Book of Abstracts

4. Simpozij o biologiji slatkih voda s međunarodnim sudjelovanjem

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Editors' remarks

The presentation of the material in this document do not imply the expression of any opinion whatsoever on the part of the Croatian association of freshwater ecologists or of other institutions involved in the organization of the Symposium. The views expressed in this volume are those of the authors of each abstract, while editors preformed technical editing and equalized the form of abstracts. The quality of the English language is the responsibility of the authors, as no substantial language editing of submitted texts was done.

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Preface

Dear colleagues,

It has been our great pleasure and honour to organise the 4th Symposium on Freshwater Biology, as the idea for this symposium stems from our enthusiasm and love for one of our greatest treasures – freshwater. The last few years have been challenging for all of us, especially in Croatia, as we have been affected not only by the pandemic but also by terrible earthquakes. Therefore, with a two-year delay, the 4th Symposium was held for the first time in the Department of Physics of the Faculty of Science, as the building of the Department of Biology is currently being reconstructed. Special thanks go to the Dean of the Faculty of Science, Prof. Mirko Planinić, PhD, and the Head of the Department of Physics, Assoc. Prof. Mihael Makek, PhD, for their generosity. Once again, our Symposium has proven to be a place where various scientists, researchers and enthusiasts of all ages meet with one goal - to improve our knowledge of freshwater ecosystems, which has been recognised by the leading organization promoting freshwater sciences, the European Federation of Freshwater Sciences (EFFS), as it has awarded our symposium a label of excellence. We are particularly pleased that we had two esteemed scientist and experts for plenary speakers, who gave us an insight into the current knowledge of freshwater ecosystems, both above and below the ground. Our young scientists also had the opportunity to present their studies to a scientific community for the first time and to improve their skills. Participants came not only from Croatia, but also from Austria, Germany, Slovenia, Serbia, France, and the Czech Republic, which proves the importance of this symposium. In this Book, which contains all the abstracts presented during the symposium, you will find a diverse and interesting range of topics on different aspects of freshwater biology, a more relevant and important discipline in biology today than ever. We hope that the 4th Symposium on Freshwater Biology has inspired you for your future research, that some new ideas have emerged and that some new colleagues and friends have been made.

We thank the sponsors and all participants with the invitation to join us again in 2025!

President of the 4th Symposium on Freshwater Biology

Vlatka Mičetić Stanković

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Dr Patrick Leitner is a senior scientist who works at the Institute of Hydrobiology and Aquatic Ecosystem Management (IHG), University of Natural Resources and Life Sciences, Vienna (BOKU). His focus in freshwater ecology is on habitat-specific analyses of macroinvertebrate assemblages under the influence of anthropogenic activities such as hydromorphological alteration, hydropeaking, pollution, climate change and siltation. The development and adaptation of multimetric indices for WFD-compliant assessment methods with respect to macroinvertebrates comprises his current research. His taxonomic expertise relates to Ephemeroptera, the Diptera family Simuliidae, aquatic Coleoptera and Crustacea.

Anthropogenically induced fine sediment deposition in streams – a severe and widely underestimated threat to benthic invertebrate communities

Sediment dynamics and composition in streams are key factors affecting stream habitat quality. Under natural conditions, the process of erosion and sedimentation creates a diversity of sediment patches in the stream bed that provide natural habitat diversity for the complex environmental needs of the biota. This sensitive balance can be disturbed by development activities in the watershed area such as agriculture, deforestation and forestry, as well as impacts of hydropower plants in the form of impoundments,reservoir flushing, hydropeaking or residual flow, which affect the quantity and quality of fine sediments and their transport and storage regime by altering the natural timing. The embedding and covering of coarse stony habitats with fine sediments not only impairs habitat structure, but also reduces oxygen concentration in the clogged hyporheic interstices and restricts the vertical connectivity. In addition, the nutritional value of periphyton in fine substrates is limited, especially when there is permanent fine sediment transport due to hydraulic stress, which further promotes macroinvertebrate drift. Consequently, these impacts result in a significant change in macroinvertebrate community structure in streams, manifested by an overall decline in species diversity and biomass, a dominance of tolerant taxa, and a decline in sensitive taxa such as Ephemeroptera, Plecoptera and Trichoptera, which in turn leads to shifts in the composition of functional feeding types. The anthropogenic deposition of fine sediments in stream beds has therefore become an increasing stress factor for rivers around the world, exacerbated by climate change phenomena like droughts or severe floods. Depending on geology and river-type, the critical grain-sizes causing these severe impacts on the biota range from fine silt and clay in lowland rivers to sand and fine gravel in mountainous streams. This inconspicuous but steady process poses a critical threat to biodiversity and masks serious ecological impairments, especially since national ecological status assessment methods compliant with the EU Water Framework Directive usually do not indicate the

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relevant consequences. The extent of fine sediment deposition and associated effects on the biota depend strongly on the hydro-morphological condition of a stream and its riparian environment. Organic structures in the riparian zone, such as roots, fallen wood or grasses, can offset the loss of diversity by providing alternative refugia for many species. The presentation highlights different fine sediment sources and the corresponding processes in different rivers types and will provide recommendations for appropriate assessment methods and small-scale restoration measures in the form of anthropogenically initiated river type specific stable structural elements such as large stones or wood, which can serve to some extend as refugial habitats and lead to a significant improvement in macroinvertebrate diversity and composition.

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Dr Asst Prof Maja Zagmajster works in the SubBioLab research group at the Department of Biology, Biotechnical Faculty, University of Ljubljana in Slovenia. Her work focuses on understanding the distribution of subterranean species and the resulting biodiversity patterns, ranging from local to large scales, as well as their ecology and necessary improvements for the conservation of subterranean habitats. She manages the SubBioDB database, the largest collection of distribution data on subterranean organisms in the Western Balkans. At the national level, she is coordinating SubBioLab activities in two projects that will incorporate data from SubBioDB: i) the Slovenian Nature Conservation Information System under the Life NarcIS project the emerging European subterranean biodiversity platform, developing under the international DarCo project funded by the EU Biodiversa mechanism. She led a CEPF-funded project SubBIOCODE, aimed at improving knowledge and conservation of subterranean biodiversity in southeastern Bosnia and Herzegovina. She coordinates activities within the Life-IP NATURA.SI project to develop a methodology for monitoring subterranean biodiversity in caves. In recent years, she has been involved in studies of interstitial, a special type of subterranean habitat near or below riverbeds. The aims are to improve knowledge of endemism and biodiversity in these poorly studied habitats and to improve their conservation, especially in the face of increasing pressures on Balkan rivers.

Groundwater biodiversity and conservation challenges

Groundwater is the most important source of drinking water, but it is also a habitat for numerous specialised animal species. In subterranean environments there is no light, there are few nutrients that come mainly from the surface, and there are no seasonal or daily climatic variations. Many animal species cope well with these conditions and live exclusively underground - some higher taxonomic groups even have no surface representatives. Due to similar ecological pressures, morphological similarities are common, while molecular analyses reveal high molecular diversity and many cryptic species. In groundwater, crustaceans are the predominant taxonomic group. In recent decades, large datasets on the distribution and molecular diversity of groundwater crustaceans have been compiled, allowing analyses of biodiversity patterns at global, European, and regional scales. The areas of greatest diversity are in the mid-latitudes, in areas of long-term high surface productivity and habitat heterogeneity. The patterns of species richness and the key environmental factors that explain them are robust to sampling differences and remain the same even when molecularly defined operational units are used instead of morphologically defined species. The Dinarides of the western Balkans are a globally exceptional region of subterranean biodiversity. Their groundwaters harbour the only subterranean representatives of some taxa in the world (hydrozoans, tubeworms, cave bivalves) and the largest subterranean amphibian in the world, the olm. New species are still being discovered in the region, and molecular analyses show high molecular diversity within many taxa. Data on subterranean species are collected in a common database, SubBioDB, which allows detailed analyses of across political boundaries. Most records are from karst groundwater, while the fauna of riverine interstitial habitats is much less studied. Groundwater species generally have very small ranges and are often

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found in only a single location, putting them at high risk of extinction. This is of particular concern in light of current development plans in the Balkans, which include the construction of numerous hydropower plants and the alteration of natural watercourses and their subterranean connections. Their negative impacts on groundwater habitats and fauna are well documented, but unfortunately rarely considered. Further research in less studied regions and groundwater habitats need to be conducted also to facilitate the protection of this globally important natural heritage.

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Hydromorphology vs. organic pollution: which is more responsible for shaping benthic invertebrate assemblages?

Lowland rivers and their biological communities are often simultaneously exposed to multiple stressors that alter environmental conditions. Hydromorphological modifications influence several environmental conditions, especially riparian vegetation, and the flow regime. Benthic invertebrates are a constituent part of river ecosystems, and they will respond to environmental alterations through structural and functional change in their assemblages. The objectives of this research were to investigate benthic invertebrate response to natural factors and anthropogenic stressors in the Bednja River, Croatia. Benthic invertebrate samples were collected in early summer 2015 at longitudinally distributed study sites, from source to mouth of the river, using AQEM multihabitat sampling method. Water quality, sub-catchment landuse, and hydromorphological modifications were quantified for each site. Several water quality parameters and nutrients followed a longitudinal gradient but hydromorphological modifications did not, showing that degradation was present along the entire river reach. Benthic invertebrate assemblages and their diversity varied between study sites and sampled microhabitats, and there was no grouping of assemblages based on river type. Assemblages on artificial technolithal differed from those on natural lithal substrate, and supported a higher share of predators. Benthic invertebrates responded best to hydromorphological modifications through flow and sediment related functional metrics, while diversity and sensitivity metrics generally correlated poorly with hydromorphological scores. The results indicate that organic pollution is a greater problem than hydromorphological degradation of the Bednja River. The response gradient shows that ecological status can be improved by lowering ammonium levels, improving riparian vegetation structure and increasing substrate size heterogeneity.

