support for food webs (Lau et al. 2009; Collins et al. 2016; Estévez et al. 2019). This is probably the result of autochthonous resources being of higher nutritional value than allochthonous resources for stream macroinvertebrates (Guo et al. 2016).

Compared to the strong differences in the macroinvertebrate community between the Krupac tributary and the mainstem sites, weaker changes were present along the mainstem continuum. Macroinvertebrate abundance increased downstream, most likely related to the fact that larger ecosystems have more food resources and consequently there is more energy to sustain a greater macroinvertebrate abundance (Thompson & Townsend 2005; Doi et al. 2009). In contrast, the community composition did not vary monotonically along the continuum (besides the increase in the percentage of grazer & collector/gatherers), only the headwater and midsection reach communities differed from those in the most downstream sites. Collector/gatherers were less abundant in the smallest sites (0 and -1), while grazers were highly abundant in the headwater and midsection sites of the mainstem (these last ones only in the mainstem). In fact, Site 7 was particularly different from other sites in the mainstem. This site was characterised by a low taxon richness, a low abundance and a very distinct community composition (Figs. 4A, 4C and 4D; Tabs. S1, S2 and S3)**.** This site is located downstream of a major tributary (Ljuta) which represents another karst spring stream. These bring large volumes of water with different physicochemical properties (i.e., different water temperature, nutrient and organic matter content) to the mainstem and create discontinuities in the environmental conditions of the mainstem continuum. Yet, they allow macroinvertebrate taxa to disperse into the nearby reaches of the mainstem (e.g., *Rhyacophila laevis*, *Rhyacophila fasciata* and *Psychomyia* spp. (Trichoptera) were only present at Site 7). Neither the Lutja tributary nor the karst spring stream could be sampled, but their characteristics are likely very similar to the Krupac tributary located a few kilometres upstream, as the downstream located Site 7 showed a lower temperature than the adjacent up and downstream sites (6 and 8), and a macroinvertebrate community similar to the Krupac tributary (Site -1; Fig. 4D), characterised by a high abundance of *Baetis* spp. (Ephemeroptera). This resembles previous studies (Bruns et al. 1984; Rice et al. 2001) that suggest that large tributaries may break the river continuum by bringing in different macroinvertebrate taxa and by creating step changes in otherwise smoother gradients of environmental conditions (i.e., water temperature, water chemistry). As specific environmental conditions are beneficial to specific macroinvertebrate taxa, such observed discontinuities demonstrate an important natural mechanism by which biodiversity is generated and maintained at a larger spatial scale in a river landscape that is so spectacularly heterogeneous as the upper course of the Neretva River.

Based on RCC predictions, we expected allochthonous assimilation to be highest in headwaters and to decrease downstream. However, average allochthonous assimilation peaked in the centre of the studied river section. This contrasted with the low allochthonous assimilation in headwaters, which could be explained by the low canopy cover by arboreous riparian vegetation and thus, probable low abundances of allochthonous food resources compared to some of the midsection sites (Tab. 1). As also shown by correlation analysis, the high average allochthonous assimilation in the centre of the studied river section coincided with higher macroinvertebrate richness and diversity. The midsection sites likely experience substantial terrestrial inputs (i.e., they showed the highest canopy cover of approx. 50%), while at the same time the river was wide enough for autochthonous resources to play a relevant role. This could translate into a resource base, that is both (i) of high quantity given allochthonous detritus as well as (ii) of high quality due to autochthonous material, that is known to often be richer in nutrients and essential nutrient components (Guo et al. 2016). This explanation would align with the observed higher allochthonous assimilation. Also, while resource samples produced inconclusive stable isotope signatures, they yielded useful C:N data. Periphytic biofilm and algae samples were found to be richer in N than leaf detritus (C:N ratio algae and biofilm = 10.8 ± 2.4, C:N ratio leaf detritus = 35.6 ± 8.2). Besides the fact that a quantitatively and qualitatively solid resource base likely benefits overall abundance and thus diversity of invertebrates, the overall greater variety of food resources may also provide multiple ecological niches and therefore further contribute to diversity. While this explanation would fit the observed relationship between richness and diversity and allochthonous assimilation, we find no supportive evidence in the patterns of total abundance, which we understand as a proxy for productivity, nor in the food web metrics derived from stable isotopes. Indeed, except for allochthonous assimilation, the food web structure and functional diversity metrics (maximum and average trophic position and isotopic richness and evenness) did not show a clear pattern in the river continuum nor were related to taxonomic diversity metrics. This result was particularly surprising given that ecosystem functionality has long been related to taxonomic biodiversity (e.g., Cardinale et al. 2012; Duffy et al. 2017). We here have to note that our study suffers from two limitations: first, our snapshot was taken in a season with intensive emergence (Graf et al. in prep.), which likely affected observable patterns of larval abundance and community composition. Second, food web patterns are ideally based on measures of secondary productivity or biomass rather than abundance. Indeed, we observed massive abundances of small-bodied early instars of several taxa (e.g., *Micrasema* sp.), suggesting limited reliability of relative abundance patterns in the context of a study on diversity and food web structure. With this in mind, we computed abundance-weighted food web and trophic functional diversity metrics (i.e., weighted average trophic position, weighted average allochthonous assimilation, weighted isotopic evenness). However, the results did not show any significant pattern.

Our study identified centrally located sites in the study section as hotspots of biodiversity. Notably, these sites were located close to the Ulog dam construction. Dams change river ecosystems dramatically and disrupt connectivity at larger spatial scales. This translates to an interference with both transport of upstream sourced organic matter and macroinvertebrate dispersal, resulting in high losses of biodiversity (e.g., Bredenhand & Samways 2009). We therefore expect that the finalisation of this large dam will have tremendous consequences for aquatic biota. This fragmentation will not only affect this specific site but the entire river given its central location in the now, still intact, physical continuum producing remarkable biotic discontinuities.

Conclusion

The results of this study highlight the good conservation status and the diversity of macroinvertebrates that inhabit the upper course of the Neretva River, making it one of the rare pristine headwater rivers remaining in Europe. The observed changes in the macroinvertebrate community indicate the presence of natural discontinuities along the river continuum, which seem to be related to the presence of tributaries fed by groundwater and the properties of the riparian vegetation, particularly the low canopy cover of the headwater and of some midsection reaches. Most of the food web structure and functional diversity metrics did not show a clear pattern along the river continuum nor were related to taxonomic diversity metrics. Only taxa richness and diversity and allochthonous assimilation peaked in the midsection reaches, probably due to a higher diversity of resources, which seem to be caused by a higher abundance of allochthonous food resources as well as a high resource quality provided by the autochthonous resources. In fact, the most diverse reaches are those where the Ulog dam is being constructed, evidencing how damaging its finalisation could be for the entire ecosystem and the urgency to conserve and protect the upper course of the Neretva River.

Povzetek

Redke preostale prosto tekoče reke v Evropi, večina izmed njih je na območju Balkana, so izjemno pomembne za razumevanje taksonomske in funkcionalne strukture združb organizmov, ki naseljujejo rečne ekosisteme. Takšen je zgornji tok reke Neretve v Bosni in Hercegovini, kjer smo vzdolž 70 kilometrov reke raziskali taksonomsko in trofično funkcionalno raznolikost zoobentosa z ocenjevanjem vzorcev sestave združb in strukture prehranjevalnih spletov, vključno s potencialnimi okoljskimi dejavniki. Z namenom rekonstrukcije lokalnih prehranjevalnih spletov smo konec junija 2022 vzorčili združbe makroskopskih nevretenčarjev in izmerili stabilne izotope (δ13C in δ15N) na devetih vzorčnih mestih. Rezultati so pokazali visoko taksonomsko raznolikost (taksonomska pestrost – v povprečju 36 – in Shannonov indeks pestrosti – povprečno 2,6) makroskopskih nevretenčarjev, visok odstotek taksonov EPT (Ephemeroptera, Plecoptera, Trichoptera; povprečno 65,2 %) in rahlo heterotrofen ekosistem (povprečna alohtona asimilacija = 54 %). Taksonomska sestava se je izrazito razlikovala med pritokom v zgornjem toku (potok Krupac), in preostalimi osmimi lokacijami, razpršenimi vzdolž glavnega debla Neretve. To je verjetno povezano z nizko temperaturo vode in visokimi koncentracijami dušika, ki sta značilni za ta pritok. Pokazali smo tudi visoko zamenjavo vrst med zgornjim (povirni in srednji del) in dolvodnim delom glavnega toka reke, večinoma povezano z nižjo relativno številčnostjo strgalcev ter drobilcev/strgalcev, a relativno večjo številčnostjo zbiralcev/nabiralcev (ki so sicer zelo redki na območjih povirja) ter strgalci in zbiralci/nabiralci. Številčnost makroskopskih nevretenčarjev in odstotek EPT sta dosegla najvišjo vrednost dolvodno (razen mesta 7 za številčnost in mesta 4 za EPT), medtem ko so srednji odseki pokazali največjo taksonomsko pestrost in raznolikost ter največjo alohtono asimilacijo. Večina funkcionalnih meritev (največji in povprečni trofični položaj ter izotopska pestrost in enakomernost) je pokazala izrazitejše diskontinuitete v prostoru vzdolž preučevanega rečnega kontinuuma. Opazovani vzorci niso v skladu z napovedmi koncepta rečnega kontinuuma. Nasprotno, naši rezultati poudarjajo edinstvenost tega odseka reke Neretve, kjer naravne diskontinuitete strukturirajo združbe makroskopskih nevretenčarjev na načine in z mehanizmi, ki jih ne enostavni ekološki koncepti ne zaobjamejo. Pritoki, ki se napajajo s podtalnico in prinašajo sladko vodo, organske vire in organizme v glavni tok, ter heterogene značilnosti obrežne vegetacije so verjetno glavne značilnosti, ki prispevajo k heterogenosti vodnega okolja in ustvarjanju zapletenih vzorcev strukture in delovanja združb. Združbe makroskopskih nevretenčarjev v zgornjem delu reke Neretve kažejo, da naravne reke v Balkanski regiji le redko sledijo konceptualnim modelom z zveznimi abiotskimi ali biotskimi gradienti, kar nakazuje, da bi morali biti varstveni ukrepi za ohranjanje teh rek, ki so pomembna evropska naravna dediščina, zasnovani širše, na večji prostorski skali.

Sažetak

Nekoliko rijeka slobodnog protoka koje su preostale u Evropi, od kojih se mnoge nalaze u regionu Balkana, veoma su vrijedne za razumijevanje taksonomske i funkcionalne strukture zajednice organizama koji naseljavaju riječne ekosisteme. Jedan od njih je gornji tok rijeke Neretve u Bosni i Hercegovini. Ovdje smo istražili taksonomsku i trofičku funkcionalnu raznovrsnost zoobentosa procjenjujući obrasce sastava zajednice i strukture mreže hrane, uključujući potencijalne pokretače životne sredine, u riječnom kontinuumu od 70 kilometara. U tu svrhu uzorkovali smo zajednicu makrobeskičmenjaka i izmjerili stabilne izotope (δ13C i δ15N) kako bismo rekonstruisali lokalne mreže hrane na devet lokacija krajem juna 2022. godine. Rezultati su pokazali visoku taksonomsku raznovrsnost (bogatstvo taksona – u prosjeku 36 – i Shannon- Raznovrsnost – u prosjeku 2,6) makrobeskičmenjaka, visok postotak EPT taksona (Ephemeroptera, Plecoptera, Trichoptera; u prosjeku 65,2 %) i blago heterotrofni ekosistem (prosječna alohtona asimilacija = 54 %). Taksonomski sastav se značajno razlikovao između pritoke koja se nalazi uzvodno (vrelo Krupac) i preostalih osam lokaliteta raspoređenih duž glavnog toka Neretve. To bi moglo biti povezano sa niskom temperaturom vode i visokim koncentracijama azota koje su karakterisale ovu pritoku. Također smo otkrili značajan promet vrsta između gornjih (izvorišni i srednji dio) tokova i nizvodnih tokova glavnog vodotoka, uglavnom povezan s nižim relativnim obiljem drobilica i sjekača, ali relativno većim brojem sakupljača (vrlo oskudni u izvorištima), strugalica i sakupljača. Brojnost makrobeskičmenjaka i postotak EPT-a dostigli su vrhunac nizvodno (osim mjesta 7 za brojnost i mjesta 4 za EPT), dok su dosezi srednjeg dijela pokazali najveće taksonomsko bogatstvo i raznovrsnost, kao i najveću alohtonu asimilaciju. Većina funkcionalnih metrika (maksimalna i prosječna trofička pozicija te izotopsko bogatstvo i ravnomjernost) pokazala je još izraženije diskontinuitete u svojim prostornim obrascima duž proučavanog riječnog kontinuma. Uočeni obrasci nisu slijedili predviđanja koncepta riječnog kontinuma. Nasuprot tome, naši rezultati naglašavaju jedinstvenost ovog dijela rijeke Neretve, gdje prirodni diskontinuiteti strukturiraju zajednice makrobeskičmenjaka na načine i mehanizme koji nisu obuhvaćeni jednostavnim ekološkim konceptima. Čini se da su prisutnost pritoka koje se napajaju podzemnim vodama koje donose svježu vodu, ali i resurse i organizme, te heterogene karakteristike priobalne vegetacije glavne karakteristike koje doprinose heterogenosti vodene sredine i stvaranju složenih obrazaca strukture i funkcije zajednice. Zajednica makrobeskičmenjaka gornje Neretve pokazuje da prirodne rijeke u regionu Balkana rijetko slijede konceptualne modele sa jednostavnim abiotičkim ili biotičkim gradijentima, što sugeriše da bi se očuvanje ovih rijeka evropskog naslijeđa trebalo dogoditi u većim prostornim razmjerima.

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Some special findings during the Neretva Science Week, clockwise from top-left: the white-clawed crayfish (*Austropotamobius pallipes*) (photo: Vladimir Tadić); *Aporia crataegi* (photo: Gernot Kunz); *Limenitis reducta* (photo: Gernot Kunz); *Herilla bosniensis* (photo: Vladimir Tadić).

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Contribution to the knowledge on butterflies and moths (Insecta: Lepidoptera) of the upper course of the Neretva River, Bosnia and Herzegovina

Branislava DUKIĆ[1], Gernot KUNZ[2]

[1]Arbor Magna - Natural Heritage Protection Society, Bulevar Vojvode Stepe Stepanovića 75a, Banja Luka, Bosnia and Herzegovina; E-mail: dukic.branislava@gmail.com
[2]Karl-Franzens-Universität, Universitätsplatz 2, Department of Biology, 8010 Graz, Austria;
E-mail: gernot.kunz@gmail.com

Abstract. The results of a biodiversity survey on butterflies and moths from the upper course of the Neretva River in Bosnia and Herzegovina are presented. This survey was conducted between 27.6. and 3.7.2022, at seven localities between Krupac and Glavatičevo. In total, 251 species were recorded, ten of which are first records for the country: *Limnaecia phragmitella* (Cosmopterigidae), *Crambus uliginosellus* (Crambidae)*, Philereme vetulata* (Geometridae), *Micropterix myrtetella (Micropterigidae), Ponometia candefacta* (Noctuidae), *Crassa unitella* (Oecophoridae), *Rhigognostis hufnagelii* (Plutellidae), *Acrobasis repandana* (Pyralidae), *Tinea trinotella* (Tineidae), *Acleris forsskaleana* (Tortricidae). Two species, namely *Diacrisia purpurata* (Erebidae) and *Aristotelia decurtella* (Gelechiidae) were rediscovered after more than 100 years. Three are on the list as Protected Species of Flora and Fauna of the Republic of Srpska and six on the European Red List of Butterflies: five having the status »near-threatened«, and one the status »vulnerable«. In this contribution, we discuss the problem of losing important habitats due to planned construction of mini hydropower plants in the region.

Key words: habitats, hydropower plants, faunistic, light trapping, Balkan Peninsula

Izvleček. Prispevek k poznavanju dnevnih in nočnih metuljev (Insecta: Lepidoptera) zgornjega toka reke Neretve, Bosna in Hercegovina – Predstavljeni so rezultati raziskave biodiverzitete dnevnih in nočnih metuljev zgornjega toka reke Neretve v Bosni in Hercegovini. Raziskava je bila opravljena med 27.6. in 3.7.2022 na sedmih lokacijah med vasema Krupac in Glavatičevo. Skupno je bilo zabeleženih 251 vrst, od katerih je 10 prvič najdenih v BiH: *Limnaecia phragmitella* (Cosmopterigidae), *Crambus uliginosellus* (Crambidae), *Philereme vetulata* (Geometridae), *Micropterix myrtetella* (Micropterigidae), *Ponometia candefacta* (Noctuidae), *Crassa unitella* (Oecophoridae), *Rhigognostis hufnagelii* (Plutellidae), *Acrobasis repandana* (Pyralidae), *Tinea trinotella* (Tineidae), *Acleris forsskaleana* (Tortricidae). Dve vrsti, in sicer *Diacrisia purpurata* (Erebidae) in *Aristotelia decurtella* (Gelechiidae), sta bili ponovno odkriti po več kot 100 letih. Tri so na seznamu zaščitenih rastlinskih in živalskih vrst Republike Srbske, šest pa na Evropskem rdečem seznamu metuljev: pet »skoraj ogroženih« ter ena »ranljiva«. V prispevku obravnavamo problem izgube pomembnih habitatov zaradi načrtovane gradnje hidroelektrarn v regiji.

Ključne besede: habitati, hidroelektrarne, favnistika, svetlobne pasti, Balkanski polotok

Apstrakt. Prilog poznavanju dnevnih i noćnih vrsta leptira (Insecta: Lepidoptera) gornjeg toka rijeke Neretve, Bosna i Hercegovina – Prikazani su rezultati istraživanja biodiverziteta dnevnih i noćnih vrsta leptira gornjeg toka rijeke Neretve u Bosni i Hercegovini. Ovo istraživanje je sprovedeno između 27.6. i 3.7.2022., na sedam lokaliteta između Krupca i Glavatičeva. Zabilježeno je ukupno 251 vrsta, od kojih je 10 prvi put zabilježeno u zemlji: *Limnaecia phragmitella* (Cosmopterigidae), *Crambus uliginosellus* (Crambidae), *Philereme vetulata* (Geometridae), *Micropterix myrtetella* (Micropterigidae), *Ponometia candefacta* (Noctuidae), *Crassa unitella* (Oecophoridae), *Rhigognostis hufnagelii* (Plutellidae), *Acrobasis repandana* (Pyralidae), *Tinea trinotella* (Tineidae), *Acleris forsskaleana* (Tortricidae). Dvije vrste: *Diacrisia purpurata* (Erebidae) i *Aristotelia decurtella* (Gelechiidae) ponovo su otkrivene nakon više od 100 godina. Tri su na listi zaštićenih vrsta flore i faune Republike Srpske, a šest na Evropskoj crvenoj listi leptira: pet ima status »skoro ugrožene«, a jedna »ugrožena«. U ovom prilogu razmatramo i problem gubitka važnih staništa zbog planirane izgradnje mini hidroelektrana u regionu.

Ključne riječi: staništa, hidroelektrane, faunizam, svjetlosne zamke, Balkansko poluostrvo

Introduction

The butterflies and moths of Bosnia and Herzegovina have been subject of interest from the late 19th century (Mitis 1882; Rebel 1904). Some parts of Bosnia and Herzegovina were systematically surveyed during the 20th century, especially by Rizo Sijarić, the most prominent lepidopterologists of the country (Lorković 1996). However, the area of the upper course of the Neretva remained completely unexplored. The closest and most frequently studied area is the area of Sutjeska National Park, which has been researched on several occasions (Rebel 1904; Schawerda 1914; Sijarić 1970; Lelo 2004; Koren 2016; Filipović & Šćiban 2017). There are also data on the presence of species from the neighboring areas of Kalinovik (Rebel 1904; Batinica 1967). The total number of recorded species in all previous surveys for the wider area of National Park Sutjeska and Kalinovik was 296. Diurnally active species have been much more widely and more systematically studied in the aforementioned surrounding area, compared to moth species for which most of the data are from the first half of the 20th century (Lelo 2004). Kalinovik and the NP Sutjeska are located approximately 25 km from the upper Neretva.

The aim of this paper is to provide first insights into the butterfly and moth fauna of the upper course of Neretva River to underline the uniqueness and richness of this area.

Materials and methods

Geographical characteristic of the region

The Neretva is a river with a total length of about 225 km, which mostly flows through Bosnia and Herzegovina (205 km). The Neretva River originates from under the mountain saddle Gredelj, part of the Jabuka Mountain, at an altitude of 1095 m a.s.l.. The Neretva River originates from five individual springs in the forests and steep slopes of the Gredelj ridge, bordering the oldest national park in Bosnia and Herzegovina, the National Park Sutjeska, with its primary Perućica forest. In its upper course, Neretva is a canyon river with numerous waterfalls and rapids. Its entire upper course, of around of around 80 km in lenght, is drinkable and for most of the year extremely clear and one of the coldest in Europe. The characteristic relief of this area, with a large height interval, diverse geological structure, and geographical position on the border of the influence of the sub-mediterranean and mountainous continental climate, offers all the conditions for diverse biodiversity (Marković 1990).

Most of the area around the upper course of the Neretva belongs to grassland and meadows that are under visible anthropogenic influence. When moving away from the river bank we encounter beech forests on limestone shallow soils on steep sides that descend into the Neretva Valley. In the parts where the slopes are less steep and the soil is a bit deeper, we find forests of sessile (*Quercus petraea*) and turkey oak (*Quercus cerris*), interspersed with forest glades and clearings.

Description of the surveys

The upper course of the Neretva River was surveyed, from Krupac to Glavatičevo, between 27.6. and 3.7. 2022. Seven localities in the area of Ulog, Nedavić, Crvanjsko Lake, Plaža, Stari most, Cerova and Krupac were sampled (Tab. 1).

Different survey methods were applied to studying diurnally active or nocturnal species. Moths were attracted with different light traps, using a mercury lamp but also super actinic UV-lights (Fig. 1). Nocturnal species were collected at localities: Ulog, Plaža, Nedavić i Crvanjsko jezero. At the Ulog locality, the light for attracting nocturnal species was set up during all seven days and throughout the night, in contrast to Crvanjsko Lake, Nedavić and Plaža locality, where the light was only set up for one night, from dusk to one hour after midnight.

Table 1. The list of localities surveyed in the area of the upper course of the Neretva River . Coordinates are given in WGS84 projection. The numbers of localities match those in the text and map.
Tabela 1. Seznam popisanih lokalitet na območju zgornjega toka reke Neretve. Koordinate so podane v projekciji WGS84. Številčne oznake lokalitet se ujemajo z oznakami v besedilu in na zemljevidu.
Tabela 1. Spisak istraženih lokaliteta na području gornjeg toka rijeke Neretve. Koordinate su date u WGS84 projekciji. Brojevi lokaliteta odgovaraju onima u tekstu i karti.

No	Name of locality	Habitat type	Elevation (m)	North	East	Dates
1	Ulog	extensive grassland	645	43.40528	18.32304	27.6.–3.7.2022
2	Plaža	riverbank vegetation	658	43.42414	18.30837	30.6.2022
3	Nedavić	forest clearings, riverbank	590	43.4581	18.32121	1.7.2022
4	Cerova	forest clearings, meadows	686	43.37887	18.35621	30.6.2022
5	Stari most	riverbank vegetation	705	43.36523	18.36999	1.7.2022
6	Krupac	extensive grassland	789	43.32942	18.42574	29.6.2022
7	Crvanjsko lake	extensive grassland	1070	43.39001	18.20425	29.6.2022

Figure 1. Light trap set at the basecamp Ulog, next to the Neretva River: 180 cm cylinder with two superactinic UV-lights.
Slika 1. Svetlobna past, postavljena v taboru v Ulogu ob reki Neretvi: 180 cm valj z dvema superaktiničnima UV-lučkama.
Slika 1. Svjetlosna zamka postavljena u baznom kampu Ulog, pored rijeke Neretve: cilindar od 180 cm sa dva superaktinička UV-svjetla.

Most of the specimens on the light trap were photographed in high quality using the Canon 1D X and Canon 7D Mark II DSLR together with the Canon EF 100mm f/2.8L Macro IS USM, the Canon MP-E 65mm f/2.8 1-5x Macro and the Canon Macro Ring Lite MR-14EX II ring flash. Pictures were then processed with Photoshop CS2 and uploaded on iNaturalist and can be verified following this link: https://www.inaturalist.org/observations?place_id=any&project_id=biodiversity-neretva&taxon_id=47157. A small fraction of pictures were photographed with different smartphones.

A few specimens were collected for accurate identification based on the male genitalia and are deposited in the first author's collection. Genitalia slides were done in a standard procedure: maceration in potassium hydroxide, washing to remove potash, dissecting and cleaning, staining, dehydrating and hardening, and mounting in Canada balsam (Jakšić 1998).

Diurnally active species were sampled with an entomological net or photographed in the wild. During the research, different types of habitats were selected: meadows, grassland, forest clearings and beaches along the Neretva River in order to cover as many different habitats as possible in the time available.

The taxonomic order and nomenclature follow Wiemers et al. (2018). The threat status in Europe is shown according to work of Van Swaay et al. (2010).

The moth families are listed in taxonomic order (Kristensen et al. 2007) while the species within each family are listed alphabetically.

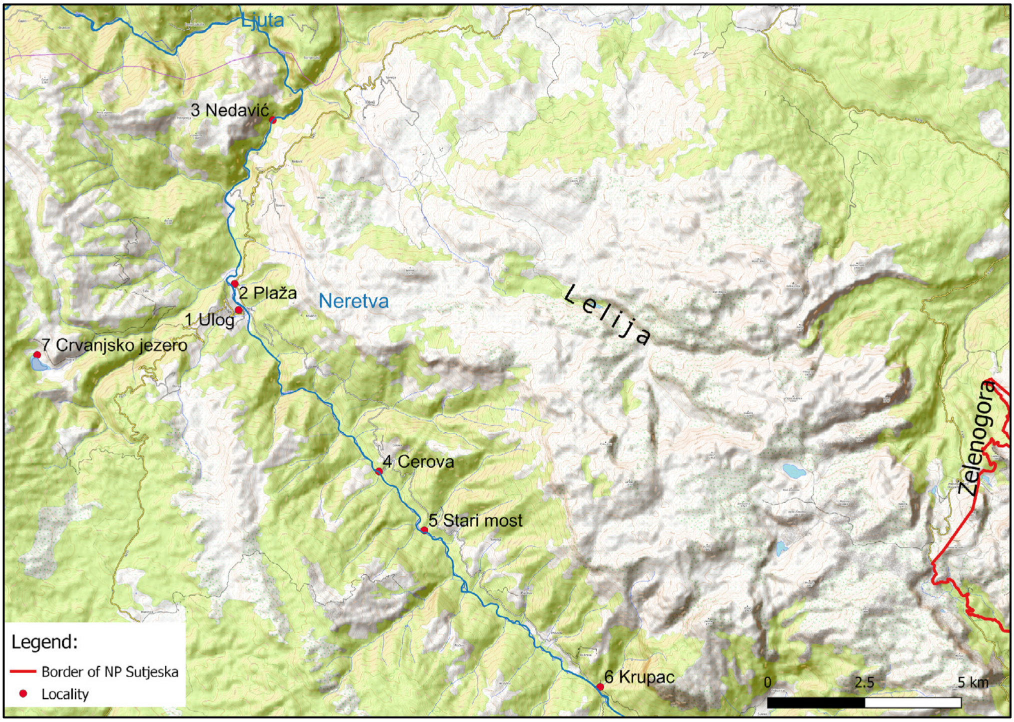

Figure 2. Position of the localities for butterfly and moth surveys on upper course of Neretva River. Locality numbers correspond to the ones given in Tab. 1.
Slika 2. Lokalitete vzorčenja dnevnih in nočnih metuljev v zgornjem toku reke Neretve. Številčne oznake lokalitet se ujemajo z oznakami na Tab 1.
Slika 2. Položaj lokaliteta za istraživanje dnevnih i noćnih vrsta leptira gornjeg toka rijeke Neretve. Brojevi lokaliteta odgovaraju onima navedenim u Tab. 1.

The topographical map was edited and processed in the QGIS software (ver. 3.22.14) and the precise coordinates were determined using GPS »Garmin e-trex 35«.

Results

A total of 55 species of butterflies (Tab. 2) were observed in the area of the upper course of the Neretva River.

Nymphalidae are the best-represented group with a total of 27 recorded species, followed by Lycaenidae with 17 species, Pieridae with six and Hesperiidae with four and Papilionidae with one species (Tab. 2.).

Table 2. A complete list of registered butterfly species during the field survey of the upper course of Neretva. The numbers of locality match those in the Tab. 1 and Fig. 2.
Tabela 2. Celoten seznam zabeleženih vrst metuljev med terenskim pregledom zgornjega toka Neretve. Številčne oznake lokalitet se ujemajo z oznakami na Tab. 1 in Sl. 2.
Tabela 2. Kompletna lista registrovanih dnevnih vrsta leptira tokom terenskog istraživanja gornjeg toka Neretve. Brojevi lokaliteta odgovaraju onima u Tab. 1 i Sl. 2.

Family	Species		Locality	Protected Species of Republic of Srpska	Conserv. status in Europe/ EU27
Papilionidae	1	*Iphiclides podalirius* (Linnaeus, 1758)	3, 5		LC
Hesperiidae	2	*Heteropterus morpheus* (Pallas, 1771)	4, 5		LC
	3	*Ochlodes sylvanus* (Esper, 1777)	6		LC
	4	*Thymelicus lineola* (Ochsenheimer, 1808)	3, 4, 6, 7		LC
	5	*Pyrgus alveus* (Hübner, 1802)	1, 5		LC
Pieridae	6	*Leptidea juvernica* Williams, 1946	6		LC
	7	*Leptidea sinapis* (Linnaeus, 1758)	3, 4, 5, 6		LC
	8	*Gonepteryx rhamni* (Linnaeus, 1758)	1, 5		LC
	9	*Colias crocea* (Geoffroy, 1785)	1, 5		LC
	10	*Aporia crataegi* (Linnaeus, 1758)	3, 4, 6, 7		LC
	11	*Pieris balcana* Lorkovic, 1970	4, 5, 6		LC
Lycaenidae	12	*Lycaena alciphron* (Rottemburg, 1775)	6		LC (NT)
	13	*Lycaena virgaureae* (Linnaeus, 1758)	1		LC
	14	*Favonius quercus* (Linnaeus, 1758)	4, 5, 6		LC
	15	*Satyrium w-album* (Knoch, 1782)	6		LC
	16	*Satyrium acaciae* (Fabricius, 1787)	6		LC
	17	*Celastrina argiolus* (Linnaeus, 1758)	6		LC
	18	*Phengaris alcon* (Denis & Schiffermüller, 1775)	6		LC (NT)
	19	*Pseudophilotes vicrama* (Moore, 1865)	4, 5, 6		NT
	20	*Cupido minimus* (Fuessly, 1775)	3, 4, 6, 7		LC
	21	*Cupido osiris* (Meigen, [1829])	6		LC
	22	*Plebejus argus* (Linnaeus, 1758)	3, 4, 6, 7		LC
	23	*Plebejus idas* (Linnaeus, 1761)	6		LC
	24	*Cyaniris semiargus* (Rottemburg, 1775)	6		LC

Family	Species		Locality	Protected Species of Republic of Srpska	Conserv. status in Europe/ EU27
	25	*Lysandra coridon* (Poda, 1761)	6		LC
	26	*Polyommatus icarus* (Rottemburg, 1775)	3, 4, 6, 7		LC
	27	*Polyommatus ripartii* (Freyer, 1830)	6		LC (NT)
	28	*Plebejus argus* (Linnaeus, 1758)	4, 5, 6		LC
Nymphalidae	29	*Limenitis reducta* Staudinger, 1901	4, 5, 6		LC
	30	*Issoria lathonia* (Linnaeus, 1758)	3, 4, 6, 7		LC
	31	*Brenthis daphne* (Denis & Schiffermüller) 1775	6		LC
	32	*Argynnis paphia* (Linnaeus, 1758)	3, 4, 6, 7		LC
	33	*Fabriciana adippe* (Denis & Schiffermüller, 1775)	3, 4, 6, 7		LC
	34	*Fabriciana niobe* (Linnaeus, 1758)	6		LC (NT)
	35	*Boloria dia* (Linnaeus, 1767)	6		LC
	36	*Apatura ilia* (Denis & Schiffermüller) 1775	3, 4, 6, 7	yes	LC
	37	*Apatura iris* (Linnaeus, 1758)	3, 4, 6, 7	yes	LC
	38	*Vanessa atalanta* (Linnaeus, 1758)	4, 5, 6		LC
	39	*Aglais io* (Linnaeus, 1758)	6		LC
	40	*Polygonia c-album* (Linnaeus, 1758)	6		LC
	41	*Nymphalis antiopa* (Linnaeus, 1758)	6	yes	LC
	42	*Nymphalis polychloros* (Linnaeus, 1758)	4, 5, 6		LC (VU)
	43	*Melitaea athalia* (Rottemburg, 1775)	3, 4, 6, 7		LC
	44	*Melitaea didyma* (Esper, 1778)	6		LC
	45	*Melitaea phoebe* (Denis & Schiffermüller, 1775)	4, 5, 6		LC
	46	*Coenonympha arcania* (Linnaeus, 1761)	3, 4, 6, 7		LC
	47	*Coenonympha glycerion* (Borkhausen, 1788)	3, 4, 6, 7		LC
	48	*Coenonympha pamphilus* (Linnaeus, 1758)	3, 4, 6, 7		LC
	49	*Lasiommata maera* (Linnaeus, 1758)	6		LC
	50	*Melanargia galathea* (Linnaeus, 1758)	3, 4, 6, 7		LC
	51	*Minois dryas* (Scopoli, 1763)	6		LC
	52	*Brintesia circe* (Fabricius, 1775)	6		LC
	53	*Satyrus ferula* (Fabricius, 1793)	6		LC
	54	*Aphantopus hyperantus* (Linnaeus, 1758)	4, 5, 6		LC
	55	*Maniola jurtina* (Linnaeus, 1758)	6		LC

During this survey we also gathered data of 196 moth species within the study area, belonging to 23 different families (Tab. 3). The most numerous are Geometridae with a total of 47 species, then Noctuidae with 37 species and Erebidae with 30 species.

Table 3. A complete list of registered moth species during field survey of the upper course of Neretva. The numbers of locality match those in the text. New species for Bosnia and Herzegovina are marked with a »**+**« sign, and species that were rediscovered after 100 years are marked with a »*****« sign.
Tabela 3. Celoten seznam zabeleženih vrst nočnih metuljev med terenskim pregledom zgornjega toka Neretve. Številčne oznake lokalitet se ujemajo s tistimi v besedilu. Z znakom »+« so označene nove vrste za Bosno in Hercegovino, z znakom »*« pa vrste, ki so bile ponovno odkrite po 100 letih.
Tabela 3. Kompletna lista zabilježenih noćnih vrsta leptira tokom terenskog istraživanja gornjeg toka Neretve. Brojevi lokaliteta odgovaraju onima u tekstu. Nove vrste za Bosnu i Hercegovinu označene su znakom »+«, a vrste koje su ponovo otkrivene nakon 100 godina označene su znakom »*«.

Family		Species list	Locality	First/ reconfirmed presence for the country
Micropterigidae	1	*Micropterix myrtetella* (Zeller, 1850)	1	+
Tineidae	2	*Monopis obviella* (Denis & Schiffermüller, 1775)	1	
	3	*Tinea trinotella* Thunberg, 1794	1	+
Plutellidae	4	*Rhigognostis hufnagelii* (Zeller, 1839)	1	+
Oecophoridae	5	*Alabonia staintoniella* (Zeller, 1850)	1	
	6	*Crassa unitella* (Hübner, 1796)	1	+
	7	*Holoscolia huebneri* Koçak, 1980	1	
	8	*Oecophora bractella* (Linnaeus, 1758)	1	
Lecithoceridae	9	*Lecithocera nigrana* (Duponchel, 1836)	1	
Cosmopterigidae	10	*Limnaecia phragmitella* (Stainton, 1851)	1	+
Gelechiidae	11	*Aristotelia decurtella* (Hübner, 1813)	1	*
	12	*Brachmia dimidiella* (Denis & Schiffermüller) 1775	1	
	13	*Dichomeris limosellus* (Schläger, 1849)	1	
	14	*Mirificarma eburnella* (Denis & Schiffermüller, 1775)	1	
Limacodidae	15	*Apoda limacodes* (Hufnagel, 1766)	1	
Zygaenidae	16	*Adscita statices* (Linnaeus, 1758)	1, 2	
	17	*Zygaena viciae* (Denis & Schiffermüller, 1775)	2	
Cossidae	18	*Cossus cossus* Linnaeus, 1758	1, 3	
	19	*Zeuzera pyrina* (Linnaeus, 1761)	1, 3	
Tortricidae	20	*Acleris forsskaleana* (Linnaeus, 1758)	1	+
	21	*Agapeta hamana* (Linnaeus, 1758)	1	
	22	*Agapeta zoegana* (Linnaeus, 1767)	1	
	23	*Aleimma loeflingiana* (Linnaeus, 1758)	1	
	24	*Archips podana* Scopoli, 1763	1	
	25	*Archips rosana* (Linnaeus, 1758)	2	
	26	*Clepsis rolandriana* (Linnaeus, 1758)	1	
	27	*Epagoge grotiana* (Fabricius, 1781)	2	
	28	*Hedya salicella* (Linnaeus, 1758)	2	
	29	*Olethreutes arcuella* (Clerck, 1759)	1	
	30	*Pseudargyrotoza conwagana* (Fabricius, 1775)	1	
	31	*Spilonota ocellana* ([Denis & Schiffermüller], 1775)	1	

Family		Species list	Locality	First/ reconfirmed presence for the country
Pterophoridae	32	*Cnaemidophorus rhododactyla* ([Denis & Schiffermüller], 1775)	1, 2	
	33	*Pterophorus pentadactyla* (Linnaeus, 1758)	1, 7	
Thyrididae	34	*Thyris fenestrella* (Scopoli, 1763)	1	
Pyralidae	35	*Acrobasis repandana* (Fabricius, 1798)	2	+
	36	*Catastia marginea* ([Denis & Schiffermüller], 1775)	3	
	37	*Hypsopygia costalis* Fabricius, 1775	1	
	38	*Hypsopygia rubidalis* (Denis & Schiffermüller, 1775)	1	
	39	*Myelois circumvoluta* Fourcroy, 1785	1	
	40	*Oncocera semirubella* Scopoli, 1763	1	
	41	*Pyralis regalis* (Denis & Schiffermüller) 1775	1	
	42	*Synaphe punctalis* Fabricius, 1775	1	
Crambidae	43	*Anania hortulata* Linnaeus, 1758	1	
	44	*Chrysocrambus linetella* Fabricius, 1781	1	
	45	*Chrysoteuchia culmella* Linnaeus, 1758	1	
	46	*Crambus uliginosellus* Zeller, 1850	2	+
	47	*Dolicharthria punctalis* (Denis & Schiffermüller, 1775)	1	
	48	*Elophila nymphaeata* Linnaeus, 1758	1	
	49	*Metasia ophialis* (Treitschke, 1829)	2	
	50	*Palpita vitrealis* Rossi, 1794	1	
	51	*Platytes cerussella* (Denis & Schiffermüller) 1775	1	
	52	*Pyrausta aurata* (Scopoli, 1763)	1	
	53	*Pyrausta despicata* (Scopoli, 1763)	2	
	54	*Sitochroa verticalis* (Linnaeus, 1758)	2	
Lasiocampidae	55	*Gastropacha quercifolia (Linnaeus, 1758)*	1, 7	
	56	*Malacosoma castrensis (Linnaeus, 1758)*	1	
	57	*Malacosoma neustria (Linnaeus, 1758)*	1, 3	
	58	*Odonestis pruni (Linnaeus, 1758)*	7	
Sphingidae	59	*Deilephila elpenor* Linnaeus, 1758	7	
	60	*Deilephila porcellus* (Linnaeus, 1758)	7	
	61	*Hyles euphorbiae* (Linnaeus, 1758)	7	
	62	*Hyles livornica* (Esper, 1780)	1, 7	
	63	*Laothoe populi* Linnaeus, 1758	1	
	64	*Marumba quercus* (Denis & Schiffermüller, 1775)	1, 3	
	65	*Smerinthus ocellata* Linnaeus, 1758	1	
Drepanidae	66	*Cilix glaucata* (Scopoli, 1763)	1	
	67	*Habrosyne pyritoides (Hufnagel, 1766)*	1	
	68	*Tethea ocularis* Linnaeus, 1767	1	
	69	*Tethea or* ([Denis & Schiffermüller], 1775)	2	
	70	*Watsonalla binaria* (Hufnagel, 1769)	1	
	71	*Watsonalla cultraria* (Fabricius, 1775)	1	

Family		Species list	Locality	First/ reconfirmed presence for the country
Geometridae	72	*Alcis repandata* (Linnaeus, 1758)	1, 7	
	73	*Aplasta ononaria* (Fuessly, 1783)	2	
	74	*Biston betularia* (Linnaeus, 1758)	1	
	75	*Bupalus piniaria* (Linnaeus, 1758)	1	
	76	*Cabera exanthemata* (Scopoli, 1763)	2	
	77	*Campaea margaritaria (Linnaeus, 1761)*	1, 7	
	78	*Catarhoe cuculata* (Hufnagel, 1767)	1	
	79	*Chiasmia clathrata* (Linnaeus, 1758)	1, 7	
	80	*Chloroclystis v-ata* (Haworth, 1809)	7	
	81	*Cleorodes lichenaria* (Hufnagel, 1767)	1	
	82	*Comibaena bajularia* (Denis & Schiffermüller) 1775	1	
	83	*Cyclophora annularia (Fabricius, 1775)*	1	
	84	*Cyclophora linearia (Hübner, 1799)*	1, 7	
	85	*Ematurga atomaria* (Linnaeus, 1758)	1	
	86	*Ennomos erosaria* (Denis & Schiffermüller,1775)	1	
	87	*Epirrhoe galiata* ([Denis & Schiffermüller], 1775)	2	
	88	*Euchoeca nebulata* (Scopoli, 1763)	2	
	89	*Euphyia frustata* (Treitschke, 1828)	1	
	90	*Fagivorina arenaria* (Hufnagel, 1767)	1, 7	
	91	*Geometra papilionaria* (Linnaeus, 1758)	1	
	92	*Heliomata glarearia* (Denis & Schiffermüller) 1775	1	
	93	*Hemistola chrysoprasaria* (Esper, [1795])	1	
	94	*Hypomecis punctinalis* (Scopoli, 1763)	1	
	95	*Hypomecis roboraria* (Denis & Schiffermüller, 1775)	1, 7	
	96	*Idaea aureolaria* (Denis & Schiffermüller) 1775	1	
	97	*Idaea aversata* (Linnaeus, 1758)	1, 7	
	98	*Idaea biselata* (Hufnagel, 1767)	1	
	99	*Idaea humiliata* (Hufnagel, 1767)	1	
	100	*Idaea ochrata* (Scopoli, 1763)	1	
	101	*Idaea trigeminata* (Haworth, 1809)	1	
	102	*Ligdia adustata* (Denis & Schiffermüller) 1775	1	
	103	*Lomaspilis marginata* (Linnaeus, 1758)	1, 3	
	104	*Macaria notata* Hulst, 1896	1	
	105	*Narraga tessularia* (Metzner, 1845)	1, 2	
	106	*Opisthograptis luteolata* (Linnaeus, 1758)	1, 7	
	107	*Ourapteryx sambucaria* (Linnaeus, 1758)	1	
	108	*Pasiphila rectangulata* (Linnaeus, 1758)	1	
	109	*Peribatodes rhomboidaria* (Denis & Schiffermüller, 1775)	1, 3	
	110	*Perizoma albulata* ([Denis & Schiffermüller], 1775)	2	
	111	*Philereme vetulata* (Denis & Schiffermüller) 1775	1	+

Family		Species list	Locality	First/ reconfirmed presence for the country
	112	*Pseudoterpna pruinata* (Hufnagel, 1767)	1	
	113	*Rhodostrophia vibicaria* (Clerck, 1759)	1	
	114	*Scopula immorata (Linnaeus, 1758)*	1	
	115	*Scopula rubiginata (Hufnagel, 1767)*	1	
	116	*Scopula tessellaria (Boisduval, 1840)*	1	
	117	*Selenia tetralunaria* (Hufnagel, 1767)	1	
	118	*Thetidia smaragdaria (Fabricius, 1787)*	1	
Notodontidae	119	*Drymonia melagona (Borkhausen, 1790)*	1	
	120	*Notodonta tritophus* (Denis & Schiffermüller) 1775	1	
	121	*Notodonta ziczac* (Linnaeus, 1758)	1	
	122	*Phalera bucephala* Linnaeus, 1758	1	
	123	*Pterostoma palpina* (Clerck, 1759)	1	
	124	*Ptilodon capucina* (Linnaeus, 1758)	1	
	125	*Spatalia argentina* (Denis & Schiffermüller) 1776	1	
	126	*Stauropus fagi* (Linnaeus, 1758)	1	
Erebidae	127	*Amata phegea* Linnaeus, 1758	1, 2, 3	
	128	*Arctia villica* (Linnaeus, 1758)	1, 3	
	129	*Arctornis l-nigrum* Müller, 1794	1,7	
	130	*Atolmis rubricollis* Linnaeus, 1761	1	
	131	*Callimorpha dominula* (Linnaeus, 1758)	1	
	132	*Calliteara pudibunda* Linnaeus, 1758	1	
	133	*Catocala fulminea* Scopoli, 1763	1	
	134	*Cybosia mesomella* Linnaeus, 1758	1	
	135	*Rhyparia purpurata* (Linnaeus, 1758)	1	*
	136	*Diacrisia sannio* (Linnaeus, 1758)	1	
	137	*Dysauxes ancilla* Linnaeus, 1767	1	
	138	*Eublemma purpurina* ([Denis & Schiffermüller], 1775)	1	
	139	*Euplagia quadripunctaria* (Poda, 1761)	1	
	140	*Euproctis chrysorrhoea (Linnaeus, 1758)*	1	
	141	*Herminia tarsicrinalis* (Knoch, 1782)	1	
	142	*Idia calvaria* (Denis & Schiffermüller) 1775	1	
	143	*Lithosia quadra* (Linnaeus, 1758)	1,3	
	144	*Lymantria dispar* (Linnaeus, 1758)	1	
	145	*Lymantria monacha* (Linnaeus, 1758)	1,7	
	146	*Miltochrista miniata* Forster, 1771	1,3	
	147	*Nudaria mundana* Linnaeus, 1761	1	
	148	*Odice suava* (Hübner, [1813])	2	
	149	*Paracolax tristalis* (Fabricius, 1794)	2	
	150	*Phragmatobia fuliginosa* (Linnaeus, 1758)	2	
	151	*Phytometra viridaria* (Clerck, 1759)	1, 3	
	152	*Polypogon tentacularia* (Linnaeus, 1758)	1	
	153	*Scoliopteryx libatrix* (Linnaeus, 1758)	2	
	154	*Euproctis similis (Fuessly, 1775)*	2	

Family		Species list	Locality	First/ reconfirmed presence for the country
	155	*Coscinia striata (Linnaeus, 1758)*	1, 3	
	156	*Zanclognatha lunalis* (Scopoli, 1763)	2	
Nolidae	157	*Bena bicolorana* (Füssly, 1775)	1	
	158	*Pseudoips prasinana* (Linnaeus, 1758)	1	
	159	*Nola cucullatella* (Linnaeus, 1758)	1	
Noctuidae	160	*Abrostola triplasia* (Linnaeus, 1758)	1	
	161	*Acontia trabealis (Scopoli, 1763)*	1	
	162	*Agrotis exclamationis* (Linnaeus, 1758)	1	
	163	*Amphipyra berbera Rungs, 1949*	1	
	164	*Apamea crenata* (Hufnagel, 1766)	1	
	165	*Apamea sublustris* Esper, 1788	1	
	166	*Atypha pulmonaris* (Esper, 1790)	2	
	167	*Autographa gamma* (Linnaeus, 1758)	1	
	168	*Callopistria juventina* (Stoll, 1782)	1	
	169	*Colocasia coryli* (Linnaeus, 1758)	1	
	170	*Cosmia trapezina (Linnaeus, 1758)*	2	
	171	*Craniophora ligustri* (Denis & Schiffermüller) 1775	1	
	172	*Deltote pygarga (Hufnagel, 1766)*	1	
	173	*Diarsia brunnea* (Denis & Schiffermüller) 1775	1	
	174	*Dypterygia scabriuscula* (Linnaeus, 1758)	1	
	175	*Euplexia lucipara* (Linnaeus, 1758)	2	
	176	*Hadena confusa (Hufnagel, 1766)*	1	
	177	*Hadena filograna (Esper, 1788)*	2	
	178	*Hadena perplexa (Denis & Schiffermüller, 1775)*	1	
	179	*Heliothis viriplaca* (Hufnagel, 1766)	1	
	180	*Hoplodrina octogenaria* (Goeze, 1781)	2	
	181	*Hoplodrina respersa* (Denis & Schiffermüller) 1775	1	
	182	*Lacanobia thalassina (Hufnagel, 1766)*	1	
	183	*Lamprosticta culta* ([Denis & Schiffermüller], 1775)	2	
	184	*Leucania comma (Linnaeus, 1761)*	1	
	185	*Melanchra persicariae* (Linnaeus, 1761)	2	
	186	*Moma alpium* (Osbeck, 1778)	1	
	187	*Mythimna (Hyphilare) ferrago (Fabricius, 1787)*	2	
	188	*Noctua comes* Hübner, [1813]	1	
	189	*Noctua fimbriata* Schreber, 1759	1	
	190	*Noctua pronuba* Linnaeus, 1758	1, 7	
	191	*Ochropleura plecta* Linnaeus, 1761	1	
	192	*Oligia strigilis* (Linnaeus, 1758)	1	
	193	*Polia nebulosa* Hufnagel, 1766	1	
	194	*Ponometia candefacta* (Hübner, 1831)	1	+
	195	*Charanyca ferruginea (Esper, 1785)*	1	
	196	*Xestia triangulum (Hufnagel, 1766)*	2	

Discussion

Until now, the butterfly and moth fauna of the area around the upper course of Neretva has not been systematically investigated. During this research, 251 species from the Lepidoptera order were registered. 55 registered species of butterflies represent 29% of the total number of butterfly species and 196 gathered moth species represent 16% of total number of registered species for Bosnia and Herzegovina (Lelo 2006, 2008, 2016) (Tabs. 2, 3).

