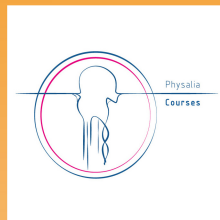


CESAMIR

SLOVAKIA
Stará Lesná

2024

July 7 - 12



SARSTEDT



Seeing beyond

LAMBDA LIFE

ISBN: 978-963-12-5936-0



Plant Science and Biodiversity Centre, Slovak Academy of Sciences
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4th CESAMIR - Stará Lesná - SLOVAKIA - July 7-12, 2024 BOOK OF ABSTRACTS

Book of Abstracts

4th Central European Symposium
for Aquatic Macroinvertebrate Research

Book of abstracts

**4th Central European Symposium for Aquatic Macroinvertebrate
Research (CESAMIR 2024)**

July 7-12 2024, Stará Lesná, SLOVAKIA

**Edited by Zuzana Čiamporová-Zaťovičová, Michaela Šamulková, Kornélia Tuhrinová
& Fedor Čiampor Jr**

Bratislava, 2024

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How to cite:

Čiamporová-Zaťovičová Z, Šamulková M, Tuhrinová K, Čiampor Jr F (eds) 2024. Book of abstracts. 4th Central European Symposium for Aquatic Macroinvertebrate Research (CESAMIR 2024). Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia, 156 pp.

ISBN 978-80-974243-1-2

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Welcome Message

Dear colleagues, friends. On behalf of the Organising committee, it is a great pleasure to welcome you to the 4th Central European Symposium for Aquatic Macroinvertebrate Research (CESAMIR), at the Congress Centre of the Slovak Academy of Sciences in Stará Lesná. We have tried our best to make your visit enjoyable and, we hope, unforgettable.

The first CESAMIR was held in Szarvas, Hungary in 2014, followed by the second in Pécs, Hungary in 2016 and the third one in Łódź, Poland in 2018. Then, unfortunately, came an unplanned break caused by the COVID pandemic. However, we have not forgotten the symposium, and although the plan to continue in 2020 didn't quite work out, we hope that in those 4 years you won't either. We hope that the 4th CESAMIR will again provide a great opportunity to present the latest results, discuss and exchange ideas not only in the sessions but also during the "after hours". We hope that you will enjoy the symposium and also the time spent in the wonderful surroundings of the High Tatras.

We would like to thank the plenary speakers for accepting our invitation and for significantly enriching the scientific programme with their plenary lectures. Of course, our thanks go to all the participants for attending and presenting a wide variety of topics in the field of aquatic invertebrate research, helping to create a rich conference programme. Last but not least our thanks go to the members of both the Scientific and Organisation Committees who helped to prepare the programme and create a pleasant background for the symposium.

Stará Lesná, 07 July 2024

Fedor Čiampor Jr & Zuzana Čiamporová-Zaťovičová
Chairs of the Scientific & Organising Committees