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Climate change and pollution reduce biomass and resource quality in freshwater food webs

Human activities are rapidly altering freshwater biodiversity and ecosystem services through the combined impacts of various stressors. In this study, we investigated the effects of increased water temperature (as a proxy for climate change), and pollution with pharmaceuticals (PhACs), and endocrine disrupting compounds (EDCs) on primary producers and first level consumers in freshwater ecosystems. We conducted a microcosm experiment with a simplified freshwater food web consisting of moss and caddisfly larvae. The experiment was designed with four treatments: control, increased water temperature, PhACs and EDCs mix, and a multiple stressor treatment. The results showed that moss exhibited a mild response to the stressors and their combination. Increased water temperature negatively affected the development of caddisfly larvae, causing earlier emergence of adults and changes in their lipidome profiles. Pollution with PhACs and EDCs had a higher impact on the protein content and metabolism of all life stages of caddisfly larvae than warming. Multiple stressor effects were recorded in caddisfly adults, causing changes in metabolic response, lipidome profiles, and a decrease in total lipid content. Additionally, sex-specific responses were observed in adults, with females being more affected in terms of metabolome, and males in terms of lipidome. This study highlights the variability of both single and multiple stressor impacts on different traits, life stages, and sexes of a single insect species. The combined effects of warming and pollution negatively affect the population dynamics of aquatic insects and indicate a reduction in biomass and resource quality for both aquatic and terrestrial food webs.

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The impact of invasive amphipod *Dikerogammarus villosus* on leaf litter decomposition in Croatian and German rivers

Native amphipod shredders are crucial for leaf litter decomposition. Invasive amphipods, like *Dikerogammarus villosus*, are replacing native shredders potentially affecting the processing of allochthonous organic matter in aquatic ecosystems. This study investigated differences in in-situ leaf litter decomposition between sites with and without the invasive *D. villosus*. The experiment was conducted at six locations, two locations in Germany and four in Croatia; two sites were compared at each location, upstream with native species and downstream where *D. villosus* was present. At each site, coarse and fine leaf bags containing 2.5 g of willow leaves were placed in the water. Leaf bags were sampled three times, macroinvertebrates were identified and measured after each sampling, and leaves were dried at 60 °C for 16 hours. At all locations two-factorial PERMANOVA indicated that shredder community differed significantly between upstream and downstream sites, due to the presence of *D. villosus*. Leaf litter decomposition rates were 0.7-7.7 times lower in fine bags. Decomposition rates in coarse bags showed opposite results: three of six upstream sites with abundant native *Gammarus* species had significantly higher rates, while three of six downstream sites, where *D. villosus* was dominant shredder, also had higher rates. Locations with higher decomposition rates at downstream sites had higher water temperatures. These results suggest that replacement of the functional role of native *Gammarus* shredders by the invasive *D. villosus* depends on water temperature and shredder community composition in invaded rivers.

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The first attempt of eDNA metabarcoding of phytoplankton in Croatia and open questions

In recent years, environmental DNA (eDNA) metabarcoding has been intensively used and developed as a novel tool for monitoring and assessment of different biological quality elements in surface waters. In this study, we attempted to do so with phytoplankton in Croatian karst lakes. Phytoplankton samples were collected monthly from April to September in 2017. Samples for microscopy were collected as direct Utermöhl samples, while samples for eDNA metabarcoding were filtered in the field and stored at -80°C until analysis. eDNA metabarcoding was performed by Ilumina sequencing of the V9 region of the 18S rRNA gene. Phytoplankton biomass and number of sequences were used as quantification units for microscopy and eDNA metabarcoding, respectively. The results of this study showed that most taxa identified by microscopy belonging to Cryptophyta, Miozoa and Ochrophyta overlapped with taxa identified by eDNA metabarcoding, e.g. *Cryptomonas spp.*, *Gymnodinium spp.*, *Gyrodinium helveticum*, *Ceratium hirundinella, Peridinium willei*, *Dinobryon spp.*, etc.. However, some most abundant taxa belonging to Bacillariophyta, Charophyta and Chlorophyta identified by microscopy were not identified by eDNA metabarcoding, e.g. *Pantocsekiella comensis*, *Synedropsis roundii, Cosmarium tenue*, *Radiococcus planctonicus, Sphaerocystis spp*., etc. The percentage of overlapping taxa per sample ranged from 0.0% to 66.7%. The greatest overlap of taxonomic composition between two methods was observed in lakes Kozjak and Prošće, while the least overlap was observed in shallow lake Vransko. This study showed that eDNA metabarcoding of phytoplankton has potential to be used as a method for biological quality assessment, but more fundamental research is needed for its implementation.

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Is the oligotrophication of the Danube real or just a random result?

Phytoplankton as a primary producer is almost exclusively associated with eutrophication and countless studies have been published on this topic. The more frequent decrease in water discharge and increase in water temperature in European rivers would lead to the conclusion that the eutrophication process is increasing, and this can be seen in the biomass and composition of phytoplankton, but instead there is some evidence of oligotrophication. In this study, phytoplankton was collected and analysed monthly from April to September in 2019 at 26 and 10 sampling sites in the Danube and the main tributaries, respectively. The results of the study showed that the highest values of chlorophyll *a* concentration (55.7 µg L^{-1}) and phytoplankton biomass (19.5 mg L^{-1}) were almost three times lower than in previous studies. The Reynold's functional group composition analysis also revealed oligotrophic indicators, such as codon X3, which occurs in half of the samples and is more abundant in the Upper Danube with good wastewater treatment. Its negative correlation to phosphorus proves that it is a good oligotrophic indicator in the Danube, but its relative biomass was not very high (max. 10.06%). This study also showed that dozens of dams in the Danube interrupt the continuity of the river, which strongly affects the biomass and composition of the phytoplankton. Therefore, continuous studies are needed to gain more certainty about whether oligotrophication is a continuous process in the Danube, the second largest European river, which is important for the 79 million inhabitants of the river basin.

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Seasonal and hydrological variability govern the taxonomic and functional diversity of periphytic ciliates in a temperate floodplain

An approach integrating both taxonomic and functional diversity is increasingly used in the studies of freshwater communities, however, such studies of periphytic ciliate communities are lacking in temperate riverine floodplains. We conducted a study in a lake positioned in one of the Danube's largest natural floodplains, Kopački Rit Nature Park, from February 2015 to September 2016, covering all seasons and hydrological phases, as well as both taxonomic and functional aspects of community analysis. Our results suggest that temperature and hydrology are principle driving variables of periphytic ciliates in floodplain lake. Hydrological changes influence ciliates mainly through reshaping the lake communities at lower trophic levels, consequently altering their food source. Higher temperature, combined with hydrological stability and high availability of suspended food during the lake isolation phase, supports the highest ciliate abundances and dominance of larger planktivorous ciliates, mostly peritrichs, which could be considered as good bioindicators for revealing the disturbance. Extreme floods strongly disturb lake planktonic communities thus reducing the resource availability for ciliates and consequently their abundance, favouring small benthivorous species more resilient to disturbance. These ciliates also dominate at lower temperatures and during hydrological phase of flow pulse. We recorded the highest taxonomic and functional diversity during the hydrological phases of flow pulse and extremely high flood pulse. Our results emphasize the importance of hydrological variability, and connectivity, for the conservation of high biodiversity in river-floodplain ecosystems and add to a growing awareness of the need for the protection of rivers' hydrological regime in its natural state.

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Beyond the lakes – Aquatic vegetation of the streams of Plitvice Lakes National Park (Croatia)

The main phenomenon of the Plitvice Lakes National Park is a series of 16 barrage lakes created by the growth of tufa barriers. However, of equal importance are streams that provide the lakes with a constant water supply. During 2021 and 2022 the aquatic vegetation of the six main tributaries (Crna Rijeka, Bijela Rijeka, Matica, Plitvica, Sartuk and Riječica) and the Korana River was investigated. In total 81 river sections were surveyed using standard Central European phytocoenological methodology. The dominant vegetation of the upper and middle reaches is species-rich bryophyte vegetation of the class *Platyhypnidio-Fontinalietea antipyreticae Philippi* 1956, associated with larger substrates, faster flow and frequent shade. By contrast, in the Korana River, the bryophyte vegetation is developed on tufa waterfalls and belongs to *Cratoneurion commutati* Koch 1928 alliance. In the lower reaches of the Bijela Rijeka, Crna Rijeka and Matica rivers, the herb vegetation with the domination of rare NATURA 2000 species *Apium repens* (Jacq.) Lag. belonging to order Nasturtio-Glycerietalia Pignatti 1953 occurs. The targeted NATURA 2000 habitat of *Ranunculion fluitantis* i *Callitricho-Batrachion* vegetation is localized to solely a few sections of the Crna Rijeka River, Plitvica Stream and Bijela Rijeka River. Finally, several river lagoons and slow-flowing sections of Plitvica Stream and Korana River are overgrown with stoneworts belonging to *Charetea fragilis* F. Fukarek ex Krausch 1964 class.