Due to the lack of past inventories, especially regarding smaller species of moths, the so-called »microlepidoptera«, ten species represent new country records. Two species, *Rhyparia purpurata* and *Aristotelia decurtella*, were last recorded for the country by Rebel in 1904, therefore we treat them as rediscovered species here (Tab. 3).

The closest systematically researched area is the Sutjeska National Park (Rebel 1904; Schawerda 1914; Sijarić 1970, 1991; Lelo 2004; Koren 2016; Filipović & Šćiban 2017). The data about butterflies and moths of NP Sutjeska were collected over a long period, during almost the entire vegetation period and carried out by several authors, in contrast to the area of the Neretva River where the survey period was limited to only seven days. Taking the above into consideration, it would be difficult to make a comparison and place it in a meaningful framework.

However, it is possible to give an overview of new and significant species recorded by this research.

Based on the reviewed available literature, the authors concluded that ten of the total number of species are new to the country. Data were also compared with available online databases (De Jong et al. 2014; https://www.gbif.org; https://lepiforum.org/). Incongruities were detected comparing the distribution data of the Fauna Europaea (De Jong et al. 2014), with the literature available to the authors (Poltavsky & Artokhin 2006; Stojanović et al. 2017). In the case of *Crambus uliginosellus* the Fauna Europaea online database (De Jong et al. 2014) lists its presence in Bosnia and Herzegovina, but the source of this record is not provided and literature untraceable. Therefore, we list this species as a new country record. Possibly, some Bosnia and Herzegovina country records displayed in the Fauna Europaea arise from unspecified localities from the former Yugoslavia. Therefore the references for some species were erroneously assigned to Bosnia and Herzegovina, even though they refer to Macedonia, for example.

All ten species were observed at the localities Ulog and Plaža. These localities are wet and marshy areas along the river, covered by hygrophilous vegetation, primarily species from the genus *Salix*, bordered by degraded oak and beech forests.

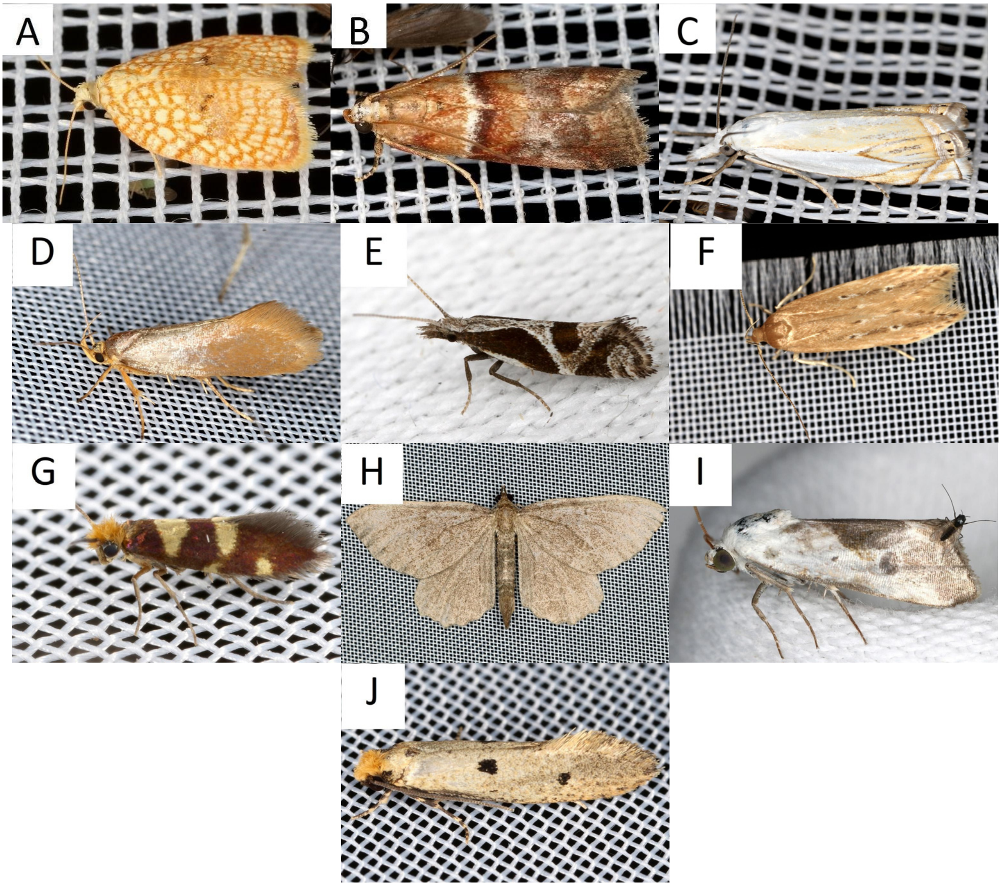

Figure 3. New moth species for Bosnia and Herzegovina found on upper course of Neretva river (see Tab. 1, Fig. 1 for details on localities): A- *Acleris forsskaleana* (Loc. 1); B- *Acrobasis repandana* (Loc. 2); C- *Crambus uliginosellus* (Loc. 2); D- *Crassa unitella* (Loc. 1); E- *Rhigognostis hufnagelii* (Loc. 1), F- *Limnaecia phragmitella* (Loc. 1); G- *Micropterix myrtetella* (Loc. 1); H- *Philereme vetulata* (Loc. 1); I- *Ponometia candefacta* (Loc. 1); J- *Tinea trinotella* (Loc. 1). Photos: B. Dukić, G. Kunz.

Slika 3. Nove vrste nočnih metuljev za Bosno in Hercegovino, najdene v zgornjem toku reke Neretve (glej tab. 1, sl. 1 za podrobnosti o nahajališčih): A- *Acleris forsskaleana* (lok. 1); B- *Akrobaza repandana* (lok. 2); C- *Crambus uliginosellus* (lok. 2); D- *Crassa unitella* (lok. 1); E- *Rhigognostis hufnagelii* (lok. 1), F- *Limnaecia phragmitella* (lok. 1); G- *Micropterix myrtetella* (lok. 1); H- *Philereme vetulata* (lok. 1); I- *Ponometia candefacta* (lok. 1); J- *Tinea trinotella* (lok. 1). Fotografije: B. Dukić, G. Kunz.

Slika 3. Nove vrste leptira za Bosnu i Hercegovinu pronađene na području gornjeg toka rijeke Neretve (vidi Tab. 1, Sl. 1 za detalje o lokalitetima): A- Acleris forsskaleana (Loc. 1); B- Acrobasis repandana (lok. 2); C- Crambus uliginosellus (Loc. 2); D- Crassa unitella (Loc. 1); E- Rhigognostis hufnagelii (Loc. 1), F- Limnaecia phragmitella (Loc. 1); G- Micropterix myrtetella (Loc. 1); H- Philereme vetulata (Loc. 1); I- Ponometia candefacta (Loc. 1); J- Tinea trinotella (Loc. 1). Fotografije: B. Dukić, G. Kunz.

Considering that this research is part of the »Save the Blue Heart of Europe« campaign, which has the task of highlighting the importance of preserving rivers and contributing to their protection, it is important to emphasize that the construction of mini-hydro power plants on the Neretva River and its tributaries would have incalculable consequences for many species and organisms, and thus indirectly or directly on the butterfly and moth fauna.

Povzetek

Na ozemlju Bosne in Hercegovine se nadaljuje dolgoletna tradicija raziskovanja metuljev in vešč, vse od leta 1882, ko je bil napisan prvi članek o dnevnih in nočnih metuljih Bosne in Hercegovine. Od takrat do danes so bila objavljena številna dela, z občasnimi prekinitvami raziskovanja. Kljub temu je območje okoli zgornjega toka reke Neretve ostalo neraziskano. Nekatera sosednja območja, kot je NP Sutjeska, so bila raziskana večkrat in s strani več avtorjev, naša študija pa je prvi vpogled v favno Lepidoptera zgornjega toka reke Neretve.

Raziskava je bila opravljena med 27. 6. in 3. 7. 2022 na sedmih lokacijah med vasema Krupac in Glavatičevo (slika 2). Pri raziskavi smo skušali zajeti čim več različnih tipov habitatov, od plaž ob bregovih reke Neretve do jas v hrastovih gozdovih. Dnevno aktivne vrste smo zbirali z entomološko mrežo in fotografirali v naravi, nočno aktivne vrste pa smo lovili s svetlobnimi pastmi.

Skupno število registriranih vrst iz reda Lepidoptera je 251, od tega je 55 vrst dnevnih in 196 vrst nočnih metuljev (tab. 2, tab. 3). Med njimi je 10 vrst prvič zabeleženih v Bosni in Hercegovini: *Limnaecia phragmitella* (Cosmopterigidae), *Crambus uliginosellus* (Crambidae), *Philereme vetulata* (Geometridae), *Micropterix myrtetella* (Micropterigidae), *Ponometia candefacta* (Noctuidae), *Crassa unitella* (Oecophoridae), *Rhigognostis hufnagelii* (Plutellidae), *Acrobasis repandana* (Pyralidae), *Tinea trinotella* (Tineidae) in *Acleris forsskaleana* (Tortricidae). Dve vrsti, in sicer *Diacrisia purpurata* (Erebidae) in *Aristotelia decurtella* (Gelechiidae), sta bili ponovno odkriti po več kot 100 letih. Tri so na seznamu zaščitenih rastlinskih in živalskih vrst Republike Srbske, šest pa na Evropskem rdečem seznamu metuljev: pet ima status »skoraj ogroženih«, eden pa status »ranljiv«.

Ta študija je bila narejena v okviru kampanje »Save the Blue Heart of Europe«, ki opozarja na pomen ohranjanja rek in prispeva k njihovemu varovanju.

Sažetak

Na području Bosne i Hercegovine postoji dugogodišnja tradicija istraživanja dnevnih i noćnih leptira koja traje još od 1882. godine, kada je napisan prvi rad o leptirima u Bosni i Hercegovini. Od tada do danas objavljeni su brojni radovi s povremenim prekidima u istraživanju. Uprkos tome, područje oko gornjeg toka rijeke Neretve ostalo je neistraženo. Neka susjedna područja poput Nacionalnog parka »Sutjeska« istraživana su u više navrata i od strane više autora, ali ova studija predstavlja prvi uvid u faunu lepidoptera gornjeg toka rijeke Nereretve.

Ovo istraživanje je sprovedeno između 27.6. i 3.7.2022., na sedam lokaliteta između Krupca i Glavatičeva (slika 2.). Tokom ovog istraživanja nastojali smo obuhvatiti što više različitih tipova staništa, od plaža uz obale rijeke Neretve do čistina unutar hrastovih šuma. Dnevno aktivne vrste sakupljene su entomološkom mrežom i fotografisane u divljini, dok su noćno aktivne vrste uhvaćene u svjetlosne zamke.

Ukupan broj registrovanih vrsta iz reda lepidoptera je 251, od čega je 55 dnevnih leptira, dok je identifikovano 196 noćnih vrsta (Tab. 2, Tab. 3). Od ukupnog broja registrovanih vrsta, 10 je novih za Bosnu i Hercegovinu: *Limnaecia phragmitella* (Cosmopterigidae), *Crambus uliginosellus (Crambidae)*, *Philereme vetulata* (Geometridae), *Micropterix myrtetella* (Micropterigidae), *Ponometia candefacta* (Noctuidae), *Crassa unitella* (Oecophoridae), *Rhigognostis hufnagelii* (Plutellidae), *Acrobasis repandana* (Pyralidae), *Tinea trinotella* (Tineidae), *Acleris forsskaleana* (Tortricidae). Dvije vrste *Diacrisia purpurata* (Erebidae) i *Aristotelia decurtella* (Gelechiidae) ponovo su otkrivene nakon više od 100 godina. Tri su na listi zaštićenih vrsta flore

i faune Republike Srpske, a šest na Evropskoj crvenoj listi leptira: pet ima status »skoro ugrožene«, a jedna »ugrožena«.

Ova studija je rađena u sklopu kampanje »Sačuvajmo plavo srce Evrope«, koja ima zadatak da ukaže na važnost očuvanja rijeka i da doprinese njihovoj zaštiti.

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Researchers used a light trap to attract butterflies, moths and other insects during the night. Clockwise from top-left: the light trap was set-up in camp for several nights (photo: Vladimir Tadić); close-up of insects attracted by the light trap (photo: Gernot Kunz); *Polygonia c-album* (photo: Gernot Kunz); Full set-up of the light trap (photo: Gernot Kunz).

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The ichthyofauna of the upper Neretva River

Jakob NEUBURG[1], Thomas FRIEDRICH[1], Elisabeth HARING[2,3], Sarah HÖFLER[4], Ana MARIC[5], Saša MARIĆ[5], Stephanie POPP[1], Predrag SIMONOVIĆ[5,6], Aleš SNOJ[7], Simona SUŠNIK BAJEC[7], Simon VITECEK[1,8], Alexandra WANKA[2], Steven WEISS[9], Kurt PINTER[1*]

[1]University of Natural Resources and Life Sciences, Institute of Hydrobiology and Aquatic Ecosystem Management, Gregor-Mendel-Straße 33, 1180 Vienna, Austria
[2]Natural History Museum Vienna, Maria-Theresien-Platz 1010 Vienna, Austria
[3]University of Vienna, Department of Evolutionary Biology, Djerassiplatz 1, 1030 Vienna
[4]Blattfisch e.U. – Consultants in Aquatic Ecology and Engineering, Leopold-Spitzer-Straße 26, 4600 Wels, Austria
[5]University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia
[6]University of Belgrade, Institute for Biological Research »Siniša Stanković« - National institute of the Republic of Serbia, Blvd. Despot Stefan 142, 11000 Belgrade, Serbia
[7]University of Ljubljana, Department of Animal Science, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[8]WasserCluster Lunz – Biologische Station GmbH, Dr. Carl Kupelwieser Promenade 5, 3293 Lunz am See, Austria
[9]University of Graz, Faculty of Natural Sciences Universitätsplatz 2, 8010 Graz, Austria
*Corresponding author. E-mai: kurt.pinter@boku.ac.at

Abstract. Fish were sampled at nine locations of the upper Neretva River, upstream of Glavatičevo. Evidence of the endangered softmouth trout (*Salmo obtusirostris*) was obtained as well as the presence of other native and non-native salmonids. Genetic analysis suggests that the native brown trout (*Salmo trutta*) in the upper Neretva is homogeneous with no significant introgression by introduced Atlantic-lineage brown trout and quantitative catch data show that their populations are healthy with densities above 1,000 ind/ha. However, the relatively low biomass estimates may be the result of overharvesting. A new haplotype of the mitochondrial control region of the bullhead (*Cottus gobio*) was detected and confirmation of native *S. trutta*, *Barbatula barbatula*, *Phoxinus* sp., and *C. gobio* in the upper reaches, underlining the importance of this river stretch. The planned expansion of hydropower plants in the upper Neretva and its tributaries will have a significant negative impact on the largely untouched ichthyofauna, as is clearly demonstrated by the conditions found in the middle course of the river, which has been dedicated to hydropower production.

Key words: fish stock assessment, electrofishing, snorkelling, eDNA, genetics, hydropower, *Salmo obtusirostris*

Izvleček. Ihtiofavna gornje Neretve – Ribe so bile vzorčene na devetih lokacijah zgornjega toka reke Neretve, nad Glavatičevom. Ugotovljena je bila prisotnost ogrožene mehkoustne postrvi (*Salmo obtusirostris*) kot tudi drugih avtohtonih in neavtohtonih salmonidov. Genetska analiza kaže, da je avtohtona potočna postrv (*Salmo trutta*) v zgornjem toku Neretve homogena, brez pomembne introgresije uvedenih atlantskih linij potočne postrvi, kvantitativni podatki o ulovu pa kažejo, da so njihove populacije zdrave z gostotami nad 1000 osebkov/ha. Vendar pa relativno nizke ocene biomase lahko izhajajo iz prekomernega izlova. Odkrit je bil nov haplotip mitohondrijske kontrolne regije glavoča (*Cottus gobio*); potrditev avtohtonih vrst *S. trutta*, *Barbatula barbatula*, *Phoxinus* sp. in *C. gobio* v zgornjih delih poudarja pomen tega odseka reke. Načrtovana širitev hidroelektrarn v zgornjem toku Neretve in njenih pritokov bo imela negativen vpliv na večinoma nedotaknjeno ihtiofavno, kar kažejo razmere v srednjem toku reke, ki je že namenjena proizvodnji hidroenergije.

Ključne besede: ocenjevanje staleža rib, elektroribolov, potapljanje, eDNA, genetika, hidroenergija, *Salmo obtusirostris*

Apstrakt. Ihtiofauna gornje Neretve – Ribe su uzorkovane na devet lokacija gornjeg toka rijeke Neretve, uzvodno od Glavatičeva. Dokazano je postojanje ugrožene mekousne pastrmke (*Salmo obtusirostris*) kao i prisutnost drugih autohtonih i alohtonih salmonida. Genetska analiza sugeriše da je autohtona smeđa pastrmka (*Salmo trutta*) u gornjem toku Neretve homogena bez značajne introgresije uvedenih atlantskih linija smeđe pastrmke, a kvantitativni podaci o ulovu pokazuju da su njihove populacije zdrave s gustoćama iznad 1.000 jedinki/ha. Međutim, relativno niske procjene biomase mogu biti rezultat prekomjernog izlova. Otkriven je novi haplotip mitohondrijske kontrolne regije peša (*Cottus gobio*). Potvrda autohtonih *S. trutta, Barbatula barbatula, Phoxinus sp.*, i *C. gobio* u gornjim tokovima naglašava važnost ovog dijela rijeke. Planirano širenje hidroelektrana na gornjoj Neretvi i njenim pritokama imaće značajan negativan utjecaj na uglavnom netaknutu ihtiofaunu, što jasno pokazuju uslovi u srednjem toku rijeke, koji je predviđen za proizvodnju hidroenergije.

Ključne riječi: procjena ribljeg fonda, elektroribolov, ronjenje, eDNA, genetika, hidroenergija, *Salmo obtusirostris*

Introduction

Rivers draining the slopes of the Western Balkans hold significant importance as biodiversity hotspots, with a high number of endemic and rare species (Freyhof & Brooks 2011; Oikonoumou et al. 2014; Schöffmann et al. 2019). Freshwater fish species diversity in this region is considered the highest in Europe (Skoulikidis et al. 2009). Unlike in many other European regions, the upper reaches of many Balkan rivers are free-flowing and relatively unaffected by major anthropogenic alterations such as channelisation or dams (Skoulikidis et al. 2009). In order to maintain the ecological integrity of the region and to foster ecosystem resilience in the face of climate change (cf. Keppel et al. 2012), management actions in these systems need to proceed with great care.

The Neretva River is one of the significant watercourses of the Western Balkan Peninsula. It originates in the Dinaric Alps and descends through rugged landscapes and a sequence of bedrock canyons and plains before entering the Adriatic Sea (Skoulikidis et al. 2009). The hydrological regime, as well as the sediment regime, are in a pristine state in the upper reaches where the river has a natural character with a pronounced diversity of habitats.

Thirty-four fish species are listed as native to the Neretva River basin, 17 of which are classified as endemic (Tutman et al. 2012; Vukić et al. 2019). Vukić et al. (2019) reported the presence of 32 introduced species. The salmonids native to the upper Neretva are the endangered softmouth trout (*Salmo obtusirostris*) (Crivelli 2006), marble trout (*Salmo marmoratus*), and native brown trout (*Salmo trutta*). Although we are aware that the taxonomic status of native Neretva trout (i.e., other than softmouth and marble trout) is complicated and uncertain, these considerations are beyond the scope of the present paper, so we will simply refer to them as (Neretva) brown trout (*Salmo trutta*). Brown and softmouth trout frequently interbreed and produce fertile hybrids. Adult purebreds are phenotypically clearly recognized and discerned from hybrids, while young specimens are more difficult to identify.

Predominantly in the impoundments of the middle reaches of the Neretva, numerous non-native species have been introduced through recreational fishing activities such as the European grayling (*Thymallus thymallus*), rainbow trout (*Oncorhynchus mykiss*), Alpine charr (*Salvelinus umbla*), brook trout (*Salvelinus fontinalis*) (Pavličević et al. 2016; Glamuzina et al. 2018; Vukić et al. 2019; Tutman et al. 2021), and non-native lineages of the brown trout (e.g., the Atlantic lineage; Razpet et al. 2007; Snoj et al. 2010). Genetic traces of the Danube lineage of brown trout have also been observed in the upper Neretva tributaries (Bernatchez et al. 1992; Razpet et al. 2007; Kalamujić et al. 2015), which raises the question of whether they naturally occur there or are the result of human-mediated translocations. Also, the fish fauna of the lower reaches of the river is currently undergoing a major transition, as seawater intrusion increases accompanied by the invasion of marine organisms (Glamuzina & Dobroslavić 2020; Tutman et al. 2021).

Studies related to fish biology and ecology on the Neretva have recently focused on the lower reaches and the estuary into the Adriatic Sea (Bartulović et al. 2004; Ivanković et al. 2010; Dulčić et al. 2017; Glamuzina et al. 2017; Glamuzina & Dobroslavić 2020). Even though studies describing the fish population in the upper reaches of the Neretva have been conducted (Razpet et al. 2007; Glamuzina et al. 2018), detailed population parameters like abundance and biomass or age class distributions are largely missing or not easily accessible to the international scientific community due to language barriers (Vegara et al. 2009; Muhamedagić et al. 2019). Molecular genetic studies on fishes of the Neretva were mainly focused on resolving genetic variation and taxonomic uncertainties within the genera *Salmo* (Bernatchez et al. 1992; Snoj et al. 2002; Razpet et al. 2007; Snoj et al. 2010; Kalamujić et al. 2015), *Cottus* (Bravničar et al. 2015), and *Phoxinus* (Palandačić et al. 2020). The majority of species in the Neretva remain understudied (ex. Tutman et al. 2017).

Here we present results of a fish ecological assessment that covers parts of the upper Neretva relying on both stock assessment and molecular genetic methods. The aims of this study were to provide a detailed stock assessment of the fish community in the upper Neretva, to provide additional insights into the systematics and the distribution of individual species as well as to potentially address some uncertainties on their native status. In addition to standard electrofishing and genetic analyses, a snorkelling survey and environmental DNA (eDNA) analysis were performed. Genetic analyses included a population genetic approach for brown trout using microsatellite markers, as well as the sequencing of mitochondrial DNA sequences for brown trout, minnow (*Phoxinus sp.*), and bullhead (*Cottus gobio*). Additionally, eDNA was extracted from water samples and analysed to try to detect softmouth trout in river sections that were either not sampled directly by other means, or where the species may have been so rare that they could have been missed with other approaches. A water sample from Glavatičevo, where softmouth trout are known to occur (Glamuzina et al. 2018), was included in the analysis as a positive control for the eDNA analysis.

Materials and methods

Study area

The upper Neretva flows roughly 100 km from its source to Jablaničko Reservoir downstream of Konjic (Fig. 1). Due to the high gradient throughout, the river's channel morphology can largely be described as constrained, with long sections running through gorges and occasional branching areas where the valley opens up. The annual mean discharge at Ulog amounts to 9 m³/s and increases to 39 m³/s at Glavatičevo (DIKTAS B&H 2012). Sampling was conducted along the main course of the upper Neretva and in two smaller tributaries, namely the Krupac, located in the upper part of the investigation area, and the lower part of the Jezernica downstream of Ulog (Fig. 1). Surveys were carried out at nine sites, eight of which are located close to the town of Ulog and one 30 km downstream at Glavatičevo, covering a river length of roughly 60 km (see also Tab. 1). The slopes at the sites Mjedenik, Confluence, Cerova, Swimming Beach, Nedavić, and Glavatičevo were 4.6%, 1.1%, 1.3%, 0.3%, 4.3%, and 0.2%, respectively.

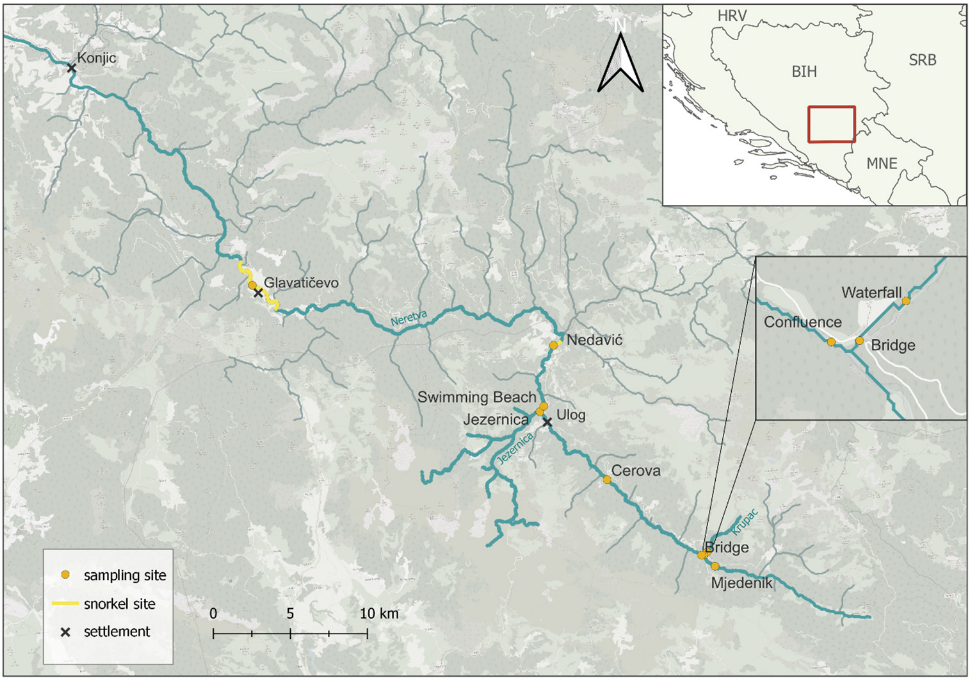

Figure 1. Study area with all respective sampling sites (orange dots) and the snorkelling site (yellow line).
Slika 1. Raziskovano območje z označenimi vzorčnimi mesti (oranžne pike) in lokacijo potapljanja (rumena črta).
Slika 1. Područje istraživanja sa svim odgovarajućim lokacijama uzorkovanja (narandžaste tačke) i mjestom ronjenja (žuta linija).

Field methods

Electrofishing

To assess the fish fauna, multiple-run depletion surveys, a quantitative method for the assessment of fish populations in wadeable streams, were conducted at three sites (Tab. 1) following Haunschmid et al. (2010). Each site was chosen to represent the typical instream habitats of the respective area. A block net with a mesh size of 20 mm was installed at the upper end of the reach to prevent fish from escaping and the length of the sampled stretch was measured using a handheld GPS. Beginning at the downstream end, three anode handlers waded upstream in parallel, with a maximum distance of 4 m between two anodes, covering the entire cross section. According to the requirements of the European Standard for the sampling of fish with electricity (EN 14011:2003) at least 50 m were covered at each site. Gasoline-powered backpack electrofishing units were used with unpulsed DC (300–600 V; 1.5–2.5 kW). All units were equipped with a 30-cm hoop anode and a cable cathode.

Table 1. Sampling sites and respective sampled length (L), width (W), area (A) and type of sampling (QN - quantitative, QL - qualitative, S - snorkelling, D - DNA analysis, eD - eDNA analysis). Quantitative and qualitative refer to electrofishing surveys.

Tabela 1. Vzorčna mesta in pripadajoče vzorčene dolžine (L), širine (W), površine (A) in tip vzorčenja (QN - kvantitativno, QL - kvalitativno, S - potapljanje, D-DNA analiza, eD - eDNA analiza). Kvantitativno in kvalitativno se nanaša na raziskave z elektro-ribolovom.

Tabela 1. Lokacije uzorkovanja i odgovarajuće uzorakovane dužine (L), širine (W), površine (A) i tip uzorkovanja (QN - kvantitativno, QL - kvalitativno, S - ronjenje, D-DNA analiza, eD - eDNA analiza).. Kvantitativno i kvalitativno se odnosi na istraživanja elektroribolovom.

Site name*	Latitude	Longitude	River	Date	L [m]	W [m]	A [m²]	QN	QL	S	D	eD
Mjedenik	43°19'09.79"	18°26'30.55"	Neretva	29.6.2022	190	2	380		●			
Waterfall	43°19'48.51"	18°25'39.83"	Krupac	29.6.2022	62	2	124		●		●	
Bridge	43°19'45.94"	18°25'32.63"	Krupac	29.6.2022	60	2	120		●			
Confluence	43°19'47.22"	18°25'25.45"	Neretva	29.6.2022	80	12	960	●			●	●
Cerova	43°22'42.43"	18°21'23.36"	Neretva	29.6.2022	66	2	132		●		●	
Swimming Beach	43°25'36.14"	18°18'39.91"	Neretva	28.6.2022	91	10	910	●	●			
Jezernica	43°25'32.90"	18°18'31.56"	Jezernica	30.6.2022	100	2	200		●			
Nedavić	43°27'28.64"	18°19'15.62"	Neretva	30.6.2022	118	11	1,298	●			●	●
				30.6.2022	464					●		
Glavatičevo	43°30'42.93"	18° 5'43.66"	Neretva	1.7.2022	4,950					●		●

* Shown in downstream direction.

Fish captured from each run were held separately in live wells at the stream margin. After the last run, all fish were identified to the lowest possible taxonomic level and measured to the nearest millimetre (Total Length (TL)). Fish biomass estimates were calculated based on existing length-weight relationships of the respective species. Population estimates were obtained using either the two-run (Seber & Cren 1967) or three-run depletion method (DeLury 1947). Estimated fish abundance and biomass were assessed for brown trout, standardised by area (hectare) and expressed as individuals/ha (ind/ha) and kg/ha. For other species, a reliable quantitative assessment was not possible due to sampling restrictions based on fish size and habitat use (e.g. cryptobenthic bullhead).

Using the same fishing gear described above, qualitative electrofishing surveys were carried out at six sites of the Neretva as well as in two tributaries (Tab. 1). At each site, selected microhabitats were sampled, aiming to supplement the species list and age class distribution. Fish were handled and measured as above. The fished area was estimated on the basis of length and width measurements.

Snorkelling survey

A snorkelling survey was conducted in two reaches of the Neretva (Tab. 1). One section was sampled downstream of Nedavić, where large boulders formed a cascade with deep pools. The second site was located at Glavatičevo, where the river had a width of >30 m and deep pools with >3 m depth. During both surveys, a high lateral visibility from shore to shore at Nedavić and of approximately 8 m at Glavatičevo provided suitable conditions. Two snorkelers drifted downstream side by side and identified, counted, and noted all visible fish following Thurow (1994). The mean number of counts per species from both snorkelers was taken as a qualitative sample. Since the primary aim was to count softmouth trout, the survey focused on habitats with a high probability of holding them. Therefore, smaller species and young age classes remain underrepresented.

DNA analyses

Fin-clips collected during electrofishing were used for genetic analyses (Tab. 3). DNA analyses were performed for three taxa (brown trout, minnow, and bullhead) in three different institutions. For clarity, despite some redundancy, laboratory procedures and data analyses are hereafter described separately for the various tasks and taxa.

eDNA analysis for the detection of softmouth trout

Analysis of eDNA samples was performed at the Central Research Laboratories of the Natural History Museum Vienna (NHM). eDNA samples were taken at three sites (Tab. 1) using a peristaltic pump and sterile disposable tubing to filter stream water through Spygen VigiDNA filters with a pore size of 0.45 µm. The mean filtration time was 27 minutes and the mean volume of water filtered was 37 L. Sampling was conducted at low flow conditions. Filters were stored in 80 mL of Spygen CL1 buffer (Pont et al. 2018) to prevent eDNA degradation. DNA extraction was carried out in the DNA clean room of the NHM following standard routines to avoid and detect contaminations. For DNA extraction, two protocols were used (1) DNeasy Blood &Tissue Kit (Qiagen) and (2) DNeasy Power Soil Pro Kit (Qiagen), and for each extraction method a negative control extraction was included to monitor contamination during DNA extraction. In addition, two fin-clip samples of softmouth trout were obtained from local fishermen and analysed to serve as a positive control. DNA extraction of those samples was performed (separated from the eDNA extractions) using the DNeasy Blood &Tissue Kit. Two marker sequences were used to test whether DNA of *Salmo* spp. could be detected in these samples: (1) partial regions of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1), (2) partial regions of the mitochondrial control region (CR). Various Polymerase Chain Reaction (PCR) primers were designed to amplify fragments of these marker sequences (Tab. 2). The regions were selected according to two criteria: (1) short fragments, and (2) that they should contain positions where *S. obtusirostris* differs from other *Salmo* species. Furthermore, species specific primers were designed for both CO1 and CR that should (with 3´-mismatches) exclude *Salmo* species other than *S. obtusirostris*. Table 2 shows the primer sequences and the corresponding amplicon lengths ranging from 162 bp to 344 bp in *Salmo* spp.

PCR reactions were performed with the Multiplex PCR Kit (Qiagen, Hilden, Germany) in a total volume of 25 µl, containing: 12.5 µl Multiplex PCR Master Mix, 0.5 µM of each primer, and 1 µl template DNA. Cycling conditions were as follows: 94 °C for 5 min; 45 cycles of 94 °C for 30 s, annealing temperature for 30 s, and 72 °C for 30 s; final extension at 72 °C for 10 min. PCRs included control reactions without template DNA as negative controls. Thermocycling and post-PCR work was performed in a separate laboratory room. PCR products were extracted from agarose gels with the QIAquick Gel Extraction Kit and sequenced (bidirectionally) at Microsynth Austria (Vienna, Austria) using the PCR primers.

Table 2. PCR primers used for the detection of *Salmo* spp. in eDNA samples. Bold primers were specifically designed for *S. obtusirostris*.

Tabela 2. PCR primerji, uporabljeni za zaznavanje *Salmo spp*. v vzorcih eDNA. Primerji, označeni krepko, so bili posebej zasnovani za *S. obtusirostris*.

Tabela 2. PCR prajmeri korišteni za detekciju *Salmo* spp. u uzorcima eDNA. Boldirani prajmeri su posebno dizajnirani za *S. obtusirostris*.

Marker sequence	Primer name	Sequence (5´-3´)	Amplicon size (bp)
CO1	SalmCO1_1+	CGTAATTGTTACAGCCCATGCC	194
	SalmCO1_2-	CTTCAACTCCAGACGAGGCT	
	Salm Co1_3+	TTATGATCGGCGGCTTTGGG	210
	Salm Co1_4-	CGGAAGCTCCTGCGTGGGCG	
CR	SalmCR_1+	CATCAGCACTAACTCAAGGT	265
	SalmCR_4-	GATATAGGAACCAAATGCCAGG	
	SalmCR_2+	CACGTGATAATAACCAACTAAG	162
	SalmCR_3-	CAATAAGAGTATGCCTACTG	
	SalmCR_5+	GATAATAACCAACTAAGTTGTC	344
	SalmCR_6-	GGGAACCCTATGCATATAAG	

DNA analysis of brown trout

This part of the molecular genetic analyses was performed at the University of Ljubljana. DNA was isolated from fish tissue following the phenol-chloroform extraction procedure (Sambrook et al. 1989).

Microsatellite analysis

Thirty-two specimens were genotyped. Of these, 18 were caught at Swimming Beach, nine at Confluence and five in the Krupac tributary above the waterfall (Waterfall; Fig. 1). All specimens were genotyped using 12 microsatellite loci (Lerceteau-Köhler & Weiss, 2006; Marić et al. 2022), which have proved as efficient for characterising the genetic diversity in different species and lineages of brown trout in the Balkans and identifying interspecific hybrids (e.g., Snoj et al. 2010; Marić et al. 2022). The protocol for PCR amplification and genotyping procedure was described in Lerceteau-Köhler & Weiss (2006) and Marić et al. (2022).

For comparison, data from various brown trout from previous studies or data of the internal database of the University of Ljubljana was included: (i) Neretva sample (Ner-ref; N=15; Neretva near Glavatičevo; Razpet et al. 2007); (ii) Danubian lineage sample (DA-ref; N=20; Panjica River (Serbia); our unpublished data); (iii) Atlantic lineage sample (AT-ref; N=15; from Czech and Danish fish farms; Snoj et al. 2010; Marić et al. 2022); and (iv) Adriatic lineage sample (AD-ref; N=21; Dragovištica River (Aegean river basin); Marić et al. 2022).

For genetic differentiation and hybrid identification, Factorial Correspondence Analysis (FCA; Benzécri et al. 1973) implemented in Genetix v. 4.05 (Belkhir et al. 1996–2004) was applied. The genetic distances between compared groups were assessed by pairwise F_{ST} values calculated in FSTAT v. 2.9.3.2 software (Goudet 1995). For each group, genetic diversity was determined by calculating heterozygosity and allelic richness using the Genetix v. 4.05 software; Neretva samples from the present study and Ner-ref were considered as one group.

Sequence analysis of the mitochondrial control region

Sequencing of the CR of brown trout was performed only for those individuals sampled at the site Waterfall, which, due to the physical isolation from the downstream population, represented the most interesting material for a phylogenetic analysis. The complete CR (ca. 1100 bp) was PCR-amplified using LRBT-25 and LRBT-1195 primers (Uiblein et al. 2001) following the PCR conditions in Marić et al. (2022). They bind within the mitochondrial genes for tRNA Thr and tRNA Phe, respectively. DNA sequences were edited in Chromas Lite v.2.6.5 (Technelysium Pty Ltd, Australia; http://technelysium.com.au/wp/chromas/) and aligned by hand. BLAST was used to compare the sequences with those deposited in the NCBI GenBank.

DNA analysis of minnow

The DNA analysis of minnow samples was performed at the NHM. DNA was extracted from tissue of seven specimens caught at Swimming Beach (Fig. 1) using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. A partial section of the CO1 gene (652 bp) was amplified using primers FishF1 and FishR1 (Ward et al. 2005) and the protocol described in Palandačić et al. (2017). PCR products were purified with Qiagen PCR purification Kit according to the manufacturer's protocol and sent for sequencing in both directions with the PCR primers to Microsynth Austria.

DNA analysis of bullhead

The DNA analysis of six *C. gobio* samples (fin clips stored in 96% alcohol) collected at Swimming Beach (Fig. 1) was performed at the Center for Genotyping of Fishery Resources in Belgrade, Serbia. Total DNA was extracted using the Quick-gDNATM MiniPrep extraction kit according to the manufacturer's instructions (Zymo Research Corporation, Irvine, CA). The mitochondrial tRNA-Pro gene and the control region (CR) were amplified using the primers CotL1 (Šlechtová et al. 2004) and HN20 (Bernatchez & Danzmann 1993) following Šlechtová et al. (2004). They bind within the mitochondrial genes for tRNA Thr and tRNA Phe, respectively. Sequencing was done at the Center for Human Molecular Genetics, University of Belgrade, Faculty of Biology. Sequences were aligned and verified with MEGA 11 (Tamura et al. 2021) and compared with existing sequences using BLAST tool from NCBI.

To assess relationships of the Neretva haplotype and other bullhead populations, available sequence data was obtained from GenBank, and MAFFT (Katoh & Standley 2013) was used to construct a 949-bp alignment comprising 149 sequences. Phylogenetic relationships were inferred under Maximum Likelihood (ML) through the IQ-Tree webserver (Minh et al. 2020; Nguyen et al. 2015; Trifinopoulos et al. 2016) with standard settings using ultra-fast bootstrap to assess branch support (Hoang et al. 2017) and automatic model selection (Kalyaanamoorthy et al. 2017) on the unpartitioned alignment.

Results

Electrofishing

In total, an area of 3,168 m² was sampled quantitatively and another 1,156 m² qualitatively. With both methodological approaches, 381 individuals belonging to four species were caught (Tab. 3). Due to the limited catchability of small individuals of minnow (TL 10 – 30 mm) this species was underrepresented. Downstream of Cerova, the minnow occurred in schools of several hundred individuals (data not shown), marking the most abundant species in this part of the Neretva. The species distribution in the stretches downstream of Cerova is then followed by brown trout, bullhead, and stone loach (*Barbatula barbatula*). At Cerova and upstream of it, the species composition was dominated by brown trout followed by bullhead. The abundance and biomass of brown trout varied among sites, ranging between 113 and 1,180 ind/ha and 11 and 40 kg/ha, respectively.

The population structure of brown trout in the upper Neretva suggests an intact demography, although deficits also become visible (Fig. 3, Tab. 4). Due to the fishing date in late June, when Young-of-the-Year (YOY) individuals of brown trout often measured less than 4 cm, they could not be caught representatively. The low number of 0+ individuals (Fig. 3) was, therefore, mainly due to methodological limitations. The 1+ cohort was well represented and showed a clear decline from headwater to lower sections (Fig. 2). Similarly, older cohorts (2++) followed the same pattern as fish of the 1+ age class.

However, individuals of brown trout captured during quantitative sampling of all age classes were smallest at Confluence, the most upstream site, and size ranges increased in a downstream direction (Tab. 4).

Stone loach was only caught during the qualitative sampling at well-structured habitats of low flow and fine substrate. The highest number of stone loach could be found in the lower parts of the Jezernica tributary (Tab. 3). Length-frequency data indicate the presence of all age classes for stone loach, bullhead, and minnow (Fig. 3). As described for the brown trout, very small individuals of these other species were not caught representatively.

Table 3. Number of caught fish per sampling site and sampling method (M1 = quantitative sampling; M2 = qualitative sampling), estimated abundance and biomass of brown trout (SE in brackets), and number of spotted fish at snorkelling sites (M3 = snorkelling). The numbers in brackets next to the captured individuals indicate the number of individuals that were sampled for genetic analysis.

Tabela 3. Število ulovljenih rib na vzorčno mesto in metodo vzorčenja (M1 = kvantitativno vzorčenje; M2 = kvalitativno vzorčenje), ocena številčnosti in biomase potočne postrvi (SE v oklepajih) ter število opaženih rib na mestih potapljanja (M3 = potapljanje). Številke v oklepajih ob ulovljenih osebkih označujejo število osebkov, vzorčenih za genetsko analizo.

Tabela 3. Broj ulovljenih riba po lokaciji uzorkovanja i metodi uzorkovanja (M1 = kvantitativno uzorkovanje; M2 = kvalitativno uzorkovanje), procijenjena zastupljenost i biomasa smeđe pastrmke (SE u zagradama) i broj uočenih riba na ronilačkim mjestima (M3 = ronjenje). Brojevi u zagradama pored ulovljenih jedinki označavaju broj jedinki koje su bile uzorakovane za genetsku analizu.

Site	**Method**	***Salmo trutta***	***C. gobio***	***Phoxinus sp.***	***Barbatula barbatula***	***S. obtusirostris***	***T. thymallus***	***O. mykiss***	***S. fontinalis***	**Total**	**ind/ha**	**kg/ha**
Confluence	M1	101 (9)	1							102	1,180 (±266)	35.2 (±10,3)
Swimming Beach	M1	39 (18)	17	23	1 (1)					80	429 (±56)	40.5 (±6,3)
Nedavić	M1	15	2 (1)	10						27	133 (±27)	11.3 (±3,4)
Total		**155**	**20**	**33**	**1**					**209**		
Mjedenik	M2	17								17		
Waterfall	M2	5 (5)								5		
Bridge	M2	15								15		
Cerova	M2	15	10 (7)	5						30		
Swimming Beach	M2	2 (2)	12 (2)	18 (2)	1 (1)					33		
Jezernica	M2	7	1	44 (3)	21 (5)					73		
Total		**61**	**23**	**67**	**22**					**173**		
Nedavić	M3	33	3	278						314		
Glavatičevo	M3	70				31	79	35	8	223		
Total		**103**	**3**	**278**		**31**	**79**	**35**	**8**	**537**		

Table 4. Delimitation of age classes of *Salmo* sp. based on TL (mm) measurements at each quantitative electrofishing site.

Tabela 4. Starostni razredi *Salmo* sp. na podlagi meritev TL (mm) na vzorčnih mestih kvantitativnega elektroribolova.

Tabela 4. Određivanje starosnih klasa *Salmo sp*. na osnovu mjerenja TL (mm) na svakom mjestu kvantitativnog elektrolovljenja.

Site name	**0+**	**1+**	**2++**
Confluence	0-79	80-149	150-229
Swimming Beach	0-99	100-189	190-269
Nedavić	0-139	140-229	230-309

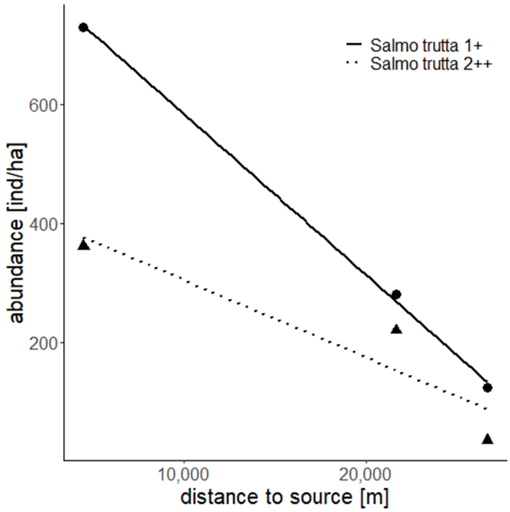

Figure 2. Abundance of 1+ and 2++ brown trout. at the respective distance from the source at the sites Confluence, Swimming Beach, and Nedavić. ● = abundance of brown trout 1+, ▲ = abundance of brown trout 2++.
Slika 2. Številčnost potočne postrvi 1+ in 2++ na posameznih razdaljah od izvira na vzorčnih mestih »Confluence«, »Swimming Beach« in »Nedavić«. ● = številčnost potočne postrvi 1+, ▲ = številčnost potočne postrvi 2++.
Slika 2. Zastupljenost smeđe pastrmke 1+ i 2++ na odgovarajućim udaljenostima od izvora na lokacijama Ušće, Plaža i Nedavić. ● = Zastupljenost smeđe pastrmke 1+, ▲ = Zastupljenost smeđe pastrmke 2++.