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Olena Bielikova

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Ondrej Vargovčík

Jarmila Lešková

Scientific Programme

SUNDAY July 7			
13:00-17:00	Registration		
18:00--	Welcome Drink		
MONDAY July 8			
8:40-9:00	Opening		
9:00-10:00	PLENARY <i>Núria Bonada</i> - Freshwater biodiversity in Mediterranean climate regions: current status and future trends		
10:00-10:30 Coffee break			
Congress hall		Business lounge	
RS1 - DIVERSITY - BIOGEOGRAPHY - PHYLOGEOGRAPHY 1		SS2 - RESPONSE TO DRYING 1	
10:30-10:50	Krzysztof Podwysocki	10:30-10:50	Petr Pařil
Environment, phylogeographic lineages or ranges – what drives the high morphological variation of the successful invader, <i>Dikerogammarus villosus</i> (Sovinsky, 1894) in European aquatic ecosystems?		Dry stream channels as attractive habitats for multiple biotic groups	
10:50-11:10	Márk Ficsór	10:50-11:10	Dorottya Hárságyi
<i>Hydropsyche</i> in the multiverse of rivers – a multi-scale, multi-model ensemble approach to explain the longitudinal distribution of larval net-spinning caddisfly species-groups		Navigating through drying histories: switching roles of connectivity in Trichoptera metacommunity organisation	
11:10-11:30	Kamil Słomczyński	11:10-11:30	Jan Sychra
Diversity of non-biting midges (Chironomidae) inhabiting common water moss (<i>Fontinalis antipyretica</i> Hedw.) microhabitats – a case study from river Rawka (central Poland)		Research of aquatic invertebrate communities in ephemeral wetlands on arable land	
11:30-11:50	Martin Drenováč	11:30-11:50	Barbora Loskotová
Fantastic beetles and where to find them? Do spring floods govern the occurrence of <i>Graphoderus bilineatus</i> in Hungary?		Role of invertebrate dispersal pathways in intermittent streams recolonisation after rewatering – an in situ experimental study	
11:50-13:00 Lunch			
RS1 - DIVERSITY - BIOGEOGRAPHY - PHYLOGEOGRAPHY 2		SS2 - RESPONSE TO DRYING 2	
13:00-13:20	Kamil Hupało	13:00-13:20	Michal Straka
Spatio-temporal niche partitioning drives a unique case of stable coexistence of two freshwater amphipods in Sicily		Disentangling environmental drivers of macroinvertebrate community structure: the role of stream drying and wastewater pollution	
13:20-13:40	Isaac Annal	13:20-13:40	Júlia Szeles
Spatial-temporal dynamic of syntopic cryptic species of the <i>Gammarus fossarum</i> complex (Amphipoda) and their ecological differentiation		The effects of drought and habitat degradation on environmental filtering and limiting similarity	
13:40-14:00	Olena Bielikova	13:40-14:00	Arnold Móra
Unveiling mitogenome of diving beetle <i>Agabus bipustulatus</i> (Linnaeus, 1767) using long-read nanopore sequencing		No way back: strong negative effects of stream intermittence on dragonfly (Odonata) assemblages	
14:00-14:20	Biljana Rimcheska	14:00-14:20	Tamás Bozóki
Are semi-mountainous and mountainous benthic communities suitable for the delineation of the 6th (Eastern Balkan) and 7th (Hellenic Western Balkan) hydrofaunistic ecoregions?		Resistance, not resilience traits, structure macroinvertebrate communities in newly drying stream sections	
14:20-15:00 Coffee break			
RS1 - DIVERSITY - BIOGEOGRAPHY - PHYLOGEOGRAPHY 3		SS2 - RESPONSE TO DRYING 3	
15:00-15:20	Didier Weber	15:00-15:20	Luka Polović
Revised overview of the subterranean aquatic fauna of Luxembourg		Comprehensive database of drying resistance and resilience traits from drying rivers across Europe	
15:20-15:40	Alice Salussolia	15:20-15:40	Anita Szloboda
From darkness to light: molecular phylogenetic analysis of the amphipod <i>Niphargus elegans</i> reveals repeated colonization of epigean environments from groundwaters		The resilience of stream food webs to drying: insights from European drying river networks	
15:40-16:00	Sebastian Prati	15:40-16:00	Gábor Várbíró
<i>Neocaridina davidi</i> , a feral shrimp in central Europe: range expansion and hidden parasites		Drought driven directional changes in presence-absence macroinvertebrates community metrics	
16:00-16:20	Didier Weber	16:00-16:20	Bernadett Boóz
First genetically confirmed discovery of <i>Niphargus</i> (Malacostraca: Niphargidae) in Germany north of the Ice Age glaciation		Flow intermittence changes the occupancy frequency distribution of stream chironomid assemblages	
PRESENTATION: Technologies for Advanced Biology Research & WORKSHOP: Nanopore Sequencing for Biologists			
16:40--			