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Distribution and ecology of *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John in Croatia

Elodea canadensis Michx. and *Elodea nuttallii* (Planch.) H. St. John, two invasive aquatic plants from North America, have coexisted in European waterways since the early 20th century. They were first documented in Croatia in 1894 and 2006, respectively. New localities of both species were discovered during the study which ran from 2016 to 2022 as part of the annual Water Framework Directive monitoring that covered the entire Croatian territory (786 sampling points in total). *Elodea canadensis* was found in 30 sampling points, mostly in rivers and *E. nuttallii* in 15 sampling points, mostly in channels. Nearly three quarters (72,5%) of all *Elodea* sampling points are in the Pannonian ecoregion, while 15% are in the Continental-Dinaric region. *Elodea canadensis* was discovered for the first time in the Mediterranean-Dinaric region in five sampling points (12,5% of records) in the river Cetina. Chemical and hydromorphological analyses were performed, as well as plant relevés. Fitting multivariate models to species abundance revealed the ecological reaction of *E. canadensis* and *E. nuttallii* to environmental descriptors.

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The importance of thermal and heated waters for aquatic plant invasions – what can we learn from *Pistia stratiotes*?

Thermally abnormal waters in Europe represent safe sites for alien invasive plants requiring warmer conditions than provided by ambient. Therefore, in such safe sites tropical and sub-tropical plants are frequent. By performing a literature review we identified at least 55 alien aquatic plant taxa from 21 families found in thermally abnormal European waters. The majority of taxa are submerged or rooted macrophytes. Six taxa are listed as quarantine pests according to EPPO. Among those, *Pistia stratiotes* is present in 7 European countries, whereby most of the records are recent. We studied *P. stratiotes* as a model species for free-floating plants in a thermally abnormal stream where a persistent population was able to survive harsh winters. Results showed air temperatures had a higher influence on *P. stratiotes* photosynthetic efficiency, than water temperature. Generally, growth and consequently surface cover can not be explained solely by thermally abnormal water temperatures. We conclude that even though the majority of thermophile alien plant occurrences resulted from deliberate introductions, thermally abnormal waters pose an invasion risk for further deliberate, accidental, or spontaneous spread, which might be more likely for free-floating macrophytes.

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Genomic approach for the conservation of an endemic crayfish species

Biodiversity of freshwaters is endangered due to climate change, habitat degradation and anthropogenic influence. Freshwater crayfish are considered keystone species of freshwater habitats. They have a strong impact on the biodiversity within their ecosystem. The idle crayfish (*Austropotamobius bihariensis*) is an endemic species distributed over a limited geographic area in the western Apuseni Mountains (Romania). Being a newly described species, the conservation status for the idle crayfish is not assessed. Reduced-representation (ddRAD) allows to gain insight into genomic diversity, population structure and phylogeographic patterns to obtain a well-grounded assessment of the species conservation status. Our samples included 235 individuals from 13 populations along the whole species distribution range. Using ddRAD sequencing we identified around 5000 genomic loci. The analyses of the genomic variants within these loci revealed moderate genetic diversity between the populations. Most of the populations have low heterozygosity and a small number of private alleles suggesting their lower adaptability to environmental changes. Individual based clustering showed signs of admixture between idle crayfish populations reflecting their close contact in the past. The genetic similarity of the individuals between the populations allows to implement translocation programmes in the conservation plans. The results of this study provide a baseline for the species management programs.

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The noble crayfish facing the crayfish plague: chronicle of a death foretold

Alien invasive species are major contributors to biodiversity loss in freshwater ecosystems. The invasive pathogen *Aphanomyces astaci* decimated multiple populations of the noble crayfish, keystone species native to European freshwaters. Temporal dynamics of the host's immune response to the pathogen infection represent a baseline knowledge for conservation actions. Here, we studied the changes in the noble crayfish immune response in a time course challenge with a highly virulent haplogroup B strain of *Ap. astaci* (1000 spores/mL). We recorded gross symptoms of the disease, dissemination of the pathogen in different host tissues, total haemocyte count (THC), gene expression profiles of key immune response regulators, and pathogen load. Our results indicate high susceptibility of noble crayfish to haplogroup B strain of *Ap. astaci*, with a mortality of 100%. Furthermore, visible symptoms of the crayfish plague disease (96 h) seem to precede the changes in the monitored immune response parameters: reduction of THC (120 h), down-regulation of immune system repressor (120 h) and up-regulation of immune system activator (192 h). All immune response parameters were significantly correlated to the pathogen load. *Ap. astaci* hyphal infiltrates were observed in the gills, abdominal muscle and heart. Taken together, these results point to the absence of a timely immune response reaction in the noble crayfish. Induction of the early immune response reaction is detrimental to the survival of the noble crayfish. Therefore, probing the trained immunity is the next major step for the conservation of this emblematic species.

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Changes in the microbiome of a successful freshwater invader along the environmental gradient

The introduction of invasive alien species into sensitive aquatic ecosystems contributes to the loss of freshwater biodiversity by altering ecosystem structure and services, and by transferring new, potentially unfamiliar microbial pathogens to native species. However, the microbes in the invaded ecosystem also interact with the microbiome of the invader, which can affect invader's physiology, immune status, health, and fitness. Additionally, these interactions between microbes can also be affected by changing environmental conditions, further affecting the invader and ultimately the invasion success. We analyzed the bacteriome and mycobiome of the signal crayfish, *Pacifastacus leniusculus*, one of the most successful freshwater invertebrate invaders in Europe. We collected the signal crayfish individuals, water and sediment samples, along this species' invasion range in the Korana River, Croatia. Using 16S and ITS rRNA amplicon sequencing, we examined the microbiomes of different crayfish tissues (exoskeleton, hemolymph, hepatopancreas, intestine), and environment (water, sediment). We investigated the differences in these microbiomes between two different microenvironments in the Korana River, i.e. upstream and downstream river segments. Our results showed significant differences between all crayfish and environmental microbiomes, confirming their uniqueness. Generally, the environmental microbiomes, and the crayfish exoskeletal microbiome (which is in continuous contact with the environment), exhibited higher taxonomic richness than the microbiomes of internal crayfish tissues. The exoskeletal and hemolymph microbiomes differed between two river segments, indicating that they are at least partly shaped by the environment. Our results provide insight into the microbiome of a successful crayfish invader and report its changes during dispersal through different microenvironments.

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Determinants of invasion success in a freshwater invader: exploring the occurrence of hepatopancreatitis and changes in the immune response in signal crayfish population in Croatia

Invasive alien crayfish threaten freshwater biodiversity and native crayfish fauna. However, the invasion success of an otherwise successful crayfish invader may be compromised by local environmental conditions or presence of local pathogens which can affect invader's physiology, immune status, health, and fitness. Here we report the occurence of idiopathic necrotizing hepatopancreatitis and changes in the immune response in the signal caryfish, one of the most successful freshwater invaders, in a recently invaded Korana River in Croatia. We sampled and histologically analysed 73 signal crayfish individuals collected in three consecutive years (2018-2020) and used several standard immune parameters (encapsulation response, hemocyte count, phenoloxidaze activity and total prophenoloxidaze) to: i) compare immune response of the signal crayfish along its invasion range, ii) analyze effects of specific predictors (water temperature, crayfish abundance and body condition) on its immune response changes. Our results show very high prevalence of lesions in hepatopancreas of signal crayfish (>91%), with 14% of individuals displaying heavy and 51% moderate necroses and/or inflammation in the tubular and interstitial structures of the organ. Heavy histopathological changes in hepatopancreas were correlated with lower organosomatic condition. Immune response exhibited significant differences along the invasion range of the signal crayfish and was mostly affected by water temperature and population abundance. Obtained results offer a baseline for elucidating the role of immunocompetence in the signal crayfish invasion in the Korana River, while aetiology of necrotizing hepatopancreatitis needs to be further investigated to determine its potential impact on signal crayfish invasion success.

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The future of crenobiont species in the era of changing climate - a cautionary tale of *Drusus croaticus* Marinković-Gospodnetić, 1971 (Insecta: Trichoptera) in the Plitvice Lake National Park

Drusus croaticus is a cold stenothermic crenobiont species found only in the springs of the mountainous Dinaric ecoregion of Western Balkans. Monthly monitoring of its emergence trends was performed from 2008 - 2021 in a spring that is part of the Plitvice Lake National Park in Croatia. Six emergence traps were continuously monitored resulting in a total of 1008 samples. The population decline was found to be so severe that the September abundance peaks dropped from 1436 individuals caught in 2011 to only 16 individuals caught in September, 2020. Forecast models built on 14 years of emergence patterns also suggest further population decline. Change point analysis showed a significant drop in both population abundance and discharge values in 2011 that coincided with a drought with the highest magnitudes reported to date for the area. After the drought, a short period of population convergence followed, but since 2013 a steady population decline is observed. The abundance of the *D. croaticus* population is generally negatively correlated with discharge (mean and maximum discharge), however GLMM found that years with significantly higher population densities coincide with years of significantly higher values of minimum discharge. In the periods after 2013, extreme maximum discharge values ("flushes") became increasingly present and seemed to be the most likely cause of population decline along with decreased minimum values. The dynamics of this population raises great concern for the crenobiont species in the region, particularly for the populations not inhabiting springs under the highest conservation regime of a National Park.

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14 years of chironomid emergence at a tufa barrier in Plitvice Lakes National Park

Emergence is the process in which the aquatic insect emerges from the water and turns into subimago or imago. Emerged aquatic insects provide an important food source to terrestrial predators. Goals of this study were an assessment of the factors influencing chironomid emergence at a tufa barrier and assessment of chironomid contribution to the ecosystem functions. At a tufa barrier Kozjak Milanovac in the Plitvice Lakes National Park adult chironomids were collected monthly from 2007 until 2020 using fixed pyramid shaped emergence traps. Only males were used in the statistical analyses. A minimum number of individuals is necessary to analyze changes in phenology because a sporadic occurrence of species can cause biases. For this reason, only species with a cumulative number of 170 individuals or more over the entire 14-year period were used in phenology assessment. The resulting 11 species accounted for almost 80 % of males' abundance of all 82 chironomid species collected. Total of 13,522 adults, of that 7,797 males were collected during the 14 years. The most abundant species was *Parametriocnemus stylatus* which was present whole year round. The most important factor influencing chironomid emergence was found to be water temperature. Average contribution of chironomids from BKM to terrestrial ecosystem was roughly estimated to be 1.3 g m^{-2}. During 14 years a change in chironomid composition at the tufa barrier was observed which might impact chironomid availability as a food source for mainly aquatic species as well as their role in sediment oxygenation.