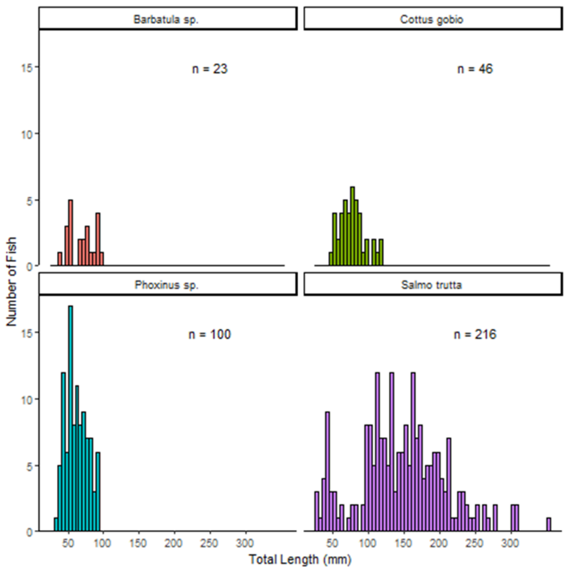

Figure 3. Population structure of species captured during quantitative and qualitative electrofishing of all sites (cumulated data).
Slika 3. Populacijska struktura vrst, ulovljenih z kvantitativnim in kvalitativnim elektroribolovom na vseh mestih (združeni podatki).
Slika 3. Struktura populacije vrsta uhvaćenih tokom kvantitativnog i kvalitativnog elektroroizlova na svim lokacijama (kumulativni podaci).

Snorkelling survey

Reaches of 464 m at Nedavić downstream of the electrofishing site and 4,950 m at Glavatičevo were surveyed through snorkelling. In total, 537 individuals from seven species were encountered. While individuals of the minnow were counted at Nedavić, they were not counted at Glavatičevo due to their high abundance and the subsequent risk of severe underestimation.

At Nedavić, a total of 33 brown trout of all age classes, three bullhead and 278 minnows with lengths between approximately five to ten cm were counted (Tab. 3). Several thousand minnow larvae and subadult fish could be identified. The most abundant species at Glavatičevo was the non-native grayling, followed by brown trout. Furthermore, 31 individuals of softmouth trout could be identified as well as rainbow trout and brook trout (Tab. 3). Around 10% of encountered brown trout could be clearly identified as stocked fish through either missing pectoral fins or gill covers. Young age classes were only observed for the brown trout. Fish from all other species ranged between 20-50 cm.

Analysis of environmental DNA (eDNA)

All primer combinations resulted in successful amplification in the positive controls. Using the primer pairs SalmCO1_1+/ SalmCO1_2-, SalmCR_1+/ SalmCR_4- , as well as SalmCR_2+/ SalmCR_3-, PCR products could be obtained from both marker sequences from all eDNA samples (both extraction methods). Sequencing revealed the presence of *Salmo* sp. haplotypes, but no haplotype specific for softmouth trout was obtained. Using the specific primers SalmCO1_3+/ SalmCO1_2- and SalmCR_4+/ SalmCR_6-, haplotypes characteristic for softmouth trout were obtained from the Glavatičevo samples for CO1, as well as CR.

DNA analysis of brown trout

Microsatellite analysis of brown trout

DNA was successfully extracted from all specimens except for one from the Confluence site. Genotyping was successful in all samples except for one from the Krupac above the waterfall, which later proved to be stone loach (see below).

FCA arranged the entire sample set into four genetically homogeneous groups: first, a Neretva brown trout group represented by most of the specimens collected in the present study including those sampled above the waterfall in the Krupac (Fig. 4; yellow), and Ner-ref (blue). The remaining three groups coincided with specimens from Adriatic (white), Danube (pink) and Atlantic (black) river basins. Six specimens from Swimming Beach deviate from the Neretva brown trout group (Fig. 4) with one located in the AT-ref group, and the others gravitating towards this group.

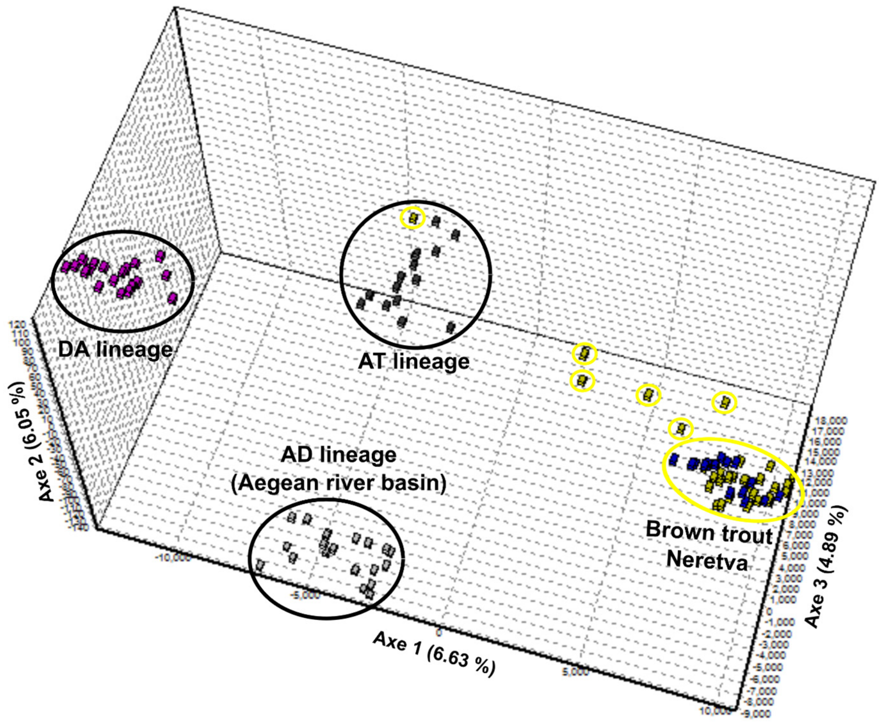

Figure 4. Factorial correspondence analysis (FCA) plot of the brown trout samples studied. AD, DA, AT stand for brown trout mitochondrial DNA lineages from the Adriatic, Danube and Atlantic river basins, respectively; for details on brown trout phylogeography, see Bernatchez et al. 1992. Genetic groups recognised by FCA are encircled. Yellow – specimens from the present study; blue –Ner-ref specimens. Individual specimens encircled in yellow are apparently introgressed with genes from Atlantic populations; one specimen (within AT-ref group) appears to be a 'pure' AT representative.

Slika 4. Diagram faktorske korespondenčne analize (FCA) vzorcev potočne postrvi. AD, DA, AT označujejo linije mitohondrijske DNK potočne postrvi iz Jadranskega, Donavskega in Atlantskega porečja; za podrobnosti o filogeografiji potočne postrvi glej Bernatchez et al. 1992. Genetske skupine, prepoznane s FCA, so obkrožene. Rumeno - primerki iz te študije; modro - primerki Ner-ref. Posamezni primerki, obrobljeni rumeno, imajo verjetno introgresirane gene atlantskih populacij; eden izmed primerkov (v skupini AT-ref) je najverjetneje 'čisti' predstavnik AT.

Slika 4. Analiza faktorske korespondencije (FCA) proučavanih uzoraka potočne pastrmke. AD, DA, AT označavaju mitohondrijalne DNK linije potočne pastrmke iz jadranskog, dunavskog i atlantskog riječnog sliva; za detalje o filogeografiji potočne pastrmke, vidi Bernatchez et al. 1992. Genetske grupe koje priznaje FCA su zaokružene. Žuta – primjerci iz ove studije; plavi –Ner-ref uzorci. Pojedinačni uzorci zaokruženi žutom bojom su očigledno introgresirani genima iz atlantskih populacija; čini se da je jedan primjerak (unutar AT-ref grupe) 'čisti' AT predstavnik.

All pairwise F_{ST} values were statistically significant (Tab. 5). The minimum value was observed between the Neretva sample from the present study and Ner-ref sample. The remaining values ranged from ca. 0.2 to 0.4; the highest values were observed in pairs with the DA-ref sample.

Table 5. Pairwise FST values between the brown trout specimens from the present study and the reference samples, i.e. Neretva reference (Ner-ref), Danubian reference (DA-ref), Atlantic reference (AT-ref) and Adriatic reference (AD-ref). Asterisks denote statistical significance (p>0.095).
Tabela 5. Parne vrednosti FST med primerki potočne postrvi iz te študije in referenčnimi vzorci, tj. referenca Neretva (Ner-ref), donavska referenca (DA-ref), atlantska referenca (AT-ref) in jadranska referenca (AD-ref). Zvezdice označujejo statistično značilnost (p>0,095).
Tabela 5. Parne FST vrijednosti između uzoraka smeđe pastrmke iz ove studije i referentnih uzoraka, tj. neretva referenca (Ner-ref), dunavska referenca (DA-ref), atlantska referenca (AT-ref) i jadranska referenca (AD-ref). Zvjezdice označavaju statističku značajnost (p>0.095).

	Neretva, present study	Ner-ref	AD-ref	AT-ref	DA-ref
Neretva, present study		**	**	**	**
Ner-ref	0.0275		**	**	**
AD-ref (Aegean river basin)	0.3081	0.2874		**	**
AT-ref	0.2516	0.2363	0.2035		**
DA-ref	0.4019	0.3957	0.4087	0.3363	

The observed parameters show a high genetic diversity for all genetic groups except for DA-ref, which reflects a medium genetic diversity (Tab. 6; see Berrebi et al. 2021).

Table 6. Genetic diversity assessment inferred from heterozygosity (He = heterozygosity expected; Ho = heterozygosity observed), and allelic richness (Ar). AD-ref = reference Adriatic lineage; AT-ref = reference Atlantic lineage; DA-ref = reference Danubian lineage.
Tabela 6. Ocena genetske raznolikosti, izpeljana iz heterozigotnosti (He = pričakovana heterozigotnost; Ho = opažena heterozigotnost) in alelna bogatost (Ar). AD-ref = referenčni jadranski izvor; AT-ref = referenčni atlantski izvor; DA-ref = referenčni donavski izvor.
Tabela 6. Procjena genetske raznovrsnosti izvedena iz heterozigotnosti (He = očekivana heterozigotnost; Ho = uočena heterozigotnost) i alelnog bogatstva (Ar). AD-ref = referentna jadranska linija; AT-ref = referentna atlantska linija; DA-ref = referentna dunavska linija.

	He	***Ho***	***Ar***
Neretva (present study +Ner-ref)	0.609	0.501	7.28
AD-ref (Aegean river basin)	0.630	0.629	5.59
AT-ref	0.744	0.743	7.74
DA-ref	0.389	0.408	3.53

Mitochondrial control region of brown trout from locality Waterfall

Sequences for the complete CR were obtained for four specimens from the Krupac tributary above the waterfall; three had a haplotype previously found in the Neretva brown trout (Delling et al. 2020; ADcr4; NCBI GenBank accession number MK184929). Another published, much shorter (309-bp) sequence (Bernatchez et al. 1993; Ad-s3; NCBI GenBank accession number: M97967) was identical in the overlapping section. The fourth specimen proved to be another species, as it exhibited a not yet described haplotype, which showed 98.14% similarity to the species *B. barbatula* from Croatia (NCBI GenBank: GU583680; Jakovlić et al. 2013).

DNA analysis of minnow

All minnow specimens exhibited the same haplotype, previously detected in the Bunica River, confluence to Neretva (GenBank accession numbers MF407681) and clustering to Clade 2 (*Phoxinus* sensu lato; clade named according to Palandačić et al. (2015)). This haplotype was also detected in Karanovac, Lepenica, Ugar and Fojnica Rivers.

DNA analysis of bullhead

A novel, not yet detected CR haplotype, Cot73 (NCBI GenBank accession number QQ379171), was obtained from all bullhead samples. In our analyses this haplotype was nested within a clade comprising haplotypes detected in the Lower Danube drainage (unpublished sequences from GenBank without more detailed geographic information), the Kolpa/Kupa River and the Tounjčica River (Šlechtová et al. 2004), which was the sister group of a well-supported clade comprising haplotypes from the Pčinja River (Fig. 5).

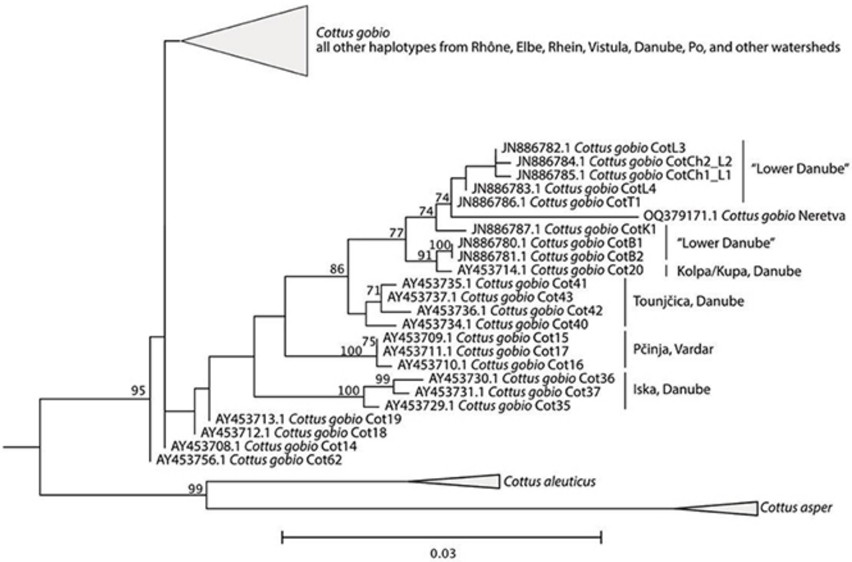

Figure 5. Relationships amongst *Cottus* spp. CR haplotypes. The haplotype representing the Neretva samples is nested within a clade comprising haplotypes from the Lower Danube drainage and haplotypes from Sava tributaries Kolpa/Kupa and Tounjčica. Besides this clade, the remaining *Cottus gobio* haplotypes are collapsed. The same is true for the outgroup species: *C. aleuticus*, and *C. asper*. For a detailed description of haplotypes, see Šlechtová et al. (2004). Alphanumeric codes before species names correspond to GenBank accession numbers, codes after species names to previously identified haplotypes (Šlechtová et al. 2004).

Slika 5. Razmerja med CR haplotipi *Cottus* spp. Haplotip, ki predstavlja vzorce iz Neretve, je vgnezden v kladu, ki zajema haplotipe iz spodnjega toka Donave in haplotipe pritokov Save Kolpe/Kupe in Tounjčice. Preostali haplotipi *C. gobio* so združeni. Enako velja za zunanje skupine vrst: *C. aleuticus* in *C. asper*. Za podroben opis haplotipov glej Šlechtová et al. (2004). Alfanumerične kode pred imeni vrst ustrezajo kodam GenBank, kode po imenih vrst pa že identificiranim haplotipom (Šlechtová et al. 2004).

Slika 5. Odnosi među CR haplotipovima *Cottus* spp. Haplotip koji predstavlja uzorke iz Neretve ugniježđen je u kladus koji obuhvata haplotipove iz donjeg toka Dunava i haplotipove iz pritoka Save Kolpa/Kupa i Tounjčica. Pored ovog kladusa, ostali haplotipovi C. gobio su kolabirali. Isto vrijedi i za vanjske vrste: *C. aleuticus* i *C. asper*. Za detaljan opis haplotipova pogledajte Šlechtová et al. (2004). Alfanumerički kodovi prije naziva vrsta odgovaraju brojevima pristupa GenBank, kodovi nakon naziva vrsta prethodno identificiranim haplotipovima (Šlechtová et al., 2004).

Discussion

This study provides a quantitative and qualitative description of the ichthyofauna of the upper Neretva, including the lower parts of two tributaries. The length-frequency diagrams showed the presence of all age classes of brown trout, bullhead, minnow and stone loach underlining the integrity of this river stretch. The number of species is low in the upper reaches and increases downstream, following the classical pattern of cold, oxygen-rich rivers with strong currents of the montane and submontane zone, with the trout region in the upper reaches (Huet 1949). With increasing distance from the source, structural composition and the species spectrum diversifies. The Neretva around Glavatičevo, which corresponds to the so-called hyporhithral of alpine rivers, was naturally colonised by the softmouth trout, marble trout, and brown trout (Glamuzina et al. 2018). As the Neretva River drainage offers the largest remaining natural habitats for the endangered softmouth trout, this species was in part a focus of our investigation.

Softmouth trout

Evidence of the softmouth trout was found in the course of the survey through both a snorkelling survey and eDNA analysis. During the snorkelling survey, the majority of softmouth trout was encountered in deep runs and pools spread out over the whole reach sampled at Glavatičevo. The DNA analysis of two softmouth trout samples showed that they carried specific haplotypes reported earlier for CR and CO1, respectively (Snoj et al. 2008; Tougard et al. 2018). Moreover, it could be shown that these haplotypes can be detected via eDNA analyses, albeit evidence was obtained only from the water sample at Glavatičevo. Thus, for future analyses, systematic sampling should be performed with a sufficient number of replicates at different localities. Yet, further optimisation of primers is necessary to increase specificity and, thus, the sensitivity of the analyses considering the potential rarity of the species in some localities. It should also be noted that hybridisation between the softmouth trout and brown trout has been reported and is relatively extensive in the Neretva basin, posing a major threat to the long-term survival of the species (Razpet et al. 2007; Snoj et al. 2007). As the typical eDNA protocol targets mitochondrial DNA only, this approach is unable to distinguish between hybridised, pure-bred, or introgressed individuals.

Neretva brown trout

As inferred from the present study, brown trout in the upper Neretva do not differ from the downstream population at Glavatičevo; together they form a genetically homogeneous unit that is clearly distinct from geographically separated genetic entities such as the brown trout lineages from the Danube and Aegean watersheds and Atlantic lineage (anthropogenically introduced into the Neretva system) brown trout. The genetic data suggest that Atlantic lineage brown trout have already invaded the upper Neretva population but have apparently not yet extended to the highest reaches as all introgressed specimens were restricted to the downstream sampling site near the village of Ulog (Swimming Beach). Stocked brown trout were only observed around Glavatičevo corresponding to previous genetic tests from the same location (Razpet et al. 2007).

The presence of stocked fish at Glavatičevo was also corroborated in real time via the snorkel survey at that locality, as typical hatchery phenotypes (missing or damaged fins, deformations and missing gill covers) of three salmonid species (brown trout, rainbow trout and brook trout) were easily observed. The stocking is likely also related to angling pressure, which is supported by the overall low total biomass (Tab. 3) and low numbers of trout exceeding 25 cm TL at all sites (Glamuzina et al. 2018). In total, low biomass, low numbers of large fish, and direct observations of remnant fishing gear, as well as hooking injuries, all potentially suggest that overfishing and harvest may be taking place.

The introgression of brown trout with non-indigenous genes was also reported to be undeniably a result of stocking (Kalamujić et al. 2015). The same is assumed for introgression from Danube populations that were observed mainly in the river Rakitnica (the right tributary of the Neretva below Glavatičevo) and in the Neretva near the confluence with Rakitnica (Razpet et al. 2007; Kalamujić et al. 2015). In this study, however, with our limited sampling campaign, no trace of a Danubian-lineage genes was detected. The question as to whether or not Danubian-lineage genes in the Neretva detected by Razpet et al. (2007) and Kalamujić et al. (2015) arrived by natural or human-mediated means remains unanswered.

Despite observations of stocked salmonids and the documented evidence of introgression in the lower courses of the surveyed reaches, the uppermost reaches of the Neretva were characterised by pristine habitat and natural population structure of all occurring species. The 1+ cohort of brown trout appears well represented and shows a typical pattern for trout populations (Unfer & Pinter 2018) in that the abundance decreases with increasing distance from the river's source. At the site Confluence, a high number of brown trout was caught, but the biomass of 35 kg/ha is low for the calculated abundance, highlighting a lack of large individuals. The results underline the importance of the upper reaches as nursery areas that support high densities of juveniles, while the lower reaches serve as feeding areas for older stages. Large fish (>25 cm TL) were mainly observed during snorkelling at Glavatičevo. In the upper reach, large individuals were predominantly captured at the lower sites Swimming Beach and Nedavić.

Non-salmonid native species

Comparing the species list from Muhamedagić et al. (2010) for the upper reach of the Neretva (source to Jablanica lake), three presumably native species, namely the European chub (*Squalius cephalus*), the gudgeon (*Gobio gobio*), and the marble trout, could not be identified during this survey. Moreover, those authors reported a relatively low abundance of minnow of only 4% (15 individuals) with respect to the whole species composition of the upper Neretva. In contrast, during this present study, several thousand minnows were observed.

Tutman et al. (2017) indicated that the upper Neretva might be inhabited by several species of stone loaches. During this survey, one specimen was identified as *B. barbatula.* However, for a more precise identification of the genetic lineage, the mitochondrial cytochrome b gene should be sequenced as it is a much more frequently used and more informative marker gene in *Barbatula* studies compared to the CR. The stone loach was only found at Swimming Beach presumably because the nearby gently flowing Jezernica tributary offered the typical habitats of stone loaches, characterised by shallow, and stagnant conditions.

Both, bullhead and minnow are considered native to the upper Neretva (Palandačić et al. 2015; Pilić et al. 2021). In the Western Balkans, highly divergent mitochondrial clades of *Phoxinus phoxinus sensu lato* have been found (Palandačić et al. 2015). The present results confirmed that the minnows sampled belong to Clade 2 of Palandačić et al. (2015), comprising populations from the lower Sava catchment and rivers flowing directly into the Adriatic Sea (Palandačić et al. 2015, 2017, 2020). Similarly, several divergent genetic clades had been identified in bullhead (Šlechtová et al. 2004; Bravničar 2012; Bravničar et al. 2015). The specimens sampled in the Neretva appeared to share an evolutionary origin with populations found in northern Balkan Danube tributaries, despite the fact that the Neretva drains towards the Adriatic basin (Bravničar et al. 2015). Phylogeographically, this would suggest colonisation of the Neretva and other North-Western Balkan rivers across major mountain systems, as previously proposed (Bravničar et al. 2021; Šlechtová et al. 2004). Detecting this unique and apparently derived haplotype in the Neretva demonstrates the significance of the stream as a refuge and source of genetic diversity in bullhead.

Non-native species

The presence of several alien species, especially the grayling, suggests potential competition with native species (Glamuzina et al. 2018). At Glavatičevo, obviously stocked individuals of brown trout, rainbow trout and brook trout were present. These specimens could be easily identified by typical symptoms of rearing environments such as missing or damaged fins, deformations, and missing gill covers. The relative abundance of grayling and rainbow trout among salmonids were higher during the present study compared to reports of Muhamedagić et al. (2010).

Summary conclusions

Through the assessment of the ichthyofauna, healthy population structures of brown trout, bullhead, minnow, and stone loach were identified as expected in a well-structured river that provides functional habitats for all age classes. The occurrence of the endangered softmouth trout and the discovery of a new mitochondrial CR haplotype of *Cottus gobio* highlight the value of the upper Neretva as a biodiversity hotspot. The construction of dams changed the natural state of the middle Neretva from a salmonid river to a chain of impoundments that is dominated by cyprinids. The subsequent restriction of longitudinal connectivity further contributed to a vast decline of salmonids in the upper reaches (Muhamedagić et al. 2010). Preserving the upper reaches of the Neretva from ecological deterioration and destruction through planned HPP expansion is paramount to preserve this river's function as a biodiversity refuge in the present human-made ecological crisis.

Povzetek

Reke zahodnega Balkana so izjemnega pomena kot vroče točke biotske raznovrstnosti, z velikim številom endemičnih in redkih vrst. Raznolikost vrst sladkovodnih rib v regiji velja za najvišjo v Evropi, tudi zaradi ohranjenosti zgornjih tokov rek, ki so pogosto prosto tekoči in brez večjih antropogenih posegov, kot so kanaliziranja in jezovi. Kljub temu so te dragocene naravne danosti močno ogrožene zaradi širitev hidroelektrarn (HE).

V Neretvi, eni najpomembnejših rek na zahodnem Balkanu, živi 34 domorodnih vrst rib, od tega 17 endemičnih. Zabeleženih je bilo tudi 32 tujerodnih vrst, ki so bile pretežno vnesene v zajezitve srednjega toka za namene rekreacijskega ribolova. Med njimi so lipan (*Thymallus thymallus*), šarenka (*Oncorhynchus mykiss*), potočna zlatovčica (*Salvelinus fontinalis*) in tujerodne vrste potočne postrvi (*Salmo trutta*), ki tekmujejo z avtohtonimi salmonidi, kot so ogrožena mehkousta prostrv (*Salmo obtusirostris*), soška postrv (*Salmo marmoratus*) in avtohtona potočna postrv. Pretekle študije rib so bile osredotočene na srednji ali spodnji tok. Študije, ki so se osredotočale na zgornji tok, niso dostopne ali nimajo podrobnih populacijskih parametrov. Namen pričujoče analize je bil preučiti stalež ribje združbe v zgornji Neretvi, zagotoviti dodatne vpoglede v sistematiko in razširjenost posameznih vrst ter odpraviti nekatere negotovosti glede statusa avtohtonosti vrst.

Uporabili smo standardni elektroribolov in napravili genetske analize, ki so zajemale populacijsko genetski pristop za potočno postrv, pisanca (*Phoxinus* spp.) in kaplja (*Cottus* spp.). Poleg tega smo se potapljali na dah in odvzeli vzorce okoljske DNK (eDNK), da bi odkrili globočka v rečnih odsekih, ki bodisi niso bili vzorčeni na druge načine bodisi bi ga zaradi njegove redkosti lahko zgrešili.

Prisotnost mehkouste postrvi je bil potrjen s potapljanjem na dah in eDNA. Vsi osebki so bili zaznani okoli Glavatičevega, njegovo morebitno pojavljanje gorvodno ostaja nepotrjeno. V prihodnosti bo potrebno sistematično vzorčenje vode za eDNA na različnih lokacijah. Poleg tega bi bilo, zaradi redkosti pojavljanja vrste, potrebno nadalje optimizirati primerje za povečanje specifičnosti in s tem občutljivosti analiz.

Potrdili smo vse starostne razrede potočne postrvi, kar kaže na ekološki pomen zgornjega toka Neretve. Številčnost in biomasa pa sta bili nizki, gibali sta se med 113 do 1180 osebkov/ha oziroma 11–40 kg/ha. Številčnost mladic potočne postrvi se je zmanjševala dolvodno, kar nakazuje pomen Zgornje Neretve za rast mladic. Genetske analize so pokazale, da so ribe iz zgornjega toka in nižje populacije okoli Glavatičevega genetsko homogena enota, ki se jasno razlikuje od geografsko ločenih genetskih entitet. Čeprav je bil atlantski izvor potočne postrvi vnesen v Neretvo, naši podatki kažejo, da se še ni razširila na območje gorvodno od Uloga. Nizka biomasa, majhno število velikih rib in neposredna opazovanja ostankov ribolovnega orodja ter poškodbe zaradi trnka potencialno kažejo na prekomerni izlov. Poleg tega so tujerodni salmonidi v okolici Glavatičevega številčno presegli avtohtone salmonide.

Sedanji rezultati so potrdili, da vzorčena pisanec in kapelj pripadata genetskim kladom, ki obsegajo populacije iz rek, ki tečejo neposredno v Jadransko morje. To nakazuje kolonizacijo Neretve in drugih rek severozahodnega Balkana prek večjih gorskih sistemov, kot so že predlagali drugi raziskovalci. Odkritje edinstvenih in izpeljanih haplotipov v Zgornji Neretvi dokazuje pomen reke kot zatočišča in vira genetske raznolikosti teh vrst. Poleg tega pojav vseh starostnih razredov nakazuje zdrave strukture populacije obeh vrst.

Rezultati ponovno dokazujejo ekološki pomen zgornje Neretve kot vroče točke biotske raznovrstnosti. V luči velikih sprememb ihtiofavne srednjega toka Neretve zaradi gradnje hidroelektrarn je nujno zaščititi gornji tok Neretve pred ekološkim osiromašenjem in uničevanjem habitatov, ki bi ga povzročila širitev HE. S tem bi ohranili funkcijo zatočišča biotske raznovrstnosti v sedanji antropogeno povzročeni ekološki krizi.

Sažetak

Neretva, jedna od najvažnijih rijeka na zapadnom Balkanu, dom je 34 autohtone vrste riba, od kojih je 17 endemskih. Zabilježene su i 32 alohtone vrste, koje su uglavnom uvedene u rezervoare srednjeg toka za potrebe rekreativnog ribolova. Među njima su: lipljen (*Thymallus thymallus*), kalifornijska pastrmka (*Oncorhynchus mykiss*), potočna pastrmka (*Salvelinus fontinalis*) i alohtone vrste potočne pastrmke (*Salmo trutta*), koje se takmiče sa autohtonim salmonidima kao što su: ugrožena mekousna pastrmka (*Salmo obtusirostris*), glavatica (*Salmo marmoratus*) i autohtona potočna pastrmka. Prethodne studije o ribama fokusirale su se na srednji ili donji tok. Studije koje se fokusiraju na uzvodno nisu dostupne ili im nedostaju detaljni parametri populacije. Cilj ove analize bio je ispitati fond riblje zajednice u gornjoj Neretvi, dati dodatni uvid u sistematiku i rasprostranjenost pojedinih vrsta, te otkloniti neke nejasnoće u pogledu statusa autohtonosti vrste.

Koristili smo standardni elektroribolov i izvršili genetičke analize koje su uključivale populacijski genetski pristup za potočnu pastrmku, pijor (*Phoxinus* spp.) i peš (*Cottus* spp.). Pored toga, ronjenjem smo prikupili i uzorke DNK iz životne sredine (eDNA) kako bismo detektovali potočnu pastrmku u dijelovima rijeke koji ili nisu uzorkovani na drugi način ili su, zbog rijetkosti vrste, mogli biti propušteni.

Prisustvo mekousne pastrmke potvrđeno je ronjenjem i eDNK. Svi primjerci su otkriveni oko Glavatičeva, moguće prisustvo vrste uzvodno ostaje nepotvrđeno. U budućnosti će biti neophodno sistematsko uzorkovanje vode za eDNK na različitim lokacijama. Osim toga, zbog rijetkosti vrste, bilo bi potrebno dodatno optimiyovati parametre kako bi se povećala specifičnost, a time i osjetljivost analiza.

Potvrđeno je prisustvo svih dobnih klasa potočne pastrmke, što odražava ekološki značaj gornjeg toka Neretve. Broj i biomasa su bili niski, u rasponu od 113 do 1180 jedinki/ha, odnosno 11–40 kg/ha. Broj mlađi potočne pastrmke se smanjio nizvodno, što ukazuje na značaj gornjeg toka Neretve za rast mlađi. Genetske analize su pokazale da su ribe gornjeg toka i niže populacije oko Glavatiča genetski homogena jedinica koja se jasno razlikuje od geografski odvojenih genetskih entiteta. Iako je atlantska linija potočne pastrve unesena u Neretvu, naši podaci pokazuju da se još nije proširila na područje uzvodno od Uloga. Niska biomasa, mali broj velikih riba i opažanja ostataka ribolovne opreme i oštećenja nastala od udica potencijalno ukazuju na prekomjeran ribolov. Osim toga, alohtonih salmonida u okolini Glavatičeva bilo je više od autohtonih salmonida.

Dosadašnji rezultati su potvrdili da uzorkovani pijor i peš pripadaju genetskim kladama koje čine populacije iz rijeka koje se ulijevaju direktno u Jadransko more. Ovo sugeriše kolonizaciju Neretve i drugih rijeka sjeverozapadnog Balkana putem većih planinskih riječnih sistema, kao što su ranije sugerirali drugi istraživači. Otkriće jedinstvenih i izvedenih haplotipova u gornjoj Neretvi pokazuje važnost rijeke kao utočišta i izvora genetske raznolikosti za ove vrste. Osim toga, pojava svih starosnih klasa ukazuje na zdravu strukturu populacije obje vrste.

Rezultati još jednom dokazuju ekološku važnost gornje Neretve kao žarišta biodiverziteta. U svjetlu velikih promjena u ihtiofauni srednjeg toka Neretve zbog izgradnje hidroelektrana, potrebno je zaštititi gornji tok Neretve od ekološkog osiromašenja i uništavanja staništa koje bi prouzrokovalo proširenje HE. Time bi se očuvala funkcija utočišta za biodiverzitet u trenutnoj ekološkoj krizi izazvanoj antropogenošću.

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Study of bats in the upper Neretva River valley (Bosnia and Herzegovina)

Maja ZAGMAJSTER[1], Vojo MILANOVIĆ[2], Behare REXHEPI[1], Ester PREMATE[1], Špela BORKO[1], Anton VORAUER[3]

[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, SubBioLab, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; E-mails: maja.zagmajster@bf.uni-lj.si, behare.rexhepi@bf.uni-lj.si; ester.premate@bf.uni-lj.si; borko.spela@gmail.com
[2]Centre for Karst and Speleology, Branilaca Sarajeva 30, 71 000 Sarajevo, Bosnia and Herzegovina; E-mail: milanovic.vojo9@gmail.com
[3]Ecotone Environmental Office, Austria; E-mail: anton.vorauer@utanet.at

Abstract. The upper Neretva River valley with river and tributaries, preserved forests, natural caves and buildings represents a very interesting area for bats. During two field expeditions in summer 2022, we collected data on bats using a variety of methods: inspecting the potential roosts, mist netting, and recording bat echolocation calls. We used manual and automatic ultrasound detectors most frequently at sites along and near the Neretva River. The morphological identification of some species was confirmed using DNA analyses. We detected at least 13 bat species in the study area extending from Krupac to Konjic. With one addition, known from the literature, 14 different bat species have been found in the upper Neretva River valley, presenting nearly a half of all bat species recorded in Bosnia and Herzegovina. We confirmed the presence of three species of the highest conservation concern in Europe, listed in the Annex II of the Habitats directive: lesser horseshoe bat (*Rhinolophus hipposideros*), greater horseshoe bat (*Rhinolophus ferrumequinum*), and greater mouse-eared bat (*Myotis myotis*). Our finding of the alcathoe bat (*Myotis alcathoe*) presents new confirmation of this species in Bosnia and Herzegovina. Also, observation of the parti-coloured bat (*Vespertilio murinus*) is one of the few records of the species in this country. The upper Neretva River valley can be considered as an area of high conservation importance for bats. Additional fieldwork, including during other seasons, would likely reveal more bat species and their sites in the area of the upper Neretva River valley.

Key words: Bosnia and Herzegovina, Neretva River, bats, echolocation, mist netting, roosts, *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros, Myotis myotis, Myotis alcathoe*

Izvleček. Raziskava netopirjev v dolini zgornje reke Neretve (Bosna in Hercegovina) – Dolina zgornje reke Neretve je z reko in pritoki, ohranjenimi gozdovi, naravnimi jamami in zgradbami zelo zanimivo območje za netopirje. Na dveh terenskih odpravah poleti 2022 smo popisovali netopirje z različnimi metodami: pregledi potencialnih zatočišč, lovom z mrežami in snemanjem eholokacijskih klicev. Na več mestih ob reki Neretvi in v njeni bližini smo uporabili ročne in avtomatske ultrazvočne detektorje. Morfološko določitev nekaterih vrst smo potrdili z analizami DNK. Skupno smo v naši raziskavi na preučevanem območju od Krupca do Konjica zabeležili vsaj 13 vrst netopirjev. Ob upoštevanju še ene, iz literature znane vrste, je bilo na območju zgornje Neretve skupno zabeleženih 14 različnih vrst netopirjev, kar predstavlja skoraj polovico vseh zabeleženih v Bosni in Hercegovini. Potrdili smo prisotnost treh vrst, ki so v Evropi deležne največje varstvene pozornosti in so navedene v Prilogi II Direktive o habitatih: mali podkovnjak (*Rhinolophus hipposideros*), veliki podkovnjak (*Rhinolophus ferrumequinum*) in navadni netopir (*Myotis myotis*). Najdba nimfnega netopirja (*Myotis alcathoe*) je nova potrditev vrste v Bosni in Hercegovini. Tudi najdba dvobarvnega netopirja (*Vespertilio murinus*) je eno redkih opažanj te vrste v državi. Dolino zgornjega toka reke Neretve lahko štejemo za območje, ki je zelo pomembno za varstvo netopirjev. Dodatno terensko delo, ki bi vključevalo tudi druge letne čase, bi razkrilo še več vrst in njihovih nahajališč na tem območju.

Ključne besede: Bosna in Hercegovina, reka Neretva, netopirji, eholokacija, lov z mrežami, zatočišča, *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Myotis myotis, Myotis alcathoe*

Apstrakt. Studija šišmiša u dolini gornje rijeke Neretve (Bosna i Hercegovina) – Dolina gornje rijeke Neretve sa rijekom i pritokama, očuvanim šumama, prirodnim pećinama i građevinama predstavlja vrlo interesantno područje za šišmiše. U dvije terenske ekspedicije početkom i krajem ljeta 2022. godine prikupljali smo podatke o šišmišima različitim metodama: pregledom potencijalnih skloništa, mreženjem i snimanjem eholokacije šišmiša. Koristili smo ručne i automatske ultrazvučne detektore na više lokacija uz i u blizini rijeke Neretve. Morfološka identifikacija nekih vrsta potvrđena je analizom DNK. Na području istraživanja, koje se proteže od Krupca do Konjica, otkrili smo najmanje 13 vrsta šišmiša. Uz još jednu, poznatu iz literature, u dolini rijeke Neretve pronađeno je ukupno 14 različitih vrsta šišmiša, što predstavlja skoro polovinu svih vrsta zabilježenih u Bosni i Hercegovini. Potvrdili smo prisustvo tri vrste koje su od najveće važnosti za očuvanje u Evropi, navedene u Aneksu II Direktive o staništima: mali potkovasti šišmiš (*Rhinolophus hipposideros*), veliki potkovasti šišmiš (*Rhinolophus ferrumequinum*) i veliki mišouhi šišmiš (*Myotis myotis*). Naš nalaz patuljastog brkatog šišmiša (*Myotis alcathoe*) predstavlja novu potvrdu prisutnosti vrste u Bosni i Hercegovini. Također, posmatranje dvobojnog šišmiša (*Vespertilio murinus*) je jedan od rijetkih zapisa ove vrste u zemlji. Gornja dolina rijeke Neretve može se smatrati područjem od velikog značaja za očuvanje šišmiša. Dodatni terenski rad, uključujući druga godišnja doba, otkrio bi više vrsta šišmiša i njihovih lokacija u tom području.

Ključne riječi: Bosna i Hercegovina, rijeka Neretva, šišmiši, eholokacija, mreže, skloništa, *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Myotis myotis, Myotis alcathoe*

Introduction

In the past two decades, knowledge on bats in Bosnia and Herzegovina (BIH) has increased. Many of the studies refer to inventories of bats at hibernation sites, mostly from caves (e.g. Presetnik et al. 2016; Mulaomerović et al. 2021), but also inventories using other methods, like mist netting and ultrasound detectors (e.g. Babić et al. 2018). To date, 31 bat species were recorded in BIH (Zagmajster et al. 2010; Babić et al. 2018), yet data on the distribution and ecology of some species remain scarce.

While a study of bat fauna was conducted in the lower Neretva River valley (Mulaomerović et al. 2015), the upper Neretva River valley in Eastern Bosnia and Herzegovina was amongst the regions with virtually no records of bats. The region is covered with well preserved pristine forests (that extend around the Neretva River's main stream and its tributaries), caves in karstic hills above the valley, pastures, and a few human settlements and buildings. All these characteristics present a high potential for a rich bat community. Yet, there were only three records for three species of bats from or in close vicinity of the upper Neretva River valley area prior to our study. In a small cave close to the village of Pridvorica u Borču (Borač under Dumoš planina), nine individuals of *Rhinolophus hipposideros* were observed on 29.9.2019 (J. Mulaomerović, pers. comm.). Mirić & Paunović (1997) reported on finding *Nyctalus leisleri* on 15.3.1968 at Boračko jezero NW from Glavatičevo, within 3 km distance from the Neretva River. The same locality and date is given for the record of *Pipistrellus pipistrellus*, which is deposited in the Natural History Museum in Belgrade (Serbia) (Zagmajster et al. 2010).

To fill this knowledge gap, a study of bats was conducted over a few days in June/July and August 2022, the first period being during the »Neretva Science Week 2022«. This event was organised to improve the knowledge on biodiversity of the area, which is threatened by a series of hydropower plants that are planned to be built on the upper Neretva River and its tributaries. Bats were studied by combining different methods: visual checking of the potential roosts in caves and buildings, recording the echolocation calls, and mist netting. Here, we present the results which revealed a rich bat community despite short time of research.

Materials and methods

Study area

We conducted the study in the upper Neretva River valley, extending from Krupac to Konjic (Fig. 1). The surrounding hills above the river and its tributaries are overgrown by forests, with few villages and individual buildings (many abandoned) present. The bedrock in the valley is formed of Cretaceous flysch at the foothills and Cretaceous carbonate, dolomite, and marl higher up on the slopes. Few caves are known from karstic hills surrounding the river valley.

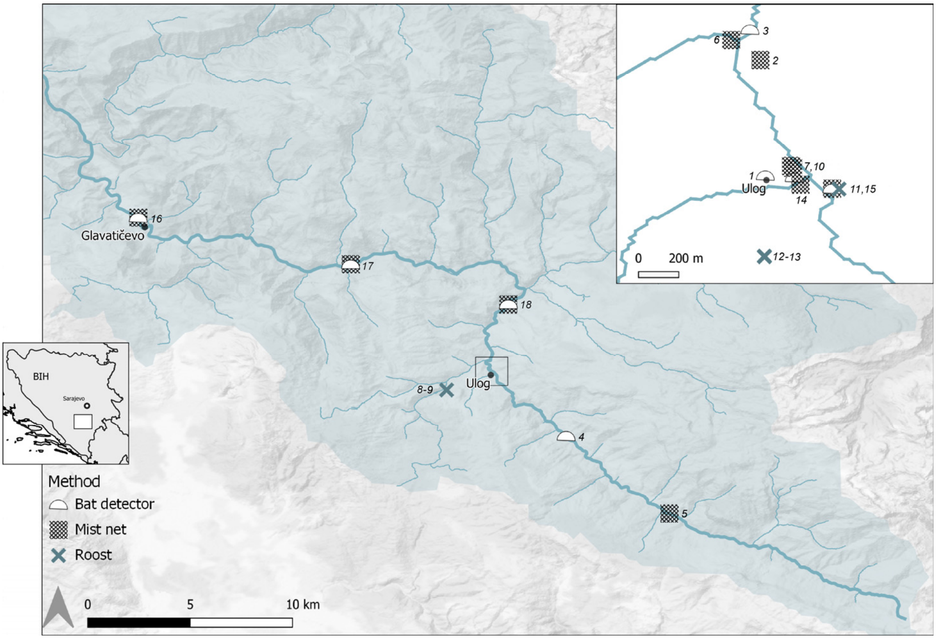

Figure 1. Map of the the upper Neretva River valley, with marked localities where we searched for bats in June/July and August 2022. Different symbols mark different study methods (explained in the legend, with details in the text). Numbers refer to localities, listed in Tab. 2. The embedded map on the left shows the position of the study area within Bosnia and Herzegovina, while the map on the top right presents a close up to Ulog area (marked with black frame on the main map).

Slika 1. Zemljevid doline zgornje Neretve z označenimi kraji, kjer smo netopirje iskali junija/julija in avgusta 2022. Z različnimi simboli so označene različne metode preučevanja (pojasnjene v legendi, podrobnosti pa v besedilu). Številke se nanašajo na lokalitete, ki so navedene v Tab. 2. Vstavljena karta na levi prikazuje položaj območja raziskave znotraj Bosne in Hercegovine, medtem ko karta desno zgoraj prikazuje približano območje okoli Uloga (označeno s črnim okvirjem na večji karti).

Slika 1. Karta doline gornje Neretve, sa označenim lokalitetima na kojima smo tražili šišmiše u junu/julu i augustu 2022. Različiti simboli označavaju različite metode istraživanja (objašnjeno u legendi, s detaljima u tekstu). Brojevi se odnose na lokalitete, navedene u Tab. 2. Ugrađena mapa pokazuje položaj istraživanog područja unutar Bosne i Hercegovine, a mapa u gornjem desnem kutu područje Uloga približano (markirano s crnim okvirom).

Checking the potential roosts

During the day, we checked two caves, »Velika Đeverđela« (the whole cave) and »Mala Đeverđela« (only the entrance part), and a few abandoned buildings in the village Ulog. We also checked one small abandoned concrete building in Ulog during the night (Tab. 2). We looked for bats by using strong headlamps or handheld lights.

Mist netting and handling of individuals

On nine nights we set polyester mist nets (mesh size 1.5 cm, and up to 3 m height) at places where we anticipated the bats' flight paths: at the bank or over the surface of the Neretva River, on the forest edge, and amongst buildings of the village Ulog (Fig. 1; Tab. 2). We set the mist nets at the time of sunset (around 20:30), and took them down at least three hours later (around midnight). Night bat inventories were carried out when there was no rain and air temperatures exceeded 10°C.

We checked the mist nets every few minutes to remove bats immediately after capture and placed them in a cloth bag. We measured the bats (weight, forearm length, other species-specific characters), determined their age class according to the presence of cartilage in the epiphysal part of the metacarpal bones (Dietz & von Helversen 2004) and determined their reproductive status (Dietz & von Helversen 2004; Dietz et al. 2009; Dietz & Kiefer 2016). In some species, we took a small patch of wing membrane tissue with a puncher (1.5 mm diameter) and stored it in 96% ethanol for subsequent molecular analyses (Wilmer & Barratt 1996). Before release, we marked each bat by cutting a tuft of hair (scapular or abdominal region) or by colouring the ear with a marker to recognise it in the case of recapture within the same night.

Molecular analyses

To confirm morphological identification in some cryptic species, we sequenced the the cytochrome C oxidase subunit I (COI) gene. We extracted the DNA from the punched wing tissue, using MagMAX DNA Multi-Sample Kit (Thermo Fisher Scientific), and amplified a standard barcoding marker, COI, using the Folmers primer pair, LCO1490 and HCO2198 (Folmer et al. 1994). After purification we sent the PCR products for sequencing to Macrogen Europe laboratory (Amsterdam, Netherlands). We handled and assembled the sequences in Geneious Prime and compared them to the available sequences of bat species in the public database GenBank, using the Basic Local Alignment Search Tool (BLAST, NCBI) as a computational method. We submitted the COI sequences from this study to GenBank and we provide them in Tab. 3.

Ultrasound detectors and sound analysis

We conducted bat surveys using ultrasound detectors on the nights without rain and with temperatures over 10°C, using two approaches: by recording bat calls manually using a hand ultrasound detector and by installing automatic ultrasound detectors (Tab. 2).

We used hand D240x ultrasound detectors (Pettersson Elektronik AB) at or in the vicinity of the mist netting sites (Tab. 2). While in heterodyne mode, we scanned the frequencies (from 20 to 110 kHz; mostly around 40 kHz) to detect bat calls. Once detected, we recorded them in 10 x time expansion mode and stored them on an external digital recorder Roland RH-09.

We set the automatic ultrasound detector (Song Meter SM4BAT FS, Wildlife Acoustics) at different sites along the Neretva River, and once in the village of Ulog (Tab. 2). The microphone was set at least 1.5 m above the ground, directed towards the river surface and its bank vegetation. The ultrasound detector recorded bats through the whole night, starting half an hour before sunset, and finishing at sunrise. The device recorded the full spectrum of sound for eight seconds with two seconds breaks, with no limits put on the sensitivity triggering the recording. We transferred all recordings to a PC and ran them through the Kaleidoscope software (Kaleidoscope Lite, Wildlife Acoustics) to produce the first separation of files with call sequences from the files with recorded noise. Due to the high number of automatic recordings, we checked only a subset of recordings per night to identify bat taxa.