TUESDAY July 9		
9:00-10:00	PLENARY <i>Astrid Schmidt-Kloiber - Walking the line from single species to information infrastructures</i>	
10:00-10:30	Coffee break	
	Congress hall	Business lounge
	RS2 - ANTHROPOGENIC IMPACTS & BIOINDICATORS 1	RS3 - MONITORING & CONSERVATION 1
10:30-10:50	Ana Previšić Bridging boundaries: exploring aquatic-terrestrial contaminant transport via emerging aquatic insects	10:30-10:50 Iva Kokotović Unravelling the complexity: exploring the combined effects of emerging contaminants and elevated temperature on caddisflies
10:50-11:10	David Boukal Bioaccumulation of chemical elements at post-industrial freshwater sites varies predictably between habitats, elements and taxa	10:50-11:10 Vojtěch Kolář Biodiversity and conservation potential of freshwater habitats in fly ash sedimentation lagoons
11:10-11:30	Violeta Berlajolli Altitude and human-induced landscape transformations as main drivers of midge communities at Balkan springs	11:10-11:30 Bart Achterkamp Habitat restoration in the Netherlands: does reintroduction of large wood facilitate macroinvertebrates?
11:30-11:50	Maciej Bonk Variability of isotopic niche in invasive spiny cheek crayfish <i>Faxonius limosus</i> in natural and anthropogenically altered riverine ecosystems	11:30-11:50 Diana Osadčaja The development of river-type-specific indices for assessing the ecological status of Lithuanian rivers using macroinvertebrates
11:50-13:00	Lunch	
	RS2 - ANTHROPOGENIC IMPACTS & BIOINDICATORS 2	RS3 - MONITORING & CONSERVATION 2
13:00-13:20	Pál Boda Long-term recovery of aquatic macroinvertebrates determined by the degree of the disturbance after an industrial disaster (Red Sludge Disaster, Hungary)	13:00-13:20 Tymoteusz Matera The benthic communities in streams of Przemyskie Foothills – issues for the planned Turnicki National Park
13:20-13:40	Khouloud Sebteoui Downsizing plastics, upsizing impact: how microplastic particle size interplays with <i>Chironomus riparius</i> bioturbation activity	13:20-13:40 Konrad Wiśniewski Preliminary results on macroinvertebrate communities in freshwater outflows of a cliff coastal zone in the Baltic Sea
13:40-14:00	Ioana Enache Small hydropower plants' impact on the ecological state of the benthic macroinvertebrate communities	13:40-14:00 Mario Rumišek Long-term research of aquatic biting midges (Diptera: Ceratopogonidae) in the Plitvice Lakes National Park
14:00-14:20	Jarosław Brodecki Aquatic invertebrates inhabiting specific anthropogenic microhabitats created in the underground river channel of Brzoza River (Łódź, central Poland)	14:00-14:20 Oxana Munju Macroinvertebrates of the Prut River basin, Republic of Moldova
14:20-14:40	Biljana Rimcheska Lake Prespa, a hotspot of freshwater biodiversity - assessment of water quality using benthic macroinvertebrates as a Biological Quality Element	14:20-14:40 Gorazd Urbanič The ecology of benthic invertebrates as a contribution to ecosystem-based management of large rivers
14:40-15:20	Coffee break	
15:20-18:00	POSTER SESSION	
19:00--	ACADEMIA BACKYARD BARBECUE	

WEDNESDAY July 10	
8:00-17:30	CONFERENCE TRIP

THURSDAY July 11		
9:00-10:00	PLENARY	Amrita Srivathsan - Filling the reference databases: rapid, large-scale barcoding using Nanopore sequencing
10:00-10:30	Coffee break	
	Congress hall	Business lounge
	RS4 - DNA BARCODING1	RS5 - ECOLOGY, CLIMATE CHANGE & PARASITES1
10:30-10:50	Michał GrabowskiMassive DNA barcoding of aquatic insects in Lake Skadar basin points to high diversity but surprisingly low faunal connectivity between waterbodies	10:30-10:50Péter BorzaInsights into the trophic ecology of invasive Ponto-Caspian mysids (Crustacea: Mysida)
10:50-11:10	Monika ŁabudzkaIntegrative taxonomy of Ephemeroptera, Plecoptera and Trichoptera of Przemyskie Foothills and Bieszczady Mountains streams	10:50-11:10Janusz ŻbikowskiCan macrophytes affect the structure of Chironomidae larvae also in nearby unvegetated zones?
11:10-11:30	Andrei-Bogdan TerecSpecies level identification of Simuliidae (Insecta, Diptera) from Romania using DNA barcode data	11:10-11:30Marija IvkovićDiptera species turnover and dominance shifts triggered by climate-driven changes
11:30-11:50	Tomasz RewiczDiversity and distribution of epigeic amphipods in Poland through DNA barcodes	
11:50-13:00	Lunch	
	SS1 - CARPATHIAN BIODIVERSITY1	RS6 - INVASIVE SPECIES1
13:00-13:20	Zoltán CsabaiFirst insight into the aquatic beetle genetic diversity of the Pannonian Ecoregion	13:00-13:20Denis Copilas-CiocianuInvasive Ponto-Caspian amphipods experience a significant trophic niche contraction outside the native range
13:20-13:40	Patrik MackoAquatic beetles in Tatra lakes – when globally small players mean a lot in terms of genetic diversity	13:20-13:40Eglė Šidagytė-CopilasFaster aerobic metabolism in invasive Ponto-Caspian vs. native amphipods
13:40-14:00	Michał GrabowskiBiodiversity of Carpathian springs - an overview of the results of the Polish-Slovak-Hungarian project	13:40-14:00Serena MuccioloExploring the osmoregulation of two Ponto-Caspian invaders through the expression of membrane proteins
14:00-14:20	Avar-Lehel DénesGenetic diversity of Diptera – DNA barcode analysis in the assessment of the ecological integrity of freshwaters from the Romanian Carpathians	14:00-14:20Yaron HersHKovitzFirst comprehensive overview of invasive aquatic macroinvertebrates in Israel
14:20-14:40	Michal RendošExploring the hidden world: first insight into diversity of epikarst invertebrates in the Western Carpathians	14:20-14:40Kęstutis ArbačiauskasIntroduction of Ponto Caspian crustaceans into a central European lake: consequences for crustacean assemblage and food web
14:40-15:20	Coffee break	
	RS7 - FAUNA OF LAKE OHRID1	RS8 - TAXONOMY & MORPHOLOGY1
15:20-15:40	Tomasz MamosDistribution, diversity, and diversification of <i>Gammarus</i> species flock in the ancient Lake Ohrid, overview of the project progress	15:20-15:40Claire DuchetStage-specific effects of warming on the larval development and temperature-size rule in <i>Sympetrum striolatum</i>
15:40-16:00	Klaudyna KrólikowskaFirst COI barcode reference library for the ancient Lake Ohrid basin – how much have we progressed?	15:40-16:00Patrik KisUnfolding the wings: predicting wing morphology from body size in aquatic beetles
16:00-16:20	Luiz F. AndradeAn overview of the lateral line organ in amphipods with emphasis on the <i>Gammarus</i> species flock from the ancient Lake Ohrid	16:00-16:20Zsolt KovácsAge-dependent variation of aedeagal morphology in <i>Agabus uliginosus</i> and the status of <i>A. lottii</i> (Coleoptera, Dytiscidae)
19:00--	CONFERENCE DINNER	