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Changes in aquatic Diptera diversity and community structure affected by climate change

The rates of global warming and climate change have influenced many habitats, especially freshwater ones, due to their greater sensitivity to stressors. Aquatic dipterans make up more than 50% of all aquatic insect species described, so the aim of this research was to identify the variations in aquatic dipteran community during the 15-year period at the tufa barrier Kozjak-Milanovac in the Plitvice Lakes National Park. We analysed monthly data collected between 2007 and 2021, where we collected adult specimens using six pyramid-type emergence traps. In total 161 taxa from 13 different families were gathered, and the data was analysed using Shannon's diversity index based on ln (H') and Pielou's evenness index (J). Non-metric multidimensional scaling analysis (NMDS), Multiple change point Analysis (MCP), and species turnover analysis were also performed. Abundance and diversity indexes showed a negative correlation, i.e., years that had higher diversity indexes had less specimens caught. NMDS analyses showed segregation of trap P2 in all years from the rest of the traps. MCP showed changes in the statistically significant species before, during and after the drought period. Species turnover showed a decrease in species number i.e., species richness. We conclude that changes in the dipteran community are not visible by looking just at the diversity indices, and they should be combined with other data like the overall abundance, the total number of species, and as well the species turnover, when conducting short-term research or long-term monitoring programs.

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Perennial phenology patterns of biting midges (Diptera: Ceratopogonidae) in Plitvice Lakes

Ceratopogonidae larvae may be found in habitats with moderate amount of moisture to the benthic areas of rivers and lakes. Good way to collect only aquatic species is by emergence traps located in rivers and lakes. Precisely this type of research was conducted in the Plitvice Lakes National Park with the aim of continuous monitoring (from 2007 to the present) of the emergence of aquatic biting midges at three locations and on another seven locations from 2007 until the end of 2008. Emergence traps were sampled once a month throughout the entire period. Out of 4800 samples from ten localities (six traps per locality), biting midges were recorded only 90 times (in only about 0.02% of samples). In total, 440 individuals were recorded in this research, represented by 33 different species within 12 genera. Using the rarefaction curve based on the Jacknife estimation method, it was determined that even after almost 15 years of research, the asymptote - the theoretically finite number of biting midges taxa in this area has not yet been found. The disjunct nature of ceratopogonid distribution and occurrence is emphasized once more with the finding of Dasyhelea gothlandica Strandberg & Johanson 2015, recorded previously only from Sweden (more than 2000 kilometres away), which is fascinating given the relatively modest flight capabilities of ceratopogonids. This finding sheds light on the vast gaps in knowledge on the ecology of this fascinating insect group.

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Emergence of Scirtids (Insecta: Coleoptera: Scirtidae) in lotic karst habitats

Water beetles are an ecological group of insects characterised by great diversity and high potential as indicators of habitat quality. Nevertheless, they are still poorly studied in south-eastern Europe. The family Scirtidae (Insecta: Coleoptera: Polyphaga) consists of representatives whose larvae live in water, whilst the adults emerge on land. They often dominate in benthic communities. So far, 15 species of the family are recorded in Croatia. The aim of the study was to investigate the population and phenological characteristics of the family in typical lotic habitats. The study was carried out in Plitvice Lakes NP at three different sites where emergence traps were placed on different substrates and with different water velocities over a period of 15 years. Three taxa were recorded: *Hydrocyphon novaki*, *H. deflexicollis* and *Elodes* sp. The genus *Hydrocyphon* was recorded at the tufa barriers, genus *Elodes* in the spring area. The sex ratio of the genus *Hydrocyphon* was in favour of males in spring and early summer, whilst shifted in favour of females towards the end of autumn. Scirtids correlated positively with water velocity and the presence of bryophytes, and water temperature played a key role in determining their abundance and emergence patterns. Emergence showed a seasonal character with the highest intensity in summer. Due to the long study period, this study is a basis for future studies of emergence patterns of water beetles, but also for monitoring freshwater ecosystems, which is of particular importance in view of the ongoing climate change.

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Annotated checklist of the family Hydraenidae (Insecta: Coleoptera) in Croatia: preliminary data

The species of the cosmopolitan family of true water beetles (Insecta: Coleoptera: Hydraenidae) mainly inhabit running waters and springs, but are also found in small ponds, hypersaline pools or hygropetric ecosystems in the Mediterranean region of Europe. As grazers, the fauna of the Hydraenidae is an important link in freshwater ecosystems about which little is known, especially in Croatia. The main objective of the study was to compile the first checklist of the family with biological and ecological notes based on a detailed review of literature, museum collections and the results of various field investigations, environmental impact studies and baseline studies. A total of 173 specimens and 76 species within 3 genera (*Hydraena*, *Ochthebius*, *Limnebius*) were identified. As they inhabit endangered freshwater ecosystems, the potential status of threatened species was determined. In addition, molecular analyses were carried out (COI, nuclear genes) to identify taxonomically interesting taxa, as the family is known for its many endemics. Our results are basis for future studies of this highly interesting family of water beetles in Croatia.

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Effects of drying on invertebrate assemblages in the hyporheic zone of the intermittent Rječina River

The hyporheic zone (HZ) of intermittent streams comprises a 3-dimensional patchwork of saturated and unsaturated sediments during dry seasons. Although HZ was proposed in 1955, numerous gaps still appeared in the recent reconceptualising HZ model. The invertebrates were collected from the HZ springbrook of the karst river Rječina near Rijeka town with an aim to determine seasonal differences in their abundance, composition and diversity in relation to the duration of channel dewatering, flow variance (the Croatian Meteorological and Hydrological Service), and basic physicochemical conditions (water temperature, DO, pH, and conductivity by WTW multi-instrument 3430 F; alkalinity and organic matter by APHA (2005) standards). A Bou-Rouch pump was used for seasonal fauna sampling by filtering water and sediment through a net (mesh size 100 µm). The fauna was stored in 96% ethanol and later identified to the lowest possible systematic level under a stereo microscope using specialist keys. The absence of surface flow resulted in an increase of the water temperature and concentration of organic matter. The diversity was highest during summer when the watercourse dried up and was lowest during winter when discharge values were between high and extreme levels. The MDS ordination based on Bray–Curtis similarities illustrated the highest similarity (≥60%) of assemblages between summer and early autumn, while the separate clusters were observed when the watercourse dried up. Hydrology, chemistry, and biological fluxes within the HZ of the Rječina River vary depending on climate extremes and, consequently, on the direction of hydrological exchange between surface and groundwater.

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Life cycle of *Drusus croaticus* Marinković-Gospodnetić, 1971 (Insecta: Trichoptera)

Life cycle of Trichoptera can last one year, several years, or certain species can have several generations in one year. Among the species of the genus *Drusus*, various life cycle durations have been determined. *Drusus rame* has a one-year life cycle, *Drusus rectus*, which lives in spring areas at higher altitudes, has a two-year life cycle. In the period from 2002 to 2003, larvae of the species *Drusus croaticus* were collected at the spring of Bijela rijeka, on the area of Plitvice Lakes National Park. The spring of Bijela rijeka is located at 714 m above sea level. Usually, caddisflies have five larval stages. Head growth happens only after molting of the larvae, which is five times in a life cycle. Therefore, observing the head measurements (width between the eyes, width of the head between the outer edges in the width of the eyes and length from the forehead to the oral apparatuses) we can determine exactly which stage the larva belongs to. A total of 3,810 larvae were measured. It was established that stage five is present in all months of the year. Also, two emergence peaks were determined, one in late spring, the other in late summer, accompanied by a large number of eggs in that period, which leads us to the conclusion that the species *Drusus croaticus* has two generations, each of which lasts one year.

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The impacts of increased temperature and pollution on the biological functions of two caddisfly species

Climate change alters surface water temperature patterns and often coincides with chemical pollution from various sources. Such a combination of stressors can impact aquatic organisms, including freshwater insects that perform important ecological functions in both freshwater and terrestrial ecosystems. To identify potential impacts of individual stressors and their combination on insects' behaviour, growth, and mortality, larvae of *Drusus croaticus* and *Allogamus uncatus* were exposed to: i) a cocktail of emerging contaminants in environmentally relevant concentrations, ii) elevated temperature, or iii) their combination for 21 days. Results suggest two species of caddisflies showed differences in behavioural patterns between sampling days 7 and 21 of the experiment. While the locomotor activity of *D. croaticus* larvae was increased on day 7 in exposure to contaminants and in elevated temperature treatments, on day 21 their locomotor activity was unaltered compared to controls. Larvae of *A. uncatus* showed unaltered locomotor activity on day 7 in all three treatments, but on day 21 in elevated temperature and multi-stress treatments, their locomotor activity was decreased. Further, *D. croaticus* larvae exposed to a cocktail of contaminants showed a significant increase in weight during the experiment. Larvae of *D. croaticus* exposed to elevated temperature suffered the highest mortality, while the mortality of *A. uncatus* was similar in all treatments. Based on the results of locomotor activity analyses, we concluded that individual stressors and their combination may contribute to a disruption of natural behaviour, which could potentially lead to higher susceptibility of caddisflies to predation.