We analysed the files with call sequences using the sound analysis program Batsound 4.0 (Pettersson Elektronik AB). We identified species or species groups based on characteristics of the echolocation calls, measured from spectrogram and power spectrum (FFT, 2048 samples, Hanning window), and using different references on bat calls (e.g. Russ 1999; Russo & Jones 2002; Barataud 2014; Middleton et al. 2014; Dietz & Kiefer 2016). We checked the shape of the calls, start and end frequency, frequency of maximum energy, and call duration for determinations (Russ 1999; Russo & Jones 2002). In some cases, recognition to species level was not possible due to similar calls between species and variation within species. In these cases, calls were attributed to species groups or genera.

Results and discussion

General overview

During the summer of 2022, we recorded at least 13 different bat species within 17 different bat taxa (Tabs. 1, 2). Groups of two or more species are given for some cases, where they could not be further identified with the methods used – but are nevertheless still informative about the presence of the bats. We confirmed the presence of two species of Rhinolophidae and at least 11 species of Vespertilionidae (Tabs. 1, 2), belonging to one and six genera, respectively.

Our results represent a considerable increase in the knowledge on bats in the area, as previously only single records of three species existed. Of those, we reconfirmed the presence of two (*R. hipposideros*, *P. pipistrellus*), while the third, *N. leisleri* (Mirć & Paunović 1997), could not be confirmed unambiguously based on echolocation calls we checked.

Table 1. Overview of bat taxa (species or species groups) recorded during summer 2022 in the upper Neretva River valley in BIH, together with abbreviations of species names and number of localities where they were observed. The list of localities is in Tab. 2.

Tabela 1. Pregled taksonov netopirjev (vrst ali skupin vrst), zabeleženih poleti 2022 v dolini zgornje Neretve v BIH, skupaj s kraticami imen vrst in številom lokalitet, kjer so bili opaženi. Seznam lokalitet je v Tab. 2.

Tabela 1. Pregled vrsta šišmiša (vrsta ili grupa vrsta) zabilježenih u ljetu 2022. godine u dolini gornje Neretve u BiH, zajedno sa skraćenicama naziva vrsta i brojem lokaliteta, na kojima su uočeni. Spisak lokaliteta nalazi se u Tab. 2.

No.	Family *Species/species group*	Abb.	No. of localities
	Rhinolophidae		
1	*Rhinolophus hipposideros* (Bechstein, 1800)	*Rhip*	5
2	*Rhinolophus ferrumequinum* (Schreber, 1774)	*Rfer*	2
	Vespertilionidae		
3	*Myotis myotis* (Borkhausen, 1797)	*Mmyo*	1
4	*Myotis nattereri* (Kuhl, 1817)	*Mnat*	1
5	*Myotis mystacinus* (Kuhl, 1817)	*Mmys*	2
6	*Myotis alcathoe* O. von Helversen & K.-G. Heller, 2001	*Malc*	1
7	*Myotis* sp.	*Myosp*	6
8	*Pipistrellus pipistrellus* (Schreber, 1774)	*Ppip*	4
9	*Pipistrellus pygmaeus* (Leach, 1825)	*Ppyg*	2
10	*Pipistrellus nathusii* (Keyserling & Blasius, 1839)	*Pnat*	1
11	*Pipistrellus kuhlii/nathusii*	*Pkuh/nat*	5
12	*Hypsugo savii* (Bonaparte, 1837)	*Hsav*	7
13	*Nyctalus noctula* (Schreber, 1774)	*Nnoc*	1
14	*Nyctalus noctula/lasiopterus*	*Nnoc/las*	2
15	*Vespertilio murinus* Linnaeus, 1758	*Vmur*	1
16	*Vespertilio/Nyctalus/Eptesicus*	*V/M/E*	6
17	*Plecotus* sp.	*Plesp*	1

Most of the findings refer to observations with ultrasound detectors at the Neretva River bank which recorded the bats on their flight paths; either when they were flying by, or were present near the point of observation whilst hunting at the bank's vegetation or above the river surface. In some cases, the bats were observed near the street lights in villages or near the houses, where many species hunt for insects attracted to light. This approach gathered a lot of information on bat presence, but in many cases exact determination to species level was not possible due to ther similarity of the echolocation calls (Barataud 2014; Middleton et al. 2014). We identified at least five different bat species with this approach (Tab. 2).

Five out of nine mist netting evenings/nights were successful in catching at least one individual bat (Tab. 2). Even though this approach is time consuming, and limited to one site per night, it allows for the most exact species identifications, as individuals are held in the hand and directly observed and allow for samples for DNA analyses to be taken. We confirmed the presence of ten different bat species using this method (Tab. 2).

Table 2. List of localities with bat taxa (species or species group) recorded in summer 2022 in upper Neretva River valley in BIH (number of individuals given in brackets in the column Taxa). Geographical coordinates are in decimal degrees (WGS84). Abbreviations: Method: OB – observation, RC – roost check, MN – mist netting, AUD – automatic ultrasound detector, HUD – hand ultrasound detector; Leg/Det. – legator/determinator: AV – Anton Vorauer, BR – Behare Rexhepi, EP – Ester Premate, SA - Stefan Andjus, ŠB – Špela Borko, VM – Vojo Milanović, MZ – Maja Zagmajster. Taxa abbreviations are in the Tab. 1.

Tabela 2. Seznam lokalitet s taksoni netopirjev (vrstami ali skupinami vrst), zabeleženimi poleti 2022 v dolini zgornje Neretve v BIH (število osebkov je podano v oklepaju v stolpcu Taxa). Geografske koordinate so v decimalnih stopinjah (WGS84). Okrajšave: Metoda: OB – opazovanje, RC – preverjanje kotišč, MN – lovljenje z mrežo, AUD – avtomatski ultrazvočni detektor, HUD – ročni ultrazvočni detektor; Leg/Det – legator/določevalec: AV – Anton Vorauer, BR – Behare Rexhepi, EP – Ester Premate, SA – Stefan Andjus, ŠB – Špela Borko, VM – Vojo Milanović, MZ – Maja Zagmajster. Okrajšave taksonov so v Tab. 1.

Tabela 2. Spisak lokaliteta vrtsa šišmiša (vrste ili grupe vrsta) evidentiranih tokom ljeta 2022. godine u dolini gornje Neretve u BiH (broj jedinki je u zagradama u koloni Taxa). Geografske koordinate su u decimalnim stepenima (WGS84). Skraćenice: Metoda: OB – posmatranje, RC – provjera skloništa, MN – mreženje, AUD – automatski ultrazvučni detektor, HUD – ručni ultrazvučni detektor; Leg/Det. – legator/određivać: AV – Anton Vorauer, BR – Behare Rexhepi, EP – Ester Premate, SA - Stefan Andjus, ŠB – Špela Borko, VM – Vojo Milanović, MZ – Maja Zagmajster. Skraćenice taksona nalaze se u Tab. 1.

No	Locality	Coordinates	Date	Method	Leg./ Det.	Taxa	Comments
1	Ulog, near Mosque/Church	43.41590, 18.31067	27.6. 2022	OB	AV/ AV	*Plesp* (1 flying)	Observed in flight
2	Ulog, downstream Camp	43.42299, 18.31037	27.6. 2022	MN	AV/ AV	*Nnoc* (1), *Vmur* (1)	Mist nets set at the river bank
3	At the right bank of the Neretva, downstream from Swimming Beach, Ulog	43.42485, 18.30973	28./29.6. 2022	AUD	MZ/ MZ	*Rfer*, *Myosp*, *Ppip*, *Ppyg*, *Pkuh/nat*, *Hsav*, *Nnoc/las*, *V/N/E*	Microphone set toward the river
4	At the right bank of the Neretva, upstream of the Cerova location	43.37879, 18.35610	29./30.6. 2022	AUD	MZ/ MZ	*Myosp*, *Pkuh/nat*, *Hsav*, *Nnoc/las*, *V/N/E*	Microphone set toward the river
5	Near the bridge over Neretva, 600 m downstream of the Krupac confluence, Southeast from the farm	43.33218, 18.41821	29.6. 2022	MN	MZ, VM, (EP, SB, BR, SA)/ MZ, VM	*Mmyo* (2), *Mnat* (1), *Mmys* (2), *Malc* (4), *Pnat* (1), *Ppyg* (1)	Five mist nets set up, one (15 m) over the river, one near the forest edge (7 m), three over small dry channel (7 m, 6 m, 10 m)
6	Ulog »beach«	43.42420, 18.30857	29.6. 2022	MN	AV/ AV	*Rfer* (1)	
7	Ulog, between two houses, south from the bar-house, near the Cinema	43.41648, 18.31223	30.6. 2022	MN	AV, VM/ AV, VM	*Ppip* (1)	Two mist nets set up
8	Velika Đeverđela cave, Ulog	43.40587, 18.28429	30.6. 2022	BR	MZ/ MZ	*Rhip* (1 humerus)	In the chamber just after the vertical drop

No	Locality	Coordinates	Date	Method	Leg./ Det.	Taxa	Comments
9	Mala Đeverđela cave, Ulog	43.40660, 18.28416	30.6. 2022	RC	MZ/ MZ	*Rhip* (1 flying)	In the entrance chamber, before the vertical drop
10	At the street light in Ulog, near the first road curve South from the Neretva	43.41578, 18.31244	30.6./1.7. 2022	AUD	MZ/ MZ	*Pkuh/nat*, *Hsav*, *V/N/E*	
11	At the right bank of Neretva River, Northeast from the bar house in Ulog	43.41509, 18.31475	1.7. 2022	MN	MZ, VM/-	/	Two mist nets: 6 m at the gravel road; 15 m above the river
				HUD	MZ/ MZ	*Myosp*, *Ppip*, *Hsav*, *Nnoc/las*, *V/N/E*	D240x Pettersoon, at the river bank, recordings
12	Ulog, above the Mosque/Church old house 1	43.41095, 18.31061	1.7.2022	RC	AV/ AV	*Rhip* (6)	Pregnant females – visual observation
13	Ulog, above the Mosque/Church old house 2	43.41091, 18.31061	1.7.2022	RC	AV/ AV	*Rhip* (8)	Pregnant females – visual observation
14	Ulog, meadow east of the Camp	43.41533, 18.31282	1.7.2022	MN	AV/-	/	
15	Abandoned small building, north from Neretva, Southeast from Ulog	43.41507, 18.31518	1.7.2022	RC	MZ/ MZ	*Rhip* (1)	Active, left the room when we checked on it (at about midnight)
16	At the right bank of Neretva River, below the Grand River Adventure Ranch, Glavatičevo	43.51044, 18.09880	2.7.2022	MN	VM/-	/	
				HUD	VM, MZ/ MZ	*Myosp*, *Pkuh/nat*, *Hsav*, *V/N/E*	
17	At the bank of Neretva River at the Brijestov bridge	43.482282, 18.226530	4.8.2022	MN	VM/-	–	Three mist nets: 9 m, 3 m, 6 m above the river
				AUD	VM/ MZ	*Myosp*, *Hsav*, *V/N/E*	
18	At the bank of Neretva River near Nedavic	43.45797, 18.32121	5.8.2022	MN	VM/ VM	*Mmys* (3)	Two mist nets: 9 m and 6 m above the river
				AUD	VM/ MZ	*Hsav*, *Pkuh/nat*, *Myosp*, *V/N/E*	

There were few observations of bats in roosts, in one case this was only based on finding bony remnants which confirmed the species' presence in the cave (Tab. 2). We recorded roosts of only one species, *R. hipposideros*, in a few buildings and caves, as this is the species that can be more easily observed due to hanging in exposed places (Dietz et al. 2009). In the short time for this research, only a few potential roosts could be checked, even though there were many more potentially suitable in the wider Neretva River valley (not only in buildings, but also in caves, rocky walls, and tree trunks).

The species richest site during our survey was at the Neretva River near the Krupac confluence, where six different bat species were caught in mist nets (Tab. 2). Mist nets were set at the edge of the forest next to the Neretva, as well as over a small side river channel next to the main river bed. This confirms that forests around the river support a very diverse bat community, including species that are typical forest species (*M. mystacinus*, *M. alcathoe*) or species hunting near riparian vegetation (*Pipistrellus* spp.). Even though other mist netting events revealed fewer bat species, one to two, they still revealed the presence of rarely encountered species. These were mist nettings at the Neretva River, both in the wider surroundings of Ulog (Tab. 2).

Measurements of bat individuals are within the typical ranges of all the species (Tab. 3). Most of the females had signs of lactation, indicating that they were rearing a juvenile. This further supports their presence in the valley and the importance of the area for reproduction of at least four bat species (Tab. 3). The tissues taken from some bats enabled exact identification to species level (see comments in the next section).

Table 3. Measurements of the bats mist netted in summer 2022 in the upper Neretva River valley in BIH. For species abbreviations see Tab. 1, for localities and dates see Tab. 2. Abbreviations: FA - forearm length, W - weight, Repr.- reproduction state, M - male, F - female, Ad - adult, Sad - subadult, D1 - length of the first finger, D5 - length of the fifth finger, P3.2 - length of the second phalang of the third finger, P3.3 - length of the third phalang of the third finger, CM^3 - length of the upper tooth row from canine to 3rd molar, P - premolar, in upper (superscripted number) or lower (subscripted number) tooth row.

Tabela 3. Meritve netopirjev, ujetih v mreže poleti 2022 v zgornjem toku reke Neretve v BIH. Za kratice vrst glej Tab. 1, za nahajališča in datume glej Tab. 2. 2. Okrajšave: FA - dolžina podlakti, W - teža, Repr.- reprodukcijsko stanje, M - samec, F - samica, Ad - odrasel, Sad - subadult, D1 - dolžina prvega prsta, D5 - dolžina petega prsta, P3.2 - dolžina druge prstnice tretjega prsta, P3.3 - dolžina tretje prstnice tretjega prsta, CM^3 - dolžina zgornje vrste zob od kanina do tretjega molarja, P - premolar, v zgornji (nadnapisana številka) ali spodnji (podnapisana številka) vrsti zob.

Tabela 3. Mjerenja šišmiša koji su uhvaćeni u mreže tokom ljeta 2022. godine u dolini gornje Neretve u BiH. Za skraćenice vrsta vidi Tab. 1, za lokalitete i datume vidi Tab. 2. Skraćenice: FA - dužina podlaktice, W - težina, Repr.- stanje reprodukcije, M - mužijak, Ž - ženka, Ad - odrasla jedinka, Sad - pododrasla jedinka, D1 - dužina prvog prsta, D5 - dužina petog prsta , P3.2 - dužina druge falange trećeg prsta, P3.3 - dužina treće falange trećeg prsta, CM^3 - dužina gornjeg zubnog reda od očnjaka do 3. kutnjaka, P - premolar, u gornjem (nadpisani broj) ili donjem (potpisani broj) zubnom redu.

Loc	Time	Species	Sex	Age	FA [mm]	W [g]	Repr.	Remarks, other measurements
2	/	*Vmur*	M	Ad	42.4	15	Inactive	D5: 48.5 mm, D1: 6,1 mm
2	/	*Nnoc*	M	Ad	54.4	30	Inactive	
5	21:40	*Mmyo*	M	Ad	59.8	29.5	Active	Enlarged testices, CM^3: 10.5 mm, strong smell, black tip on tragus
5	21:41	*Mmyo*	M	Ad	60.9	28.0	Active	Enlarged testices, CM^3: 10.2 mm, strong smell, black tip on tragus
5	21:15	*Mnat*	M	Ad	38.1	7.0	Inactive	Black epididymis; GenBank Acc.No. OR487194

Loc	Time	Species	Sex	Age	FA [mm]	W [g]	Repr.	Remarks, other measurements
5	22:00	*Mmys*	F	Ad	34.4	7.0	Lactating	D1: 6.0 mm, Tibia: 17.8 mm, dark ear, face brownish-pale
5	22:26	*Mmys*	F	Sad	34.8	6.1	Nuliparous	D1: 6.0 mm, Tibia: 17.6 mm, Foot: 5.6 mm, dark ear, greyish, young
5	21:15	*Malc*	M	Ad	33.1	4.5	Active	Enlarged testices, D1: 5.3 mm, Tibia: 15.6 mm, Foot: 4.4 mm, Upper teeth row: no cing obvious (Dietz et al. 2009). Species first identified as Mmys, but allocated to *Malc* according to DNA; GenBank Acc.No. OR487193
5	21:43	*Malc*	F	Ad	32.8	5.5	Lactating	D1: 4.3 mm, Tibia: 14.5 mm, Foot: 4.3 mm, bright face; GenBank Acc. No. OR487195
5	22:00	*Malc*	F	Ad	32.3	5.0	Lactating	D1: 4.1 mm, Tibia: 14.9 mm, Foot: 4.4 mm, light face, bright ears; GenBank Acc.No. OR487196
5	22:00	*Malc*	M	Ad	31.6	4.0	Inactive	D1: 4.9 mm, Tibia: 14.5 mm, Foot: 5.3 mm, light face, black epididymis; GenBank Acc.No. OR487197
5	22:30	*Pnat*	M	Ad	34.3	8.5	Active	Enlarged testices, epidydimis slightly black, D5: 43.8 mm, upper teeth like *Pnat* (Dietz & von Helversen 2004); Ring: BIHA0140
5	21:15	*Ppyg*	F	Ad	30.5	5.0	Lactating	D5: 37.5 mm, P3.2: 7.7 mm, P3.3: 6.7 mm, internal edge of the ear: 7.6 mm, orange glands (buccal and around vagina)
6	/	*Rfer*	M	Ad	54.7	24	Inactive	
7	23:00	*Ppip*	F	Ad	30.0	6.0	Lactating	Wing membrane as in Dietz et al. (2004) for *Ppip*, echolocation upon release – around 45 kHz
18	21:10	*Mmys*	F	Sad	36.0	6.0	Nuliparous	No protocone, $P^3 < 2/3\ P^2$; $P_3 < P_2$; dark ears, face; greyish fur, cartilage in joints; Ring: BIHA0114; GenBank Acc.No. OR487200
18	/	*Mmys*	F	Ad	34.9	5.0	Lactated	No protocone, $P^3 \sim 2/3\ P^2$; $P_3 < P_2$; dark ears, face; brownish fur; Ring: BIHA0115; GenBank Acc.No. OR487201
18	21:20	*Mmys*	M	Sad	35.9	5.5		No protocone, $P^3 < 2/3\ P^2$; $P_3 < P_2$; dark ears, face; greyish fur, cartilage in joints; Ring: BIHA0116; DNA did not amplify

Comments to bat species

Rhinolophidae, *Rhinolophus* spp.

We recorded two species of the genus during our study, the smallest (*R. hipposideros*) and the largest (*R. ferrumequinum*) of this family in Europe (Dietz et al. 2009). Both are known to use caves and parts of buildings as shelters. In our study we only found roosts of *R. hipposideros* by recording individuals or small groups of individuals in some abandoned buildings and a cave (Tab. 2). The groups of individuals in some of the buildings in Ulog (Tab. 2) indicate that these were females forming nursery colonies. Individuals had fat bellies and looked to be close to gestation period (Fig. 2).

We caught only one male of *R. ferrumequinum* in the mist net set near the Neretva River near Ulog (Tab. 2), but did not find any roosts of the species. Its presence in the region was further supported with the species echolocation calls detected at one site near the Neretva River near Krupac (Tab. 2). The species is known to feed in riparian habitats (Dietz et al. 2019), and considering the preserved habitats it could be expected to be more common in the Neretva River valley. There was an old record of the species from the town of Nevesinje (Felten et al. 1977), but our observations confirm its current presence in the upper Neretva River valley.

Figure 2. A group of Lesser horseshoe bats (*Rhinolophus hipposideros*) observed in an abandoned building in Ulog. Due to fat bellies of the individuals, and typical behaviour in forming summer groups, this is very likely a group of females forming a nursery colony (Photo: A. Vorauer).

Slika 2. Skupina malih podkovnjakov (*Rhinolophus hipposideros*) opazovana v zapuščeni stavbi v Ulogu. Zaradi debelih trebuhov osebkov in značilnega vedenja pri oblikovanju poletnih skupin je zelo verjetno, da gre za skupino samic v porodniški koloniji (Foto: A. Vorauer).

Slika 2. Grupa malih potkovastih šišmiša (*Rhinolophus hipposideros*) uočena u napuštenoj zgradi u Ulogu. Zbog debelih trbuha jedinki i tipičnog ponašanja pri formiranju ljetnih grupa, vrlo je vjerovatno da je riječ o grupi ženki koja formira prodničnu koloniju (Foto: A. Vorauer).

Vespertilionidae, *Myotis* spp.

The biggest species of this genus, *Myotis myotis*, uses caves and abandoned buildings as roosts, and feeds mostly in the forest openings or along forest roads (Dietz et al. 2019). We caught one male (Fig. 3) at the Neretva River near Krupac, flying out of the forest at the river bank.

Figure 3. The greater mouse-eared bat (*Myotis myotis*) caught in the mist net close to the Krupac confluence with Neretva (photo: Ester Premate).
Slika 3. Navadni netopir (*Myotis myotis*), ujet v mrežo v bližini sotočja Krupca z Neretvo (foto: Ester Premate).
Slika 3. Veliki mišouhi šišmiš (*Myotis myotis*) uhvaćen u mrežu u blizini ušća Krupca u Neretvu (foto: Ester Premate).

Myotis nattereri is a medium sized species of the genus, being a typical forest species, dwelling in crevices in tree trunks, and feeding among the forest vegetation (Dietz et al. 2019). We caught one male at the Neretva River near Krupac, flying out of the forest at the river bank (Tab. 2). Due to a suitable habitat for roosting and feeding, it can be predicted that this species is resident here and common in the upper Neretva River area.

There were two sites at which individuals of the smallest species of the genus were mist netted, belonging to the *Myotis mystacinus* species complex (*M. mystacinus, brandtii, alcathoe, davidii*, Dietz & Kiefer 2016). It is a group of morphologically similar species, where discrimination is not always unambiguous. All species of the complex are forest species, roosting in tree hollows, and feeding in the forest (Dietz et al. 2019). *M. alcathoe*'s description has been supported by using molecular differences (von Helversen et al. 2001; Dietz et al. 2009). In order to reliably identify species, we used DNA barcoding to help identify the species of this complex.

One of the animals first morphologically identified as *M. mystacinus* in the field was genetically identified as *M. alcathoe*, proving the difficulty in morphological discriminations of the species (Tab. 3). Also on the European level, distribution of this species is full of knowledge gaps, mainly due to under-sampling of the habitats of this bat species and misidentifications during fieldwork (Jan et al. 2010; Bashta et al. 2011). For example, the presence of *M. alcathoe* in Portugal was also confirmed using DNA barcoding, after an individual, morphologically determined as *M. mystacinus*, was analysed molecularly (Rebelo et al. 2020).

Myotis alcathoe was confirmed only at Neretva near Krupac site, flying in the net set at the forest edge near Neretva. The first record of the species in the country comes from Sarajevo area, and was based on morphological characters only (Babić et al. 2018). Later, the species was confirmed for eastern Bosnia and Herzegovina also with molecular analyses (Verhees et al. 2021). As *Myotis alcathoe* is a species bound to riparian and forest habitats, more findings of the species in the upper Neretva River could be expected. Proper management of the forest is crucial for preservation of the species (von Helversen et al. 2001; Coronado et al. 2017).

The other bat species of the complex are here referred to as *Myotis mystacinus* (Tabs. 1,2). However, in a recent study, it has been suggested that *Myotis davidii* populations may have historically replaced the *M. mystacinus* s.str. in the Balkans, where even hybridising zones between the species may exist (Çoraman et al. 2020). Discrimination between two species is difficult both morphologically or based on mitochondrial DNA (Benda et al. 2016; Dietz & Kiefer 2016; Çoraman et al. 2020). We report *M. mystacinus* s.l. here, but its exact species status, not only in the upper Neretva River valley but probably in the whole Bosnia and Herzegovina, remains to be resolved.

The species of the genus *Myotis* sp. have characteristic frequency modulated shape echolocation calls, which are very similar to one another and species can only rarely be identified to species/species group level. The calls recorded near the Neretva River surface most likely belong to the species group *M. daubentonii/capaccinii;* species that typically hunt over water surfaces (Dietz et al. 2009). Due to this, their calls show typical interruptions due to the reflection from the water surface (Russ 1999) (Tab. 2). Further analyses of bat calls, as well as additional field work, including mist netting, may reveal further bat species of this genus in the area.

Vespertilionidae, *Pipistrellus* spp. & *Hypsugo savii*

Pipistrellus pipistrellus and *P. pygmaeus* are morphologically very similar, and were only recognised as separate species three decades ago (Jones & van Parijs 1993; Barlow & Jones 1997). The two species can be distinguished based on echolocation calls (Russo & Jones 2000; Barataud 2014). In our study, we detected *P. pygmaeus* more often, whilst both species were caught in the mist nets. *Pipistrellus pygmaues* is a species that is more bound to riparian habitats, and indeed it was recorded at sites near the Neretva River (Tab. 1, 2).

It was less than ten years ago that the species *P. nathusii* was confirmed to occur in BIH (Karapandža et al. 2014), with a few records in subsequent years (Husanović & Presetnik 2021). Our finding of the species at the Krupac site near the Neretva River is therefore one of the rare findings in the country. Part of the reason that the species distribution is poorly known could be the fact that its echolocation calls overlap with the echolocation calls of the species *P. kuhlii,* to such an extent that discrimination is not possible (Kalko 1995; Russo & Jones 1999; Barataud 2014; Dietz & Kiefer 2016). As the two species cannot be reliably discriminated based on echolocation calls, such observations are allocated to a species group *P. kuhlii/nathusii*, which was recorded in almost all sites where ultrasound detector observations were made (Tabs. 1, 2). Social calls of the species are species-specific, and they were used to confirm the presence of *P. nathusii* during autumn in Mostar (Husanović & Presetnik 2021).

Hypsugo savii was confirmed at all sites where bats were recorded with ultrasound detectors, showing the species is generally widespread in the region. The species typically roosts in rocky walls, but also in building walls, which are both present in the Neretva River valley.

Vespertilionidae, other bat taxa recorded

Species of the group *Vespertilio murinus*, *Nyctalus* spp., *Eptesicus* spp. (»V/N/E« in Tabs. 1, 2) use echolocation with a lot of overlap, therefore we conservatively used this call determination category for such cases. Even though this category includes the genus *Eptesicus*, we cannot confirm its presence based on our results, whilst we did detect species of the other two genera in our study (Tabs. 1, 2).

In some cases we were able to identify species of the group *Nyctalus noctula/Nyctalus lasiopterus*, due to two characteristic types of echolocation calls, and low call frequency (Russo & Jones 2002; Barataud 2014). We decided to remain conservative in separating the two species, due to large frequency overlap, and kept the group category. However, some studies have shown that discrimination between the two species is possible in some cases (Estók & Siemers 2009). Considering that we mist netted the male of *N. noctula*, it is most probable that most of the echolocation calls belong to this species.

Based on the amount of recording analyses for this study, we could not yet unambiguously confirm the presence of *N. leisleri*. The species is expected in the area as it is bound to large forested areas. The only reliable confirmation of species presence so far is the report from near Boračko jezero, close to Glavatičevo (Mirić & Paunović 1997).

Reliable discrimination of *V. murinus* is possible only based on male display song calls (Zagmajster 2003), but they were not recorded during our study, as they are typically emitted during the mating season in autumn. The confirmation of the species presence in the Neretva river valley was therefore possible due to a successful mist netting event close to the Ulog region. The species typically feeds in riparian zones, finding many suitable habitats within the Neretva River valley (Dietz et al. 2009).

The observation of the *Plecotus* bat is so far based on the visual observation of the flying individual made by AV. It is highly likely that the species of this genus are present in the area, yet the only data existing to date are from about 14 km from Ulog, close to the region of Zalomka – where echolocation calls of *Plecotus auritus* were recorded (Mulaomerović 2022).

Conservation notes

We detected at least 13 bat species in the study area extending from Krupac to Konjic. With one additional species, known from the literature, 14 different bat species have been found in the upper Neretva river valley, presenting nearly a half of all bat species recorded in Bosnia and Herzegovina. The rich bat community in the Neretva River valley, despite the short time of research, proved that this area is potentially very rich in bat species and of high importance for conservation. All bat species are protected under the Bern convention, which is the basis for the designation of Emerald protection sites - since 2023, the upper Neretva River valley has been amongst such proposed sites (EEA 2023). Bats as migratory species are protected under the Bonn Convention (OJ L 1982), and a special agreement of this convention dedicated specifically to bat protection (Eurobats 1991), which was also officially signed by Bosnia and Herzegovina in 2021.

In European Union (EU) member states, one of the most powerful international documents for the protection of bats is the EU Habitats Directive (OJ EC 1992). As accession to the EU is also a path being taken by Bosnia and Herzegovina, under this directive, one of the obligations shall be the list of proposed Sites of Community Importance. In this EU document, all bats are protected (Annex IV), but there are some species of even higher conservation concern, which need designation of areas for protection (Annex II). In our study, we recorded three bat species with the highest conservation concern in the Neretva River valley: *R. ferrumequinum*, *R. hipposideros* and *M. myotis*. According to the EU Habitats Directive, these species need the designation of special areas for their conservation. The upper Neretva River valley could be such an area for bats and should be included in such an European conservation network.

Planned constructions in the Neretva River valley, including a set of hydropower plants and their corresponding reservoirs, accompanied by forest cuts due to the building processes, threaten to significantly change the pristine nature of the area. This will consequently change the habitats for bats, which have been shown to suffer from hydropower constructions and related changes of natural habitats (Zortéa et al. 2021). Considering the rich bat community in the area, hydropower plants on the Neretva River present a significant threat to bats in the area. It is also highly important to conduct further studies of bats in the area, including other seasons, to get a more complete overview of the bat diversity in the area.

Povzetek

V BIH je bilo doslej zabeleženih 31 vrst netopirjev (Zagmajster et al. 2010; Babić et al. 2018), vendar je podatkov o razširjenosti in ekologiji nekaterih vrst malo. Za tri vrste netopirjev so bili z območja zgornje doline reke Neretve ali iz njene neposredne bližine zabeleženi le trije zapisi: *Rhinolophus hipposideros* je bil opažen 29.9.2019 (J. Mulaomerović, pers. comm.). Mirić & Paunović (1997) poročata o najdbi *Nyctalus leisleri* 15.3.1968 pri Boračko jezero SZ od Glavatičeva, v oddaljenosti 3 km od reke Neretve. Isto nahajališče in datum sta navedena tudi pri zapisu o *Pipistrellus pipistrellus*, ki je shranjen v Prirodoslovnem muzeju v Beogradu (Srbija) (Zagmajster et al. 2010). Da bi zapolnili to vrzel v znanju, smo v juniju/juliju in avgustu 2022 opravili raziskavo netopirjev, pri čemer je bilo prvo obdobje v času »Neretvanskega tedna znanosti 2022«.

Raziskavo smo opravili v zgornjem delu doline reke Neretve, ki se razteza od Krupca do Konjica (Sl. 1). Čez dan smo pregledali dve jami, »Velika Đeverđela« (celotno jamo) in »Mala Đeverđela« (samo vhodni del), ter nekaj zapuščenih stavb v vasi Ulog. Devet noči smo postavili poliestrske mreže za meglo na mestih, kjer smo predvidevali preletne poti netopirjev (Sl. 1; Tab. 2). Za potrditev terenskih določitev pri nekaterih kriptičnih vrstah smo iz tkiva, odvzetega nekaterim netopirjem, sekvencirali gen COI. Preiskave netopirjev z ultrazvočnimi detektorji smo izvajali v nočeh brez dežja in ob temperaturah nad 10 °C, in sicer z ročnimi in avtomatskimi ultrazvočnimi detektorji (Tab. 2).

Med 17 različnimi taksoni netopirjev smo zabeležili vsaj 13 različnih vrst netopirjev (Tab. 1, 2). Skupine dveh ali več vrst so navedene v nekaterih primerih, ko jih z uporabljeno metodo ni bilo mogoče natančneje določiti - vendar so še vedno informativne glede prisotnosti netopirjev. Potrdili smo prisotnost dveh vrst iz družine Rhinolophidae (*Rhinolophus hipposideros*, *Rhinolophus ferrumequinum*) in vsaj 11 vrst iz družine Vespertilionidae znotraj 15 različnih taksonov (*Myotis myotis*, *Myotis nattereri*, *Myotis mystacinus* s.l., *Myotis alcathoe*, *Myotis sp.*, *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*, *Pipistrellus nathusii*, *Pipistrellus kuhlii/nathusii*, *Hypsugo savii*, *Nyctalus noctule*, *Nyctalus noctula/lasiopterus*, *Vespertilio murinus*, *Vespertilio/Nyctalus/Eptesicus* spp., *Plecotus* sp.) Zabeležili smo tudi nekaj redko opaženih vrst v BIH (*Myotis alcathoe*, *Vespertilio murinus*).

Večina ugotovitev se nanaša na opazovanja z ultrazvočnimi detektorji. V petih od devet noči, ko smo lovili z mrežami, smo uspešno ujeli vsaj enega netopirja (Tab. 2). S to metodo smo potrdili prisotnost desetih različnih vrst netopirjev (Tab. 2). Le pri eni vrsti, *R. hipposideros*, smo zabeležili zatočišča v nekaj stavbah in jamah, od katerih so nekatere najverjetneje kotišča. Vrstno najbogatejša lokacija je bila na reki Neretvi pri sotočju s Krupcem, kjer smo v mreže ujeli šest različnih vrst netopirjev.

Bogata združba netopirjev, ki smo jo odkrili v dolini reke Neretve kljub kratkemu času raziskave, je pokazala, da je to območje potencialno zelo bogato z netopirji in zelo pomembno za njihovo ohranjanje. Vse vrste netopirjev so zavarovane. V skladu z EU Habitatno direktivo (OJ ES 1992) je treba za nekatere vrste netopirjev predlagati potencialno območje, pomembno za Skupnost (Priloga II). V naši raziskavi smo v dolini reke Neretve zabeležili tri take vrste netopirjev, ki so najpomembnejše za varstvo: *R. ferrumequinum, R. hipposideros* in *M. myotis*.

Načrtovane gradnje v dolini reke Neretve, vključno z nizom hidroelektrarn in pripadajočih zadrževalnikov, ki jih spremlja krčenje gozdov, predstavljajo nevarnost, da se bistveno spremeni neokrnjena narava območja. To bo posledično spremenilo habitate za netopirje, na katere ima gradnja hidroelektrarn dokazano negativen vpliv (Zortéa et al. 2021). Glede na bogato vrstno pestrost lahko dolino zgornjega toka reke Neretve štejemo za območje velikega varstvenega pomena za netopirje.

Sažetak

U BiH je do sada zabilježena 31 vrsta šišmiša (Zagmajster et al. 2010; Babić et al. 2018), ali podataka o rasprostranjenosti i ekologiji nekih vrsta je malo. Za tri vrste šišmiša iz ili u neposrednoj blizini gornje doline rijeke Neretve zabilježena su samo tri zapisa: *Rhinolophus hipposideros* uočeni su 29.9.2019 (J. Mulaomerović, pers. comm.). Mirić & Paunović (1997) izvještavaju o pronalasku *Nyctalus leisleri* 15.3.1968 na Boračkom jezeru SZ od Glavatičeva, na udaljenosti od 3 km od rijeke Neretve. Isti lokalitet i datum dat je i za zapis *Pipistrellus pipistrellus,* koji se nalazi u Prirodnjačkom muzeju u Beogradu (Srbija) (Zagmajster et al. 2010). Kako bi se popunila ova praznina u znanju, nekoliko dana u junu/julu i augustu 2022. godine provedeno je istraživanje šišmiša, a prvi period je bio tokom »Neretvanske sedmice nauke 2022.«.

Istraživanje smo proveli u dolini gornje Neretve, koja se proteže od Krupca do Konjica (Sl. 1). Tokom dana smo proverili dve pećine, »Velika Đeverđela« (cela pećina) i »Mala Đeverđela« (samo ulazni deo), i nekoliko napuštenih objekata u selu Ulog. Devet noći smo postavljali poliesterske mreže na mjestima gdje smo predviđali puteve leta šišmiševa (Sl. 1; Tab. 2). Da bismo potvrdili terenska određivanja kod nekih kriptičnih vrsta, sekvencirali smo COI gen iz tkiva uzetog od nekih vrsta. Istraživanja vršili smo ručnim ili automatskim detektorima u noćima bez kiše i pri temperaturama preko 10°C (Tab. 2).

Zabilježili smo najmanje 13 različitih vrsta šišmiša unutar 17 različitih taksona šišmiša (Tab. 1, 2). Za neke slučajeve date su grupe od dvije ili više vrsta, gdje se nisu mogle dalje identificirati s korištenom metodom - ali su i dalje informativne o prisutnosti šišmiša. Potvrđeno je prisustvo dvije vrste Rhinolophidae (*Rhinolophus hipposideros, Rhinolophus ferrumequinum*) i najmanje 11 vrsta iz porodice Vespertilionidae u okviru 15 različitih taksona (*Myotis myotis*, *Myotis nattereri*, *Myotis mystacinus*, *Myotis alcathoe*, *Myotis* sp., *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus, Pipistrellus nathusii, Pipistrellus kuhlii/nathusii, Hypsugo savii*, *Nyctalus noctula*, *Nyctalus noctula/lasiopterus*, *Vespertilio murinus*, *Vespertilio/Nyctalus/Eptesicus*. Zabilježili smo i neke rijetko opažene vrste u BiH (*Myotis alcathoe, Vespertilio murinus).*

Većina nalaza odnosi se na opažanja ultrazvučnim detektorima. Pet od devet pokušaja lova s mrežom bilo je uspješno u hvatanju najmanje jedne jedinke šišmiša (Tab. 2). Ovom metodom smo potvrdili prisustvo deset različitih vrsta (Tab. 2). Zabilježili smo skloništa samo jedne vrste, *R. hipposideros*, u nekoliko zgrada i pećina, od kojih su neke najvjerovatnije porodiljske kolonije. Vrstama najbogatije nalazište tokom našeg istraživanja zabilježeno je na rijeci Neretvi kod ušća Krupca, gdje je u mrežama uhvaćeno šest različitih vrsta šišmiša.

Bogata zajednica šišmiša otkrivena u dolini rijeke Neretve i pored kratkog vremena istraživanja, dokazala je, da je ovo područje potencijalno vrlo bogato vrstama šišmiša i od velikog značaja za njihovo očuvanje. Sve vrste šišmiša su zaštićene različitim konvencijama. Prema Direktivi EU o staništima (OJ EC 1992), neke vrste šišmiša trebaju odrediti područja za zaštitu (Aneks II). U našem istraživanju zabilježili smo tri vrste šišmiša od najvećeg značaja za očuvanje u dolini rijeke Neretve: *R. ferrumequinum, R. hipposideros* i *M. myotis*.

Planirana gradnja u dolini rijeke Neretve, uključujući niz hidroelektrana, njihovih odgovarajućih akumulacija, praćena sječom šuma, predstavlja prijetnju netaknuti prirodi područja. To će posljedično promijeniti staništa šišmiša, za koje se pokazao negativni utjecaj hidroenergetskih konstrukcija i sličnih promjena prirodnih staništa (Zortéa et al. 2021). S obzirom na bogatstvo vrsta, dolina gornje Neretve može se smatrati područjem od velikog značaja za očuvanje šišmiša.

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Leading the way – presence of brown bear *Ursus arctos*, lynx *Lynx lynx* and grey wolf *Canis lupus* underlines the integrity and corridor function of the upper Neretva Valley in Bosnia and Herzegovina

Manuela HABE

Vienna, Austria; E-mail: habemanu@gmail.com

Abstract. Despite its connecting character to the Sutjeska National Park in the North-East, the course of the upper Neretva (Ulog and Borač valley) in Bosnia and Herzegovina (BiH), has to date not been an area of focus for carnivore research. With the aim of contributing to an improved understanding of the present distribution of large and endangered carnivores in this area, a field survey was conducted during the Neretva Science Week 2022. Opportunistic data collection was carried out along a predefined road transect (20 km) and six line transects (13 km) tracked by foot. Additionally, reports of mammal signs for six corresponding research sites along the Neretva were registered. Altogether, at least 15 mammal species could be distinguished. From the identified carnivores, four are listed in Annex II of the Habitats Directive. Furthermore, the signs of lynx presence, in addition to brown bear and grey wolf, highlight the connectivity value of the upper Neretva in its current state. Based on information from local practitioners, the main threats to large carnivores are hunting pressure and a negative perception as competitors. A likely decline in habitat quality and fragmentation, due to the planned hydropower plants expansion and logging activities, exacerbate these threats. Consequently, further systematic monitoring is recommended to assess the possible impact of human driven environmental changes.

Key words: large carnivores, Eurasian lynx, connectivity, habitat integrity, Neretva

Izvleček. Utiranje poti – pojavljanje rjavega medveda *Ursus arctos*, risa *Lynx lynx* in sivega volka *Canis lupus* kaže na pomen in povezovalno funkcijo doline zgornje Neretve v Bosni in Hercegovini – Kljub povezanosti habitatov z nacionalnim parkom Sutjeska na severovzhodu, tok zgornje Neretve (dolini Ulog in Borač) v Bosni in Hercegovini (BiH) doslej ni bil predmet raziskovanja velikih zveri. Z namenom, da bi prispevali k boljšemu razumevanju trenutne razširjenosti velikih in ogroženih zveri na tem območju, smo v okviru Tedna znanosti na Neretvi 2022 opravili terensko raziskavo. Oportunistično zbiranje podatkov je potekalo po vnaprej določenem cestnem transektu (20 km) in vzdolž šestih prehojenih linijskih transektov (13 km). Poleg tega smo zabeležili znake pojavljanja sesalcev na šestih raziskovalnih lokacijah ob Neretvi. Skupno smo zaznali vsaj 15 vrst sesalcev. Od identificiranih zveri so štiri navedene v Prilogi II k direktivi o habitatih. Poleg tega znaki pojavljanja risa, poleg rjavega medveda in sivega volka, poudarjajo povezljivostno vrednost zgornje Neretve v njenem trenutnem neokrnjenem stanju. Na podlagi informacij lokalnih strokovnjakov sta glavni grožnji za velike zveri lov in negativno mnenje ljudi. Verjeten upad kakovosti habitatov in razdrobljenost zaradi načrtovane širitve hidroelektrarn in dejavnosti sečnje te nevarnosti še povečujeta. Zato priporočamo nadaljnje sistematično spremljanje, s katerim bi ocenili možni vpliv antropogenih okoljskih sprememb na velike zveri in druge sesalce.

Ključne besede: velike zveri, evrazijski ris, povezljivost, celostnost habitata, Neretva

Apstrakt. Utirući put – prisustvo mrkog medvjeda *Ursus arctos*, risa *Lynx lynx* i sivog vuka *Canis lupus* naglašava integritet i koridornu funkciju doline gornje Neretve u Bosni i Hercegovini – Uprkos vezi sa Nacionalnim parkom »Sutjeska« na sjeveroistoku, tok gornje Neretve (dolina Uloga i Borača) u Bosni i Hercegovini (BiH) do sada nije bila područje fokusa istraživanja mesoždera. S ciljem da se doprinese boljem razumijevanju sadašnjeg rasprostranjenja velikih i ugroženih zvijeri na ovom području, u toku »Sedmice nauke na Neretvi« 2022. godine obavljeno je terensko istraživanje. Oportunističko prikupljanje podataka obavljeno je duž unaprijed definisanog putnog transekta (20 km) i šest linijskih transekata (13 km) pređenih pješice. Osim toga, na šest istraživačkih lokacija duž Neretve zabilježili smo znakove prisutnosti sisara. Ukupno smo otkrili najmanje 15 vrsta sisara. Od identifikovanih mesoždera, četiri su navedena u Aneksu II Direktive o staništima. Nadalje, znakovi prisutnosti risa, pored mrkog medvjeda i sivog vuka, ističu vrijednost povezanosti gornjeg toka Neretve u njenom sadašnjem netaknutom stanju. Na osnovu informacija lokalnih stručnjaka, glavne prijetnje velikim karnivorima su pritisak zbog lova i negativna percepcija kao konkurenata. Vjerovatni pad kvaliteta staništa i fragmentacija, zbog planirane gradnje hidroelektrana i sječe šume, pogoršavaju ove prijetnje. Shodno tome, preporučuje se dalje sistematsko praćenje kako bi se procijenio mogući uticaj ekoloških promjena uzrokovanih ljudskim djelovanjem.

Ključne riječi: velike zvijeri, euroazijski ris, povezanost, cjelovitost staništa, Neretva

Introduction

Nowadays, it is widely recognised that large carnivores (LCs) play an irreplaceable role in nature conservation by providing direct, as well as indirect, ecosystem services and upholding biodiversity. Overall, the cascading effects that could result from a change in the status of apex predators are context dependent and difficult to predict (May et al. 2008; Ripple et al. 2014; Beschta & Ripple 2018; Ausilio et al. 2021; Giergiczny et al. 2022; Malhi et al. 2022). Current EU and international regulations thus favour a preventive approach of upholding the protection of large carnivores by recommending a range of protective measures applying to the species and its remaining habitats (European Parliament et al. 2018; Trouwborst 2019).

While BiH has one of the greatest diversities of animals and plant species in Europe, nature conservation is not considered a national priority which is why systematic and reliable data, necessary for adequate planning and management, is often lacking or incomplete (IMPAQ Int. 2020).

Unfortunately, this is also true for the »Gornji tok Neretve« (the upper Neretva river), which has been officially nominated as a candidate Emerald Site for BiH (EEA 2023), whilst its integrity continues to be threatened through planned hydropower constructions and a lack of structured spatial management. Therein, information on species distribution is essential for effective large-scale conservation and management (ENETWILD et al. 2020). In the absence of sound knowledge, perceptions of large carnivores in the region and the emotions associated with them, are likely to influence the acceptance of their persistence (Lescureux et al. 2011; Rode et al. 2021).

Although there was continuous communication with local scientists and hunting associations, before and during the week, specific information was difficult to obtain. Local practitioners (forest workers, hunters, and wildlife managers) confirmed the presence of brown bear (*Ursus arctos*) and grey wolf (*Canis lupus*), but not for lynx (*Lynx lynx*). The presence of mesocarnivores such as otters, wildcats, and red foxes was reported. As for the European wildcat, no proof was provided. Potentially abundant prey species were chamois, different mice species, European hare, roe deer, and wild boar. Regarding perceptions of large carnivores, people indicated they had no problem with the presence of bears but favoured a declining wolf population and preferred the absence of lynx, as they are seen as potential competitors for existing game species. To our knowledge, no research has yet focused on the presence of large carnivores along the opper Neretva River. Therefore, the aim of this study is to contribute to a better understanding of the current distribution of large and endangered carnivores in this area.

Materials and methods

Most state-of-the-art techniques for the thorough study of large mammals require preliminary information, manpower, funding, and long sampling times (e.g. camera trapping, genetics). Still, field work should be adapted to the on-the-ground conditions (Breitenmoser et al. 2006; ENETWILD et al. 2020; Hočevar et al. 2020). In this case, limited information, time, and capacity required rigorous context-dependent sampling considerations. The method of choice was opportunistic sampling along track transects (road, trails). Underlying this is the fact that bear, wolf and lynx tend to have similar habitat preferences and use existing infrastructure for travels within a given habitat (May et al. 2008; Filla et al. 2017).

Field work

Fieldwork was conducted during the Neretva Science Week (NSW) 2022, from 27.6. to 3.7., along the upper course of the Neretva River. The research area spanned over the border of the two autonomous entities of the Federation of Bosnia and Herzegovina and República Srpska and included three municipalities: Gacko (Rep. Srpska), Kalinovik (Rep. Srpska) and Konjic (Federacija BiH). In selecting the research transects in accordance with the corresponding main research sites of the NSW, a special focus was given to the upper Neretva, the part above Ulog (Fig. 1).

The Main Research Sites covered along the Neretva were Krupac Confluence, Cerova, Uolgski Buk, Swimming Beach (Ulog,) and Brijestov Bridge. The edges of each research site were tracked by foot once and the corresponding research transects (13 km) were supplemented with additional research transects along the Klištica river, accordingly. Since Brijestov Bridge is quite far from the focal area including the road transect, a longer research transect (6 km) was chosen to account for these circumstances.