FRIDAY July 12		
9:00-10:00	PLENARY	Arne Beermann - It takes two to tango: on the benefits and limits of molecular approaches for assessing freshwater biodiversity
10:00-10:30	Coffee break	
	Congress hall	
	RS9 - DNA METABARCODING	1
10:30-10:50	Ondrej Vargovčík	Invertebrate DNA metabarcoding sheds light on the environmental gradients of the Tatra Mountain lakes
10:50-11:10	Till-Hendrik Macher	A new tool in the toolbox: evaluating the potential of DNA metabarcoding for the monitoring of macroinvertebrates in regulatory context
11:10-11:30	Robin Schütz	TrendDNA: studying long-term biodiversity change using environmental DNA contained in the German Specimen Bank
11:30-11:50	Patrik Macko	One step closer to Biomonitoring 2.0: a case study reinforcing the perspective of routine use of metabarcoding data in the multimetric assessment of the ecological quality of freshwater ecosystems
11:50-12:30	AWARDS, CLOSING	
12:30-14:00	Lunch	

Programme at a glance

	Sunday July 7	Monday July 8	Tuesday July 9	Wednesday July 10	Thursday July 11	Friday July 12
8:40 - 9:00		Opening		Mid-Conference Trip		
9:00 - 10:00		PLENARY Session I	PLENARY Session II		PLENARY Session III	PLENARY Session IV
10:00 - 10:30		Coffee break	Coffee break		Coffee break	Coffee break
10:30 - 11:50		RS1-1 SS2-1	RS2-1 RS3-1		RS4-1 RS5-1	RS9-1
11:50 - 13:00		Lunch	Lunch		Lunch	Awards, Closing
13:00 - 14:20	Registration	RS1-2 SS2-2	RS2-2 RS3-2		SS1-1 RS6-1	Lunch
14:20 - 15:00		Coffee break	Coffee break		Coffee break	
15:00 - 16:20		RS1-3 SS2-3	Poster Session		RS7-1 RS8-1	
18:00 - :-)	Welcome Drink	Presentation & Workshop	Academia Backyard Barbecue		Conference Dinner	

General Information

Venue – [Congress center ACADEMIA](#) (Stará Lesná, SK-059 60, Tatranská Lomnica, SLOVAKIA) which belongs to [Slovak Academy of Sciences](#), is located in beautiful surroundings of the Slovak part of Tatra Mountains, at the border of the Tatra National Park ([TANAP](#)), the oldest national park in Slovakia. In “ACADEMIA” building all oral and poster presentations, Opening/Closing ceremonies, Welcome Drink, and lunches will be held, as well as accommodation for around 100 participants. For the conference scientific sessions, two rooms, a Congress Hall and a Business Lounge are available in the Congress centre. The poster session has a specific block in the programme, however the posters can be installed throughout the whole conference, so there will be plenty of space for fruitful discussions. In addition to the conference activities, the hotel offers a Relax Centre and Wooden Gazebo. During the conference, free WiFi and parking is ensured.