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Benthic macroinvertebrates in an intermittent stream: dynamics of recovery and recolonization patterns

Intermittent streams are habitats that periodically stop flowing. Cessation was historically natural, but climate change has affected many streams, turning them into intermittent type. Rising temperatures and changing rainfall patterns are the main causes of such habitat interruptions. In the recovery of affected areas, the dynamics of benthic community recolonization in relation to environmental conditions plays a key role. We investigated the recovery of the benthic community after the rewetting of a streambed in a small intermittent stream in the Kordun region, Croatia, with a dominant clay substrate. This is a rarely studied habitat type, especially in relation to flow. We divided the studied stream into four different sections depending on morphology and anthropogenic influences. We sampled at 11 sites, 6 times a year, depending on the dynamics of the water flow, measured environmental parameters. The main objective of the study was to assess the dynamics and characteristics of the benthic community colonization depending on the environmental conditions and substrate type. As the river sections differed in terms of the extent of anthropogenic impact and seasonality, the community composition responded in the same way. Larvae of Chironomidae were most dominant, followed by adult Amphipoda, Isopoda and Oligochaeta. Indicator specimens such as larvae of Ephemeroptera, Plecoptera, Trichoptera and Coleoptera were present in the stream studied. According to our analyses, Oligochaeta, Amphipoda and Plecoptera are good recolonization pioneers. This research will provide comprehensive, systematic data on the colonization of benthic macroinvertebrates in intermittent habitats and show how anthropogenic impacts affect the community and how long it takes to return to its original state.

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Transfer of waterborne endocrine disrupting compounds in riparian food webs

Endocrine disrupting compounds (EDCs) are found in freshwaters worldwide, mainly originating from wastewater effluents. These contaminants can accumulate in aquatic organisms and, upon emergence of aquatic insects, can be transferred to terrestrial food webs. Data on such contaminant flux is very scarce and extent of the lateral transport through food webs in the riparian zone is still unexplored. To investigate how food web linkages affect the transfer of EDCs from aquatic to terrestrial ecosystems, an *in situ* study was conducted on location impacted by wastewater. Sampling included water, biofilm, macrophytes, aquatic insects, terrestrial invertebrates, and soil. Terrestrial fauna was sampled at different distances from the river (0-1 m and 1-3 m distance from the waterline, respectively). Ultra-performance liquid chromatography (UPLC) was used to screen all samples for 25 compounds. The study found that soil had the highest total EDCs concentration, while Diplopoda had highest concentration compared to all invertebrates. The presence and concentrations of individual compounds varied among different sample types, but bioaccumulation was generally increased with proximity to the river. Results suggest that EDCs can be transferred to the riparian zone not only through aquatic insects but also through detritivores and soil exposed to flooding, as Diplopoda and Lumbricidae stand out with highest differences in concentrations between two terrestrial transects with higher concentrations closer to the river. This study provides new insights into the food web flux of EDCs and the lateral extent of their subsiding in the riparian zone, contributing to our understanding of cross-ecosystem flux of EDCs.

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Effects of climate change and wastewater effluent on aquatic macroinvertebrates: Insights from a mesocosm experiment

Freshwater ecosystems are impacted by complex chemical mixtures from multiple sources, including wastewater effluents. Additionally, climate change causes changes in, e.g., water temperatures due to long-term shifts in global weather. Freshwater biodiversity is particularly sensitive to such stressors. Moreover, stressors often interact, resulting in additive or interactive effects that are difficult to study and understand in terms of their impacts. Studies of the multiplicative effects of climate change, e.g., changing water temperatures, and chemical pollutants on freshwater biodiversity and their consequences are limited. Accordingly, the aim of the current study was to investigate the individual and combined effects of wastewater effluent and increased water temperature on model freshwater communities. A mesocosm experiment was conducted using a simplified freshwater food web containing non-vascular macrophytes (moss) and aquatic macroinvertebrates that feed as shredders and grazers. Samples were collected at the beginning and end of the experiment, whereas emerging animals were collected daily. Analyses such as total lipid content, metabolome and lipidome profiling were performed to evaluate the response of non-model aquatic macroinvertebrates to selected stressors at the molecular level. Our results demonstrate a species-specific response to selected stressors and their combination, as well as different modes of action of multiple stressors. Our study highlights the variability in the effects of both individual and multiple stressors on different traits and life stages of various macroinvertebrates in model communities.

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Mayfly assemblages in lentic phase of karst intermittent rivers in the Mediterranean

Intermittent rivers are hydrologically extreme freshwater habitats which periodically dry out, and are characterized by interchange of lotic, lentic, and terrestrial phases. They are most common in semi-arid regions of the world, where they represent an important water resource. Despite the increase in research interest in intermittent rivers, community ecology studies of such habitats are still rare, and most so far have focused on lotic habitats. Mayflies are often among the most abundant benthic invertebrates in freshwater ecosystems. Since mayflies show high sensitivity to degradation of their habitats, they are used in bio-monitoring programs worldwide. This study aimed to test mayfly habitat preferences during the lentic phase of karst intermittent rivers in the Mediterranean. Three karst intermittent rivers in Croatia (the Krčić, Čikola and Miljašić Jaruga rivers) were included in this study. A total of seven or eight pools were sampled from each river using a Surber sampler, and the physico-chemical water properties were measured at each pool. Species richness was comparable among rivers (i.e. four or five species), while abundance was lower in the Miljašić Jaruga River compared to the other two rivers, most probably due to rather high water temperature in the pools of this river (mean value was 27.3°C), which was, together with conductivity, the main environmental variable shaping mayfly assemblages in the lentic phase of studied rivers. In such harsh habitats, i.e. relatively small temporary pools with warm water (mean temperature >19.7°C), even some species (*Procloeon pennulatum* and *Nigrobaetis niger*) considered rare in Croatian freshwater habitats were able to survive, indicating conservation value of habitats in the Mediterranean intermittent rivers.

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Differences in vertical distribution of physico-chemical parameters in deep and shallow lakes - A case study from Plitvice Lakes National Park, Croatia

In a changing climate, freshwater ecosystems are continuously monitored in order to establish patterns of chemical and biological shifts. Cascading Plitvice lake`s system, apart from larger and deeper lakes such as Kozjak and Prošćansko Lake, incorporates smaller, shallower lakes, with specific dynamic processes described through changes of physico-chemical and biological parameters. Lakes Prošćansko, Kozjak, Gavanovac and Milanovac were monitored during 2015 in April, May, August and October. Water column measurements included water temperature, dissolved oxygen concentration (DO), oxygen saturation, pH and electrical conductivity measured with probes. Thermal stratification with thermocline characterized deeper lakes especially pronounced in August, while shallow lake Milanovac was mainly thermally homogenous through the monitored period. Deeper lakes noted increased DO concentration in August at 5 and 15 m of depth, which could be connected with a phenomenon of deep chlorophyll maxima. Electrical conductivity was negatively correlated with other parameters, and measured values decreased in August, especially observed for last monitored Lake Milanovac. This can be a consequence of tufa precipitation through the lake`s system. Principal component analysis (PCA) separated lakes with similar characteristics according to their depth, while Prošćansko Lake, with anoxic conditions in deep-water column in October, separated from other lakes. Comparison of temporal data between 1985 and 2015 for deeper lakes showed increased water temperature, and for Prošćansko Lake decreased DO in 2015 as an opposite to values in 1985 at 30 m of depth. This research points to differences between lakes with regard to their depth and spatial position in the cascading system.

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Freshwater gastropod assemblages in Mediterranean intermittent rivers: the influence of physico-chemical water properties

Gastropods are a key component of freshwater macrozoobenthos, inhabiting a wide array of both perennial and intermittent water bodies. Despite a growing interest in intermittent river ecology, very few studies have focused on gastropod assemblages and their ecological preferences in this dominant type of lotic habitats in the Mediterranean. Encompassing four karstic intermittent rivers of Croatia, this study aims: 1) to compare taxonomic assemblage metrics of freshwater gastropods among the rivers and 2) to assess the relationships between abiotic factors and gastropod assemblage composition (using pooled data). Gastropod sampling was performed in April 2021 during the lotic phase, using a standard Surber sampler. Simultaneously, physico-chemical water properties were measured at each location, and water samples were taken for chemical analysis. Six gastropod taxa were recorded in total. Statistically significant differences in gastropod assemblage metrics were found between two rivers, Miljašić Jaruga and Krčić, whereas the other two (Čikola and Guduča) harboured intermediate values. Abundance and species richness peaked in Miljašić Jaruga, showing positive correlations with water temperature, dissolved oxygen concentration and conductivity. Higher values of these factors are likely related to dense macrophyte vegetation and the prevalence of fine sediments, possibly enabling gastropods to survive the dry period. In contrast, assemblage metrics and dominant species exhibited a negative correlation with water velocity, suggesting a preference for microhabitats with lower water current. Our results show that Mediterranean intermittent rivers can provide suitable habitats for freshwater gastropods during the lotic phase, with assemblage composition strongly influenced by physico-chemical water properties.