The road transect was based on geographic information, movement considerations and suitability for tracking, the dirt road that runs on the right side of the Neretva River, from Ulog to the Krupac Confluence, was selected as the road transect (20km). In the course of the NSW, the road transect was surveyed three times (mornings; MON, WED, FRI) focusing on wet and sandy sections. All field signs of medium and large sized mammals were systematically recorded, with special attention given to carnivores and potential prey species (PP).

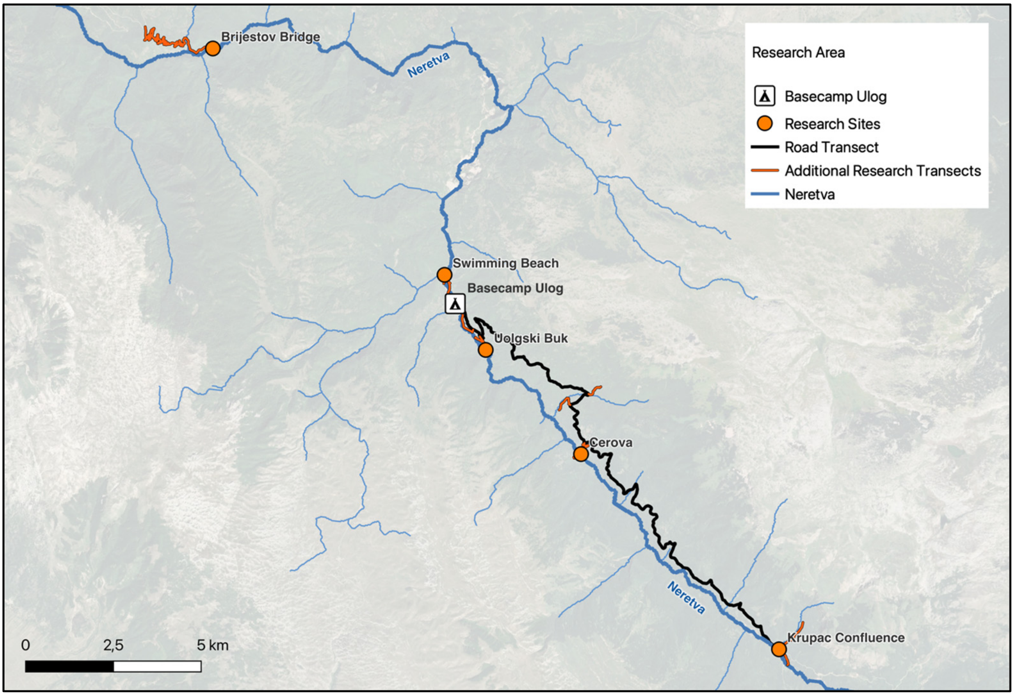

Figure 1. Map of the research area in the upper Neretva valley, with marked point locations and transect lines where large carnivores were searched for.

Slika 1. Zemljevid raziskovanega območja v dolini gornje Neretve z označenimi točkami in transektnimi linijami, kjer smo iskali velike zveri.

Slika 1. Karta istraživanog područja u dolini gornje Neretve, sa označenim tačkama i linijama transekata na kojima su tražene krupne zvijeri.

Records were categorised for: direct observation by scientists (DO), documented track (T), Faeces (F) or Animal (A). Spatial data analysis and map creation was carried out using QGIS (Version 3.28.0).

Results

From the 45 documented records (Fig. 2), at least 15 mammal species could be distinguished, as in some cases, we could only reliably determine the genera (Tab. 1). Four of them are listed in Annex II of the FFH Directive (OJ EC 1992), namely grey wolf (*Canis lupus*), European otter (*Lutra lutra*), Eurasian lynx (*Lynx lynx)* and brown bear (*Ursus arctos*).

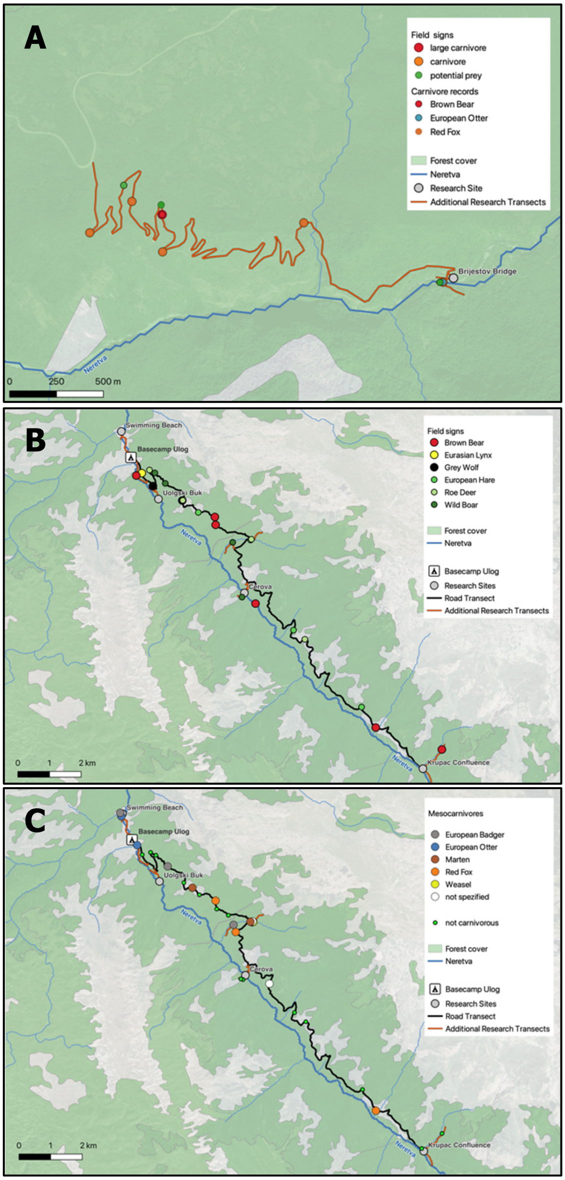

Figure 2. Registered signs of mammal species. A – Recorded field signs at the transect near research site Brijestov Bridge. B – Records of large carnivores and three potential prey species in the focus area around Ulog. C – Summarized field signs of mesocarnivores. The coordinates can be retrieved from the author upon request.

Slika 2. Zabeleženi znaki prisotnosti sesalcev. A – Znaki prisotnosti na transektu blizu lokacije Brijestov most. B – Podatki o velikih zvereh in treh potencialnih vrst plena na območju okoli Uloga. C – Znaki prisotnosti mezo-plenilcih. Koordinate so na zahtevo na voljo pri avtorici.

Slika 2. Snimljeni znakovi prisutnosti sisavca. A – Znakovi prisutnosti na transektu blizu lokaliteta Brijestov most. B – Podaci o velikih karnivorih i treh vrsta potencijalnog plijenu za područje oko Uloga. C – Terenske znakovi prisutnosti mezokarnivora. Koordinate se mogu preuzeti od autora na zahtjev.

Table 1. List of the recorded Mammalian Taxa (in alphabetical order) and Category of Proof (direct observation (DO), documented Tracks (T), Faeces (F) or Animal (A)).
Tabela 1. Seznam zabeleženih taksonov sesalcev (po abecednem vrstnem redu) in kategorija dokaza (neposredno opazovanje (DO), dokumentirane sledi (T), iztrebki (F) ali žival (A)).
Tabela 1. Spisak zabilježenih taksona sisara (po abecednom redu) i kategorija dokaza (direktno posmatranje (DO), dokumentovani tragovi (T), izmet (F) ili životinja (A)).

Species	Scientific name	Proof	Number of sites
Dormouse	*Glis glis*	DO	1
European badger	*Meles meles*	T, F	4
Brown bear	*Ursus arctos*	T, F	5
Grey wolf	*Canis lupus*	T, F	2
European hare	*Lepus europaeaus*	DO, T, F	5
European mole	*Talpa europaea*	A	1
European otter	*Lutra lutra*	T, F	2
Eurasian lynx	*Lynx lynx*	T	1
Marten	*Martes spp.*	T, F	3
Muskrat	*Ondatra zibethicus*	T	1
Red fox	*Vulpes vulpes*	T, F	7
Roe deer	*Capreolus capreolus*	DO, T	4
Weasel	*Mustela spp.*	F	1
White-breasted hedgehog	*Erinaceus roumanicus*	A	1
Wild boar	*Sus scrofa*	T, F	5

While the presence of brown bear and wolf could be reconfirmed, the record of putative lynx tracks close to Ulog complements the existing data for signs of the presence of lynx (Fig. 3). Due to conflicting expert opinions on the recorded lynx track, we categorised this observation as C3 after the SCALP criteria (Molinari et al. 2021).

Discussion

Being aware of the limitations of this expedition approach and subsequent evaluation, the uncertainty of some distinguished mammal tracks, indicate the weakness of the chosen method for detecting carnivores in unknown/unexplored terrain. In addition, training local practitioners (e.g. game wardens, forest workers, etc.) and researchers, working on other topics at the same time, in data sampling and LC trail detection can improve results. In a remote area like the upper Neretva Valley equipping trained people with camera traps, might be one of the few options to carry out a systematic and prolonged camera survey. Involving hunters in monitoring can result in enhanced trust and management (Lescureux et al. 2011; Hočevar et al. 2020).

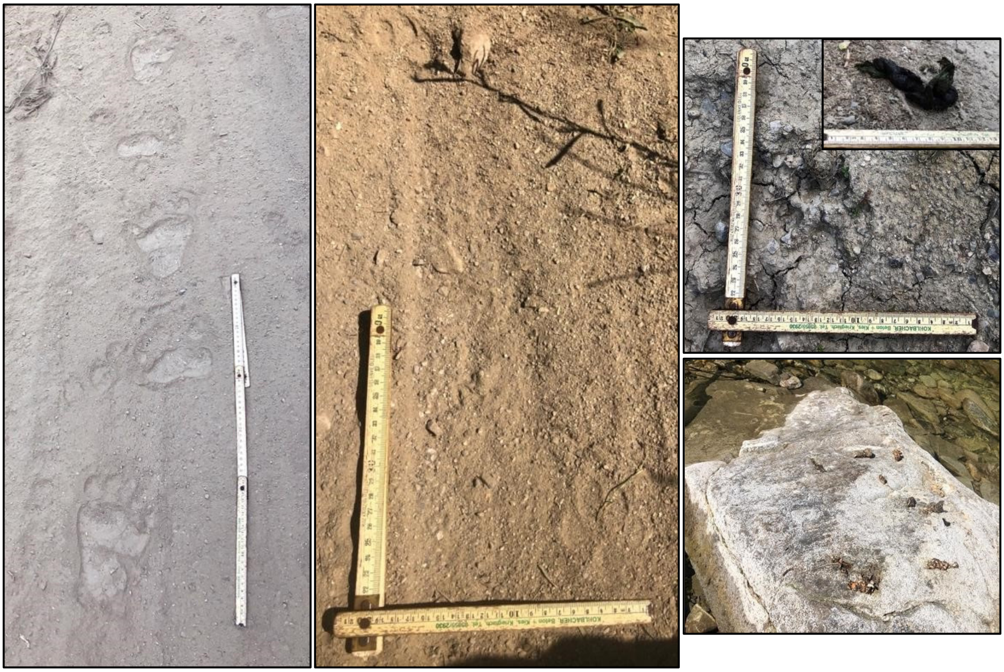

Figure 3. Pictures of the field signs documented for brown bear (left), lynx (middle), otter (bottom right) and wolf (top right).

Slika 3. Slike dokumentiranih znakov pojavljanja rjavega medveda (levo), risa (sredina), vidre (desno spodaj) in volka (desno zgoraj).

Slika 3. Slike tragova sa terena dokumentovani za mrkog medvjeda (lijevo), risa (u sredini), vidre (dolje desno) i vuka (gore desno).

Considering that the area around Maglic and Zelengora is a zone where the Dinaric and Balkan lynx populations might merge (Kaczensky et al. 2013; Kunovac et al. 2018; Kaczensky et al. 2021), this lynx record might serve as an indication for lynx migration along the course of the upper Neretva. As shown by Dobbert et al. (2021) the area is also one of the remaining areas in Europe suitable to support a lynx population, with forest cover and human disturbance being the most limiting factors. While human induced mortality and habitat fragmentation are generally within the major threats for LC persistence (Boitani et al. 2015; Behnke 2019) the known extended effect of dam construction (Alho 2011) holds the potential to substantially exacerbate these threats. In conclusion, the author highly recommends further and broader surveys of the course of the upper Neretva.

Povzetek

Kljub svojemu povezovalnemu značaju z nacionalnim parkom Sutjeska na severovzhodu, tok zgornje Neretve (dolini Ulog in Borač) v Bosni in Hercegovini (BiH) doslej ni bil osrednje območje raziskovanja zveri. Dandanes je splošno priznano, da imajo velike zveri nenadomestljivo vlogo pri ohranjanju narave (Ripple et al. 2014; Malhi et al. 2022). Informacije o pojavljanju in distribuciji teh vrst pa so bistvenega pomena za učinkovito širše ohranjanje in upravljanje ekosistemov (ENETWILD et al. 2020). Zato je bil cilj raziskave prispevati k boljšemu razumevanju trenutne razširjenosti velikih in ogroženih zveri na obravnavanem območju. Posledično je bila v okviru Tedna znanosti na Neretvi 2022 (27. 6. – 3. 7.) opravljena terenska analiza. Oportunistično zbiranje podatkov je potekalo vzdolž vnaprej določenega cestnega transekta (20 km) in šestih prehojenih linijskih transektov (13 km). Poleg tega smo zabeležili znake pojavljanja sesalcev na šestih raziskovalnih lokacijah ob Neretvi. Dokumentirali smo 45 znakov pojavljanja, iz katerih je bilo mogoče razločiti vsaj 15 vrst sesalcev. Od identificiranih zveri so štiri navedene v Prilogi II k Direktivi o habitatih, in sicer rjavi medved (*Ursus arctos*), vidra (*Lutra lutra*), evrazijski ris (*Lynx lynx*) in sivi volk (*Canis lupus*). Znaki pojavljanja risa, poleg rjavega medveda in sivega volka, poudarjajo povezljivostno vrednost zgornje Neretve v njenem trenutnem neokrnjenem stanju. Umrljivost, ki jo povzroči človek, in razdrobljenost habitata sta na splošno med glavnimi grožnjami obstoju velikih zveri (Boitani et al. 2015; Behnke 2019). Medtem ko lokalni strokovnjaki vidijo lovski pritisk in negativno dojemanje zveri kot glavni grožnji za velike zveri v regiji, imajo načrtovana širitev hidroelektrarn in dejavnosti sečnje potencial, da znatno povečajo te grožnje, na primer z znanim razširjenim učinkom gradnje jezu (Alho 2011). Zato priporočamo nadaljnje sistematično spremljanje stanja v obravnavanem območju, s katerim bi ocenili možni vpliv antropogenih okoljskih sprememb na velike zveri in druge sesalce.

Sažetak

Uprkos svojoj povezanošču sa Nacionalnim parkom »Sutjeska« na sjeveroistoku, gornji tok Neretve (dolina Uloga i Borača) u Bosni i Hercegovini (BiH) do sada nije bio područje fokusa istraživanja karnivora. Kako je danas široko poznato da veliki karnivori igraju nezamjenjivu ulogu u očuvanju prirode (Ripple et al. 2014; Malhi et al. 2022) informacije o distribuciji vrsta su ključne za učinkovito šire očuvanje i upravljanje (ENETWILD et al. 2020). Cilj ove studije bio je doprinijeti boljem razumijevanju sadašnjeg rasprostranjenja velikih i ugroženih zvijeri na ovom području. Shodno tome, provedeno je terensko istraživanje u sklopu »Sedmice nauke na Neretvi« 2022. godine (27. jun – 3. jul). Oportunističko prikupljanje podataka obavljeno je duž unaprijed definisanog putnog transekta (20 km) i šest dodatnih linijskih transekata (13 km) pređenih pješice. Osim toga, na šest istraživačkih lokacija duž Neretve zabilježili smo znakove prisutnosti sisara. Dokumentovali smo 45 znakova prisutnosti, od kojih se moglo izdvojiti najmanje 15 vrsta sisara. Od identifikovanih mesoždera, četiri su navedena u Aneksu II Direktive o staništima, a to su: smeđi medvjed (*Ursus arctos*), evropska vidra (*Lutra lutra*), evroazijski ris (*Lynx lynx*) i sivi vuk (*Canis lupus*). Nadalje, znakovi prisutnosti risa, pored mrkog medvjeda i sivog vuka, ističu vrijednost povezanosti gornje Neretve u njenom trenutnom netaknutom stanju. Smrtnost uzrokovana ljudskim djelovanjem i fragmentacija staništa uopšteno su jedne od glavnih prijetnji za opstanak velikih mesoždera (Boitani et al. 2015; Behnke 2019). Dok lokalni stručnjaci vide pritisak zbog lova i negativnu percepciju konkurencije kao glavne prijetnje za velike karnivore u ovoj regiji, planirana gradnja hidroelektrana i sječa šume, imaju potencijal da značajno pogoršaju ove prijetnje, npr. kroz poznati prošireni efekat izgradnje brane (Alho, 2011). U skladu s tim, preporučuje se dalje sistematsko praćenje kako bi se procijenio mogući uticaj ekoloških promjena uzrokovanih ljudskim djelovanjem.

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During the Neretva Science Week, Vanja Lazic translated the observations of the scientists in beautiful drawings. From top to bottom: a stonefly (*Perla marginata* - larva); drawing created by Vanja from the stonefly; Vanja painting t-shirts for all participants on location in Ulog. Photos: Vladimir Tadić.

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Differential controls on CO_2 and CH_4 emissions from the free-flowing Neretva River, Bosnia and Herzegovina

Martin DALVAI RAGNOLI, Thea SCHWINGSHACKL, Serafine KATTUS, Julius LISSY, Elisabeth WENINGER, Gabriel SINGER

Department of Ecology, University of Innsbruck, 6020 Innsbruck, Austria. Correspondence: Martin Dalvai Ragnoli; E-mails: martin.dalvai-ragnoli@uibk.ac.at and Gabriel Singer; E-mail: gabriel.singer@uibk.ac.at

Abstract. Streams and rivers emit methane (CH_4) and carbon dioxide (CO_2), two greenhouse gasses contributing to global warming. Estimates for diffusive gas emissions can be obtained by multiplying the concentration gradient between water and atmosphere with the gas transfer velocity. The latter is purely physically constrained, yet spatially highly variable. And - in a flowing water ecosystem - the local concentration gradient is the result of a dynamic balance between upstream evasion and resupply. The collection of representative emission data is thus challenging and emissions of river ecosystems are rarely assessed considering temporal variability and spatial dependence at network scale. In this study, we uncover spatial heterogeneity and controls of concentrations and emission fluxes of the two greenhouse gasses, CH_4 and CO_2, along a 50 km length of a pristine river system, the Neretva River in Bosnia and Herzegovina. This remote river network has so far remained barely influenced by human activities and the hydromorphological status is to date not altered. The Neretva can therefore serve as a reference for similar systems in the region. This seems to be particularly important as rivers in the Western Balkans, including the Neretva, are currently experiencing a surge in hydropower development and damming, which is known to strongly affect riverine greenhouse gas emissions. We found high emissions as a result of co-occurrence of high concentration with high exchange velocity, but we identified different underlying mechanistic processes driving the evasion of the two gasses. CH_4 was strongly supply-limited: elevated concentrations were exclusively measured in a large pool (0.84 µmol L^{-1} compared to a median concentration of 0.005 µmol L^{-1} in the entire study section). This resulted in CH_4 evasion being four orders of magnitude higher in the turbulent reach following the pool (22 mmol m^{-2} d^{-1}) compared to the median evasion at network scale (0.06 mmol m^{-2} d^{-1}). In contrast, CO_2 evasion was more variable in time and equally dependent on CO_2 and gas exchange velocity. The construction of dams intended in this area would lead to reservoirs of slowly flowing or standing water with similar habitat conditions as the observed CH_4-hotspot. The concomitant increase in residence time and higher retention of organic material will lead to an increase of CH_4 production replacing aerobic respiration. Consequently, CH_4 emissions can be expected to drastically increase by orders of magnitude. This greenhouse gas footprint of hydropower generation may counteract the promised climate benefits in terms of renewable energy production.

Key words: dams, gas transfer velocity, greenhouse gas, greenhouse gas footprint of hydropower, emission fluxes, pristine reference river

Izvleček. Različni mehanizmi kontrol emisij CO_2 in CH_4 iz prostotekoče reke Neretve v Bosni in Hercegovini – Potoki in reke oddajajo metan (CH_4) in ogljikov dioksid (CO_2): toplogredna plina, ki prispevata h globalnemu segrevanju. Ocene difuzne emisije plinov izračunamo tako, da zmnožimo koncentracijski gradient med vodo in atmosfero ter hitrost prenosa plina. Slednja je izključno fizikalno omejena, vendar prostorsko zelo spremenljiva. Lokalni koncentracijski gradient v tekočem vodnem ekosistemu je rezultat dinamičnega ravnovesja med izgubo vodika in ponovnim polnjenjem. Zato je zbiranje reprezentativnih podatkov o emisijah zahtevno opravilo, ocene emisij rečnih ekosistemov pa le redko upoštevajo časovno spremenljivost in prostorsko odvisnost na ravni celotne mreže. Študija analizira prostorsko heterogenost in kontrolne mehanizme koncentracij in emisijskih tokov toplogrednih plinov CH_4 in CO_2 vzdolž 50 km dolgega odseka neokrnjenega rečnega sistema reke Neretve v Bosni in Hercegovini. Ta odročni del rečnega sistema do sedaj skorajda ni bil izpostavljen človekovim posegom in je, za zdaj, hidromorfološko še vedno nespremenjen v prvotnem naravnem stanju. Zgornja Neretva zato lahko rabi kot referenca za podobne sisteme v regiji. To je še posebej pomembno, saj se na rekah zahodnega Balkana, vključno z Neretvo, trenutno hitro razvija hidroenergetika in gradijo jezovi, ki znatno vplivajo na emisije toplogrednih plinov iz rek. V študiji smo odkrili visoke emisije, ki so posledica hkratnega pojavljanja visoke koncentracije in visoke izmenjave. Identificirali smo različne mehanistične procese, ki vodijo do emisij obeh plinov. CH_4 je bil močno omejen na viru: povišane koncentracije smo izmerili izključno v velikem bazenu (0,84 µmol L^{-1} v primerjavi s srednjo koncentracijo 0,005 µmol L-1 v celotnem odseku). Posledično so bile emisije CH_4 v turbulentnem delu, ki sledi bazenu, višje za kar velikostne razrede (22 mmol m^{-2} d^{-1}), v primerjavi s srednjimi emisijami na ravni celotnega omrežja (0,06 mmol m^{-2} d^{-1}). Nasprotno pa je bila emisija CO_2 bolj časovno spremenljiva in enako odvisna od koncentracije CO_2 in hitrosti izmenjave le tega. Načrtovana gradnja jezov na tem območju bi vodila do rezervoarjev počasi tekoče ali stoječe vode s podobnimi habitatnimi razmerami, kot so bili opaženi na vroči točki CH_4. Spremljajoče povečanje časa zadrževanja in večje zadrževanje organskega materiala bo vodilo do povečano proizvajanje CH_4, ki bo zamenjalo aerobno dihanje. Posledično lahko pričakujemo, da se bodo emisije CH_4 drastično povečale. Toplogredni odtis proizvodnje hidroenergije bo tako najverjetneje izničil obljubljene podnebne koristi v smislu proizvodnje obnovljive energije.

Ključne besede: toplogredni plin, emisijski tokovi, hitrost prenosa plina, neokrnjena referenčna reka, jezovi, toplogredni odtis hidroelektrarn

Apstrakt. Različiti mehanizmi kontrole emisija CO_2 i CH_4 iz slobodnotekuće rijeke Neretve, Bosna i Hercegovina – Ekosistemske funkcije su okosnica usluga ekosistema koje rijeke pružaju ljudskim zajednicama. Funkcionisanje ekosistema proizlazi iz interakcije između bioloških zajednica i njihovog okruženja. Pošto se uslovi životne sredine u rijekama mijenjaju duž njihovog longitudinalnog kontinuma, mijenja se i funkcionisanje. Ponekad ove promjene ne prate glatke gradijente već velike diskontinuitete. Ovo može biti slučaj u krečnjačkim, kraškim rijekama zbog iznenadnih masivnih unosa podzemnih voda duž pejzaža, što je tipičan fenomen za balkanske rijeke. Uprkos velikom geodiverzitetu i velikoj ekološkoj vrijednosti, balkanske rijeke su i dalje nedovoljno istražene. Ovdje smo istražili kako funkcioniše ekosistem i njihova raznovrsnost (procijenjena kao multifunkcionalnost) se mijenja duž kontinuma kraške rijeke Neretve koja slobodno teče u Bosni i Hercegovini. U tu svrhu izmjerili smo podskup osnovnih funkcija ekosistema (bruto primarna proizvodnja ekosistema, neto primarna proizvodnja biofilma i enzimske aktivnosti, razgradnja organske materije) u jedanaest riječnih tokova od izvora Neretve do riječnih dijelova uzvodno od akumulacije Jablanica. Otkrili smo da su različite funkcije dostigle svoj maksimum u različitim dijelovima Neretve zavisno o unosu hranjivih materija. Dok je razgradnja organske materije bila najveća u glavnim vodama zbog unosa nutrijenata iz priobalne vegetacije, enzimska aktivnost biofilma je imala najveće vrijednosti u srednjim dijelovima zbog unosa NH^{4+}-N u podzemne vode, a primarna proizvodnja je bila najveća u većini nizvodnih dijelova zbog akumulacije NO^{3-}-N i PO_4^{3-}-P sa slivnom površinom. Kao rezultat toga, prosječna multifunkcionalnost je dostigla vrhunac na lokacijama s najvećom koncentracijom nutrijenata preko kontinuma rijeke Neretve, što ukazuje na jači uticaj unosa nutrijenata od položaja mreže. Iskonski uslovi Neretve rezultiraju oligotrofnim uslovima duž njenog gornjeg toka. Naši rezultati naglašavaju veliku osjetljivost funkcionisanja ekosistema u Neretvi na unos nutrijenata i ekološkog diskontinuiteta, bilo prirodne ili ljudske. Potencijalni veliki, dugoročni uticaji na područje mogu promijeniti postojeće gradijente životne sredine, a time i funkcionisanje ekosistema u rijekama na lokalnom i regionalnom nivou.

Ključne riječi: staklenički plinovi, emisijski tokovi, brzina prenosa gasa, netaknuta referentna rijeka, brane, staklenički plinovi iz hidroelektrana

Introduction

Streams and rivers are important sources of gaseous carbon to the atmosphere. Out-gassing of carbon from streams to the atmosphere occurs mostly in the form of methane (CH4) and carbon dioxide (CO2), which are also the two most important greenhouse gasses (GHG) contributing to global warming (Aufdenkampe et al. 2011; Stanley et al. 2016). A recent estimate suggests global carbon out-gassing from inland waters amounts to 3.9 PgC yr^{-1} (Drake et al. 2018), where streams and rivers may be responsible for more than 85% of CO_2 (Raymond et al. 2013) and for 50% of CH4 (Stanley et al. 2016) evasion. However, emissions from streams and rivers are considered to not be well quantified on a global scale (Hotchkiss et al. 2015; Lupon et al. 2019) as substantial uncertainties remain due to the heterogeneity, temporal variability and badly constrained surface areas of small headwaters. Rivers are also highly dynamic systems and difficulties associated with continuous monitoring hinder collection of representative emission data. Taken together, uncertainties to estimate global surface area of streams and rivers, lack of dissolved gas concentration measurements and gas transfer velocity measurements still hinder accurate global upscaling of gas emissions. Especially small streams and rivers are under-represented in literature; yet, these appear to have the highest gas exchange velocities, often also the highest CO_2 partial pressures, and highest surface-to-volume ratios (Aufdenkampe et al. 2011; Butman & Raymond 2011; McGinnis et al. 2016).

Aquatic gas evasion in streams can vary at the scale of meters. Besides the partial pressure of a gas in the water phase, evasion is regulated by physical processes linked to water turbulence, which determine the vertical gas transfer velocity (k; m d^{-1}) (Crawford et al. 2013). Current upscaling approaches mostly use estimates of gas partial pressure at a large scale combined with a finer scaled estimate of gas transfer velocity, thereby poorly accounting for actual process variability at small spatial scale (Crawford et al. 2017; Duvert et al. 2018). More importantly, the resulting flux estimates, achieved by multiplication of a concentration term and the gas transfer velocity, may be erroneous as these two variables are not independent from each other in flowing water ecosystems: gas concentration is the result of a dynamic balance between resupply to the system versus evasion (Rocher-Ros et al. 2019). Depending on the limiting factor, gas evasion can be either supply-limited or transfer-limited. While the former happens at low concentrations, the latter happens in sections with low gas transfer velocities (Rocher-Ros et al. 2019).

CO_2 can be produced and consumed by in-stream processes: the production of CO_2 by heterotrophs is counteracted by the consumption of CO_2 by primary producers. This means that CO_2 concentration may be subject to widespread day-night changes because of the absence of photosynthetic activity at night (Peter et al. 2014; Gómez-Gener et al. 2021). While organic matter is preferentially degraded through aerobic respiration, anaerobic pathways are not unusual. In fact, some studies report the potential for benthic in-stream CH_4 production in anoxic sediments with enhanced organic content (Crawford & Stanley 2016). In the presence of anoxic (or generally low-redox) microzones, aerobic CO_2 and anaerobic CH_4 production may occur simultaneously in the streambed sediment (Baker et al. 1999). This way, CH_4 resulting from CO_2 reduction has even been observed in oxygenated headwater streams (Flury & Ulseth 2019). Notably, in-stream produced CH_4 may be quickly consumed by methanotrophic bacteria, which use this highly reduced organic compound as energy and carbon source in zones well supplied with oxygen (Bastviken 2009; Stanley et al. 2016). Indeed, high methane oxidation rates typically substantially depress otherwise high methane emissions (Bastviken 2009).

GHGs, however, also originate from surrounding ecosystems, specifically soils and groundwater, and can be delivered to streams by subsurface soil flow paths or by groundwater input (Lupon et al. 2019). Groundwater input, which is a key connection between terrestrial and aquatic environments (Leach et al. 2017), is often characterised by a change in stream water chemistry (Horgby et al. 2019) as it can deliver large amounts of carbon (DIC, DOC and gaseous C) to the stream (Crawford et al. 2015; Duvert et al. 2018; Ledesma et al. 2018; Lupon et al. 2019). Thereby, sourced organic carbon can be respired within a very short distance (Rasilo et al. 2017), supporting high mineralisation rates and elevated concentrations in carbon gases (Horgby et al. 2019). Groundwater input also directly brings carbon gases, which may quickly outgas from small streams with high gas exchange velocities. This combination of localised input and outgassing can create notably small-scale spatially variable concentrations of CO_2 and CH_4 (Lupon et al. 2019). Such point source inputs with chemically distinct properties and which potentially result in local hot spots for GHG emissions, may be of particular importance in streams draining karstic terrain, where underground flow paths can be long and water reaches rivers predominantly through high-volume springs (Valdes et al. 2007; Operta & Pamuk 2015).

The goal of our study was to investigate CH_4 and CO_2 concentrations and assess the drivers of respective emission fluxes along the continuum of the upper course of the Neretva River in Bosnia-Herzegovina. This river section is characterised by high landscape diversity, including upland forested reaches with limited alder-covered floodplains, several steep canyon reaches, and a meandering alluvial reach where multiple channels appear. Anthropogenic influence, however, is minimal, making this river section a potential reference for similar systems in the region. The river is further influenced by multiple spatially discontinuous inputs of karstic springs that can reach substantial discharge. Thus, the studied river section presents itself as a naturally heterogeneous river system, where ecological concepts describing river continua are challenged and the existing discontinuities of river morphology, lateral input and in-stream functioning may dominate fluxes of CO_2 and CH_4. Consequently, we expect a high spatial variation in dissolved gas concentration and spatially distinct hotspots of CO_2 and CH_4 related to karstic inputs. We further expect spatial patterns to not overlap between the two gases, reflecting that underlying processes and drivers for emission fluxes along this free-flowing river section are not identical for CO_2 and CH_4.

Material and methods

Study area and study design

The Neretva has a catchment size of more than 10,000 km^2 and flows from the Dinaric Alps in Bosnia and Herzegovina into the Adriatic Sea in Croatia over a length of 220 km (Djedjibegovic et al. 2010). We studied 50 km of the upper course of the Neretva River from the Krupac confluence (10 km upstream of the village of Ulog, (43°19'20.5"N, 18°26'02.4"E) until a point upstream of the Jablanica reservoir near Konjic (43°37'55.4"N, 17°59'58.3"E). The hydromorphological status of the upper Neretva River is defined as near natural (Chamberlain

2018) and human influence is minimal due to the absence of big settlements and industrial areas.

Hydrogeologically, the studied section of the Neretva River can be divided into two parts: The stretch upstream of Ulog is characterised by fissured aquifers with low permeability, whereas the section downstream of Ulog until Konjic contains karst aquifers of high permeability (Grego 2020). The mean annual flow rate amounts to roughly 9 $m^3 s^{-1}$ in Ulog and to about 58 $m^3 s^{-1}$ in Konjic (DIKTAS B & H 2012).

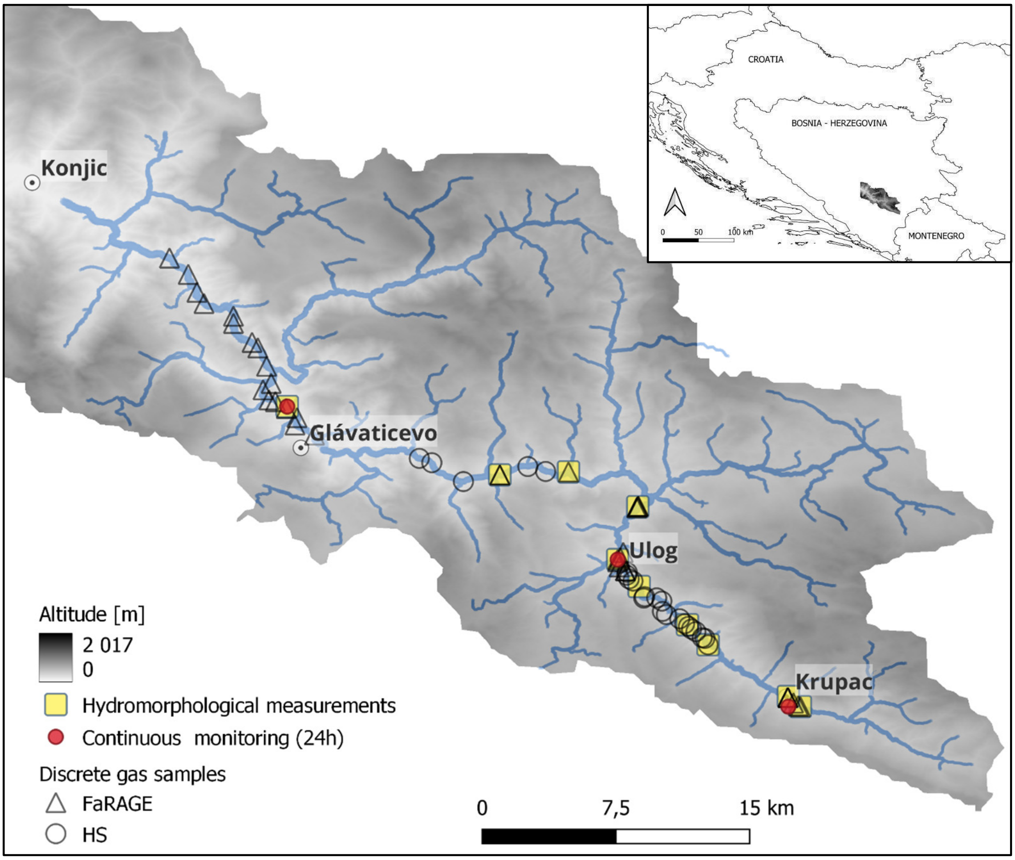

Figure 1. Studied section of the upper Neretva River. Here shown are sampling sites for hydromorphological measurements, the sites selected for continuous monitoring over 24 h and sites used for discrete gas samples. The latter were either performed with the self-built equilibration system (FaRAGE) or with the headspace technique (HS). Major settlements Konjic, Glavatičevo and Ulog are highlighted as well as the confluence of the Krupac tributary.

Slika 1. Preučevani odsek zgornje Neretve. Prikazana so vzorčišča za hidromorfološke meritve, mesta, izbrana za neprekinjeno 24-urno spremljanje, in mesta, uporabljena za posamične vzorce plina. Zadnje smo opravili z lastno izdelanim ekvilibracijskim sistemom (FaRAGE), ali pa z metodo plinske faze (HS). Izpostavljena so glavna naselja Konjic, Glavatičevo in Ulog ter sotočje pritoka Krupac.

Slika 1. Proučavani dio gornjeg toka rijeke Neretve. Ovdje su prikazana mjesta uzorkovanja za hidromorfološka mjerenja, mjesta izabrana za kontinuirano praćenje tokom 24 sata i mjesta korištena za uzimanje diskretnih uzoraka plina. Ovi posljednji su obavljeni ili sa samostalno izgrađenim sistemom za ekvilibraciju (FaRAGE) ili tehnikom faze plina (HS). Naglašena su i glavna naselja Konjic, Glavatičvo i Ulog, kao i ušće pritoke Krupac.

During a ten-day field campaign in July 2022, we performed single point measurements of dissolved CH_4 and CO_2 at 60 sites (Fig. 1). In addition to these measurements taken at arbitrary time points during daylight hours (from 08:00 to 18:00), we took three series of discrete single point measurements spread over 24 h at three selected sites.

Hydromorphology and auxiliary field data

At ten sites spanning the entire study section (Fig. 1), we measured discharge and characterised the river's morphology. For discharge (Q), we measured depth (d) and flow velocity (v) at a minimum of ten regularly spaced (0.5-2.5 m) locations along a cross-section with measured width (w). Flow velocity was measured at 40% depth using a flow meter (FlowSens, SEBA Hydrometrie, Germany). Discharge was then calculated as the product of width, average depth and average flow velocity. At site 1, which was located after a confluence, we calculated discharge from the measured discharge of one tributary and conductivities at both tributaries and the mainstem by mass balance. Additionally, at each of these ten sites, we selected a 50-meter-long river reach to measure depth and flow velocity representing a river's average morphological character better than a single cross-section. To randomise sampling points, we walked from shore to shore in a zig-zag pattern and took a total of 15-50 measurements every three steps.

Catchment area and slope at network scale were derived from a digital elevation model (Copernicus 2022) with a resolution of 30 meters using the R package *watershed* (Talluto 2020) for delineation of the watershed. Hydraulic parameters used for computation of k_{600} at reach-scale were derived from hydraulic scaling relations for river depth (d) and flow velocity (v) as functions of discharge (Leopold et al. 1964):

$$d = c_d Q^{e_d} \tag{1}$$
$$v = c_v Q^{e_v} \tag{2}$$

Parameters of these power law relationships were obtained by fit to hydraulic measurements and compared with values reported in literature. To predict discharge, we used a simple linear model of measured Q to catchment area, which in turn is derived from a digital elevation model (DEM).

Surface water temperature, conductivity, dissolved oxygen, and pH were measured using a WTW handheld probe (Xylem, Weilheim, Germany) at 10 cm depth of a well-mixed location. Additionally, a water sample was taken at the 10 main sites to characterise the chemical composition of the surface water for dissolved organic carbon (DOC) and nutrient concentration. Methods for water chemical analysis are described in detail elsewhere (Del Campo et al., this issue).

Dissolved gas concentration

We used two different methods to measure dissolved gas concentrations of CO_2 and CH_4. The headspace technique was used to measure grab samples collected at remote locations and during the continuous monitoring of gas concentrations over time, at three sites. The equilibrator-based method, which is needed for *in-situ* application of the measuring instrument, was used for easily accessible locations and for sampling from a raft in the lower sections of the study area. Gas concentration was always measured using a micro portable greenhouse gas analyser (MGGA, Los Gatos Research, USA). The MGGA uses laser absorption spectroscopy and detects CO_2 and CH_4 with a precision of 6 ppm and 4 ppb, respectively.

We computed gas saturation using local measurements of water temperature and atmospheric pressure as well as the mean of CO_2 and CH_4 atmospheric background concentration measured at arbitrary locations and points in time during our sampling campaign.

To measure concentrations of CH_4 and CO_2 by the headspace technique, samples were taken with a 60 mL syringe by collecting 30 mL of water from 10 cm below the water surface and filling up the syringe with background air (30 mL). To enhance equilibration between both phases the syringe was intensely shaken for approximately two minutes. From the gaseous headspace approximately 25 mL were transferred into pre-evacuated 20 mL gas vials and stored with overpressure until further analysis. The MGGA was then used in an environment with stable temperature and pressure conditions to measure small sample volumes in a closed-loop configuration (Wilkinson et al. 2018). After temperature acclimation and pressure equalisation to local conditions, the gas vials were directly measured by incorporating them into the closed loop using three-way valves (Fig. 2). This closed loop method requires consideration of sample dilution by ambient gas inside the loop by calculating a volume ratio between sample volume and loop volume. For this purpose, a sample of gas standard with known gas composition was analysed at *in-situ* pressure and temperature conditions. From the resulting headspace concentration, the equilibrium concentration of the water phase was computed using Henry's law of solubility. The original water concentration is finally computed by summing the number of moles in the equilibrated phases and accounting for the background concentration. Detailed computation steps can be found in the supplementary information.

To measure dissolved CO_2 and CH_4 concentration *in-situ*, we built a fast equilibrator similar to the Fast-Response Automated Gas Equilibrator (FaRAGE) described by Xiao et al. (2020). This flow-through system equilibrates a continuous flow of fresh water with a small closed volume of air and allows continuous measurement of dissolved gas concentrations. We modified the original FaRAGE system to better suit our needs, specifically higher portability and lower maintenance and application costs (Fig. 2). Briefly, water from the river is continuously pumped into the system using a peristaltic pump (Eijkelkamp, Netherlands). The water phase is mixed in the mixing unit with an internally recirculated gas phase. Gas bubble size is decreased using a pumice stone. After mixing, the gas-water mixture travels through a coiled tubing (2 m length) to enhance equilibration. Degassing occurs at the separation unit, when the mixture enters a chamber with a larger diameter and the flow velocity is decreased. From there, water is discharged back into the river by flowing freely downwards. The gas phase, however, is recirculated in a closed loop by sending it back to the mixing unit using a gas-tight membrane pump. From the separation unit a gas sample is drawn using the flow rate of the MGGA, bypassed through the analyser to continuously measure the gas concentration, and returned to the separation unit. During use, this equilibrator system was submerged in the river to allow equilibration at water temperature.

Complete equilibration is reflected in a stable concentration plateau measured by the MGGA. All gas calculations are hereafter explained for CH_4, but work analogously for CO_2. Dissolved gas concentration can be directly computed from equilibration concentration by applying Henry's law of solubility:

$$C_{CH_4\,w} = p_{CH_4} KH_{CH_4}(T_w) \quad (3)$$

Where $C_{CH4\,w}$ is the dissolved concentration of CH_4 (mol L^{-1}), p_{CH4} the partial pressure (atm) computed from the molar fraction measured (in ppm) by the MGGA and the ambient pressure, and KH_{CH4} the temperature dependent Henry constant (mol L^{-1} atm^{-1}). KH can be calculated for CO_2 and CH_4 according to IHA (IHA 2010). In the field, ambient pressure was measured using a multifunctional watch (Suunto core, Finland) and water temperature was measured with the WTW handheld probe (Xylem, Weilheim, Germany).

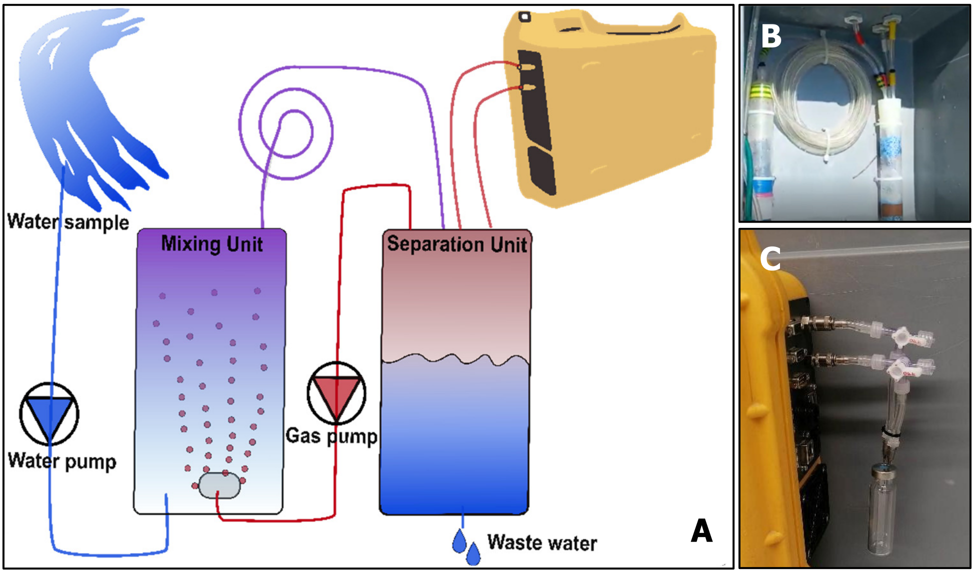

Figure 2. Schematic illustration of the equilibrator system (A): main components are a water pump, a mixing unit, a separation unit, a gas pump and the MGGA. The red colour marks the flow of the gaseous head space, blue the water flow and purple the gas-water mixture. Picture of the mixing unit and separation unit of the equilibrator system (B) and picture of the used closed loop system for headspace samples (C).

Slika 2: Shematski prikaz ekvilibracijskega sistema (A): Glavne komponente so vodna črpalka, mešalna enota, separacijska enota, plinska črpalka in MGGA. Rdeča barva označuje tok plinske faze, modra vodni tok, vijolična pa mešanico plina in vode. Slika mešalne enote in separacijske enote ekvilibracijskega sistema (B) ter slika uporabljenega zaprtega sistema za vzorce plinske faze (C).

Slika 2: Šematski prikaz sistema ekvilibratora (A): Glavne komponente su vodena pumpa, jedinica za mješanje, separacijska jedinica, plinska pumpa i MGGA. Crvena boja označava tok gasne faze, plava boja tok vode, a ljubičasta boja mješavinu gasa i vode. Slika jedinice za mješanje i separacijske jedinice sistema ekvilibratora (B) i slika korištenog zatvorenog sistema za uzimanje uzoraka faze plina (C).

Calculation of GHG flux to the atmosphere

The diffusive gas flux can be calculated for known dissolved gas concentration according to:

$$F_{CH_4} = k_{CH_4}\ \left(C_{CH_4\,w} - C_{CH_4\,EQ}\right) \tag{4}$$

Here, F_{CH4} is the gas flux of CH_4 (mol m^{-2} d^{-1}), k_{CH4} the gas exchange velocity (m d^{-1}), $C_{CH4\ W}$ the measured dissolved gas concentration (mol L^{-1}), and $C_{CH4\ EQ}$ the hypothetical gas concentration that the water phase would have in complete equilibrium with the ambient air (mol L^{-1}). The latter can be computed from the water temperature and the atmospheric partial pressure using Henry's law of solubility. The gas transfer velocity can be computed for any gas and temperature from the standardised gas transfer velocity (k_{600}) and Schmidt number scaling (Raymond et al. 2012):

$$k_{CH_4} = k_{600} \cdot \left(\frac{600}{Sc_{CH_4}(T_W)}\right)^{0.5} \tag{5}$$

Here, the Schmidt number is calculated with the water temperature and given the specific parameterisation of Raymond et al. (2012), the exponent of 0.5 is commonly used for turbulent water (Jähne et al. 1987). k_{600} was estimated by an ensemble-modelling approach from channel hydraulics (i.e., stream velocity, slope, depth and discharge) using the average of predictions of equations 1-7 in Raymond et al. (2012), the necessary hydromorphological parameters were predicted for each site based on the fitted hydraulic scaling relationships.