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Spatial variabiliy of Diptera assemblages in lotic phase of karst intermittent rivers in the Mediterranean

Intermittent rivers are characterized by periodical flow cessation, and occur mostly in semi-arid regions of the world, such as the Mediterranean. Organisms inhabiting intermittent rivers are exposed to highly variable environmental conditions, mainly due to repeated hydrological changes from the lotic to lentic and terrestrial phases. The aim of this study was to investigate Diptera assemblages of the lotic phase of three karst intermittent Mediterranean rivers in Croatia – the Čikola, Krčić and Miljašić Jaruga Rivers. At each river, Diptera larvae were sampled at three study sites, in four replicates each, and physico-chemical water properties were also measured. Specimens were identified to the family level. A total of nine Diptera families was recorded. With eight families, the Čikola River had the highest taxa richness of Diptera due to the greatest habitat heterogeneity. However, this river also had the lowest abundance of dipterans. The Miljašić Jaruga River had the lowest taxa richness with only four families, most likely due to the greatest anthropogenic impact on this river. The opportunist non-biting midges (Chironomidae) were the dominant family in the study area, which can be attributed to their adaptation to diverse and even extreme environmental conditions. The Diptera assemblages showed a positive response to higher water temperatures and conductivity, as well as lower dissolved oxygen concentration in water. In conclusion, our results show that Diptera assemblages in the studied intermittent rivers are influenced by river morphology, microhabitat composition and physico-chemical water properties.

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Better understanding of drying river networks through project DRYvER using drying resistance and resilience traits

Drying River Networks (DRNs) are highly vulnerable freshwater ecosystems representing over a half of the world's waterways. They have unique physical, chemical, and biological properties due to their fluctuating hydrology, which supports freshwater metacommunities with a high number of specialists. The ability of freshwater organisms to survive and recover from drying events is a critical feature of DRNs. Therefore, it is essential to understand the unique traits of freshwater organisms inhabiting DRNs for proper management and conservation of these ecosystems. To concentrate efforts and to understand and mitigate the effects of climate change on DRNs, DRYvER project was initiated (www.dryver.eu). The project aims to develop a comprehensive meta-system framework that will improve our understanding of the effects of drying and changes in the dry periods on river network biodiversity and ecological integrity. The work includes creating a database compiled from the existing and newly gathered data containing taxonomic information on bacteria, fungi, diatoms, macroinvertebrates, and fish across Europe, with attributed traits such as capsule production in bacteria, mycelial growth in fungi or diapause in macroinvertebrates, present or unique in each group. This knowledge will be used to create predictive models that can accelerate the development of effective conservation strategies to maintain and restore the ecological integrity of DRNs. Additionally, understanding the biological responses of DRNs to environmental change can help predict the impacts of climate change on freshwater ecosystems globally.

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Metal(loid)s accumulation and oxidative stress responses in brown trout under the influence of wastewater in a karst catchment

Besides estimating metal exposure in the dynamic river medium, risk assessment usually involves biological responses of aquatic organisms, that reflect long-term changes in the environment. Therefore, the impact of industrial and municipal effluents in the Krka River was estimated in the spring of 2021 by measuring (ICP-MS) Cd, Cu, Zn, Tl, Mn, Fe, Mo, As, Cs, Rb, K, Mg, Na, and Ca concentrations in the intestines of native fish species, brown trout (*Salmo trutta* Linnaeus, 1758), from the Krka River source (reference site, KRS), downstream from the wastewaters (KRK) and in the Krka National Park, Brljan Lake (KBL). In addition, the multi-biomarker approach included measurement (spectrophotometry) of biomarkers of oxidative stress (malondialdehyde, MDA) and antioxidant capacity (total glutathione, GSH; catalase activity, CAT). Macroelements did not show statistically significant spatial differences, while elements such as Cu, Mn, and Zn were the highest in KRK, suggesting wastewater influence, which was confirmed by element concentrations in water, especially of those used in industry. Exceptions, although not always significant, were the highest concentrations of Mo in fish from KBL and of Cd, Cs, Tl, and Rb in fish from KRS. Significantly higher MDA concentrations in fish from KBL and KRK compared to the reference site pointed to oxidative stress, which was confirmed by the need of enhanced levels of both antioxidants (GSH and CAT) at these two sites. Evident effects on the biota, even in the national park, indicated potential risks to the protected area and emphasized the need for continuous and rigorous monitoring.

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Trichoptera of Vojvodina district - new findings and future expectations

Trichoptera (caddisflies) are small, holometabolous, mostly nocturnal insects, with life cycles dependent on freshwater, where their larval stages complete development. Caddisflies inhabit a very wide range of freshwater ecosystems, from small ponds and springs, to large rivers, bogs and lakes. These insects are very important water quality indicators, and their absence from these habitats is mostly related to ecosystem pollution. Currently, there is more than 16 000 described species, with highest biodiversity in tropical regions. In fauna of Serbia, about 220 species are known so far, but considering that there are many unexplored areas, number of species is expected to be significantly higher. Vojvodina district is the northern part of Serbia, and it belongs to Pannonian basin. With total area of about 21 500 km², Vojvodina is rich in large international rivers, like Danube and Tisza, many lakes, springs and artificial canals, all of which are habitats of great importance for Trichoptera biology. In terms of Trichoptera biodiversity, Vojvodina is almost completely unexplored. So far, we collected 45 Trichoptera species in Vojvodina, with some interesting specimens still undetermined. We detected five new species for the fauna of Serbia: *Agraylea sexmaculata* Curtis, 1834; *Oxyethira flavicornis* Pictet, 1834; *Orthotrichia tragetti* Mosely, 1930; *Ironoquia dubia* Stephens, 1837; *Ceraclea senilis* Burmeister, 1839. Main goal in our study is to complete Trichoptera list of Serbia, by searching fauna of Vojvodina. Also, very important part of our work is to associate larval and adult stages of caddisflies life cycles, using morphological keys and DNA barcoding methods.

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Aquatic plants diversity in the Lika River Basin

The Lika River is located in the central part of the Dinaric karst of Croatia. It is 78 km long and it has a basin with an area of approximately 1570 km^2. The aquatic plants of the Lika River Basin have never before been systematically studied. Here, we present the results of a floristic study of aquatic plant diversity of the Lika River and its tributaries Bogdanica, Jadova and Novčica. This study is based on fieldwork carried out during the spring and summer of 2022. It was established that there were 34 species of autochthonous vascular aquatic plants, classified into 28 genera and 23 families. The families with the greatest number of species were buttercup family (Ranunculaceae, 11.8%), umbellifers (Apiaceae, 8.8%) and grasses (Poaceae, 8.8%). The life-forms spectrum is dominated by hemicryptophytes (52.9%) and hydrophytes (44.1%) which is characteristic of moist and aquatic habitats. We recorded the occurrence of four endangered plant species: *Hippuris vulgaris*, *Menyanthes trifoliata* and *Ranunculus lingua* are endangered (EN), while *Glyceria fluitans* are vulnerable (VU). These endangered four species are also statutorily protected plants, as well as the species *Utricularia vulgaris*. The results of this research show that the Lika River Basin is an essential enrichment of plant diversity, indicating the need to preserve and protect the area investigated.

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Which habitat characteristics promote growth of brown trout (*Salmo trutta* L. 1758)?

Fish production is a direct measure of habitat quality. Physical characteristics of the habitat, as well as the quality and quantity of available food, can promote or slow the fish growth. Brown trout populations were monitored from April to October (2015) at three study sites with different habitat characteristics. Brown trout growth parameters (biomass, production, overall growth quality) and environmental parameters (water conductivity, suspended particles, prey biomass, prey diversity, pH, dissolved oxygen, etc.) were correlated to examine their relationship (CCA). One of the sites (Belosavac), the most productive, had high conductivity and was rich in prey, with domination of Gammaridae, but with low prey diversity and evenness. The other two sites (Rasina and Lomnica) had higher prey diversity and evenness but significantly lower prey abundance, with one of the two sites (Lomnica) having very low water conductivity and suspended particles concentration and the lowest prey abundance. High brown trout biomass and production were found to be positively correlated with high water conductivity, amount of suspended particles, prey abundance and diversity. Overall growth quality was negatively correlated with high prey diversity and evenness found at a site with very low prey abundance. According to this study, high water conductivity, as found in calcareous streams, promotes high prey production and consequently high trout production, while high prey diversity does not positively affect trout production when its abundance is low.

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Assessment of ecological potential of the Medjuvršje reservoir based on benthic macroinvertebrates

The Medjuvršje reservoir is a highly modified water body on the Zapadna Morava River by the construction of a hydroelectric power plant. According to the national regulation it belongs to type 2 watercourses, i.e. large rivers with mineral substrates of medium grain size. The aim of this study was to evaluate the potential of the Medjuvršje reservoir using bioindicative properties of benthic macroinvertebrates, taking into consideration their community composition. Macrozoobenthos were collected from two sites in the littoral zone of the reservoir using a standard hand net following the standard methodology (EN 27828). Specimens were sorted and identified under a stereo microscope using the appropriate identification keys. Assessment of the reservoir's ecological potential was carried out based on the class boundaries for Type 2 rivers using several biotic parameters calculated from AQEM (2002). The representatives of Oligochaeta, Mollusca, Odonata and Chironomidae were most abundant in the benthic community. The larval stages of the insects Ephemeroptera, Heteroptera, Coleoptera and Trichoptera were recorded only from the microhabitats with abundant macrophyte vegetation. Within this study, we also recorded the first finding of a freshwater sponge for this type of water body. Based on the macrozoobenthos composition and according to analyzed indices (saprobic index Zelinka & Marvan, BMWP and ASPT score, Diversity index Shannon-Weaner, Total number of taxa, share of Oligochaeta-Tubificidae) the ecological potential was assessed as good (II class of Water quality). However, when the EPT index was considered, the status deteriorated to IV (site 1) or V class (site 2) as poor or bad status.