Data analysis

To investigate the underlying processes for gas evasion of both gases, we computed Spearman's correlation between (i) evasion flux and concentration gradient, and (ii) evasion flux and gas specific transfer velocities (using the *rcorr()* function from the R package *Hmisc* version 4.8-0). To compare the importance of the concentration gradient and gas evasion velocity as drivers of evasion flux of the two gases in our dataset, we also computed fractions of explained variance in a multivariate additive model that results from a log-transformation of the diffusive flux equation (4):

$$log\ F_{CH_4} = log\ k_{CH_4} + log\left(C_{CH_4\,w} - C_{CH_4\,EQ}\right) \tag{6}$$

For temporal correlation we examined the data of the three sampling sites used for continuous monitoring. For spatial correlation we considered all measurements taken along the investigated study section at arbitrary times. We further investigated the dynamic range of evasion flux, gas concentration and gas transfer velocity by calculating the coefficient of variance (CV) over space and time.

We did all statistical analyses in R (version 4.1.1) using the *lm()* function for linear regressions and ggplot2 (version 3.3.5) for visualisation. Maps of dissolved gas concentration, gas flux and gas transfer velocity were created using the program QGIS (version 3.28.2).

Results and discussion

Scaling relationships for hydromorphological variables

The investigated river section has no gauging station to monitor discharge or water level. During the field campaign, we observed a stable water level at Ulog and no rainfall. Personal observations of local inhabitants of Ulog confirmed identical conditions during the week prior to our field campaign, which is supported by the nearest meteorological weather station (https://www.wunderground.com/history/daily/ba/sarajevo/LQSA). There is no longer influence of snowmelt at this time of the year (July). We thus assumed constant discharge during our ten-day field campaign at all investigated sites. Discharge ranged from 0.50 $m^3\ s^{-1}$ at the most upstream site (above the confluence with Krupac tributary) to 12.27 $m^3\ s^{-1}$ close to Glavatićevo (Tab. 1).

We fitted a simple linear model to predict discharge (Q, in $\frac{m^3}{s}$) from the catchment area (A, in km^2) (R^2 = 0.91, with a proportional factor of $10^{-8}\frac{m^3}{s\ km^2}$) at any location along the whole investigated river section. Additionally, we used measured hydromorphological variables to fit hydraulic scaling relationships using a linear model with log-transformed variables. We used the root mean square error (RMSE) between model predictions and the measured hydromorphological measurements to compare model parameterisations from our fit and parameters reported in literature (Raymond et al. 2012; Ceola et al. 2014) (Tab. 2). While model parameters reported in Raymond et al. (2012) are derived from a large global dataset and are consequently generalised to predict hydromorphological variables at a larger scale, our parameters, along with parameters from Ceola et al. (2014), were obtained by fitting a single river network. Values for estimated exponents from our study are quite close to values reported from Ceola et al. (2014), who investigated the Ybbs catchment in Austria, which is dominated by calcareous and dolomitic limestone and karstic terrain in the higher mountainous areas. Similarities in our model parameters to parameters from Ceola et al. (2014) can be explained by geological and hydrological similarities of the two catchment areas. Lastly, we used model parameters obtained in this study to compute flow velocity and channel depth along the whole study section.

In an ensemble-modelling approach, we used Q, d and v from our models, in combination with river slope extracted from the DEM, to estimate k_{600} for the whole investigated river section and to identify reaches with high vs. low gas transfer potential (Fig. 3). k_{600} was spatially heterogeneous along the investigated river section, with a high dynamic range (CV of 94 %). This is largely due to variation of slope and results in a few reaches of very high gas transfer velocity (> 100 m d^{-1}), of which two reaches had k_{600} even above 150 m d^{-1}. Compared to these steep reaches, gas transfer in flatter, slower flowing sections was approximately 100 times lower (lowest value of 1.7 m d^{-1}), resulting in a range of k_{600} spanning three orders of magnitude. Mean k_{600} at our sampling sites was 37.6 ± 35.5 m d^{-1}, with a distribution slightly skewed towards higher values (median of 28.8 m d^{-1}) and a maximum value of 164.5 m d^{-1}.

Table 1. Measured hydromorphological variables along the main stem of the Neretva River. Site 1.0 is directly after the confluence with the Krupac tributary and discharge is computed from the mass balance of both tributaries. Depth and flow velocity are reported as mean with standard deviation from a sample size of 15 to 43 measurements.

Tabela 1. Izmerjene hidromorfološke spremenljivke vzdolž glavnega toka reke Neretve. Mesto 1.0 je tik pod sotočjem s pritokom Krupac. Pretok je tu izračunan kot seštevek obeh pritokov. Globina in hitrost toka sta navedeni kot povprečje s standardnim odklonom iz vzorca 15 do 43 meritev.

Tabela 1. Izmjerene hidromorfološke varijable duž glavnog toka rijeke Neretve. Mjesto 1.0 je odmah nakon ušća sa pritokom Krupac i protok je izračunat iz balansa mase obe pritoke. Dubina i brzina toka prikazani su kao prosjek sa standardnom devijacijom iz uzorka veličine od 15 do 43 mjerenja.

Site	Latitude	Longitude	Q ($m^3 s^{-1}$)	Depth (m)	Flow velocity ($m s^{-1}$)
0	43.32240	18.43400	0.50	0.30 ± 0.21	0.30 ± 0.21
1	43.32942	18.42574	0.91	0.27 ± 0.18	0.36 ± 0.18
2	43.36523	18.36999	0.88	0.34 ± 0.36	0.55 ± 0.36
3	43.37887	18.35621	0.81	0.52 ± 0.13	0.22 ± 0.13
4	43.40527	18.32304	1.04	0.54 ± 0.21	0.26 ± 0.21
5	43.42414	18.30837	0.99	0.87 ± 0.32	0.41 ± 0.32
6	43.45800	18.32121	1.04	0.53 ± 0.40	0.40 ± 0.40
7	43.48435	18.27429	5.92	0.59 ± 0.26	0.59 ± 0.26
8	43.48227	18.22665	6.05	0.55 ± 0.22	0.67 ± 0.22
10	43.52945	18.08061	12.27	0.55 ± 0.44	0.59 ± 0.44

Dissolved methane (CH_4) concentrations and fluxes

We identified a single hotspot of high CH_4 concentration in an exceptionally large pool in the section upstream of Ulog (Fig. 4 A). All three measurements taken directly in the pool revealed elevated CH_4 concentrations (0.84, 0.54 and 0.63 µmol L^{-1}) compared to measurements in flowing sections. Notably, methane concentration after the turbulent section immediately following the pool was an order of magnitude lower than in the pool (0.087 µmol L^{-1}). Such spatial clustering of high CH_4 concentrations was previously observed in stream reaches enriched in organic matter and lake transitions, in combination with elevated abundance of methanogens (Crawford et al. 2017). In fact, across various types of streams, highest CH_4 concentrations were reported downstream of eutrophic lakes (Crawford & Stanley 2016). This indicates that a change in flow conditions with a resulting longer residence time and an increased trapping of organic material creates an environment prone to methanogenesis, compared to turbulent and free-flowing river sections.

Mean methane concentration along the entire investigated river section was 0.05 ± 0.15 µmol L^{-1}, with a distribution highly skewed towards lower values (median 0.005 µmol L^{-1}). With the exception of measurements associated with one large natural pool, these concentrations are still mostly above saturation but unexpectedly low for the highly vegetated area that likely generates substantial input of organic carbon (with mean DOC of 0.42 ± 0.08 mg L^{-1} in the study reach; Del Campo et al., this issue). In fact, CH_4 concentrations were within the range reported from low-productivity Alpine headwater streams with poorly developed soils and low DOC (Flury & Ulseth 2019) and other low-order streams (Stanley et al. 2016; Wallin et al. 2018). 12% of our measured values were even undersaturated in terms of CH_4 (given a mean measured atmospheric concentration of 2.07 ± 0.11 ppm), turning the river into a local sink of atmospheric CH_4. The respective locations were evenly spread along the whole study section.

Table 2. Parameters of power law relationships for hydraulic scaling of this study, from Raymond et al. (2012) and from Ceola et al. (2014). The latter fitted w, d and v separately and additionally by constraining the sum of the exponents equal to one (labelled as c). Also reported here are the root mean square error (RMSE) between model prediction (after application of the respective function on our data) and observed data (i.e. the site-mean of respective measurements).

Tabela 2. Parametri razmerij moči za hidravlično skaliranje te študije, vzeti iz Raymond et al. (2012) in Ceola et al. (2014). Slednji so prilagajali w, d in v ločeno in dodatno z omejevanjem vsote eksponentov je enako ena (označeno kot c). V tem primeru je zabeležen tudi koren srednjega kvadrata napake (RMSE) med napovedjo modela (po uporabi ustrezne funkcije na naših podatkih) in opaženimi podatki (tj. povprečjem meritev na posameznem mestu).

Tabela 2. Parametri zakona moći za hidrauličko skaliranje ove studije, iz Raymond et al. (2012) i iz Ceola et al. (2014). Ovi posljednji su prilagodili w, d i v zasebno i dodatno ograničavajući zbir eksponenata da bude jednak jedan (označeno kao c). Također su prikazane greške srednjeg kvadrata (RMSE) između modelne predikcije (nakon primjene odgovarajuće funkcije na naše podatke) i zabilježenih podataka (tj. srednjeg mjesta odgovarajućih mjerenja).

	c_d	e_d	$RMSE_d$	c_v	e_v	$RMSE_v$
This study	-0.804	0.133	0.16	-1.025	0.257	0.09
Raymond et al. (2012)	0.4	0.29	1.43	0.19	0.28	1.11
Ceola et al. (2014)	-1.031	0.285	0.20	-0.935	0.317	0.13
Ceola et al. (2014) (c)	-1.156	0.274	0.21	-1.232	0.288	0.12

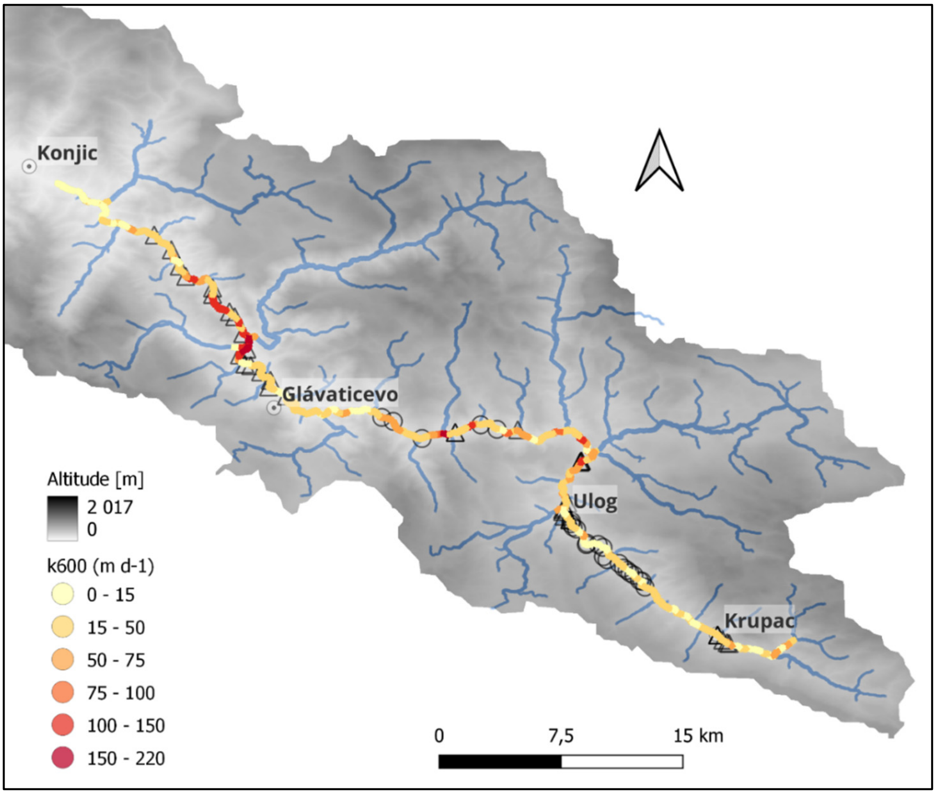

Figure 3. Modelled k_{600} for the whole investigated river section. Sampling sites for discrete gas samples and important locations are shown for orientation.

Slika 3. Modelirani k_{600} za celotni preučevani odsek reke. Za orientacijo so prikazana vzorčišča za posamične vzorce plina in pomembne lokacije.

Slika 3. Modelovani k_{600} za cijeli istraženi dio rijeke. Za orijentaciju su prikazana mjesta uzorkovanja za diskretne uzorke plina i važne lokacije.

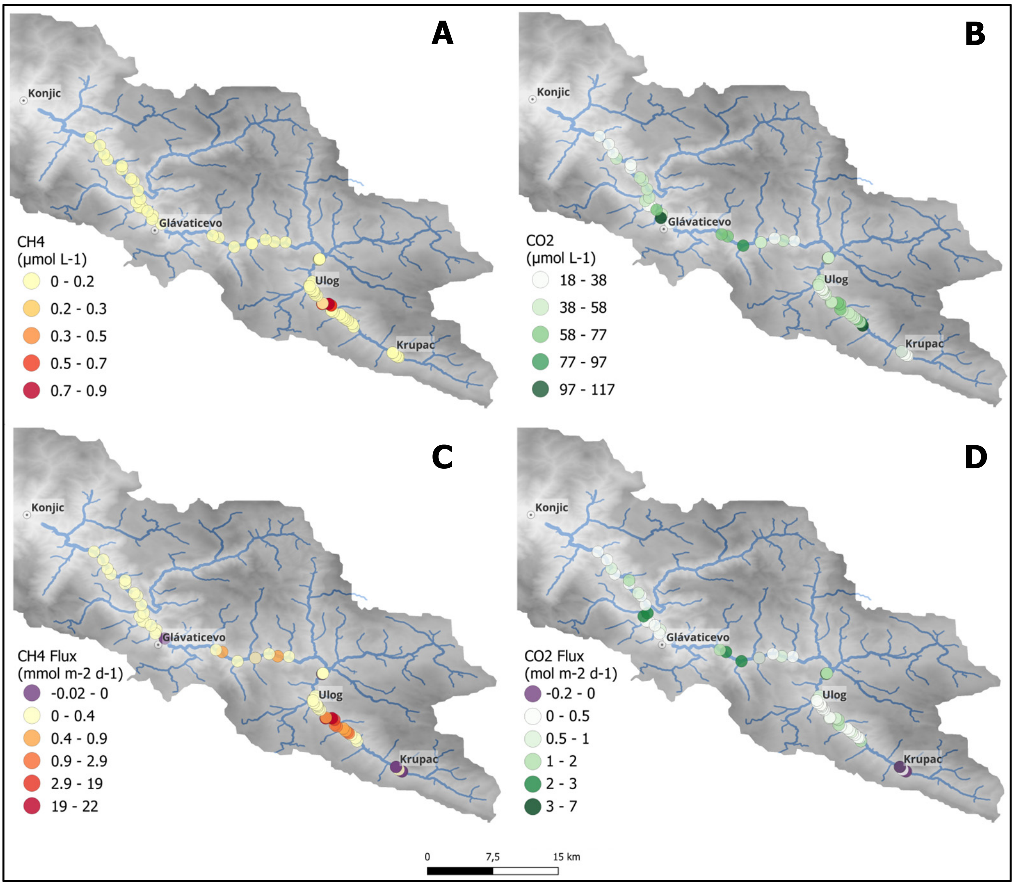

Figure 4. Spatial distribution of CH_4 concentration in µmol L^{-1} (A) and CH_4 Flux (C) as well as CO_2 concentration in µmol L^{-1} (B) and CO_2 Flux (D) along the investigated study section. Locations where the river acted as a sink of atmospheric CH_4 or CO_2 are highlighted as purple dots. Important locations are shown for orientation. Note: CH_4 Flux is given in mmol m^{-2} d^{-1}, while CO_2 Flux in mol m^{-2} d^{-1}.

Slika 4. Prostorska distribucija koncentracije CH4 v µmol L-1 (A) in toka CH_4 (C) ter koncentracije CO_2 v µmol L-1 (B) in toka CO_2 (D) vzdolž preučevanega odseka. Lokacije, kjer je reka delovala kot ponor atmosferskega CH_4 ali CO_2, so označene kot vijolične pike. Za orientacijo so prikazane pomembne lokacije. Opomba: Tok CH_4 je naveden v mmol m^{-2} d^{-1}, medtem ko je tok CO_2 v mol m^{-2} d^{-1}.

Slika 4. Prostorna distribucija koncentracije CH_4 u µmol L-1 (A) i flux CH_4 (C) kao i koncentracija CO_2 u µmol L-1 (B) i flux CO_2 (D) duž istraživanog dijela studije. Mjesta gdje se rijeka ponašala kao ponor atmosferskog CH_4 ili CO_2 označena su ljubičastim tačkama. Za orijentaciju su prikazane važne lokacije. Napomena: CH_4 Flux je dat u mmol m^{-2} d^{-1}., dok je CO_2 Flux u mol m^{-2} d^{-1}.

Our assessment of CH_4 concentration is based on grab samples collected at arbitrary times of the day. This may bias our data given the potential temporal variability and limit comparability across sites. To address this issue, we collected samples over a 24-hour time series at three sites. A clear diurnal cycle was found for CH_4 only at the most downstream of those monitoring sites (Site 10, Fig. 5A), with the highest variation in concentration: temporal coefficients of variation were 35% at Site 10 compared to 26% and 14% at Site 1 and 5, respectively. The highest CH_4 concentration at Site 10 was recorded during morning hours and coincided with lowest temperature and thus highest gas solubility. In fact, the maximum concentration difference at Site 10 was 0.14 µmol L^{-1} (with 0.08 and 0.22 µmol L^{-1} as minimum and maximum values respectively). However, excluding higher concentrations measured at nighttime, when no discrete sampling was done anywhere else, the maximum concentration difference at this site is reduced to 0.04 µmol L^{-1} and the temporal coefficient of variation is reduced from 35% to 16%. For upstream Sites 1 and 5, CH_4 concentration was stable throughout the whole day with mean values of 0.02 ± 0.005 and 0.05 ± 0.007 µmol L^{-1}. Such absence of a strong daily variation in methane concentrations indicates that our grab sample-based measurements are representative even though samples were collected at different daytimes. For further downstream sites, our data suggest caution may be appropriate for estimates of daily mean concentration and evasion fluxes, which may be underestimated given our daytime sampling. However, as all our grab samples were collected during a daytime time window, for which only limited temporal variability could be observed across all sites, we are confident that our data allows unbiased assessment of spatial variation over the whole study section.

Both temporal and spatial variation in CH_4 evasion are strongly correlated to dissolved CH_4 concentration, but not to the gas transfer velocity (Tab. 3). Correlation to concentration becomes even more dominant when data with temporal variation at the three selected monitoring sites are used. Here, minor changes in k_{CH4} result only from changes in water temperature. For spatially variable data, we found ΔC_{CH4} to be the main driver for methane evasion (with R^2 of 0.74 in a simple linear regression), while the river morphology (hence the gas transfer coefficient) had only a minor influence on the amount of gas flux ($R^2 < 0.01$, Fig. 6A). In line with this result, in a multiple linear regression using log-transformed variables, 74% of the variation of CH_4 evasion flux could be attributed to the concentration gradient, but only 16% to the spatially varying gas transfer coefficient. This indicates supply limitation for CH_4 flux in our study system and is further confirmed by CH_4 evasion being at least ten times more variable in space (CV of 353%) than in time (CV of 28, 14 and 33% for Sites 1, 5 and 10 respectively).

The highest evasion flux (22 mmol m^{-2} d^{-1}) was computed at the transition from the large pool with high concentrations to the following turbulent section (Fig. 4C). This is a result of high supply (from CH_4 produced in the pool) and high transfer potential in the downstream flowing section. High CH_4 concentrations are however restricted locally to a couple of hundred meters after their source due to rapid oxidation and/or rapid outgassing. These results are consistent with reported measurements of spatially distinct CH_4 peaks resulting from groundwater input with rapid decline along the consecutive 150 m (Lupon et al. 2019). Additionally, the river can locally act as a sink of atmospheric CH_4 (Fig. 4C).

Mean CH_4 flux to the atmosphere in our study area (1.15 ± 4.06 mmol m^{-2} d^{-1}, computed including sites acting as sinks as well as sources) is within the range reported from mountain headwater streams (Crawford et al. 2015; Stanley et al. 2016; Flury & Ulseth 2019). However, using the mean flux to upscale methane evasion for the whole study area would result in an overestimation of evasion from the system, as high fluxes related to the large pool greatly contribute to a high mean compared to the median CH_4 flux (0.06 mmol m^{-2} d^{-1}).

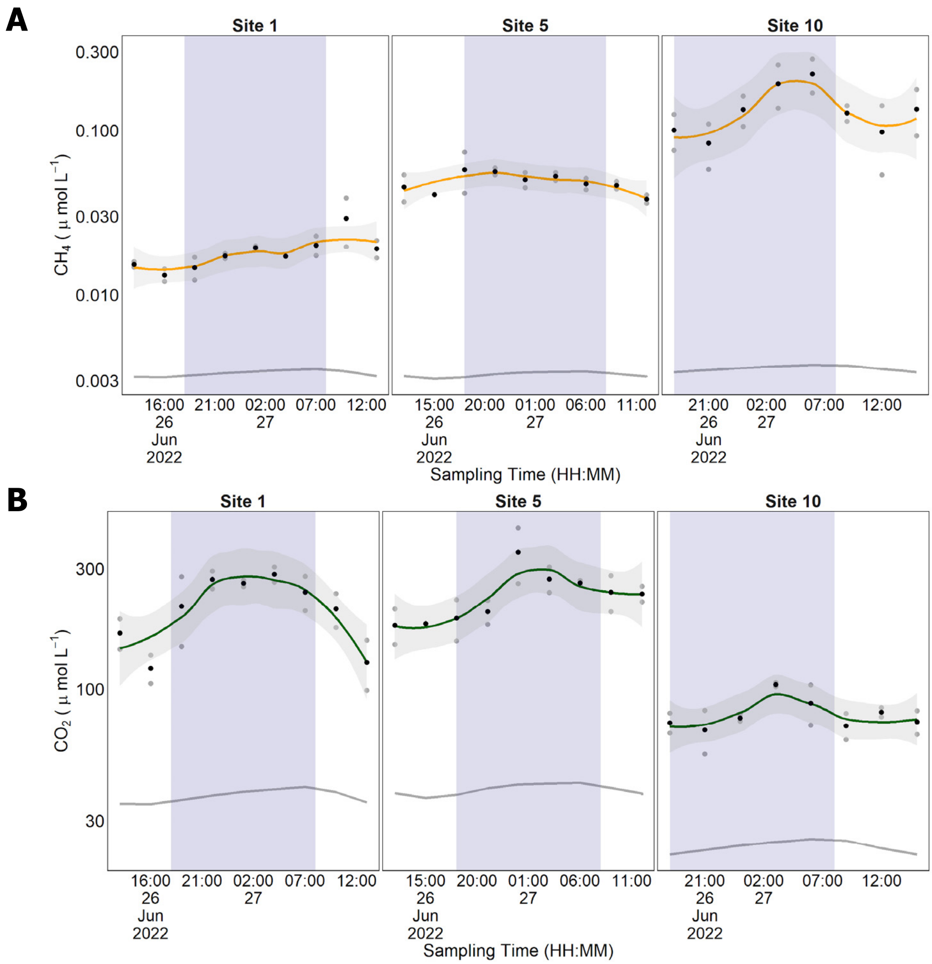

Figure 5. Diurnal concentration trend for CH_4 (A) and CO_2 (B) for Sites 1, 5 and 10. Gray dots represent individual measurements. Black dots represent the average of measurements taken in duplicate. The orange line and green line represent the smoothed trend line using a polynomial regression and the gray area represents the 95% confidence interval of the regression model. The gray line represents the equilibration concentration computed from the atmospheric background and the water temperature. Nighttime, when no samples were taken for discrete gas sample analysis along the continuum, is shaded in blue. Note: The y-axes are in logarithmic scale.

Slika 5. Dnevni trend koncentracije za CH_4 (A) in CO_2 (B) na lokacijah 1, 5 in 10. Sive pike ponazarjajo posamezne meritve, črne pa povprečje podvojenih meritev. Oranžna in zelena črta ponazarjata gladko trendno črto z uporabo polinomske regresije, sivo območje pa 95-odstotni interval zaupanja regresijskega modela. Siva črta predstavlja ravnotežno koncentracijo, izračunano iz atmosferskega ozadja in temperature vode. Nočni čas, ko ni bilo odvzetih vzorcev plina za analizo vzdolž kontinuuma, je modro osenčen. Opomba: osi y so v logaritemski skali.

Slika 5. Dnevni trend koncentracije za CH_4 (A) i CO_2 (B) za mjesta 1, 5 i 10. Sive tačke predstavljaju pojedinačna mjerenja, a crne tačke predstavljaju prosjek mjerenja uzetih u dupliciranim uzorcima. Narandžasta linija i zelena linija predstavljaju izglađenu trend liniju koristeći polinomsku regresiju, a siva oblast predstavlja 95% interval povjerenja regresijskog modela. Siva linija predstavlja koncentraciju ekvilibracije izračunatu iz atmosferske pozadine i temperature vode. Noćno vrijeme, kada se nisu uzimali uzorci za analizu diskretnog plinskog uzorka duž kontinuma, je označeno plavom bojom. Napomena: Y-ose su na logaritamskoj skali.

Dissolved carbon dioxide (CO_2) concentrations and fluxes

Distribution of CO_2 concentration was less skewed towards one side, with mean CO_2 values of 46.4 ± 20.3 and a median of 40.6 μmol L^{-1} (Fig. 4B). Maximum measured CO_2 concentration was 117 μmol L^{-1} and only 7% of measured concentrations were undersaturated compared to the atmosphere (given a mean measured atmospheric concentration of 515 ± 30 ppm). Sites with CO_2-undersaturation were all measured at the upstream end of the study section close to the confluence with the Krupac tributary. Additionally, turbulent sections (with $k_{600} \geq 100$ m d^{-1}) generally had lower CO_2 concentrations (maximum of 42 μmol L^{-1}), while less turbulent sections did support a broad range of concentrations (with a variation of 7% with a mean of 40 ± 3 μmol L^{-1} for turbulent and 45% with a mean of 47 ± 21 μmol L^{-1} for less turbulent sections). These findings are supported by aggregated data from a worldwide stream water chemistry data set (Hartmann et al. 2014), where CO_2 concentration was always low at high k_{600} but showed large variations at lower gas transfer velocities.

Table 3. Spearman correlation (and asymptotic p-values) of spatial and temporal variations between evasion flux F_i, stream concentration and gas specific exchange velocities. The i stands for CH_4 for correlation with C_{CH4} and k_{CH4} and for CO_2 for correlation with C_{CO2} and k_{CO2}. For spatial correlation, discrete measurements taken at arbitrary points in time are considered (n=60). For temporal correlation, measurements taken at the three sites of continuous monitoring over 24 hours (each with n=9) are independently analysed and we report mean and standard deviation of correlation coefficients. Changes in k_{CH4} and k_{CO2} on the temporal scale result only from changes in water temperature and were always insignificant.

Tabela 3. Spearmanova korelacija (in asimptotske p-vrednosti) prostorskih in časovnih variacij med tokom emisij Fi, koncentracijo toka in specifičnimi hitrostmi izmenjave plina. i označuje CH4, korelacijo s C_{CH4} in k_{CH4} ter CO_2 za korelacijo s C_{CO2} in k_{CO2}. Pri prostorski korelaciji smo upoštevali posamezne meritve, opravljene ob poljubnem času (n=60). Pri časovni korelaciji smo neodvisno analizirali meritve, opravljene na treh mestih neprekinjenega spremljanja v 24 urah (vsaka z n=9), tu poročamo o povprečju in standardnem odklonu korelacijskih koeficientov. Spremembe v k_{CH4} in k_{CO2} skozi čas so zgolj posledica sprememb v temperaturi vode in so bile vselej neznačilne.

Tabela 3. Spearmanova korelacija (i asimptotske p-vrijednosti) prostornih i vremenskih varijacija između fluxa izbjegavanja Fi, koncentracije toka i specifičnih brzina razmjene gasa. »I« predstavlja CH_4 za korelaciju sa C_{CH4} i k_{CH4} i za CO_2 za korelaciju sa C_{CO2} i k_{CO2}. Za prostornu korelaciju, razmatraju se diskretna mjerenja uzeta u proizvoljnim vremenima (n=60). Za vremensku korelaciju, mjerenja uzeta na 3 mjesta kontinuiranog praćenja tokom 24 sata (svaka sa n=9) se analiziraju nezavisno i prijavljujemo srednju vrijednost i standardnu devijaciju koeficijenata korelacije. Promjene u k_{CH4} i k_{CO2} na vremenskoj skali rezultiraju samo promjenama u temperaturi vode i uvijek su bile nebitne.

		C_{CH4}	k_{CH4}	C_{CO2}	k_{CO2}
Spatial	Corr. Coeff with F_i	0.84	0.08	0.55	0.60
	p	0.00	0.57	0.00	0.42
Temporal	Corr. Coeff with F_i	0.96 ± 0.04	-0.34 ± 0.21	0.96 ± 0.05	-0.57 ± 0.34

At all three sites where gas samples were taken over 24 hours, we found diurnal fluctuations in CO_2 (Fig. 5B), with higher concentrations during nighttime when photosynthesis is absent and respiration prevails. The most downstream site (Site 10) experienced the lowest diurnal fluctuation in CO_2, which might be a result of greater dilution of the benthic primary production signal by the 10-fold higher discharge compared to upstream sites. CO_2 concentration over the investigated time period varied for 29%, 23% and 15% for Sites 1, 5 and 10, respectively, which equals a maximum concentration difference of 164, 170 and 35 μmol L^{-1}. If we consider variation and maximum concentration difference during the restricted sampling window for discrete gas samples (from 08:00 to 18:00) the variation decreases to 26%, 15% and 6% for Sites 1, 5 and 10, respectively and maximum concentration difference is reduced to 86, 63 and 9 μmol L^{-1}. These results indicate that our data supports comparisons of CO_2 concentrations and fluxes across sites of the entire study section, yet daily mean values will suffer from systematic underestimation due to high nighttime CO_2 concentrations.

Evasion of CO_2 was dependent on both ΔC_{CO2} and k_{600}, with R^2 in a simple linear regression of 0.31 and 0.36, respectively (Fig. 6B). In a multiple linear regression using log-transformed variables, 38% of the spatial variation of CO_2 evasion flux could be attributed to the concentration gradient and 62% to the gas transfer coefficient. Compared to spatial variation, temporally variable CO_2 evasion at the three selected sites was strongly correlated to the dissolved concentration rather than to the gas transfer velocity (Tab. 3). This is likely due to high daily variations in the dissolved gas concentration, while on a temporal scale, k_{CO2} changed only due to changes in temperature. Nevertheless, CO_2 evasion was four times more variable in space (CV of 136%) compared to temporal variations at any of the selected three sites (CV of 32, 25 and 18% for Sites 1, 5 and 10 respectively), suggesting external CO_2 point sources and/or locally enhanced mineralization rates as drivers of elevated evasion rates.

Mean computed CO_2 flux among all sampling sites was 0.77 ± 1.04 mol m^{-2} d^{-1} (equal to 9.2 ± 12.5 g_C m^{-2} d^{-1}), with a median of 0.4 and a maximum flux of 6.44 mol m^{-2} d^{-1} (corresponding to 4.8 and 77.3 g_C m^{-2} d^{-1}, respectively). This high flux is a result of the maximum measured CO_2 concentration (117 µmol L^{-1}) spatially co-occurring with an above average k_{600} value (69 m d^{-1}). Although the mean CO_2 evasion is within the range reported from boreal and arctic streams (Lupon et al. 2019; Rocher-Ros et al. 2019), our maximum exceeds the evasion reported from those systems. Uptake of atmospheric carbon, due to undersaturation of the water phase was negligible in our study section.

Conclusion: Hydromorphological implications on GHG concentration and fluxes

Contrary to expected we did not detect any proof for point source input of CH_4 due to groundwater inflow from karstic springs. Influence of point source input on CO_2 concentration cannot be excluded. However, sites with elevated dissolved CO_2 values could not be linked to karstic flow paths. As a result, we found no indication of karstic inputs to dominate GHG concentration and flux patterns in the river continuum in its current pristine form.

We acknowledge that our concentration measurements and computed fluxes represent snapshot estimates taken during hydraulic base flow conditions present during our field campaign in July 2022. A change in flow conditions and extreme hydrological events, can drastically alter the nature and magnitude of GHG emissions from water bodies (Johnson et al. 2007; Dinsmore & Billett 2008). We note that such event-driven pulses cannot be captured with our approach.

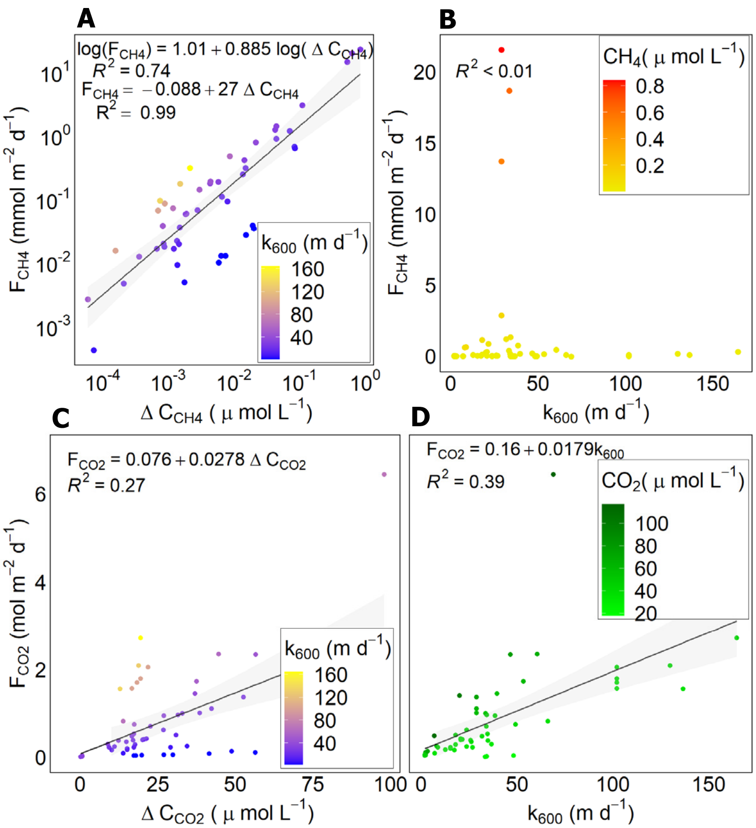

Figure 6. Relationship between CH_4 evasion rate (in mmol m^{-2} d^{-1}) to ΔC_{CH4} (A) and to k_{600} (B) and between CO_2 evasion rate (in mol m^{-2} d^{-1}) to ΔC_{CO2} (C) and to k_{600} (D) in a simple linear regression. ΔC is computed as the difference between dissolved and atmospheric concentration. The black lines represent a linear regression with the gray area showing 95% confidence intervals for the linear model. Fitting parameters are found in the model equation of respective plots. Note: the axes of plot A have a logarithmic scale and model equations for F_{CH4} as function of C_{CH4} are given for log-transformed and non-log transformed values.

Slika 6. Razmerje med stopnjo izparjevanja CH_4 (v mmol m^{-2} d^{-1}) do ΔC_{CH4} (A) in do k_{600} (B) in med stopnjo izparjevanja CO_2 (v mol m^{-2} d^{-1}) do ΔC_{CO2} (C) in do k_{600} (D) v enostavni linearni regresiji. ΔC je izračunan kot razlika med raztopljenimi in atmosferskimi koncentracijami. Črne črte ponazarjajo linearno regresijo, siva območja pa prikazujejo 95-odstotni interval zaupanja za linearni model. Parametre prileganja najdemo v modelni enačbi ustreznih grafikonov. Opomba: osi grafa A so v logaritemski skali, modelne enačbe za F_{CH4} kot funkcijo C_{CH4} pa so podane za logaritemsko transformirane in ne-transformirane vrednosti.

Slika 6. Odnos između stope izbjegavanja CH_4 (u mmol m^{-2} d^{-1}) do ΔC_{CH4} (A) i do k_{600} (B) i između stope izbjegavanja CO_2 (u mol m^{-2} d^{-1}) do ΔC_{CO2} (C) i do k_{600} (D) u jednostavnoj linearnoj regresiji. ΔC je izračunat kao razlika između rastvorene i atmosferske koncentracije. Crne linije predstavljaju linearnu regresiju sa sivom oblasti koja pokazuje 95 % interval povjerenja za linearni model. Parametri prilagođavanja nalaze se u modelnoj jednačini odgovarajućih plotova. Napomena: ose zapleta A su na logaritamskoj skali i modelne jednačine za F_{CH4} kao funkciju C_{CH4} su date za logaritamski transformirane i netransformirane vrijednosti.

Rather than CH_4 originating from groundwater inputs, we found indications that CH_4 is locally produced if conditions allow. Although these two origins are not mutually exclusive, we found a single CH_4 hotspot in an exceptionally large pool upstream of Ulog, where CH_4 concentrations were at least an order of magnitude higher than the measured median for the whole river section. In the pool, standing water and the concomitant increase in residence time in combination with higher sediment and organic matter accumulation likely create a habitat favorable for CH_4 production. As a result of constant CH_4 supply from the pool and high gas transfer velocity due to high water turbulence, evasion of CH_4 was four orders of magnitude higher in the turbulent section immediately following the pool (22 mmol m^{-2} d^{-1} compared to median evasion at network scale of 0.06 mmol m^{-2} d^{-1}). Additionally, CH_4 concentration after this turbulent section was an order of magnitude lower than in the pool, suggesting spatially restricted high outgassing rates, potentially supported by fast CH_4 oxidation. As a result of this single CH_4 hotspot, variation of emissions was an order of magnitude higher in space (353%) than in time (max variation of 33%). On the river network scale, this resulted in the CH_4 concentration to be the critical determinant for spatial variation in CH_4 emissions. The river morphology (hence the gas transfer coefficient) had only little influence on the magnitude of CH_4 flux, indicating a strong supply limitation for CH_4 flux in our study system. In contrast, temporal variation in CO_2 concentration was higher at a diurnal time scale and CO_2 concentration varied widely in space but with less pronounced concentration hot spots. Additionally, sections with high turbulence featured generally lower CO_2 concentrations, while less turbulent sections supported a wider concentration range (with a variation of 7% for turbulent and 45% for less turbulent sections). Spatial variability of CO_2 concentration can originate from external CO_2 and/or heterogeneous sources or accumulation of organic matter, which lead to enhanced mineralisation rates, or from local conditions which favour higher photosynthesis rates. Our results indicate that high CO_2 evasion is due to a combination of both underlying parameters (k_{600} and ΔC_{CO2}) but turbulent reaches can be limited in supply, as the absence of high C_{CO2} in those reaches can be a result of supply rates being lower than evasion rates.

River morphology influences gas transfer potential and although in our study section it had little influence on the magnitude of CH_4 flux, there is no limitation for potential outgassing due to transfer limitation (high dynamic range of k_{600}). However, hydromorphological features play an important role in the creation of environments favoring CH_4 production. Additionally, a change in the flow regime from flowing to standing water also increases possibilities for additional emission pathways (Crawford et al. 2014). Not accounting for these can lead to an underestimation of CH_4 flux of certain features of the river. Converting a free-flowing river reach into a standing water reservoir does not only lead to production and emission of CH_4 instead of CO_2, but also to an activation of additional emission pathways besides diffusion (Maeck et al. 2013; Yang et al. 2014; McGinnis et al. 2016). Specifically, CH_4 ebullition, which can contribute to 80% of emissions in a dammed river (McGinnis et al. 2016) and emissions from periodically inundated riparian vegetation areas must therefore also be considered. These additional emissions are sustained by the trapping of organic material in the reservoir reaches (Maeck et al. 2013). In fact, direct comparison between riverine and reservoir reach revealed the latter to be the major methane source and could directly link these emissions to sediment accumulation even for small dams (Maeck et al. 2013). In alignment with our own findings of high CH_4 fluxes in the turbulent reach immediately downstream of the CH_4-concentration hotspot, reservoirs are known to support high CH_4 fluxes in post-turbine flowing water sections, especially when deeper water bodies are released and brought into contact with the atmosphere (Yang et al. 2014). These mechanisms must be expected to produce higher CH_4 emissions from the Ulog hydropower plant that is currently under construction in the center of our investigated river section.

Povzetek

S kombinacijo meritev vodnega prostora in uporabo sistema za uravnoteženje, ki smo ga izdelali sami, smo lahko določili prostorsko heterogenost koncentracije CH_4 in CO_2 vzdolž 50 km dolgega odseka neokrnjenega rečnega sistema zgornjega toka reke Neretve v Bosni in Hercegovini. Ta odročni del rečnega sistema do sedaj skorajda ni bil izpostavljen človekovim posegom in je, za zdaj, hidromorfološko še vedno nespremenjen v prvotnem naravnem stanju. Za oceno emisij plinov iz lokalnih meritev koncentracije plinov in za oceno dejavnikov zadevnih emisijskih tokov smo uporabili skalirne modele celotnega omrežja za hidromorfološke parametre v kombinaciji z naklonom in povodjem, pridobljenimi iz digitalnega modela višin. Te smo uporabili za izračun hitrosti prenosa plinov. Vzdolž celotnega raziskanega odseka reke smo našli eno žarišče povišane koncentracije CH_4. Visoka koncentracija najverjetneje izvira iz lokalnega nastajanja CH_4, ki je posledica hidromorfoloških razmer, kot je počasi tekoča oziroma stoječa voda, v kateri se kopiči organski material. Tej vroči točki nastajanja CH_4 je sledil turbulenten odsek reke, ki je omogočil visoke tokove izločanja plina. Naši rezultati kažejo, da je izločanje CH_4 v reki Neretvi omejeno z viri. Nasprotno so za izločanje CO_2 odgovorni drugi osnovni mehanistični procesi, ki so bolj spremenljivi v času in prostoru. Na več mestih v preučevanem rečnem odseku se je izločanje CO_2 povečalo, ko sta bila tako k_{600} kot ΔC_{CO2} visoka, kar nakazuje, da ima omejitev prenosa pomembnejšo vlogo poleg oskrbe. Zlasti pri oceni emisij CO_2 dodatno oviro povzroča časovna variabilnost, ki lahko privede do podcenjevanja emisij, če se vzorci jemljejo le podnevi. Naši rezultati tudi poudarjajo pomen diskontinuitete kontrolnih faktorjev za tokove emisij toplogrednih plinov. Hipoteza rečnega kontinuuma, ki ustvarja predvidljiv gradient tokov emisij CO_2 ali CH_4, torej ni podprta v naravni reki, kot je Neretva. Visoki emisijski tokovi katerega koli toplogrednega plina so odvisni od sočasnega pojava fizične priložnosti, ki jo poganja geomorfologija, specifičnih procesov nastajanja toplogrednih plinov ter njihovih virov - habitatov, bogatih z nakopičenim organskim materialom. Kraški izviri bi lahko bili tak vir organskega materiala, ki bi se nadalje presnavljal v reki. Vendar nismo našli znakov, da bi kraški vnosi vplivali na koncentracijo toplogrednih plinov in vzorce pretoka zgornjega toka reke Neretve.

Na splošno lahko rečemo, da emisije toplogrednih plinov preučevanega rečnega odseka trenutno nimajo velikega vpliva na globalno segrevanje. V svojem skoraj naravnem stanju preučevani odsek reke Neretve izpušča večinoma nizke količine CO_2. Edini pomemben vir CH_4 je velik bazen, za katerega je značilna stoječa voda. Projekti hidroelektrarn, načrtovani na tem območju, bi spremenili pogoje pretoka iz tekočih odsekov v velike bazene stoječe vode s podaljšanim časom zadrževanja in kopičenjem organskega materiala. Tudi manjši rezervoarji imajo lahko podobne habitatne pogoje kot opazovana vroča točka CH_4. Posledično pričakujemo višje koncentracije CH_4 in višje emisije CH_4 iz takšnih umetnih, lentičnih okolij kot tudi iz postturbinskih odsekov neposredno nizvodno, kjer se višje hitrosti izmenjave plinov ujemajo z višjimi koncentracijami. Zaradi 28-krat večjega potenciala segrevanja CH_4 v primerjavi s CO_2 bi te spremembe drastično spremenile odtis toplogrednih plinov preučevanega odseka reke Neretve. Spreminjanje hidromorfološkega stanja in toka te naravne reke bo prispevalo h globalnemu segrevanju. Z znanstvenega vidika bi bilo glede na biogeokemične posledice nujno ustaviti razvoj hidroelektrarn na zgornji Neretvi, in namesto tega zaščititi ta rečni odsek kot naravni referenčni sistem, ki nam lahko pomaga izboljšati znanje o vlogi rek v svetovnem ciklu ogljika.

Sažetak

Našom kombinacijom mjerenja vodenog prostora i mjerenja pomoću samostalno izgrađenog ekvilibracijskog sistema, bili smo u mogućnosti otkriti prostornu heterogenost koncentracije CH_4 i CO_2 duž 50 kilometara gornjeg toka rijeke Neretve, izolovanog riječnog sistema koji je do sada bio pod malim uticajem ljudskih aktivnosti. Da bismo procijenili emisije gasa iz lokalnih mjerenja koncentracije gasa i ocijenili pokretače odgovarajućih emisijskih tokova, koristili smo modele mrežnog skaliranja za hidromorfološke parametre u kombinaciji s nagibom i površinom sliva izračunatim iz digitalnog modela elevacije za izračunavanje brzine prenosa gasa. Duž cijelog istraživanog riječnog dijela, pronašli smo jedno žarište povećane koncentracije CH_4. Visoka koncentracija najvjerojatnije potiče iz in situ proizvodnje CH_4, koja je lokalno favorizovana zbog hidromorfoloških uslova, poput sporotekuće do stajaće vode i akumulacije organskog materijala. Ovom žarištu proizvodnje CH_4 slijedilo je turbulentno područje koje omogućava visoke tokove ispuštanja gasova. Naši rezultati stoga sugerišu da je izostajanje CH_4 u rijeci Neretvi snažno ograničeno snabdjevanjem. Suprotno tome, različiti osnovni mehanistički procesi pokreću izostajanje CO_2, koje se pokazalo više varijabilnim u vremenu i prostoru. Izostajanje CO_2 povećalo se kada su na više mjesta u proučavanoj riječnoj dionici bili visoki i k_{600} i ΔC_{CO2}, sugerišući da ograničenje prenosa igra važniju ulogu pored snabdjevanja Pogotovo pri procjeni emisija CO_2, vremenska varijabilnost je dodatna prepreka i može dovesti do potcjenjivanja tokova kada se uzorci uzimaju samo za vrijeme dnevnih sati. Naši rezultati takođe ističu važnost diskontinuiteta kontrolnih faktora za emisije stakleničkih plinova. Ideja o riječnom kontinumu koji stvara predvidljivi gradijent emisija CO_2 ili CH_4 nije podržana u prirodnoj rijeci poput Neretve. Visoki emisijski tokovi bilo kojeg stakleničkog plina zavise o koegzistenciji fizičke prilike pokretane geomorfologijom, podršci procesima proizvodnje i izvora specifičnih za stakleničke plinova koji uključuju staništa bogata akumuliranim organskim materijalom. Kraški izvori mogu dostaviti organski materijal rijeci koji se dalje metabolizira. Međutim, nismo pronašli nikakve indikacije da kraški ulazi utiču na koncentraciju i uzorke tokova stakleničkih plinova gornjeg toka rijeke Neretve. Sveukupno, možemo reći da su emisije stakleničkih plinova proučavanog riječnog dijela trenutno ograničene u smislu njihovog potencijala globalnog zagrijavanja. U svom gotovo prirodnom stanju, proučavani dio rijeke Neretve uglavnom emituje CO_2 pri niskim vrijednostima. Jedini značajan izvor CH_4 je veliki bazen sa stajaćom vodom. Zanimljivo je, hidroenergetski projekti planirani u tom području promijenili bi uslove toka iz tekućih dijelova u velike bazene stajaće vode s povećanim vremenom zadržavanja i akumulacijom organskog materijala. Zapravo, čak bi i manji rezervoari mogli imati slične uslove staništa kao što je promatrano žarište CH_4. Stoga očekujemo veće koncentracije CH_4 i veće tokove snabdjevanja CH_4 iz takvih umjetnih, lentitskih okruženja, kao i iz dijelova neposredno ispod turbine, gdje se veće brzine razmjene gasova podudaraju s većim koncentracijama. Zbog 28 puta većeg potencijala zagrijavanja CH_4 u odnosu na CO_2, ove modifikacije bi drastično promijenile okvir stakleničkih plinova proučavanog dijela rijeke Neretve. Promjena hidromorfološkog stanja i toka ove prirodne rijeke doprinijeće će globalnom zagrijavanju. Sa naučnog stajališta, zaustavljanje izgradnje hidroelektrana na gornjoj Neretvi je čin odgovornosti s obzirom na potencijalne biogeohemijske implikacije. Umjesto toga, bolje bi bilo zaštititi ovaj dio rijeke kao prirodni referentni sistem koji nam može pomoći da poboljšamo naše znanje o ulozi rijeka u globalnom ciklusu ugljenika.

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Drone shots overlooking the Neretva Valley. Top: upstream from Ulog; bottom; close to Glavatičevo. Photos: Vladimir Tadić.

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Nutrient inputs shape ecosystem functioning gradients along the pristine, upper Neretva River, Bosnia and Herzegovina

Rubén DEL CAMPO*, Barbara JECHSMAYR, Veronika SETTLES, Melanie STRÖDER, Gabriel SINGER

Department of Ecology, Fluvial Ecosystem Ecology, University of Innsbruck, Austria
*Corresponding author. E-mail: ruben.del-campo@uibk.ac.at

Abstract. Ecosystem functions are the backbone of ecosystem services that rivers provide to human societies. Ecosystem functioning emerges from the interaction between biological communities and their environment. As environmental conditions in rivers change along their longitudinal continuum, so does functioning. Sometimes, these changes do not follow smooth gradients but rather great discontinuities. This can be the case in calcareous, karstic rivers due to the sudden massive inputs of groundwater along the landscape, a typical phenomenon for Balkan rivers. Despite their high geodiversity and their great ecological value, Balkan rivers remain understudied. Here, we investigated how ecosystem functions and their diversity (estimated as multifunctionality) change along the continuum of the karstic, free-flowing Neretva River in Bosnia and Herzegovina. For this purpose, we measured a subset of fundamental ecosystem functions (ecosystem gross primary production, biofilm net primary production and enzymatic activities, organic matter decomposition) in 11 river reaches from the Neretva headwaters to river sections upstream of the Jablanica reservoir. We found different functions reached their maximum in different sections of the Neretva depending on nutrient inputs. While organic matter decomposition was highest in headwaters due to the input of nutrients from riparian vegetation, biofilm enzymatic activity expressed highest values at middle sections due to groundwater inputs of NH_4^+-N. Primary production was highest at the most downstream sections due to the accumulation of NO_3^--N and PO_4^{3-}-P within the catchment area. As a result, average multifunctionality peaked at sites with the highest nutrient concentration across the Neretva river continuum, indicating a stronger influence of nutrient inputs than network position. The pristine conditions of the Neretva result in oligotrophic conditions along its upper course. Our results emphasize the great sensitivity of ecosystem functioning in the Neretva to nutrient inputs and environmental discontinuities, either natural or human-made. Potential major, long-term impacts in the area might alter existing environmental gradients and thus ecosystem functioning in rivers at local and regional scale.

Key words: ecosystem functioning, functional diversity, functional indicators, karst, dissolved organic matter, river networks

Izvleček. Vnos hranil oblikuje gradiente delovanja ekosistema vzdolž nedotaknjenega gornjega toka reke Neretve, Bosna in Hercegovina – Ekosistemske funkcije so hrbtenica ekosistemskih storitev, ki jih reke zagotavljajo človeški družbi. Delovanje ekosistema izhaja iz interakcije med biološkimi združbami in njihovim okoljem. Ko se okoljske razmere v rekah spreminjajo vzdolž kontinuuma, se spreminja tudi delovanje ekosistema. Včasih te spremembe niso zvezne, ampak zelo neenakomerne. To se lahko zgodi v apnenčastih, kraških rekah zaradi nenadnega velikega dotoka podzemne vode vzdolž pokrajine, kar je značilen pojav za balkanske reke. Kljub veliki geodiverziteti in veliki ekološki vrednosti so balkanske reke še vedno premalo raziskane. Raziskovali smo, kako se spreminjajo funkcije ekosistemov in njihova raznolikost (ocenjena kot multifunkcionalnost) vzdolž kontinuuma kraške, prosto tekoče reke Neretve v Bosni in Hercegovini. V ta namen smo izmerili podmnožico temeljnih ekosistemskih funkcij (bruto primarno proizvodnjo ekosistema, neto primarno proizvodnjo biofilma in encimske aktivnosti, razgradnjo organske snovi) v enajstih rečnih delih od povirja Neretve do rečnih odsekov gorvodno od akumulacije Jablanica. Ugotovili smo, da so različne funkcije dosegle svoj maksimum v različnih delih Neretve, odvisno od vnosa hranil. Medtem ko je bila razgradnja organske snovi najvišja v povirju zaradi vnosa hranil iz obrežne vegetacije, je bila encimska aktivnost biofilma najvišja v srednjih odsekih zaradi vnosa NH4+-N v podtalnico, primarna proizvodnja pa je bila največja v večini dolvodnih odsekov zaradi kopičenja NO3--N in PO43--P z zbirno površino. Posledično je povprečna multifunkcionalnost dosegla vrh na mestih z najvišjo koncentracijo hranil v kontinuumu reke Neretve, kar kaže na močnejši vpliv vnosa hranil kot položaja v omrežju. Neokrnjeno stanje Neretve povzroča oligotrofne razmere vzdolž njenega zgornjega toka. Naši rezultati poudarjajo veliko občutljivost delovanja ekosistema v Neretvi na vnose hranil in okoljske prekinitve, bodisi naravne bodisi človeške. Morebitni večji, dolgoročni vplivi na tem območju bi lahko spremenili obstoječe okoljske gradiente in s tem delovanje ekosistemov v rekah na lokalni in regionalni ravni.

Ključne besede: delovanje ekosistema, funkcionalna pestrost, funkcionalni indikatorji, kras, raztopljena organska snov, rečna omrežja

Apstrakt. Unosi nutrijenata oblikuju gradijente funkcionisanja ekosistema duž netaknutog gornjeg toka Neretve, Bosna i Hercegovina – Ekosistemske funkcije su okosnica usluga ekosistema koje rijeke pružaju ljudskim zajednicama. Funkcionisanje ekosistema proizlazi iz interakcije između bioloških zajednica i njihovog okruženja. Pošto se uslovi životne sredine u rijekama mijenjaju duž njihovog longitudinalnog kontinuma, mijenja se i funkcionisanje. Ponekad ove promjene ne prate glatke gradijente već velike diskontinuitete. Ovo može biti slučaj u krečnjačkim, kraškim rijekama zbog iznenadnih masivnih unosa podzemnih voda duž pejzaža, što je tipičan fenomen za balkanske rijeke. Uprkos velikom geodiverzitetu i velikoj ekološkoj vrijednosti, balkanske rijeke su i dalje nedovoljno istražene. Ovdje smo istražili kako funkcioniše ekosistem i njihova raznovrsnost (procijenjena kao multifunkcionalnost) se mijenja duž kontinuma kraške rijeke Neretve koja slobodno teče u Bosni i Hercegovini. U tu svrhu izmjerili smo podskup osnovnih funkcija ekosistema (bruto primarna proizvodnja ekosistema, neto primarna proizvodnja biofilma i enzimske aktivnosti, razgradnja organske materije) u jedanaest riječnih tokova od izvora Neretve do riječnih dijelova uzvodno od akumulacije Jablanica. Otkrili smo da su različite funkcije dostigle svoj maksimum u različitim dijelovima Neretve zavisno o unosu hranjivih materija. Dok je razgradnja organske materije bila najveća u glavnim vodama zbog unosa nutrijenata iz priobalne vegetacije, enzimska aktivnost biofilma je imala najveće vrijednosti u srednjim dijelovima zbog unosa NH4+-N u podzemne vode, a primarna proizvodnja je bila najveća u većini nizvodnih dijelova zbog akumulacije NO3--N i PO43--P sa slivnom površinom. Kao rezultat toga, prosječna multifunkcionalnost je dostigla vrhunac na lokacijama s najvećom koncentracijom nutrijenata preko kontinuma rijeke Neretve, što ukazuje na jači uticaj unosa nutrijenata od položaja mreže. Iskonski uslovi Neretve rezultiraju oligotrofnim uslovima duž njenog gornjeg toka. Naši rezultati naglašavaju veliku osjetljivost funkcionisanja ekosistema u Neretvi na unos nutrijenata i ekološkog diskontinuiteta, bilo prirodne ili ljudske. Potencijalni veliki, dugoročni uticaji na područje mogu promijeniti postojeće gradijente životne sredine, a time i funkcionisanje ekosistema u rijekama na lokalnom i regionalnom nivou.

Ključne riječi: funkcioniranje ekosistema, funkcionalna raznolikost, funkcionalni indikatori, krš, otopljena organska tvar, riječne mreže

Introduction

Ecosystem functioning is the base for ecosystem services that rivers provide to human societies (von Schiller et al. 2017). A healthy, functional river maintains fluxes of water, organisms, and resources along its longitudinal continuum but also with its terrestrial surroundings. The main energy source of most rivers is allochthonous organic matter (OM) originating from terrestrial surroundings (Allan & Castillo 2009). High terrestrial primary production can lead to low light availability within the river channel and therefore a low primary production. Thus, in areas with high canopy cover and small stream size, decomposition of plant litter material can be the main subsidy of energy for aquatic communities (Graça et al. 2001). In turn, within river sections with high light availability, clear water and stable flow, primary production can be the main source of energy (Rodríguez-Castillo et al. 2019).

Major environmental changes in rivers occur along their longitudinal axis from the source to the outlet. Still today, our understanding of how rivers change along their longitudinal continuum is associated with the hypotheses developed in the river continuum concept (RCC) (Vannote et al. 1980). The RCC assumes that the continuous growth of river size controls light availability, the alteration of basal resources of food webs, and therefore ecosystem functioning. Thereby a river can be divided into several functional zones. In other words, the river ecosystem »specializes« into certain functions depending on the position within the fluvial network. For instance, upstream sections are specialized in the heterotrophic degradation of terrestrial OM, while larger downstream sections become more specialized in the in-stream production of OM. This zonation can be broken by natural environmental discontinuities like massive groundwater inputs to the system or by human impacts, for instance flow regulation by dams. Surface-groundwater exchanges can be especially important in karstic ecosystems where they contribute to strong temporal and/or spatial variations in flow regimes, water chemistry and nutrient supply (Bonacci 2015; Rugel et al. 2016).

Ecosystem functioning arises from the combined contribution of several individual ecosystem functions. Every ecosystem function plays a unique role in the ecosystem. A change or a disturbance of one function cannot simply be compensated by the increase of another one. Indeed, spatial changes in the performance of ecosystem functions (e.g. from a heterotrophic system to an autotrophic one) associated with changes of environmental conditions and/or biological communities, can have major implications for the energy fluxes within an ecosystem (Peter et al. 2011). Similar to the concept of species diversity, individual functions can be integrated within the concept of »functional diversity« or multifunctionality (Byrnes et al. 2014), which can indicate the overall performance of an ecosystem (Manning et al. 2018). Traditionally, the assessment of ecosystem functioning along river networks only focused only on single functions (Naiman et al. 1987; Tiegs et al. 2009; Rodríguez-Castillo et al. 2019; Feio et al. 2021). Instead, exploring changes in single functions and multifunctionality can offer a more holistic perspective of the functional integrity of rivers; for instance, to their capacity to respond to environmental fluctuations or disturbances (Perkins et al. 2015).

Our understanding of environmental and functional changes of rivers along their longitudinal continuum is also limited to northern-temperate biomes from Central Europe or North-America. Indeed, river systems from Southern countries in Europe like the Balkan Peninsula are much less investigated and can differ greatly in their functioning due to their heterogeneous geology. For instance, the dominance of calcareous lithologies can induce drastic changes across river networks in karstic regions due to the greater degree of surface-subsurface water exchange between rivers and groundwater. Considering this, we investigated changes in ecosystem functions and multifunctionality and their environmental drivers along 11 sites distributed along the upper course of the karstic Neretva River. We compared various linear models (monotonic and non-monotonic) to describe the spatial variation of ecosystem functions and multifunctionality along the upper Neretva. If ecosystem functioning along the upper Neretva is driven by environmental gradients as expected from the RCC (e.g. increased light availability with an increase of river size), we predict functions to show smooth monotonic trends along the river size continuum. In contrast, we predict that discontinuities in water chemistry due to large groundwater inputs would result in non-monotonic trends of ecosystem functions along the river continuum (e.g. as could be captured by polynomial models).

Materials and methods

Study sites

Sampling took place along the upper course of the Neretva River in Bosnia and Herzegovina during a ten-day field campaign during base flow conditions between the end of June and beginning of July 2022. Originating in the Dinaric Alps, the Neretva River flows through a karstic area distinguished by its high groundwater exchange (Operta & Pamuk 2015) and a sub-alpine Mediterranean climate. The Neretva River has a length of 220 km with a catchment size of more than 10.000 km² (Djedjibegovic et al. 2010), which drains into the Adriatic Sea on the coast of Croatia. In this work, we sampled 11 sites from the confluence between the Neretva River and Krupac stream in the upper part of the network to large further downstream sections of the Neretva River, with the most downstream sampling site upstream of Glavatičevo (Fig. 1).

Experimental design

At each sampling site, we measured three autotrophic and two heterotrophic ecosystem functions related to primary production at ecosystem-scale, biofilm functioning and the decomposition of particulate OM (Tab. 1), as well as their main environmental drivers (nutrient concentration, dissolved OM, discharge and catchment area).

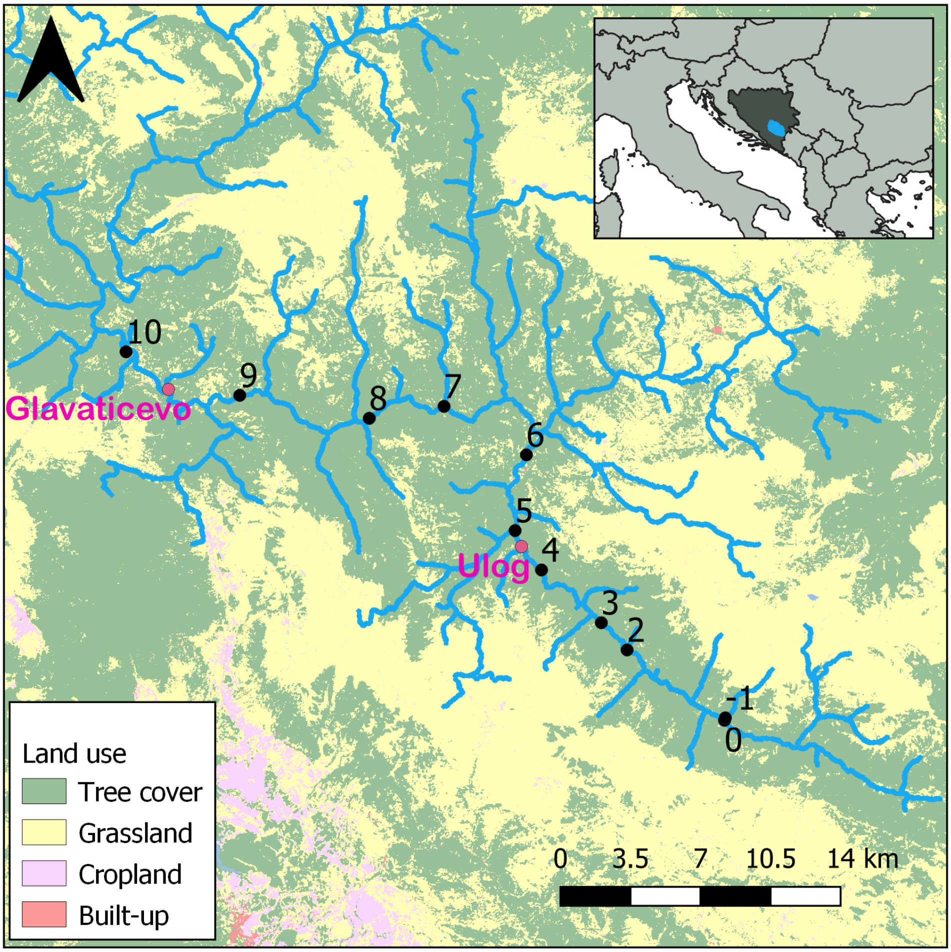

Figure 1. Sampling sites along the upper course of the Neretva River in Bosnia and Herzegovina. Sites are numbered from -1 to 10 following an up- to downstream order (i.e. site 10 is the most downstream site). Site -1 corresponds to the Krupac stream, a tributary to the Neretva in its upper section, while site 0 is the Neretva mainstem, upstream of the confluence with Krupac. Land use in the catchment is mainly natural, dominated by forest and grasslands. Land use data extracted from »© ESA WorldCover project [2021]«.

Slika 1. Vzorčna mesta vzdolž gornjega toka reke Neretve v Bosni in Hercegovini. Mesta so označena s številkami od -1 do 10 po vrstnem redu od zgoraj navzdol (tj. mesto 10 je najbolj dolvodno). Lokacija -1 ustreza potoku Krupac, pritoku Neretve v njenem zgornjem delu, medtem ko je lokacija 0 glavni tok Neretve, gorvodno od sotočja s Krupcem. Raba tal v porečju je pretežno naravna, prevladujejo gozd in travniki. Podatki o rabi zemljišč so pridobljeni iz vira »© ESA WorldCover project [2021]«.

Slika 1. Mjesta uzorkovanja duž gornjeg toka rijeke Neretve u Bosni i Hercegovini. Lokacije su numerisane od: 1 do 10 prema redosledu od gornjeg toka do nizvodno (tj. lokacija 10 je najnizvodnija lokacija). Lokacija 1 odgovara potoku Krupac, pritoci Neretve u njenom gornjem dijelu, dok je lokacija 0 glavni izvor Neretve uzvodno od ušća u Krupac. Korištenje zemljišta u slivu je uglavnom prirodno, dominiraju šume i travnjaci. Podaci o korištenju zemljišta izvučeni iz »© ESA WorldCover project [2021]«.

Table 1. Overview of sampled ecosystem functions and applied methods. GPP = gross primary production, NPP = net primary production.
Tabela 1. Pregled spremljanih ekosistemskih funkcij in uporabljenih metod. GPP = bruto primarna produkcija, NPP = neto primarna produkcija.
Tabela 1. Pregled uzorkovanih funkcija ekosistema i primijenjenih metoda. GPP = bruto primarna proizvodnja, NPP = neto primarna proizvodnja.

Aspects of ecosystem functioning	Method	Autotrophic functioning	Heterotrophic functioning
Ecosystem primary production	O_2 and light loggers	GPP	
Biofilm functioning	Light chamber incubation	NPP	
	Enzymatic activities	Phosphatase	Phenol oxidase
Particulate OM decomposition	Decomposition of cotton strips		Decomposition rate

Environmental drivers

At every site we measured a range of surface water physico-chemical variables, such as nutrient supply, dissolved organic carbon (DOC) and the composition of dissolved OM (DOM); additionally, river discharge and catchment area were measured as possible environmental drivers of ecosystem functioning. Conductivity, pH and water temperature were measured in the field with a WTW multi-probe (Xylem). Discharge was estimated from cross-section profiles of depth, width and flow velocity measurements by multiplying mean velocity with the cross-sectional area. Flow velocity was measured at 40% of depth using a flow meter (FlowSens, SEBA Hydrometrie, Germany). Catchment area was derived from a digital river network model (Copernicus DEM, https://doi.org/10.5270/ESA-c5d3d65) with a resolution of 30 meters using watershed R package (Talluto 2020, version 0.4.9, https://github.com/flee-group/watershed).

Surface water samples for nutrients, DOC and DOM were collected using plastic syringes and filtered through pre-rinsed 0.2 µm membrane filters (Sartorius). Additionally, we took groundwater samples at a subset of 5 sites from 60 to 90 cm depth from the riverbed surface using a Bou-Rouch pump (Tab. S2). Water samples were stored in the dark and cooled until analysis in the laboratory. Nutrients (Cl^-, NO_3^--N, SO_4^{2-}, Na^+, K^+, Mg^{2+}, Ca^{2+}) were analysed with an Ion Chromatograph. Additionally, NH_4^+-N and soluble reactive phosphorus (indicator of ortho-phosphate PO_4^{3-}-P concentration) were analysed using colorimetric methods based on absorbance (Hitachi U2000 Spectrophotometer). DOC concentration was determined with a TOC Analyzer (Shimadzu).

DOM was characteriised by spectroscopic analysis. Fluorescence intensities were measured at excitation wavelengths ranging from 250 to 450 nm (5 nm increments) and emission wavelengths from 350 to 550 nm (4 nm increments) using a Fluoromax-4 Spectrofluorometer (Horiba). Absorbance was measured with a Spectrophotometer (Hitachi). The absorbance scan was performed at wavelengths from 700 to 200 nm. Baseline and blanks were done with MilliQ water. The baseline of absorbance was corrected for the remaining instrumental drift by subtracting the mean absorbance at wavelengths between 680 and 700 nm. For fluorescence, the blank was subtracted from each sample and corrected for the inner filter effect

(Kothawala et al. 2013). Raman normalisation was applied after removing and interpolating the scattering area. From fluorescence and absorbance data representative peaks and indices were determined. All data processing and spectroscopic analyses were done using the R package staRdom following indications of Pucher et al. (2019). The fluorescence peaks B and T indicate the presence of protein-like compounds, while peaks A and C are indicators for humic-like compounds and peak M for microbially-derived humic compounds (Coble 1996). Fluorescence index (FI) distinguishes terrestrial and microbial sources of fulvic acids (McKnight et al. 2001). Humification index (HIX) determines the degree of humification and thus potential stability of OM (Ohno 2002). The biological index (BIX) determines autotrophic productivity and autochthonous DOM origin (Huguet et al. 2009). SUVA254 is estimated as the ratio between decadal absorbance at 254 nm (divided by cell path) and DOC concentration and it is an indicator for DOM aromaticity (Weishaar et al. 2003). Changes in the spectral slope from 275 to 295 nm (S275-295) and from 350 to 400 nm (S350-400) and their ratio (SR) are related to the molecular weight of DOM. S350-400 indicates changes in the weight induced by photochemical shifts (Helms et al. 2008).

Primary production at river ecosystem scale

We measured primary production at the river ecosystem scale using the one-station approach for stream metabolism (Odum 1956) using oxygen loggers (MiniDOT, PME) and light loggers (HOBO Pendant Temperature/Light, Onset). Both loggers were placed at each site during the first two days of the campaign and exposed for 7–9 days. Oxygen loggers were submerged at spots with clearly flowing water in the thalweg of the river. Light loggers were placed out of the water at spots where light conditions were considered representative for the whole reach. Data were recorded at ten-minute intervals. Gross primary production (GPP) was modelled using the inverse-modelling approach by a maximum likelihood estimation variant with the specific application named »m_np_oi_tr_plrckm.nlm« from the R package streamMetabolizer (Appling et al. 2018). The model was fit to a time series of observed changes in dissolved oxygen (DO) using water temperature and light as continuous environmental forces, and the scalars average depth (z) and by-site estimates of the gas exchange rate coefficient (k*600*). In this model, the change of DO over time equals the oxygen produced by (GPP) minus the oxygen consumed by ecosystem respiration (ER) and the coefficient of gas exchange rate between water and the air (K). K was normalised by depth and converted to a common Schmidt number of 600 to get k600 (m d^{-1}). k600 was calculated using an empirical equation formulated to predict gas exchange velocities based on stream velocity and slope (equation 3 from Tab. 2 in Raymond et al. 2012). The model specified GPP to respond proportionally to light using a non-linear minimization fitting process, where maximum GPP is estimated from the maximum values of empirical GPP-light relationships in the data. We discarded ER estimates from the metabolism model due to the great influence of groundwater input on the results. We excluded negative values of GPP as they are physically impossible and associated with errors in model estimations (Appling et al. 2018).

Biofilm-related measurements

We measured epilithic biofilm net primary production (biofilm NPP) using chamber incubations under light conditions. For each site, various stones were collected randomly and placed into three transparent glass chambers together with a propeller-mixing device, filled up with river water, and closed without air inclusion. All chambers were placed over the riverbed totally immersed in stream water to keep temperature stable amongst chambers. During incubation, changes in DO were measured with a fibre optic trace oxygen metre (Microx 4, PreSens). Incubations lasted until a clear increase in DO was visible. Net production of DO (mg DO L^{-1}) was computed by linear regression, modelling the change in DO over time. This volumetric rate was converted to al flux (mg DO h^{-1}) using the water volume in the chambers during incubation (L), incubation time (h) and surface area of sampled stones (cm^2). NPP was expressed as specific NPP (mg DO μg^{-1} Chla h^{-1} cm^{-2}) through normalisation by chlorophyll a mass (µg Chl a). Specific NPP is an ecophysiological feature rather than a productivity measure largely driven by algae biomass. This normalisation was necessary because of the low biomass of biofilm across all study sites.

After the chamber incubations, biofilm was scraped from stones to produce a slurry with river water. Two subsamples (each 10 ml) were filtered on pre-weighted GF/C filters (Whatman) and frozen in the field for later analyses of ash-free dry mass (AFDM) and Chla in the laboratory. For AFDM, filters were dried, weighed and combusted at 450 °C to assess total organic mass. To determine Chla, filters with biofilm slurry were extracted in acetone overnight. Afterwards, absorbance at 664 nm and 750 nm was measured with a Spectrophotometer (Hitachi) before and after addition of hydrochloric acid to correct for pheophytin (Steinman et al. 2017).

Whilst in the field, the remaining biofilm slurry was used to measure phosphatase and phenol-oxidase enzymatic activities using a fluorometric assay with artificial fluorogenic substrata (methylumbelliferone-substrate or MUF) and a colorimetric assay resulted from the oxidation of L-dihydroxyphenylalanine (L-DOPA), respectively. Phosphatase activity is associated with the uptake of phosphorus (mainly by autotrophic organisms in epilithic biofilm), while phenol-oxidase participates in the degradation pathway of terrestrial DOM by heterotrophic organisms. 3 ml of biofilm slurry and river water were mixed with 1 ml of enzymatic substrate (MUF-phosphatase or L-DOPA) and incubated in a water bath at ambient stream temperature and dark conditions together with MUF standards, abiotic degradation controls for both enzymatic substrates and quench controls following standard protocols (Hendel & Marxen 2020). Incubations of phosphatase samples lasted for 1 hour and were stopped by adding glycine NaOH buffer and freezing. Phenol-oxidase incubation was stopped after a 2 h incubation by directly freezing the samples. To estimate enzymatic activities, thawed samples were analysed in the laboratory. Phosphatase fluorescence was measured at 365/455 nm excitation/emission nm with a Fluoromax-4 Spectrofluorometer (Horiba). Phosphatase activities were calculated from fluorescence of MUF in the samples using MUF calibration curves and corrected by controls. For phenol-oxidase, absorbance at 460 nm was measured with a Spectrophotometer (Hitachi) and estimated as the amount of DIQC released by dividing the corrected absorbance by the extinction coefficient ($k = 1.66$ mM^{-1}). Results of enzyme measurements were expressed as activities per g of biofilm AFDM, and per biofilm surface area and incubation time as µmol MUF h^{-1} cm^{-2} g $AFDM^{-1}$ and nmol DIQC h^{-1} cm^{-2} g $AFDM^{-1}$ for phosphatase and phenoloxidase activities respectively (Hendel & Marxen 2020). Note that, similar to NPP, enzymes were normalised both by biomass and surface area due to the very low biomass of biofilm across study sites.

Decomposition of cotton strips

We used cellulose fabric cotton strips as a standardised material to measure microbial decomposition of particulate OM (Tiegs et al. 2013). Three cotton strips were fixed under water to a metal rebar together with a temperature logger at each site and incubated for 7 - 9 days. After incubation, cotton strips were soaked in 96% ethanol and stored in a fridge until further analysis. In the laboratory, strips were washed gently with tap water, dried at 50°C for two days and weighed to the nearest 0.001 mg. To correct for inorganic material attached to the cotton strips after exposition, we measured ash mass of a 1 × 1.5 cm sub-sample of each cotton strip after combustion at 450°C for 4 hours. The decomposition rate of cotton strips was computed using exponential decay models corrected by the sum of average daily temperature over incubation period at every site (Bärlocher 2020).

Data analysis

Multifunctionality as a measure for diversity of ecosystem functions at each sampling site was assessed by applying the averaging and single threshold approaches of Byrnes et al. (2014). The averaging approach indicates the average level of multiple functions in a site. It consists of averaging the standardised values of multiple functions per site. To this aim, before averaging, values of each ecosystem function are standardised by their observed maximum. Averaging then yields a multifunctionality index between 0 and 1. The single threshold approach indicates the number of functions exceeding a certain threshold. For this, thresholds at 25, 50 and 75% of the maximum observed value per function were determined. Contrary to the averaging approach, raw, non-negative data (instead of standardised) were used for the calculations. To avoid the influence of possible outliers, the maximum value for each function was estimated as the average of the three highest values.

Principal component analyses (PCA) were used to explore main changes in the environment (water physico-chemistry and discharge) and DOM composition across sampling sites with the R package »FactoMineR« (Lê et al. 2008). Variables were z-standardised before the analyses. Amongst all the environmental covariables, we considered nutrients (NO_3^--N, NH_4^+-N and PO_4^{3-}-P), DOC and light to be the main potential drivers of ecosystem functions along the studied river continuum. Thus, linear or non-linear changes of those drivers along the catchment area gradient were explored by fitting various types of linear models (null, linear, quadratic, cubic, exponential, logarithmic and power-law). Then, the Akaike information criterion corrected for small sample size (AICc) was used to choose the most parsimonious model. The same approach was used to explore the relationship between catchment area and every individual ecosystem function or average multifunctionality. Since the multifunctionality index derived from the threshold approach is a count variable (i.e. number of functions above a certain threshold), we used linear models with a quasipoisson error distribution and a log link function to model the effect of the catchment area, following recommendations of Byrnes et al. (2014). This different approach is necessary due to the abundance of zero values in the study response, and the likely overdispersion of the data.

Furthermore, we performed redundancy analysis (RDA) to explore multivariate relationships between ecosystem functions and environmental variables that we considered as potential main drivers (catchment area, NO_3^--N, NH_4^+-N, PO_4^{3-}-P, DOC, DOM composition and light). DOM composition was represented by the first two axes of the DOM PCA to summarise the multivariate chemical information of spectroscopic indexes. Due to the small sample size (11 sites), we needed to impute missing values of various functions (phenoloxidase, decomposition and GPP) with the median of each function to carry out the RDA. All ecosystem functions were z-standardised before the analyses. Significances of the overall model, individual RDA axes and explanatory variables were assessed by permutation tests. RDA was performed with the vegan package (Oksanen et al. 2020). All data analyses were carried out with R software, version 4.1.3 (R Core Team 2022).

Results

Environmental changes along the river continuum

Nutrient and DOC concentrations were very low across all study sites (maximum concentrations of 529, 250 and 4 µg /L^{-1} for DOC, NO_3^--N and PO_4^{3-}-P, respectively). This resulted in high values of nutrient ratios, with C:N (DOC : NO_3^--N) and N:P (NO_3^--N : PO_4^{3-}-P) ranging from 1.4 to 20.26, and 39 to 1075, respectively. All the main environmental drivers (light, DOC, NO_3^--N, NH_4^+-N and PO_4^{3-}-P) changed significantly with catchment area (Fig. 2, Tab. S1). To facilitate the interpretation of spatial patterns of bioreactive solutes like nutrients or DOC, we also explored the effect of catchment area on a conservative element like Cl^-. Cl^- increased monotonically with catchment area following a proportional increase with discharge ($p < 0.001$, $R^2 = 0.92$). In contrast to the conservative Cl^-, the other environmental drivers, NO_3^--N, NH_4-N, PO_4^{3-}-P and DOC, showed non-monotonic changes with catchment area following cubic models. NO_3^--N and PO_4^{3-}-P showed highest concentrations in the Krupac stream (site -1) and at most downstream sites, while the lowest nutrient content was reached in middle sections. DOC followed the opposite pattern, with maximum values in mid-sections and lowest concentrations at the uppermost site and in lower sections. NH_4-N followed a similar pattern to DOC but increased again at most downstream sites, following a strong cubic trend. Water temperature followed a similar pattern to NH_4-N, that is, peaking at middle section sites (0 to 6), then dropping at site 7, and finally increasing again towards most downstream sites. Although the spatial changes of water temperature were best described by the cubic model ($R^2 = 0.84$), the power-law model resulted in the lowest AICc despite its worser fit ($R^2 = 0.16$, see Tab. S1). This disagreement could be due to the small sample size (11 observations) to fit rather complex models. Light availability changes with catchment area were best described by a power-law model, increasing mainly from uppermost to mid-sites.

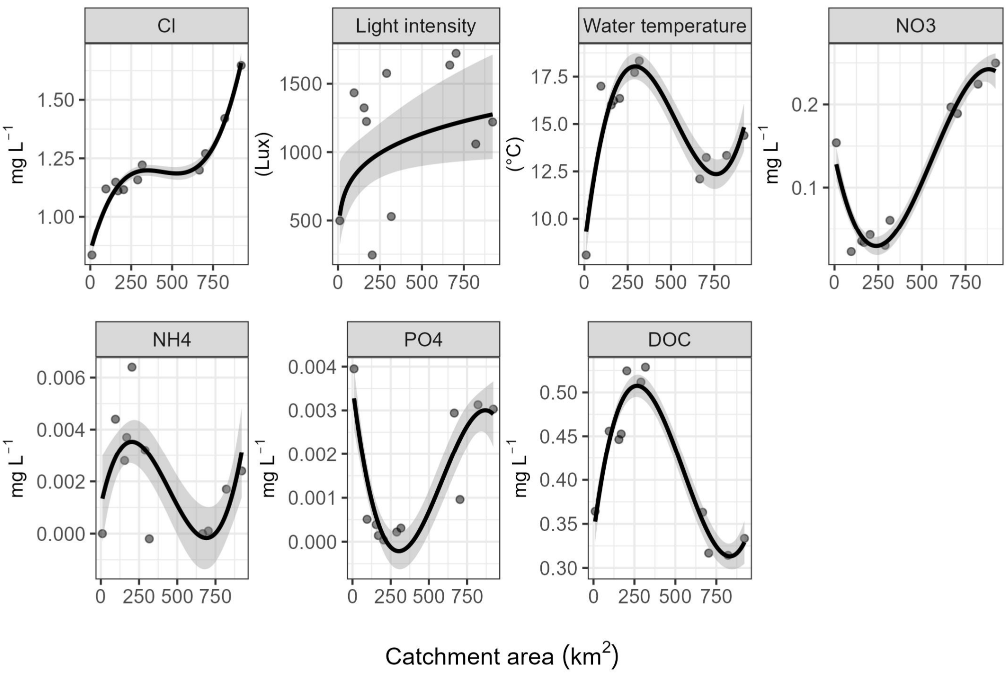

Figure 2. Most parsimonious models describerd the relationships between principal environmental drivers (light and the concentration of DOC and nutrients in river water) and catchment area. All parameters were best described by cubic models except light intensity (power-law model). Results for chloride (Cl^-) are included as a reference to compare spatial changes of bioreactive solutes like nutrients with catchment area, where a conservative element was only subjected to changes in concentration due to the increase in discharge along sampling sites. Model fits and performance are described in Tab. S1.

Slika 2. Najbolj parsimonični modeli, ki opisujejo razmerja med glavnimi okoljskimi dejavniki (svetloba in koncentracija DOC in hranil v rečni vodi) in površino porečja. Vsi parametri so bili najbolje opisani s kubičnimi modeli, razen jakosti svetlobe (»power-law« model). Rezultati za klorid (Cl-) so vključeni za primerjavo, da se prostorske spremembe hranil s površino porečja lahko primerjajo z elementom, ki je izpostavljen le spremembam koncentracij zaradi večanja pretoka med vzorčnimi mesti. Prileganje in delovanje modela sta opisana v tabeli S1.

Slika 2. Najštedljiviji modeli koji opisuju odnose između glavnih pokretača životne sredine (svjetlo i koncentracija DOC i nutrijenata u riječnoj vodi) i slivnog područja. Svi parametri su najbolje opisani kubičnim modelima osim intenziteta svjetlosti (model stepena). Rezultati za hlorid (Cl-) uključeni su kao referenca za poređenje prostornih promjena bioreaktivnih otopljenih materija poput nutrijenata sa slivnom površinom sa konzervativnim elementom koji je samo podvrgnut promjenama koncentracije zbog povećanja ispuštanja duž mjesta uzorkovanja. Modeli i performanse opisani su u tabeli S1.

The PCA used to describe environmental changes across sites explained up to 77.2% of the variance along the first two dimensions (Fig. 3A). The first dimension explained 51.5% of the variance and was mainly associated with the concentrations of nutrients (NO_3^--N and PO_4^{3-}-P), DOC and ions Na^+ and K^+. The second dimension explained 25.7% of variance and was related to the ions Mg^{2+} and SO_4^{2-}, discharge and conductivity. Sampling sites were principally distributed along the first dimension creating two main groups, downstream sites (sites 7 - 10) with higher concentrations of NO_3^--N and PO_4^{3-}-P but also higher discharge, and sites from upper and mid sections (sites 0 and 2 to 6) characterised by very low nutrient concentrations but high content in Na^+ and Ca^{2+} ions. Site -1 (Krupac stream) was completely differentiated from the rest of the sites with very particular conditions (spring-fed, forested stream with very low light and water temperature, low concentration in cations, but high content in NO_3^--N and PO_4^{3-}-P). A second PCA with water chemistry of groundwater samples (Fig. S1) revealed very similar patterns to the environmental PCA. Upstream site 0 had high values of NH_4^+-N and PO_4^{3-}-P, sites of middle sections (3 and 5) presented the highest values for SO_4^{2-} and Na^+, while most downstream sites (8 and 10) had the highest values of Mg^{2+} and NO_3^--N.

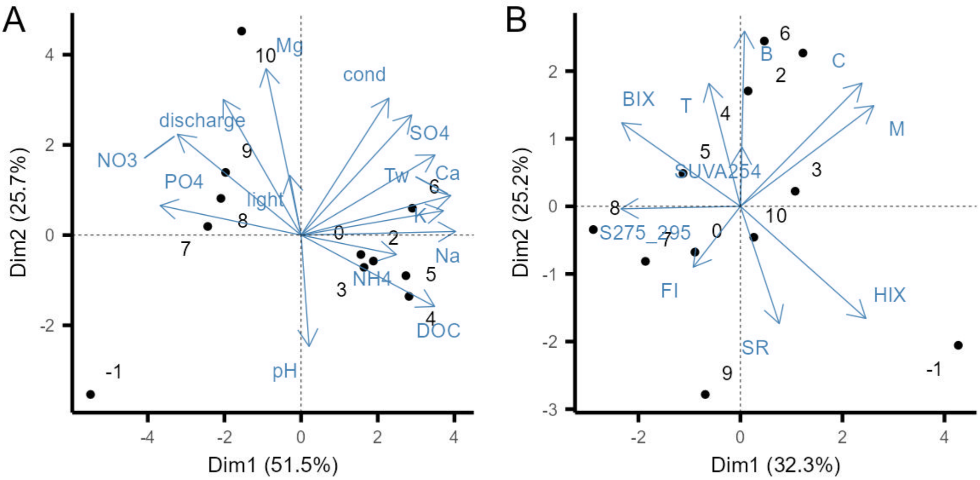

Figure 3. Principal component analyses (PCA) of environmental variables (A) and DOM composition (B) across study sites. Sites are numbered from -1 to 10 following an up- to downstream order. »Cond« in A stands for electric conductivity. DOM indices and peaks in B are explained in section environmental drivers.

Slika 3. Analiza glavnih komponent (PCA) okoljskih spremenljivk (A) in sestave DOM (B) na lokacijah študije. Mesta so označena s številkami od -1 do 10 po vrstnem redu od zgoraj navzdol. »Cond« v A pomeni električno prevodnost. Indeksi DOM in vrhovi v B so pojasnjeni v razdelku »Environmental drivers«.

Slika 3. Analiza glavnih komponenti (PCA) varijabli sredine (A) i sastava DOM (B) na mjestima istraživanja. Lokacije su numerisane od: 1 do 10 po redosledu od gornjeg toka do nizvodno. »Cond« u A označava električnu provodljivost. DOM indeksi i vrhovi u B objašnjeni su u dijelu »Environmental drivers«.

The PCA of DOM composition explained up to 57.5% of the variance along the first two dimensions (Fig. 3B). The first dimension explained 32.3% of the variance and was mainly associated with the spectral slope S275-295 (proxies for DOM molecular size) and the fluorescence intensity of peak M and C (humic-like compounds). The second dimension explained 25.6% of variance and was related to HIX (indicator of terrestrial DOM signatures), BIX (indicator of in-stream produced DOM) and peaks B and T (protein-like peaks). The first dimension configured a molecular weight gradient, separating mainly downstream sites (7, 9, 10, but also site 0) characterised by small DOM compounds, from upstream sites (-1, 2 and 3) characterised by high-molecular weight, but also more humic DOM. The second axis configured another gradient based on DOM origin, separating mainly site -1 with old, terrestrially-derived DOM, from other sites in the middle and downstream sections with fresher DOM, mainly produced in-stream.

Changes of ecosystem functions and multifunctionality along the river continuum

All the studied functions except for phenoloxidase activity showed significant changes along the catchment size gradient (Fig. 4, Tab. S2). Functions related to autotrophic functioning (ecosystem GPP, biofilm NPP and phosphatase activity) showed monotonic trends, with low values in the upper sections, and a big increase with catchment area towards most downstream sites. Still, each function was best described by different models. GPP resulted in a monotonic cubic model. The biofilm NPP, linear, quadratic and cubic models showed a similar performance according to AICc (Tab. S2). Phosphatase was described similarly by exponential and power-law models. Contrarily to autotrophic functions, microbial decomposition rates showed a U-shaped pattern along the Neretva continuum, with a strong decrease from the uppermost site to the middle sections, and then an increase towards the most downstream sites. Decomposition was similarly described by quadratic and logarithmic models. Phenoloxidase activity showed very low values across the upper Neretva, presenting higher values only at one site in the upper section, but no other noticeable spatial patterns across the network, thus resulting in a non-significant effect of catchment area for any model.

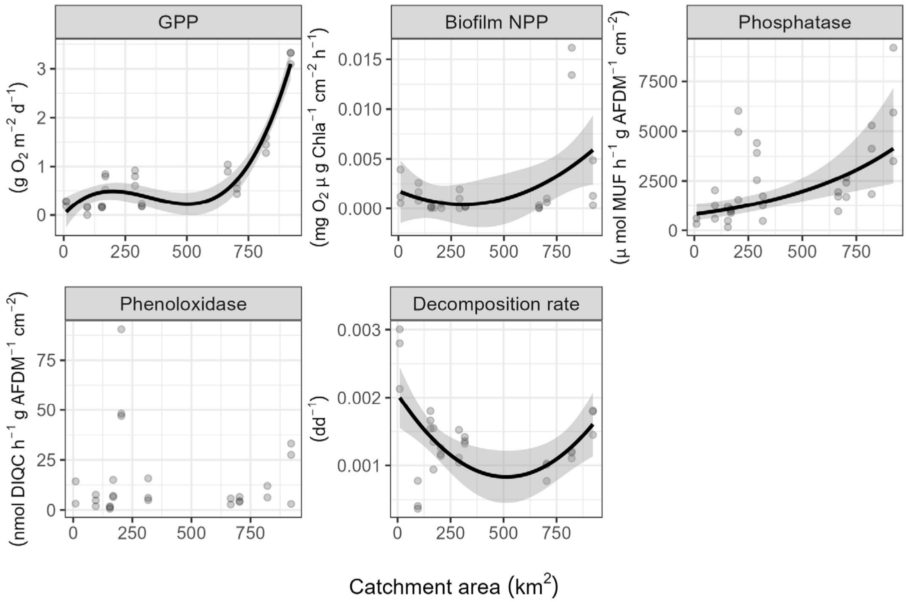

Figure 4. Most parsimonious models described the relationships between the studied ecosystem functions and catchment area. Decomposition and biofilm NPP were best described by quadratic models, GPP by cubic model, and phosphatase by exponential model. Linear fit and confidence intervals are only represented for significant models. Model fits and performance are described in Tab. S2.

Slika 4. Najbolj parsimonični modeli, ki opisujejo razmerja med preučevanimi ekosistemskimi funkcijami in površino porečja. Razpad in NPP biofilma so najbolje opisali kvadratni modeli, GPP kubični model, fosfatazo pa eksponentni model. Linearno prileganje in intervali zaupanja so predstavljeni samo za statistično značilne modele. Prileganje in delovanje modela sta opisana v Tabeli S2.

Slika 4. Najštedljiviji modeli koji opisuju odnose između proučavanih funkcija ekosistema i područja sliva. NPP dekompozicije i biofilma najbolje su opisali kvadratni modeli, GPP kubni model, a fosfataza eksponencijalni model. Intervali linearnog uklapanja i pouzdanosti predstavljeni su samo za značajne modele. Odgovaranje modela i performanse opisani su u tabeli S2.

The RDA analysing the effect of nutrients, DOM composition, DOC, light and catchment area on the studied functions resulted in an overall significant model ($F = 4.76$, $p = 0.007$) with an adjusted R^2 of 0.75 (Fig. 5A). The environmental variables included in the RDA explained 95% of the variation of studied ecosystem functions across sites. Even so, only the first canonical axis of the RDA resulted significance (RDA1: $F = 42.95$, $p = 0.010$) due to the main effects of the catchment area ($F = 9.98$, $p = 0.002$), NH_4^+-N ($F = 7.78$, $p = 0.005$), NO_3^--N ($F = 4.86$, $p = 0.031$), DOC ($F = 5.34$, $p = 0.019$) and DOM PC1 ($F = 4.40$, $p = 0.018$). All ecosystem functions (except phosphatase) were highly correlated with RDA1, resulting in an ordination of sites along this axis, especially separating sites 4, 9 and 10 from the rest. According to variance inflation factors (VIF), multicollinearity of the RDA model was too high (square root of VIF > 2), therefore forward selection was used to select only the best explanatory variables. This resulted in a second significant model ($F = 4.39$, $p < 0.001$, adjusted $R^2 = 0.50$), with sites separated similarly than the original RDA but only catchment area, NH_4^+-N and DOM PC1 as significant predictors (Fig. S2).