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Diversity and distribution of Simuliidae (Insecta: Diptera) in the Balkan Peninsula

The aim of this study was to investigate the diversity and distribution of blackflies (Insecta: Diptera, Simuliidae) fauna in the Balkan Peninsula. Samples were collected from 2013 to 2021 at 256 sites. The distribution analysis and ecological differentiation of species was done according to altitudinal categories, water body type, catchment area and hydro-ecoregion. A total of 46 blackflies taxa were identified. The predominant species was *Simulium ornatum* (detected at 119 sites, with 7,630 individuals examined - 19% of all individuals collected). *Simulium reptans*, *S. variegatum*, and *S. balcanicum* were also found to be frequent. The only species recorded in all hydro-ecoregions was *S. reptans*. Most of the recorded species were euryvalent with respect to altitude and water body type. *Prosimulium hirtipes* was common in small rivers, while *S. brevidens* was a crenobiont found at altitudes above 800 m. The highest diversity (Species richness, Shannon index and Equitability) of blackflies was found in small and medium-sized water bodies at altitudes of 500-800 m. High beta diversity characterized all simuliid communities, which was reflected in high values of species turnover and low values of nestedness.

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The application of Chironomid Pupal Exuvial Technique (CPET) to assess water quality in reservoirs in Croatia

The need for electrical power, irrigation or water supply often leads to river damming and the construction of reservoirs. These artificial lakes have a specific hydrological regime and environmental conditions, which is reflected in different biotic communities. Chironomidae (Diptera) is one of the most important family of aquatic insects and an important biotic indicator. In 2019, we sampled 23 reservoirs in Croatia and used the CPET method to assess chironomid diversity and water quality. CPET is method for assessing chironomid diversity by collecting exuviae in efficient, harmless and economic way to evaluate water quality of investigated sites. Sampling net (mesh size 300µm) was used to skim the water surface and to collect exuviae along a 10 m shoreline transect. Each sample was preserved in 4% formaldehyde. Further analysis consisted of separation and mounting exuviae in Berlese mounting medium, and identification. Physico-chemical water parameters and substratum size were assessed *in situ*. A total of 7059 individuals belonging to 116 chironomid taxa were identified. The percentage share of subfamilies and tribes in the community composition was as follows: 59.54% Tanytarsini - with *Paratanytarsus bituberculatus* as the most abundant of all recorded species, 18.67% Orthocladiinae, 12,42% Chironomini, and 9.04% Tanypodinae - with *Procladius choreus* as the most frequent species in the reservoirs. Diamesinae were present with only 0.33%. The most diverse sites were HEPP reservoirs Dubrava with a Shannon index value of 2.914, followed by Varaždin and Čakovec. The majority of studied reservoirs were characterized by good water quality. CPET has proven to be a very useful chironomid bioassessment method.

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Long-term phenology patterns and ecology of *Ibisia marginata* (Fabricius, 1781) (Diptera, Athericidae) at Plitvice Lakes National Park

The family Athericidae, more precisely the species *Ibisia marginata* is an interesting and under-researched component of freshwater ecosystems. The life cycle of *Ibisia marginata* consists of egg, larva, pupa and imago. Adults inhabit marginal vegetation, while larvae live exclusively in the water medium. The goals of this study were to identify the abundance and occurrence of *Ibisia marginata,* to identify the phenological and ecological patterns, the sex ratio and the preference for the microhabitat. During 16 years, monthly, from 2007 until the end of 2022, emergence traps at tufa barriers Labudovac and Kozjak-Milanovac collected adults of *I. marginata*. Additionally, from 2007 until the end of 2008 specimens were collected at tufa barrier Novakovića Brod, Stream Plitvica and Korana River within the Plitvice Lakes National Park. A total of 374 individuals of *I. marginata* were collected during the study period. In general, *I. marginata* prefers lower discharge values, their number was lower when the discharge prior to emergence was higher and emerged from June to August, with the emergence peak in July. Moss was the preferred substrate for *I. marginata* individuals to emerge from.

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Restored tufa-depositing streams: a dynamic interface between terrestrial and lotic ecosystems

A comprehensive assessment of stream restoration success should link changes in habitat structure not only to changes in taxonomic diversity, but also to functional diversity that affects ecosystem functioning. In this study, we compared the taxonomic and functional composition of periphytic ciliates between restored and unrestored (control) streams for two different immersion periods to: i) identify species with indicator potential; ii) identify functional traits that differ between the two stream types; and iii) examine the effects of environmental parameters on species and functional trait composition. In addition, we compared the two stream types in terms of microhabitat heterogeneity through the deposition of organic and inorganic matter (tufa). The periphyton of restored streams is colonized by species that reach high abundance not only in inland waters but also in soil, such as *Platyophrya vorax*, which was identified as a potential indicator species for restored streams of the Skradinski buk barrier, which has implications for the importance of carbon cycling and organic matter cycling in restored streams. In restored streams, better competitors prevail, i.e., omnivorous, and bacterivorous free-swimming ciliates that utilize a wider range of different niches created by the greater complexity of the microhabitat due to leaching of the surrounding soil, precipitation of calcite crystals, i.e., tufa, and retention of allochthonous organic matter particles. The relationship between microhabitat heterogeneity, ciliate functional traits and organic matter dynamics suggests that stream restoration affects ecosystem functioning through its influence on functional components, suggesting that restored streams are the link between terrestrial and lotic ecosystems.

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Impact of seasonal variations on phototrophs and water quality in cave stream

It is well known that aquatic habitats are influenced by environmental parameters and that ecological parameters in these habitats follow seasonal patterns, as does the living world. In contrast, cave environments are characterized by the absence of seasons, but sites near the entrance are influenced by external conditions. Planktonic and benthic cyanobacterial and algal communities were studied in winter, spring, and summer 2020 at six sites along the Trnavski stream in Stopić Cave (Serbia), which is characterized by a large entrance and a short tourist trail. In addition, a detailed analysis of benthic diatoms was conducted, as they are considered excellent bioindicators. Multivariate analyses were conducted to separately examine the effects of seasonality on these communities. Of the 132 planktonic and benthic cyanobacteria and algae, more than half could be assigned to one of the three seasons; most taxa were assigned to spring only (>35), 32 to winter, and seven to summer. For benthic diatoms (181 taxa after detailed analysis), 25 and 24 taxa were assigned to winter and spring, respectively, and 18 to summer. In both cases, most taxa were assigned to winter and spring and the fewest to summer. Water quality of Trnavski stream, based on diatoms, ranged from high to bad, depending on the season and sampling site. The best water quality was noticed in winter, and the poorest in summer.

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Water beetles in the area of Biljski rit and Kopački rit Nature Park

Flood areas in Biljski rit and Nature Park Kopački rit are important habitats for many water beetle species that are recognized as indicators of biodiversity. The main objective of this research was to take inventory of the water beetles in the area of Biljski rit and the monitoring of water beetles in the area of the Kopački rit Nature Park. The research took place from year 2020 to 2022, during the summer months, from May to September. Two sampling methods were used: the method of active netting and trap method with an attractant. In the area of the Biljski rit a total of 1328 individuals were collected. They were grouped into 5 families and 39 species. During the two years of research in the area of the Kopački rit Nature Park, a total of 1404 individuals were collected, which were grouped into 7 families and 42 species. The highest numbers of water beetles were recorded during July in all years of research. The protected species *Graphoderus bilineatus* (De Geer, 1774) was recorded at all research locations, and the total number of specimens sampled was 48 in the area of the Biljski rit and 35 in the area of the Kopački rit Nature Park. Although it was expected that the larger number of collected individuals of water beetles would be in the area of the Kopački rit Nature Park, the absence of floods and dry periods during summer of 2022 significantly reduced the number of collected individuals.

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Cadmium accumulation and biomarker responses in the digestive glands of *Unio crassus* Philipsson in Retzius, 1788 following cadmium exposure

Cadmium accumulation above a certain threshold can adversely affect aquatic organisms. To evaluate the effects of Cd exposure on aquatic organisms, we monitored changes in the responses of the molecular biomarkers acetylcholinesterase (AChE), catalase (CAT), glutathione S-transferase (GST), malondialdehyde (MDA), metallothioneins (MT), total glutathione (tGSH), and total proteins (TP) in the digestive glands of *Unio crassus* mussels. In addition, the changes in concentrations of total and cytosolic Cd, Cu, and Zn in the digestive glands of the mussels were monitored. Mussels were exposed to 50 µg/L of Cd for 7 days. Metals were determined using a high-resolution inductively coupled plasma mass spectrometer, and biomarkers were measured spectrophotometrically. The results showed almost 30-fold higher total and cytosolic cadmium concentrations in the digestive glands of Cd-exposed mussels than in control mussels. Furthermore, the activities of biomarkers AChE and CAT were 50% and 70% lower, respectively, in exposed group than in control group. In addition, tGSH concentrations were 20% higher in exposed group than in control group. Other biomarkers showed similar activity/concentration trends in both studied mussel groups. The lack of MT induction in the presence of high Cd accumulation suggests possible toxic effects due to exceeding cellular inducible/defence capacity. Although our study examined only one concentration and exposure duration, the results suggest that Cd exposure can induce significant changes in metal accumulation and biochemical responses in mussels. Nevertheless, further studies are needed to determine the effects of Cd at different concentrations and exposure times and to clarify the mechanisms underlying toxic effects.

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Distribution of the North-American signal crayfish in river Drava basin in Slovenia in 2022

The North-American signal crayfish (*Pacifastacus leniusculus*) has been recorded in many European countries. It is a very problematic invasive species because it transmits crayfish plague (*Aphanomyces astaci*), a fungal parasite lethal to native European crayfish species and because it negatively affects aquatic communities. We report that the population is increasing in the river Drava in Slovenia. Until our study, the distribution of the *P. leniusculus* in the Drava River was known only from the town of Dravograd on the Austrian border downstream to the artificial accumulation lake near the town of Ptuj. Here we report recent surveys of *P. leniusculus* in the Slovenian Drava River (from the Austrian to the Croatian border) using submerged crayfish traps and visual inspections at 29 locations in July and August 2022. Additionally, we surveyed 19 tributaries of the Drava River. Monitoring of environmental conditions included measurement of water temperature, pH, flow, and habitat type. Our results show that *P. leniusculus* was found in the Drava River at 19 sites, some of which were new. No specimens were found in the tributaries. Our results indicate that populations of *P. leniusculus* in the Drava River are persistent and vital.