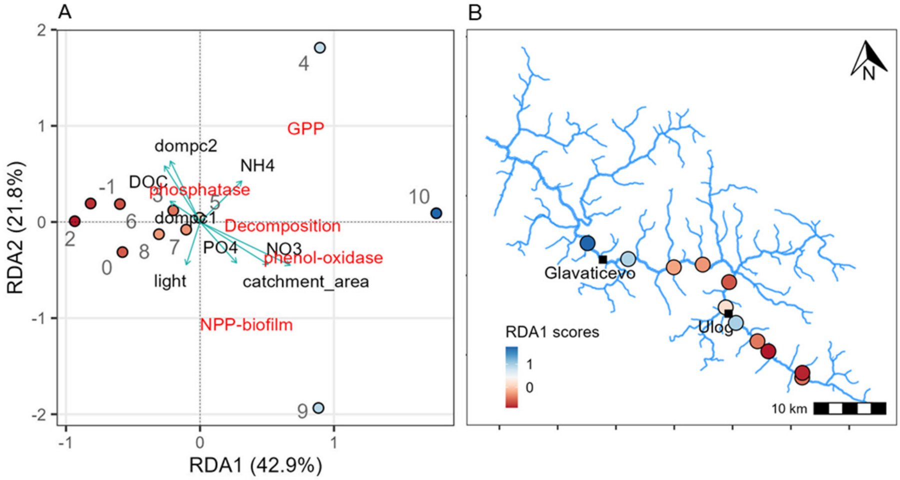

Figure 5. (A) RDA triplot showing multivariate relationships between ecosystem functions (red text) and principal environmental drivers (NH_4^+-N, NO_3^--N and PO_4^{3-}-P, DOC, DOM composition, light and catchment area defined by blue arrows and black text) across study sites (coloured dots). DOM composition is summarised by the first two components of DOM PCA from Fig. 2. The scaling of the RDA shows the effects of environmental explanatory variables. The length of the arrow indicates the strength of the environmental driver explaining ecosystem functions, while the direction indicates the correlation amongst drivers. The map in (B) shows site scores of RDA1 as a colour gradient across the upper course of the Neretva, showing sites with highest average functioning with blue colours.

Slika 5. (A) Graf RDA, ki prikazuje multivariatne odnose med ekosistemskimi funkcijami (rdeče besedilo) in glavnimi okoljskimi spremenljivkami (NH_4^+-N, NO_3^--N in PO_4^{3-}-P, DOC, sestava DOM, svetloba in površina porečja; označeni z modrimi puščicami in črnim besedilom) na vzorčnih mestih (barvne pike). Sestava DOM je povzeta po prvih dveh PCA komponentah DOM s Sl. 2. Merilo RDA prikazuje velikost učinka okoljskih spremenljivk. Dolžina puščice označuje pomen okoljskih dejavnikov pri pojasnjevanju ekosistemskih funkcij, medtem ko smer nakazuje korelacijo med dejavniki. Zemljevid v (B) prikazuje vrednosti RDA1 na vzorčnih mestih v zgornjem toku Neretve, prikazane z barvnim gradientom, mesta z najvišjim povprečnim delovanjem so obarvana modro.

Slika 5. (A) RDA triplot koji prikazuje multivarijantne odnose između funkcija ekosistema (crveni tekst) i glavnih varijabli okoline (NH_4^+-N, NO_3^--N in PO_4^{3-}-P, DOC, DOC, DOM sastav, svjetlo i područje sliva definisano je plavim strelicama i crnim tekstom) preko mjesta istraživanja (tačke u boji). DOM sastav je sažet sa prvih dvih komponenata DOM PCA sa Sl. 2. Skaliranje RDA pokazuje efekte objašnjavajućih varijabli okoline. Dužina strelice ukazuje na snagu varijabli okoline u objašnjavanju funkcije ekosistema, dok pravac ukazuje na korelaciju među učesnicima. Karta (B) prikazuje rezultate lokaliteta RDA1 kao gradijent boja preko gornjeg toka Neretve, prikazujući lokacije s najvišim prosječnim funkcionisanjem sa plavom bojom.

Additionally, we explored the spatial distribution of scores of the RDA1 axis of the general model (all environmental predictors) across the Neretva River network map (Fig. 5B) and observed the same patterns as for the average multifunctionality (Fig. 6C). Indeed, average multifunctionality and RDA1 site scores were highly correlated (Fig. S3), suggesting a strong combined effect of nutrients and catchment area on average functioning across the upper Neretva.

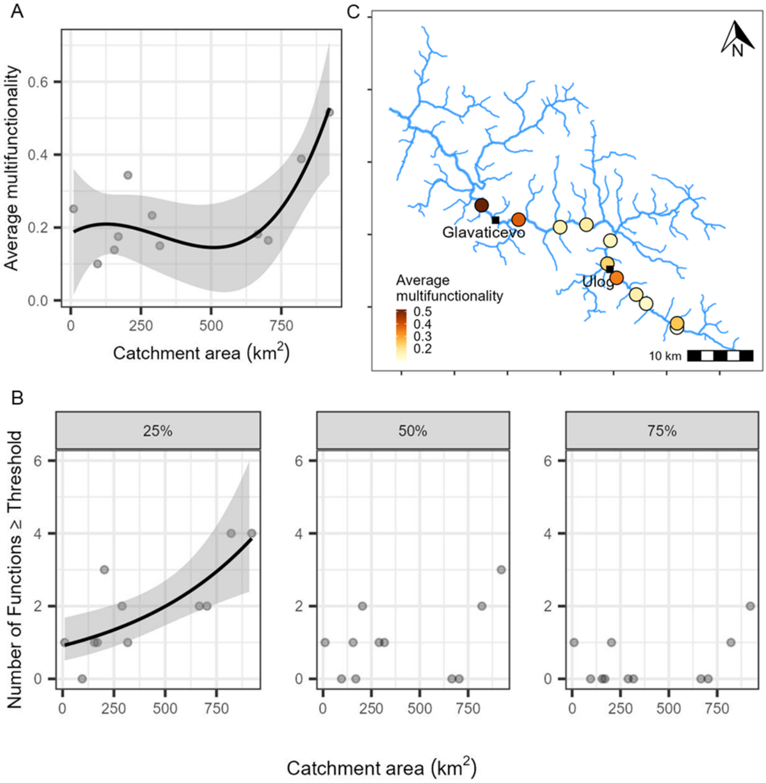

Figure 6. Spatial changes in multifunctionality across the upper course of the Neretva. Linear models in (A) and (B) show the relationship between multifunctionality and catchment area, whilst the map in (C) shows with a colour gradient how average multifunctionality varies across sampling sites distributed along the Neretva River continuum. In (A) and (C), multifunctionality is estimated as the average of the standardised value of the studied functions. In (B), multifunctionality is estimated through the single threshold approach, defining the number of ecosystem functions that surpass thresholds at 25%, 50% and 75% regarding the maximum value observed per function. Linear fit and confidence intervals are only represented for significant models.

Slika 6. Prostorske spremembe multifunkcionalnosti v zgornjem toku Neretve. Linearni modeli v (A) in (B) prikazujejo razmerje med multifunkcionalnostjo in velikostjo porečja, medtem ko zemljevid v (C) z barvnim gradientom prikazuje, kako povprečna multifunkcionalnost variira med vzorčnimi mesti vzdolž reke Neretve. V (A) in (C) je multifunkcionalnost ocenjena kot povprečje standardizirane vrednosti preučevanih funkcij. V (B) je multifunkcionalnost ocenjena s pragom, ki definira število funkcij, ki presegajo prag 25 %, 50 % in 75 % najvišje vrednosti posamezne funkcije. Linearno prileganje in intervali zaupanja so predstavljeni samo za statistično značilne modele.

Slika 6. Prostorne promjene multifunkcionalnosti preko gornjeg toka Neretve. Linearni modeli (A) i (B) pokazuju odnos između multifunkcionalnosti i slivnog područja, dok karta (C) pokazuje gradijentom boje kako prosječna multifunkcionalnost varira na mjestima uzorkovanja koja su raspoređena duž kontinuma rijeke Neretve. Kod (A) i (C) multifunkcionalnost se procjenjuje kao prosjek standardne vrijednosti proučavanih funkcija. Kod (B), multifunkcionalnost se procjenjuje kroz pristup jedinstvenog praga, definišući broj funkcija ekosistema koje premašuju pragove od 25%, 50% i 75% u odnosu na maksimalnu vrijednost koja je uočena po funkciji. Intervali linearnog uklapanja i pouzdanosti predstavljeni su samo za značajne modele.

Average multifunctionality presented very low values across the upper Neretva, ranging from 0.1 to 0.5 (given a maximum of 1). Average multifunctionality increased slightly with catchment area (Fig. 5A); however, this relationship was mainly pulled by the two most downstream sites with the highest values for multifunctionality (Fig. 5C). When considering multifunctionality from the single threshold approach, the effect of catchment area was only significant for the 25% threshold ($F = 10.4$, $p = 0.010$), not for 50 or 75% (50% $F = 1.68$, $p = 0.227$; 75% $F = 1.63$, $p = 0.233$) (Fig. 5B). In other words, the increase of average multifunctionality along the Neretva was related to functions performing at a low level: i.e., regarding the maximum values found in the network.

Discussion

Longitudinal environmental and geomorphological changes along the upper course of the Neretva had a great impact on water chemistry, nutrients and the composition of DOM. Cl^- and Mg^{2+} increased from up- to downstream in clear correlation with the catchment area. General increases of these elements further downstream could be caused by additional input from groundwater (Gomi et al. 2002; Allan & Castillo 2009) and by the accumulation of tributaries. These results are indeed supported by the drastic drop in water temperature at site 7 associated with a sizable input of cold water. At this site, this input could only come from groundwater or a major tributary like the Jezernica stream. Moreover, due to the scarce human activity in the upper part of the Neretva River, the concentrations of nutrients (NO_3^--N and PO_4^{3-}-P) were very low across the studied area. All sites were considerably oligotrophic with a $N:P > 16$, suggesting a potential phosphorus limitation all along the upper Neretva, but especially so in the middle sections where nutrient concentrations were lower (Fig. 2). The increase of water accumulation and human-land use at the most downstream sites promoted an increase of nutrient concentrations. In contrast, the further upstream sites (sites 0 to 6, except site -1) were more enriched in Ca^{2+}, K^+ and Na^+ than downstream (Fig. 3). These results may be explained by a stronger influence of rock weathering in the upper part of the network, which is especially characteristic for karstic systems (Grasby & Hutcheon 2000; Han & Lui 2004). Rock and water interactions usually lead to the dissolution of evaporite and carbonate minerals. Additionally, in headwaters, ions can also originate from atmospheric processes (Grasby & Hutcheon 2000). Groundwater chemistry presented similar spatial patterns to surface water, but also higher concentrations than surface water, which might support an upwelling movement of water from groundwater to the surface (Fig. S1, Tab. 4). DOC in surface water was also very low, peaking at the middle sections. DOM was mainly of terrestrial origin as suggested by very low BIX and FI values (< 0.7 values for both) at all sites (Smith & Kaushal 2015). Even so, DOM showed significant changes along the Neretva River continuum, with the strongest humic-like signatures at the uppermost sites (especially site -1 due to the close interaction with riparian canopy) and a shift towards more in-stream produced DOM with low molecular weight at the downstream sites. These longitudinal changes followed similar patterns as shown in previous studies, both in karstic (Ni et al. 2020) and non-karstic rivers (Casas-Ruiz et al. 2020; Hosen et al. 2020), suggesting that the connectivity between the river and the surrounding land dominate over geology to shape longitudinal changes in DOM composition. Even so, we found evidence for discontinuities in DOM composition gradients at mid-sections, where we observed the strongest signal of protein-like compounds from microbial sources (peaks T and B, see Tab. S5). As well as the highest DOC and NH_4^+-N concentrations. These changes in DOM and nutrients might be associated with inputs of groundwater rich in labile DOM associated with subterranean microbial activity (Simon et al. 2010; McDonough et al. 2022). Due to the likely great relevance of surface-groundwater exchanges in the Neretva, future studies should further investigate those fluxes and chemical composition of groundwater at much finer spatial resolution than in our study.

All studied ecosystem functions except phenoloxidase enzymatic activity showed important changes along the upper course of the Neretva. Mainly, autotrophic functioning (measured as ecosystem-level GPP, biofilm NPP and phosphatase activity) increased monotonically with the catchment area (Fig. 4). According to RDA models, these spatial changes in ecosystem functioning were mainly associated with the increase of nutrient concentration (PO_4^{3-}-P, NO_3^--N and NH_4^+-N) from upstream to downstream. The only exceptions were sites -1 and 4, where external nutrient inputs from riparian vegetation or groundwater boosted microbial decomposition and biofilm enzymatic activity, respectively. In line with our results, Rodríguez-Castillo et al. (2019) also found GPP to increase with catchment area, mainly as a response to NO_3^--N concentrations. Contrary to expectations, we found no relationship between GPP (nor biofilm NPP) and average light intensity during the study period. This is surprising as light is considered the most important driver of GPP in ecosystem metabolism studies (see Bernhardt et al. 2022). Indeed, previous studies have linked the increase of GPP with catchment area mainly to the increase of light availability (Mejia et al. 2018; Segatto et al. 2021). Similarly, Feio et al. (2021) found highest epilithic biofilm production rates to occur at middle sections of a river continuum in Portugal as a response to light. Even so, nutrients can become the main regulator of stream metabolism under situations of stable flow and no light limitation (Bernhardt et al. 2018). Indeed, most study sites (except for sites -1 and 4) were wide, open reaches with high light availability, potentially explaining the stronger influence of nutrients than light on primary production along the upper course of the Neretva.

Similar to whole-ecosystem GPP, we found biofilm functioning to be mainly mediated by nutrient concentration. Biofilm NPP and phosphatase responded mainly to changes in NO_3^--N concentration while phenoloxidase increased with NH_4^+-N. Surprisingly we found no response of biofilm functioning to PO_4^{3-}-P concentration despite high N:P and C:P suggested strong limitation by phosphorous. To maximise the efficiency of energy consumption, microbial communities produce appropriate enzymes only when they are limited by the availability of the respective nutrients (Sinsabaugh et al. 2002). Indeed, we found relatively high values of phosphatase enzymatic activity across all sites, likely as a response to the phosphorous limitation. In fact, previous studies analysing functioning in stromatolite biofilm formations within oligotrophic calcareous streams have found similar results (Romaní & Sabater 1998). Previous studies have shown stromatolite biofilm communities are well adapted to low nutrient and DOC concentrations by maximising the retention and recycling of organic carbon and nutrients (Sabater et al. 2000). A major dependence of the heterotrophic communities within epilithic biofilm on microbial-derived carbon (e.g. algal exudates) would explain the very low values of phenoloxidase activity found along the upper course of the Neretva (Bianchi 2011; Rier et al. 2014). Phenoloxidase is associated with the degradation of complex molecules like lignin, and its activity can be used to infer the recalcitrant compounds in a river (Peter et al. 2011; Rier et al. 2014). Despite the main terrestrial origin of DOM in upper Neretva, DOC concentration was very low at all sites (< 1 mg L^{-1}). No relationship was found between phenoloxidase and DOC or any descriptor of humic DOM, reinforcing the idea that epilithic biofilm was independent of terrestrial carbon during the time of the sampling campaign.

Phenoloxidase only showed higher values under an increased NH_4^+-N concentration, likely due to the preference of NH_4^+-N as the nitrogen source of certain biofilm communities (Borchardt 1996, von Schiller et al. 2007).

Microbial decomposition of cotton strips showed a U-shaped pattern along the catchment area gradient. Particulate OM (e.g. leaf litter) decomposition rates usually reach maximum values in headwaters and decrease along the river continuum, mainly due to the analogous decrease of shredder density (Graça et al. 2001). The decomposition of cotton strips is mainly mediated by microbial communities and shredders barely have any influence (Tiegs et al. 2013). In this case, the increase of decomposition rates at downstream sites can be explained by the increase in PO_4^{3-}-P concentration as evidenced by its strong correlation with decomposition rates. Heterotrophic functions like decomposition are positively influenced by a higher concentration of nutrients as microbes are more active and stimulate decomposition rates (Escoffier et al. 2018).

To sumarise, we found ecosystem functions can show both monotonic and non-monotonic trends along the upper course of the Neretva. In particular, autotrophic functions did follow the expected patterns from the RCC, increasing rather monotonically along the catchment area gradient. In contrast, heterotrophic functioning did not. Main spatial changes in OM decomposition were mediated by PO_4^{3-}-P, while phenoloxidase activity was mainly affected by NH_4^+-N. Average multifunctionality was strongly correlated to the first axis of the RDA model (Fig. S3), that was mainly pulled by the catchment area, but also NO_3^--N and NH_4^+-N concentration. These results suggest that the spatial zonation of functioning across the upper course of the Neretva may arise from the combined effect of nutrient inputs and river size. In fact, multifunctionality presented the highest values in sites with greater inputs of nutrients, that is, most downstream sites (9 and 10), but also at site 4, where groundwater inputs may result in higher NH_4^+-N concentration than anywhere else. These results suggest that natural discontinuities mediated by groundwater inputs may have an important influence on ecosystem functioning under such oligotrophic conditions. We acknowledge, however, that our experimental design was inappropriate to clearly discern the exclusive influence of groundwater inputs on ecosystem functioning from other possible drivers. Additionally, our study was only carried out at a single time during baseflow conditions, thus we omitted how seasonal changes in the hydrological connection between the river and surrounding terrestrial ecosystems (e.g. decreasing or increasing the water exchange during low / high flow conditions) might translate into temporal variations of ecosystem functioning in the Neretva (Lupon et al. 2023). Future studies should put more effort to replicate spatial surveys of water chemistry and ecosystem functioning at large spatial scale during different hydrological conditions to accurately evaluate the role of natural discontinuities in functioning within the upper Neretva. Furthermore, we acknowledge that a holistic understanding of ecosystem multifunctionality requires analysing microbial diversity (Peter et al. 2011, Perkins et al. 2015). Indeed, microbial communities and their diversity are the backbone of river ecosystem functioning (Altermatt et al. 2020). Future studies should explore spatial changes in diversity (not only of microbial communities, but also macrozoobenthos) and their linkage to ecosystem functioning. Undisturbed, pristine rivers in the Balkans are considered refugia for declining freshwater biodiversity (Bakrac et al. 2021; Smeti et al. 2023). The conservation of these systems is pivotal, not only to protect biodiversity, but also to understand its fundamental role in ecosystem functions and services.

Ecosystem functioning, and therefore, the ecological integrity of the pristine upper course of the Neretva River are threatened by the construction of dams. Dams alter the flow regime (Bernhardt et al. 2018) and thus disrupt spatial and temporal variability of water flow (Cardinale et al. 2002), drastically affecting biogeochemical cycles (Wang et al. 2018). Our results show that ecosystem functioning in the Neretva is strongly linked to nutrient inputs due to the great oligotrophic conditions; thus, a disruption of nutrient dynamics in the catchment would severely alter ecosystem functioning at a large spatial scale. Furthermore, we found different drivers for autotrophic and heterotrophic functions. Different functions exert different contributions to energy and matter fluxes within river ecosystems, thus a damage of one function by human impacts cannot simply be compensated by another function. The disruption of functioning spatial patterns by the construction of dams would therefore exert a dramatic alteration of energy fluxes in the Neretva River, which might cascade into negative effects on biological communities and also ecosystem services.

Povzetek

Podobno kot vsi živi organizmi morajo reke pridobivati energijo z različnimi biološkimi procesi, imenovanimi ekosistemske funkcije; na primer proizvodnja nove organske snovi s fotosintezo ali recikliranje mrtve organske snovi z razgradnjo. Te funkcije ekosistema so rezultat interakcije med organizmi v reki (kot so mikrobne skupnosti, bakterije, ličinke žuželk ali celo ribe) in njihovim okoljem (kemična sestava vode, značilnosti rečnega sedimenta ...). Okoljske razmere reke se naravno spreminjajo na njeni poti od povirja do dolvodnih odsekov. Na primer, povečanje širine reke, ko se reka spušča, povzroči postopno povečanje razpoložljivosti svetlobe v strugi, kar lahko nato spodbudi alge in rastline k fotosintezi in s tem primarni proizvodni funkciji. Te naravne spremembe vzdolž dolžine reke običajno sledijo gladkim gradientom (tj. počasnim, a neprekinjenim spremembam). V kraških rekah, kakršna je reka Neretva, se lahko ti okoljski gradienti prekinejo z nenadnim vdorom velikih količin podzemne vode z drugačno kemično sestavo kot rečna voda. Te nenadne spremembe lahko posledično vplivajo na funkcije ekosistema v reki. Reke na Balkanskem polotoku se zaradi neokrnjenosti in raznolike geologije zelo razlikujejo od drugih sistemov v Evropi. V tej študiji smo raziskali, kako se okoljske razmere in funkcije ekosistema spreminjajo vzdolž zgornjega toka reke Neretve v Bosni in Hercegovini. Junija 2022 smo vzorčili in analizirali fizikalno-kemijsko sestavo rečne vode in podzemne vode ter različne funkcije ekosistema na enajstih rečnih odsekih, razporejenih od povirja do dolvodnih odsekov gorvodno od akumulacije Jablanica. Zlasti smo raziskali pet funkcij, povezanih z različnimi biološkimi skupnostmi v reki: primarno proizvodnjo na ravni celotnega ekosistema z uporabo zapisovalcev kisika in neposredno iz biofilmskih skupnosti, ki rastejo na kamnih z uporabo inkubacijskih komor; fosfatazne in fenoloksidazne encimske aktivnosti biofilma; in mikrobna razgradnja organske snovi z uporabo bombažnih trakov. V celotnem zgornjem toku Neretve smo ugotovili zelo nizke koncentracije hranil. Posledično je večina funkcij ekosistema na splošno pokazala nizko aktivnost in se je povečala šele, ko se je zaradi zunanjih vnosov povečala koncentracija hranil. Ugotovili smo, da so različne funkcije dosegle največjo aktivnost na različnih položajih ob reki Neretvi in odvisno od vnosa različnih hranil. Na primer, razgradnja organske snovi je bila najvišja v povirju zaradi vnosa nitratov in fosfatov iz obrežne vegetacije. Encimska aktivnost biofilma je bila največja na srednjih odsekih reke zaradi vnosa amonija iz podzemne vode. Končno je bila primarna proizvodnja reke največja na večini dolvodnih odsekov zaradi kopičenja nitratov in fosfatov iz gorvodnega dela porečja. Medtem ko nekatere od teh sprememb v funkcijah ekosistema vzdolž Neretve sledijo podobnim vzorcem, ki jih najdemo v drugih rekah drugje, druge ne. Na primer, primarna proizvodnja je sledila tipičnemu vzorcu in se povečala od zgoraj navzdol. Nasprotno pa se je razgradnja organske snovi razlikovala od pričakovanj in je zaradi večje razpoložljivosti hranil pokazala največjo aktivnost v zgornji vodi, pa tudi na večini dolvodnih območij. Na splošno naši rezultati kažejo, da je Neretva sistem, omejen s hranili, ki je lahko zelo občutljiv za spremembe okoljskih razmer. V takšnih okoliščinah lahko nenadni vdori velikih količin podzemne vode iz okoliškega kopenskega sistema močno vplivajo na delovanje ekosistema Neretve.

Kljub temu bi morale prihodnje študije ponoviti podobna vzorčenja kemije vode in funkcij ekosistema, vendar z veliko večjo prostorsko ločljivostjo in v različnih letnih časih, da bi potrdili to pomembno vlogo vnosa podtalnice v Neretvo. Naši dokazi o delu je edinstveno delovanje Neretve v primerjavi s tipičnimi srednjeevropskimi rekami zaradi njene kraške geologije, pa tudi njenega še vedno nedotaknjenega in edinstvenega ohranjenega stanja vzdolž njenega zgornjega toka. Večje človeške spremembe v reki ali porečju bi lahko sprožile drastične spremembe v delovanju ekosistema tako v lokalnem kot v velikem prostorskem merilu, kar bi lahko posledično motilo ekosistemske storitve, ki jih reka Neretva zagotavlja lokalnim skupnostim.

Sažetak

Slično svakom živom organizmu, rijeke trebaju dobiti energiju kroz različite biološke procese koji se nazivaju funkcije ekosistema na primjer: proizvodnja nove organske materije putem fotosinteze ili recikliranje mrtve organske materije kroz razgradnju. Ove funkcije ekosistema rezultat su interakcije između organizama u rijeci (kao što su zajednice mikroorganizama, bakterije, larve insekata ili čak ribe) i njihovog okruženja (hemijski sastav vode, karakteristike riječnog sedimenta,…). Uslovi životne sredine rijeke mijenjaju se prirodno na njenom putu od izvora do nizvodnih dijelova. Na primjer, rijeka se širi idući niyvodno što rezultira postupnim povećanjem dostupnosti svjetlosti u koritu, što onda može stimulisati alge i biljke na fotosintezu, a time i primarnu proizvodnu funkciju. Ove prirodne promjene duž rijeke obično prate glatke nagibe (tj. spore, ali konstantne promjene). U kraškim rijekama poput rijeke Neretve, ovi ekološki gradijenti mogu biti prekinuti iznenadnim unosom velikih količina podzemnih voda drugačijeg hemijskog sastava od riječne vode. Ove nagle promjene mogu uticati na funkcije ekosistema u rijeci. Rijeke na Balkanskom poluostrvu se veoma razlikuju od drugih sistema u Evropi zbog svog netaknutog stanja i njihove raznovrsne geologije. U ovoj studiji smo istraživali kako se mijenjaju uslovi životne sredine i funkcije ekosistema duž gornjeg toka rijeke Neretve u Bosni i Hercegovini. U junu 2022. godine uzorkovali smo i analizirali fizičko-hemijsku strukturu riječne i podzemne vode, kao i različite funkcije ekosistema na jedanaest lokaliteta raspoređenih od izvora do nizvodnih dijelova uzvodno od akumulacije Jablanica. Konkretno, istražili smo pet funkcija vezanih za različite biološke zajednice u rijeci: primarnu proizvodnju na nivou cijelog ekosistema pomoću loggera kisika i direktno iz zajednica biofilma koje rastu na kamenju korištenjem inkubacionih komora; enzimske aktivnosti biofilma fosfataze i fenoloksidaze i mikrobno razlaganje organske materije pomoću pamučnih traka. Našli smo vrlo niske koncentracije nutrijenata duž gornjeg toka Neretve. Kao posljedica toga, većina funkcija ekosistema je generalno pokazala nisku aktivnost i samo se povećala kada je koncentracija nutrijenata porasla zbog vanjskih inputa. Otkrili smo da su različite funkcije dostigle svoju maksimalnu aktivnost na različitim pozicijama duž rijeke Neretve i zavisno o unosu različitih nutrijenata. Na primjer, razgradnja organske materije bila je najveća u izvorišnim vodama zbog unosa nitrata i fosfata iz priobalne vegetacije. Enzimska aktivnost biofilma bila je najveća na srednjim dijelovima rijeke zbog unosa amonijaka iz podzemnih voda. Konačno, riječna primarna proizvodnja bila je najveća na većini nizvodnih dionica zbog akumulacije nitrata i fosfata iz uzvodnog sliva. Neke od ovih promjena u funkcijama ekosistema su slične drugim rijekama, a neke ne. Na primjer, primarna proizvodnja slijedila je tipičan obrazac i povećavala se od izvora prema nizvodno. Nasuprot tome, razgradnja organske materije se razlikovala od očekivanja i pokazala je najveću aktivnost u izvorištu, ali i na većini nizvodnih lokacija zbog veće dostupnosti nutrijenata. Uopšteno, naši rezultati pokazuju da je Neretva sistem s ograničenim nutrijentima, koji može biti jako osjetljiv na promjene uslova životne sredine. U takvim okolnostima, nagli unos velikih količina podzemnih voda iz okolnog kopnenog sistema može uveliko uticati na funkcionisanje ekosistema Neretve. Ipak, buduće studije bi trebale ponoviti slična uzorkovanja hemije vode i funkcija ekosistema, ali s mnogo većom prostornom rezolucijom i u različitim godišnjim dobima kako bi se potvrdila ova važna uloga unosa podzemnih voda u Neretvi. Dokaz o našem radu »Jedinstveno funkcioniranje Neretve« u poređenju s tipičnim srednjoevropskim rijekama zbog svoje karstne geologije, ali i njenog još uvijek netaknutog i jedinstvenog očuvanog stanja duž njenog gornjeg toka. Veliki uticaj ljudskih faktora na rijeku ili sliv mogle bi pokrenuti drastične promjene u funkcionisanju ekosistema kako na lokalnom nivou tako i na velikim prostornim razmjerima, što bi zauzvrat moglo poremetiti usluge ekosistema koje rijeka Neretva pruža lokalnim zajednicama.

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NAVODILA AVTORJEM

Poslanstvo revije **NATURA SLOVENIAE** je objavljati rezultate terensko-bioloških raziskav in podatke, ki prispevajo k razumevanju zgodovine flore in favne srednje in jugovzhodne Evrope. Dobrodošli so prispevki s pomembnimi in novimi podatki o razširjenosti vrst vseh kraljestev, pregledni seznami vrst ter ekološke, biogeografske, biodiverzitetne in naravovarstvene študije. Prispevki so objavljeni v angleškem ali slovenskem jeziku.

Revijo v imenu Biotehniške fakultete Univerze v Ljubljani in Nacionalnega inštituta za biologijo izdaja Založba Univerze v Ljubljani.

Naslov glavne urednice: Maja Zagmajster, Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, Večna pot 111, SI1000 Ljubljana; maja.zagmajster@bf.uni-lj.si

PREDLOŽITEV ROKOPISOV
Rokopisi naj bodo oddani prek spletnega portala Založbe Univerze v Ljubljani: https://journals.uni-lj.si/NaturaSloveniae.

Vse rokopise bo recenziral vsaj po en recenzent. Avtorji lahko predlagajo imena in kontakte potencialnih recenzentov, uredništvo pa si pridržuje pravico, da izbere kateregakoli drugega. Po pregledu s strani recenzenta(-ov), avtor ali avtorji sami pripravijo novo verzijo rokopisa. Popravljen rokopis je treba vrniti z označenimi spremembami (lahko uporabite orodje Track changes) skupaj z različico brez označenih sprememb ali pripomb in spremnim dopisom, v katerem so pojasnjene opravljene spremembe.

Predloženi rokopis ne sme biti predhodno objavljen v nobeni obliki in ne sme biti hkrati predložen drugam (v drugo revijo, bilten ali kongresno publikacijo). Z oddajo rokopisa podelijo založniku pravico do prve izdaje članka po licenci Creative Commons Attribution 4.0 International.

FORMAT IN OBLIKA PRISPEVKA
Rokopisi so lahko predloženi v obravnavo kot znanstveni članek, kratka znanstvena vest in terenska notica. Dobrodošli so tudi drugi formati prispevkov (pregledni članki, komentarji, mnenjski članki), vendar se je treba o tem predhodno posvetovati z urednikom.

Znanstveni članek je popoln opis izvirne raziskave, vključno z uvodnim pregledom ozadja in stanja poznavanja tematike. Struktura sledi principu »IMRAD« (uvod, material in metode, rezultati, razprava), sledijo sklepi (neobvezno), zahvala (neobvezno), literatura, povzetek, dodatno gradivo (neobvezno).
Kratka znanstvena vest je izvirno delo, ki poroča o manjšem naboru podatkov ter o delnih ali predhodnih rezultatih raziskav. Struktura sledi principu »IMRAD«, vendar je poenostavljena, na primer z enotnim razdelkom »rezultati in razprava«. Glavnemu besedilu sledijo zahvala (neobvezno), literatura, povzetek in dodatno gradivo (neobvezno).
Terenska notica je kratko poročilo o novih in zanimivih najdbah, ki izhajajo iz biološkega terenskega dela ali so z njim povezane. Vsebuje glavno besedilo, zahvalo (neobvezno) in literaturo.

Naslov prispevka mora biti informativen, jasen in jedrnat. Naslovu morajo slediti imena in priimki ter polna imena avtorjev s poštnimi in elektronskimi naslovi. Lahko so dodane številke ORCID.

Vsak prispevek mora vsebovati **izvleček**, ki vključuje zgoščeno predstavitev ciljev, uporabljenih metod, rezultatov in zaključkov. Izvleček naj ne bo daljši od 250 besed za znanstvene članke, 200 besed za kratke znanstvene vesti in 100 besed za terenske notice.

Avtorji naj vključijo **pet do največ deset ključnih besed**, zapisanih po abecednem vrstnem redu, ki morajo odražati področje raziskav in vsebino, zajeto v prispevku. Terenska notica ne vsebuje ključnih besed.

Znanstveni članki in kratke znanstvene vesti morajo vsebovati **povzetek**, ki sledi glavnemu besedilu rokopisa. Namen povzetka je zagotoviti izčrpne informacije za slovensko govoreče bralce, kadar je prispevek napisan v angleščini, ali neslovensko govoreče, kadar je prispevek v slovenščini. Povzetek naj ne bo le ponovitev izvlečka, ampak naj vsebuje več informacij o ciljih, metodah, rezultatih, razpravi in lahko vključuje navedbe literature ter sklice na slike in tabele. Priporočena dolžina povzetka je približno 500 besed za znanstvene članke in 300 besed za kratke znanstvene vesti. Pisci, ki ne govorijo slovensko, lahko pripravijo angleški izvleček, ključne besede in povzetek, te pa bo uredništvo prevedlo v slovenščino. Terenska notica nima povzetka.

Rokopisi morajo biti predloženi v enem od razširjenih formatov za urejanje besedila, kot sta Microsoft Word (docx, doc) ali tekstovni dokument ODF (odt), po možnosti s pisavo »Times New Roman« velikosti 12, levo poravnavo in 3 cm robovi na A4 straneh. Med vrsticami naj bo dvojni razmik, vrstice naj bodo oštevilčene neprekinjeno po celem rokopisu. Naslov prispevka ter naslovi poglavij in podpoglavij morajo biti napisani s krepko pisavo velikosti 14. Znanstvena imena vseh rodov in vrst morajo biti zapisana v ležečem tisku.

SLIKE IN TABELE
Prispevki lahko vsebujejo do deset slik in/ali tabel. Tabele in slike, vključno z legendami, je treba umestiti v rokopis na želeno mesto. Ločljivost slik v rokopisu je lahko nižja, da se zmanjša velikost oddane datoteke. Če je rokopis sprejet v objavo, je treba slike predložiti ločeno kot visokokakovostne vektorske ali rastrske grafike v formatih pdf, svg, jpg ali tiff. Slike morajo biti pripravljene brez elementov, kot so robovi in podnapisi; te lahko dodate v rokopis z uporabo urejevalnika besedil. Če potrebujete pomoč pri pripravi grafike ustrezne kakovosti, se obrnite na urednika.

Slike in tabele morajo biti oštevilčene zaporedno (Slika 1, Slika 2 ..., Tabela 1, Tabela 2 ...), v tekstu se je treba vsaj enkrat sklicevati na vsako sliko in tabelo v skrajšani obliki (Sl. 1 ali Sl. 1, 2; Tab. 1 ali Tab. 1, 2).

Tabele in slike morajo skupaj s naslovi/podnaslovi in legendami vključevati dovolj podrobnosti, da so razumljive same po sebi. Naslovi/podnaslovi naj bodo v obeh jezikih (angleščini in slovenščini), ne glede na glavni jezik besedila. Za neslovensko govoreče avtorje bo za slovenske prevode poskrbelo uredništvo.

Če so vključene fotografije, je treba v oklepaju navesti ime in priimek avtorja.

DRUGA NAVODILA ZA OBLIKOVANJE

Vsi datumi se pišejo s številkami, ne glede na jezik prispevka, gre za obliko: Dan.Mesec.Leto, na primer 23.5.2000, 16.6.2015.

Domačih imen ne pišemo z veliko začetnico, izjema je poimenovanje po osebi (npr. Savijev netopir).

Kadar se domače in znanstveno ime uporabljata skupaj, velja naslednje: znanstveno ime sledi domačemu brez oklepajev le v naslovu, v besedilu pa ga je treba navesti v oklepaju. Obe imeni skupaj naj se uporabljata le pri prvi omembi v besedilu prispevka, pozneje pa naj se dosledno uporablja le ena oblika. Vsako znanstveno ime naj bo vsaj enkrat zapisano v celoti, tj. vključno z avtorjem in letnico opisa.

Koordinate lokalitet naj bodo v WGS84 decimalnih stopinjah. Za Slovenijo so lahko tudi v veljavnem koordinatnem sistemu ETR89. Koordinatni sistem mora biti jasno označen.

LITERATURA
Navajanje literature in seznam literature naj bosta v skladu s slogom »Council of Science Editors« (CSE) (https://www.councilscienceeditors.org/scientific-style-and-format), z uporabo sistema »Name-Year«, z nekaterimi spremembami (navedenimi v nadaljevanju): https://www.mcgill.ca/library/files/library/cse-name-year-citation-style-guide.pdf

V besedilu:
V besedilu sta priimek avtorja in letnica objave navedena v oklepaju takoj za besedilom, na katerega se nanaša:

Večina samic odlaga jajca v prvi polovici junija (Fritz 2003) in...

Če ima vir dva avtorja, sta navedena oba priimka med katerima je znak »&« (to se razlikuje od navodil na zgornji povezavi!). Pri delih s tremi ali več avtorji se navede samo priimek prvega avtorja, ki mu sledi »et al.«:

...označene želve z marginalnim vrezovanjem (Vamberger & Kos 2011)...
...živi v spodnjem toku reke Save na Hrvaškem (Šalamon et al. 2013)...

Če se navaja več virov hkrati, jih je treba navesti v kronološkem zaporedju in po abecednem redu, če so viri objavljeni v istem letu strani istega avtorja/-jev, med sabo pa jih ločiti s podpičjem. Dve ali več del, ki jih je napisal isti avtor v istem letu, je treba letnici dodati oznako (a, b, c ...). Enake oznake se navedejo v seznamu literature.

... (Müller 1921; Seifert 2007a, 2007b; Ionescu-Hirsch et al. 2009; Lapeva-Gjonova & Kiran 2012; Wiezik & Wieziková 2013).

Če je avtor reference organizacija, inštitucija, univerza itd., se v besedilu uporabi skrajšana oblika imena, tako da se ohrani prva črka besed v imenu ali priznana kratica:

... (ARSO 2022).

Seznam literature:
Seznam literature naj sledi spodnjim primerom, a za dodatne primere naj se sledi prej navedenim smernicam za citiranje:

[ARSO] Agencija Republike Slovenije za okolje. 2022. Podnebne značilnosti oktobra 2022. Ljubljana (SI): Agencija Republike Slovenije za okolje, Ministrstvo za okolje, podnebje in energijo. https://meteo.arso.gov.si/met/sl/climate/current/climate_month/ [accessed on 26.11.2022]

Balestrieri A, Remonti L, Prigioni C. 2015. Towards extinction and back: Decline and recovery of otter populations in Italy. In: Angelici FM, editor. Problematic Wildlife. Springer International. Switzerland. p. 91-105. https://doi.org/10.1007/978-3-319-22246-2_5

Gregorc T, Nekrep I. 2010. Poročilo skupine za vidro. In: Vinko D, editor. Raziskovalni tabor študentov biologije Most na Soči 2010. Ljubljana (SI): Društvo študentov biologije. p. 12-21.

Kruuk H, Conroy JWH, Glimmerveen U, Ouwerkerk EJ. 1986. The use of spraints to survey populations of otters (*Lutra lutra*). Biological Conservation. 35: 187-194. https://doi.org/10.1016/0006-3207(86)90050-9

Gorički Š, Stanković D, Snoj A, Kuntner M, Jeffery WR, Trontelj P, Pavic M, Grizelj Z, Năpăruș-Aljančič M, Aljančič G. 2017. Environmental DNA in subterranean biology: Range extension and taxonomic implications for *Proteus*. Scientific Reports. 7: 1-11. https://doi.org/10.1038/srep45054

Navajanje zakonodajnih dokumentov:
Slovenska zakonodaja:
Navajanje v besedilu: (Ur. l. RS 2002) ali (Ur. l. RS 2004a) ali (Ur. l. RS 2004b)
Seznam literature:

Ur. l. RS. 2002. Pravilnik o uvrstitvi ogroženih rastlinskih in živalskih vrst v rdeči seznam. Uradni list RS, št. 82/02, 42/10.

Ur. l. RS. 2004a. Uredba o zavarovanih prostoživečih živalskih vrstah. Uradni list RS, št. 46/04, 109/04, 84/05, 115/07, 32/08 – odl. US, 96/08, 36/09, 102/11, 15/14, 64/16, 62/19.

Zakonodaja EU, mednarodne konvencije:
Navajanje v besedilu: (UL ES 1992)
Seznam literature:

OJ EC. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities L 206, 22.7.1992. p. 7-50.

INSTRUCTIONS TO AUTHORS

The mission of **NATURA SLOVENIAE** is to foster the understanding of the natural history of Central and Southeastern Europe by publishing the results of field-biological research and data. Manuscripts reporting significant and new distributional records, species lists from all kingdoms as well as ecological, biogeographical, biodiversity and conservation studies are welcome. Papers are published in English or Slovenian language.

The journal is being published by University of Ljubljana Press on behalf of the Biotechnical Faculty of the University of Ljubljana and the National Institute of Biology (Slovenia).

The address of the Editor in Chief: Maja Zagmajster, Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI1000 Ljubljana; maja.zagmajster@bf.uni-lj.si

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Manuscripts should be submitted via the web portal of the University of Ljubljana Press:
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All manuscripts will be subject to peer review by at least one referee. Authors are invited to suggest the names and contacts of potential referees, although the editor reserves the right to select any other. Once reviewed by the referee(s), the manuscript should be revised by the author or authors themselves. The revised manuscript should be returned with marked changes (Track changes tool can be used) along with a clean version (no marked changes or comments) and a rebuttal letter explaining the changes made.

The submitted manuscript must not have been previously published in any form and must not be simultaneously submitted elsewhere (in other journals, bulletins or congress publications). By submitting a manuscript, the authors the authors grant the publisher right of first publication with the work simultaneously licensed under the Creative Commons Attribution 4.0 International License.

TYPES AND FORMAT OF CONTRIBUTIONS
Manuscripts may be submitted for consideration as Scientific Papers, Short Communications and Field Notes. Other formats of contributions are also welcome (review articles, comments, opinion papers), but should be discussed beforehand with the editor.

Scientific Paper is a complete description of original research including an introductory overview of the state-of-the-art. The structure is typically IMRAD (Introduction, Material and Methods, Results, Discussion), followed by Conclusions (optional), Acknowledgements (optional), References, Summary, Supplementary material (optional).
Short Communication is an original paper reporting on a smaller dataset as well as partial or preliminary research results. The structure follows the IMRAD logic but is simplified, for example by fusing the Results and Discussion sections. The main text is followed by Acknowledgements (optional), References, Summary, and Supplementary material (optional).
Field Note is a short report on new and interesting findings coming from or related to biological field work. It contains information in the main text, Acknowledgements (optional) and References.

The **title of the contribution** should be informative, clear and concise. The title should be followed by the **name(s) and full affiliations of the author(s)**, with postal and e-mail addresses. ORCID numbers are optional.

Each contribution should contain the **abstract** which includes concise information about the objectives, methods used, results and conclusions. The abstract should not exceed 250 words for Scientific Papers, 200 words for Short Communications and 100 words for Field Notes.

Authors should include **five to maximum ten keywords**, written in alphabetical order, which must accurately reflect the field of research and content covered in the paper. A Field Note does not contain key words.

Scientific Papers and Short Communications should include a **Summary** following the main text of the manuscript. The purpose of the Summary is to provide comprehensive information for Slovenian or non-Slovenian speaking readers when the contribution is written in English or Slovenian, respectively. The Summary should not be a repetition of the abstract but contain more information on objectives, methods, results, discussion, and may include citations from the reference list or mentions of figures and tables. The recommended length is about 500 words for Scientific Papers and 300 words for Short Communications. Non-Slovenian speaking writers can provide English Abstract, Keywords and Summary. These will be translated into Slovenian by the editorial team. A Field Note does not contain a Summary.

Manuscripts should be submitted in one of the major text editing formats such as Microsoft Word (docx, doc) and ODF text document (odt), preferably using »Times New Roman« size 12 font, align left and margins of 3 cm on A4 pages. Double spacing should be used between lines, which should be numbered continuously for the whole manuscript. The manuscript title and headings of chapters and subchapters should be written in bold font size 14. The scientific names of all genera and species must be written in italic.

ILLUSTRATIONS AND TABLES
Papers should contain up to ten figures and/or tables. Tables and figures, including legends, should be inserted in the manuscript at the desired position. The resolution of figures in the manuscript may be reduced to ensure a manageable file size. If the manuscript is accepted for publication, figures should be submitted separately as high-quality vector or raster graphics, in pdf, svg, jpg, or tiff formats. Figures should be prepared without graphical elements such as borders and captions; those can be added in the manuscript using the word processor instead. Please contact the editor if you need assistance with preparing graphics of sufficient quality.

Figures and tables should be numbered consecutively throughout the manuscript (Figure 1, Figure 2 …, Table 1, Table 2 …). Each Figure and Table should be referred to at least once in the main manuscript text, in abbreviated form (Fig. 1 or Figs. 1, 2; Tab. 1 or Tabs. 1, 2).

Tables and figures along with their titles and legends should contain enough details to be self-explanatory. Titles should be given in both languages (English and Slovenian), regardless of the language of the main text. For non-Slovenian speaking writers, Slovenian translations will be provided by the editorial team.

When photographs are included, the author's name and surname should be given in brackets.

OTHER FORMAT GUIDELINES
All dates are written with numbers, no matter the language of the contribution, it is the form: Day. Month. Year, for example 23. 5. 2000, 16. 6. 2015.

Vernacular names should not be capitalized, an exception being naming after a person (e.g. Savi's pipistrelle). When vernacular and scientific name are used together, the following applies: the scientific name follows the vernacular without brackets only in the title, while in the text it should be given in parentheses. Both names together should be used only at the first mention in the contribution text, while later only one form should be used consistently. Each scientific name should at least once be written in full, i.e. including taxonomic authority and year of description.

Coordinates of localities should be given in WGS84 decimal degrees format. For Slovenia, they can also be in the valid ETR89 coordinate system. The coordinate system must be clearly indicated.

REFERENCES
Citing of the references and the format of the reference list should follow the Council of Science Editors (CSE) style (https://www.councilscienceeditors.org/scientific-style-and-format), using Name-Year system, with some modifications (listed below): https://www.mcgill.ca/library/files/library/cse-name-year-citation-style-guide.pdf

In the text:
The author's surname and the year of publication are enclosed in parentheses immediately following the text to which it refers:

Most females lay eggs in the first half of June (Fritz 2003) and…

If a reference has two authors, both surnames are included separated by »&« (this is different to instructions in the link!). For works with three or more authors, only the first author's name is included, followed by et al.:

…marked turtles by marginal notching (Vamberger & Kos 2011)…
…does live downstream along the Sava River in Croatia (Šalamon et al. 2013)…

If several sources are cited at once, they should be listed in chronological order and alphabetically among references published in the same year, separated by a semicolon. Two or more works written by the same author in the same year should be marked by a designator (a, b, c…) to distinguish them. The same designators are used in the reference list.

… (Müller 1921; Seifert 2007a, 2007b; Ionescu-Hirsch et al. 2009; Lapeva-Gjonova & Kiran 2012; Wiezik & Wieziková 2013).

If the author of a reference is an organization, institution, university, etc., an abbreviated form of the name is used in the in-text citation, by retaining the first letter of each word in the name, or some other recognized abbreviation:

… (FAO 2007).

Reference list:
The reference list should follow the below examples, but see the above given citation guidelines:

[ARSO] Agencija Republike Slovenije za okolje. 2022. Podnebne značilnosti oktobra 2022. Ljubljana (SI): Agencija Republike Slovenije za okolje, Ministrstvo za okolje, podnebje in energijo. https://meteo.arso.gov.si/met/sl/climate/current/climate_month/ [accessed on 26.11.2022]
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Citing legislation documents:
Slovenian legislation:
Citation in the text: (Ur. l. RS 2002) or (Ur. l. RS 2004).
Reference list:
Ur. l. RS. 2002. Pravilnik o uvrstitvi ogroženih rastlinskih in živalskih vrst v rdeči seznam. Uradni list RS, no. 82/02, 42/10.
Ur. l. RS. 2004. Uredba o zavarovanih prosto živečih živalskih vrstah. Uradni list RS, no. 46/04, 109/04, 84/05, 115/07, 32/08 – odl. US, 96/08, 36/09, 102/11, 15/14, 64/16, 62/19.

EU legislation, international conventions:
Citation in the text: (OJ EC 1992)
Reference list:
OJ EC. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities L 206, 22.7.1992. p. 7-50.