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The first record of the trematode parasite *Aspidogaster conchicola* in the freshwater mussel *Unio pictorum* in Croatia

Aspidogaster conchicola von Baer, 1826 is a common and widely distributed parasite of freshwater molluscs in the temperate zones of the Nearctic and Palearctic. Nevertheless, knowledge about its distribution and hosts in Croatia is still insufficient. Therefore, the aim of our research was to investigate the presence of *A. conchicola* in the freshwater mussel *Unio pictorum* (Linnaeus, 1758). This species from the family Unionidae is widely distributed in the rivers of Europe and Russia. The study was conducted in the Mrežnica River, where *U. pictorum* is a common species. Five specimens of *U. pictorum* were collected by scuba diving in October 2021. The dissection of mussels was carried out in the laboratory, and all tissues were inspected under a stereo microscope. Determination of the parasite was performed based on the number and arrangement of alveoli. The results showed that the prevalence of infection was 40 % (two out of five mussels were infected), and the intensity of infestation was 2 - 3 specimens/mollusc, which is higher than the values known so far. All parasites were detected in the mussels' pericardial cavity. In conclusion, this is the first record of *A. conchicola* in *U. pictorum* in the Mrežnica River, and the first finding in this mussel species in Croatia. Further investigation is needed to elucidate its distribution among other hosts.

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Do the recreational sectors in Lake Palić differ in their ecological status?

Lake Palić is a natural aeolian lake in northern Serbia, divided into four sectors. The first one is the main collector of purified municipal water, while the others are used for recreational purposes. The largest, fourth sector is a subject to the strongest anthropogenic influences. This study was conducted to determine if there are differences among recreational sectors in water quality assessed by microalgae. Phytoplankton and epiphytic diatoms were sampled in the second, third, and fourth sectors during spring, summer, and autumn. Qualitative and quantitative analyses of microalgae, chlorophyll *a* estimation and calculation of diatom indices were performed to obtain parameters for the ecological status assessment according to national regulations. IPS diatom index was fairly consistent across sectors and seasons, indicating good ecological status. Phytoplankton parameters varied among sectors, with the greatest differences occurring in spring: very bad ecological status was observed for the fourth sector, moderate for the third, and even good for the second. The main causes of poor water quality in the fourth sector in spring were the phytoplankton abundance (2362985 cells/ml) and the percentage of cyanobacteria (58.2%). These parameters occurred even higher in autumn and especially in summer. Compared to the second and third sectors with similar values of phytoplankton parameters, the fourth sector is characterized by significantly higher values, which shows that a stronger anthropogenic influence can significantly contribute to the water quality deterioration.

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New findings of rare and threatened non-diatom freshwater algae in NP "Kopaonik", Serbia

In the summer of 2022, a total of 22 algae samples were collected from 16 sites in the Kopanik Mt. area as part of the Kopaonik National Park Wetland Habitats Monitoring Project. All algae samples were fixed with formaldehyde. The algal preparations were identified using a Zeiss AxioImager M.1 microscope with AxioVision 4.9 software. A total of 135 taxa from seven phyla of algae were determined. For the first time two algae which are characteristic of high mountain regions *Dolichospermum zinserlingii* and *Staurastrum tohopekaligense* were recorded on the territory of Serbia. The freshwater red alga *Hildenbrandia rivularis* was found in the Gvozdac River. According to Serbian legislation *H. rivularis* is a strictly protected species in Serbia. Another freshwater red alga, *Lemanea rigida*, was found in the Bistrička River. Data on the distribution of this alga in southeastern Europe are very scarce. Species *Lemanea rigida* was found in Croatia (Koletić et al. 2020) and in Serbia at the Božička, Golema and Jelašnica rivers (Mitrović et al. 2020). The cold-water stenotherm alga *Hydrurus foetidus* was found in the Gvozdac River and in the Bistrička River. In Serbia, *H. foetidus* is found mainly in streams and rivers in mountainous regions. In this study, one species from the genus *Draparnaldia*, a heterotrichous green alga, was found. So far, the species of this genus have been found in four locations: the Jabučka Plain, Stanjanska River, Trkoviški Timok and Svrljiški Timok (Simić 2002).

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Response of zooplankton assemblages in the Adriatic coastal ponds to different restoration methods

Freshwater ecosystems are under significant anthropogenic pressure, often resulting in biodiversity loss. Therefore, it is important to investigate and understand the response of different communities to restoration. Zooplankton is an important component of lentic ecosystems, and an excellent indicator of ecosystem change. The main objectives of this study were: (i) to explore the response of zooplankton to restoration by comparing zooplankton assemblages between unrestored Adriatic coastal ponds of anthropogenic origin and those subjected to different restoration methods (macrophyte restoration, drainage of sediment), in terms of trophic groups and ecological types; and (ii) to determine the main drivers of zooplankton composition in each category of ponds. Shallow ponds restored by macrophyte reduction had the highest zooplankton abundance, while the lowest abundance of zooplankton was observed in shallow water bodies without restoration methods applied. Unrestored ponds were dominated by planktonic rotifers and algivorous-feeding, predatory copepods, while restored ponds had many littoral detritivorous cladocerans. Zooplankton assemblage structure was mainly influenced by conductivity, concentration of suspended organic matter, macrophyte structure complexity, and phytoplankton biomass. The results of this study confirm the potential of zooplankton as bioindicator of restoration methods and the importance of appropriate restoration measures in preserving biodiversity and good ecological status of shallow water bodies.

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Benthic macroinvertebrates of the upper stretch of the Neretva River

The aim of this study was to identify taxa richness of benthic macroinvertebrates region of the Neretva River, which is under the potential impact of human activities. The "Neretva Science Week" study area is located in eastern Bosnia and Herzegovina and it covers the upper region of the Neretva River, between its confluence with the Krupa River and the city of Konjic. In the summer 2022, aquatic macroinvertebrates were collected from a total of nine sites: seven sites on the Neretva River and two on its tributary Krupa River. Kick and Sweep hand net sampling was performed following the EN 27828:1994 standard. In total, 101 taxa belonging to 13 taxonomic groups were recorded. Based on our results, Diptera and Ephemeroptera were most taxa rich (17 and 18 taxa, respectively) and most abundant taxa (with 27.19% and 34.79% of collected individuals, respectively), followed by Trichoptera (24 taxa; 12.98% of collected individuals) and Plecoptera (6 taxa, 15.95% of collected individuals) At the lower taxonomic level, the most abundant taxa were mayfly *Ecdyonurus alpinus* Hefti, Tomka & Zurwerra, 1987 and Simuliidae (Diptera) larvae. Overall, our results indicate high benthic macroinvertebrate taxa richness, and domination of insect orders that typically occur in mountain rivers, such as the upper reaches of the Neretva River. In order to preserve the pristine character of the river, as well as its biota, it is essential to maintain the near-natural state.

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New record of exotic diatom *Capartogramma crucicula* (Grunow) Ross in Europe

During hydrobiological survey of the Danube River in Serbia, exotic diatom *Capartogramma crucicula* (Grunow) Ross was recorded for the first time. The species has pantropical distribution. Also, it has been reported in Europe, in the freshwaters of France, Spain, Portugal and Germany, usually as rare and exotic. Throughout our perennial research of the Danube River, epilithic diatom samples were collected, processed, and analyzed using standardized procedures. Nonindigenous diatom *C. crucicula* was identified in the sample collected in September 2018 at the locality Radujevac, situated downstream the dam Iron Gate II. Its morphological features are clearly seen using light microscopy, with noticeable "x" shaped stauros, thus it could be hardly overlooked or misidentified during previous investigations. Relative abundance of *C. crucicula* in the sample was low, about 1%. Presence of diatom species of tropical origin in European temperate region may be linked with climate change and warming of inland waters. Further algological research will reveal if *C. crucicula* will become a common member of diatom community of the Danube River in Serbia.

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Longitudinal profile of benthic macroinvertebrate communities of the Mirna River with special emphasis on chironomid larvae

The Mirna River is the longest river in Istria with a length of 53 km. Benthic macroinvertebrates are a community of macroscopically visible aquatic invertebrates that spend most of their lives on and in the bottom of the water body. The objective of this study was to compare the benthic macroinvertebrate composition along the course of the Mirna River. Sampling was conducted once in May 2022 at 3 sites along the Mirna River. Samples were collected using a 25 x 25 cm hand net and stored in 80 % ethanol. Basic physical and chemical parameters were measured at each station. The highest diversity was recorded at the most upstream station, Kamenita Vrata, where 14 taxa of benthic macroinvertebrates were recorded, of which the order Ephemeroptera and the family Chironomidae had the highest abundance. In the sample from the next downstream station, Portonski Most, 10 taxa were detected, with Amphipoda being the most abundant. At the station near the mouth, Dionzijev Most, the lowest diversity was recorded, with 8 taxa, and the Polychaeta group was the most numerous. The highest species richness of chironomids was recorded at the station Kamenita Vrata. Chironomid species *Halocladius variabilis* (Staeger, 1839), characteristic of salt or brackish water, was recorded only at the station Dionizijev Most. These differences in the composition of benthic macroinvertebrate communities are the result of differences in physical and chemical water properties at the individual stations, and the strongest influence of sea water is manifested in the high conductivity at the Dionizijev Most station.

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