Language – the official language of the symposium is English. There will be no provision for simultaneous translation into other languages.

Registration & information – all participants, including speakers, must register at the information desk and will receive an official conference package, the invoice, a “Certificate of attendance” and a name badge, and there will be an opportunity to upload their presentation file. Attendees will be requested about social events options.

Opening hours of the registration and information desk:

Sunday, July 7, 13:00–17:00

Monday & Tuesday, July 8–9, 08:30–19:00

Thursday & Friday, July 11–12, 08:30–9:00, during coffee breaks

Name badges – every attendee should wear her/his official name badge visibly all time to enter the sessions, meals and social events.

Meals – the conference fee covers lunch on all days, Welcome Drink (Sunday) and Conference Dinner (Thursday, included only in the FULL package). During the Mid-Conference field trip lunch will be served on site.

Accompanying persons – accompanying persons are also welcome on this meeting. Their registration package includes Welcome Drink, Mid-Conference field trip, and Conference Dinner.

Social Events

Welcome Drink – all participants are invited to attend the Opening & Welcome Drink beginning at 18:00 on Sunday, July 7, at the conference venue.

Barbecue (optional program) – all participants are invited to attend the informal Barbecue from 20:00 to 24:00 on Tuesday, July 9, in the Congress Centre garden.

Mid-Conference field trip – on Wednesday, July 10, (FULL, STANDARD and ACCOMP. PERSON Packages) we prepared an all-day-long field trip. There and back we will go by buses, we meet at 8:00 at the KC Academia. There are two options:

- **Dunajec river on wooden barges**

Zamagurie is the northernmost part of the Spiš region, located north-east of the Tatra Mts. This outwardly harsh and inhospitable region has a unique folk architecture, numerous historical monuments and natural beauties. The symbol of Zamagurie are the Pieniny Mts. Its steep rocks are washed by the Dunajec river, which drains water into the Baltic Sea. The white waters of the Dunajec have shaped a beautiful canyon approximately 9 km long, which is the largest natural canyon in Central Europe.

- **Alpine lakes in the High Tatras**

High mountain hike through Veľká Studená dolina valley from Hrebienok to Zbojnícka chata chalet. This option is suitable for more hiking oriented participants in adequate physical condition, which are ready to hike for about 3 hours (ca 5.6 km) upwards, and overcome about 700 meters of elevation. You will be rewarded with a view of the breathtaking scenery of the Tatra peaks, valleys and glacier remains – pearls of the Tatras – alpine lakes, called “plesá”. And, last but not least, with refreshment at Zbojnícka chata chalet 1960 m a.s.l., named after outlaws (outlaw = “zbojník”) who were once frequent in these areas.

Conference Dinner – on Thursday, 11 July in the evening, a Conference Dinner is prepared for the conference participants (FULL registration) in “*Panorama Bachledka*” restaurant in Bachledova dolina. In a unique architecture hidden in the heart of nature you will be able to enjoy a traditional folklore program and delicious food.

Dinner is planned for 19:00, guests will be transported by buses from the conference centre. The restaurant is reached by cable car, from which you can enjoy beautiful views of the Tatra landscape.

Guidelines for Presenters

Scientific Programme – there are 3 types of presentations: plenary lectures (performed in the Congress Hall each morning), normal oral presentation and poster presentation. The programme will be arranged as parallel and non-parallel regular sessions (Congress Hall, Business Lounge) and poster session (Lecture Hall foyer).

Oral presentations

Talks except plenaries will be scheduled in 20-minute slots. We strongly advise all presenters not to speak for more than 15 minutes to allow 5 minutes for discussion and questions from the audience. Please note that the time limit will be strictly enforced by the session chairs to facilitate keeping the time schedule of the symposium.

Speakers are required to provide the **final version of their presentation** on a USB flash drive (memory stick) / external hard drive and upload it to the respective computer in advance: ideally during on site registration or a day before presentation, but latest in the morning for (morning sessions), or during lunch break (afternoon sessions).

Acceptable formats for presentations are **Power-Point (*.ppt, *pptx)** or **Adobe (*.pdf)**, both 4:3 and 16:9 formats are supported. During the on-site submission, CESAMIR 2024 technical staff will be available to assist you. After uploading your presentation, please check it to make sure that all fonts, images, animations and/or audio/video clips are working properly. Please, name your files as follows (according to final program): **"Session_slot_Last name"** (e.g. RS1-1_Mamos).

Conference rooms will be equipped with **laptop computers, data projectors, screens, laser pointers, microphones** (optional). Personal laptops cannot be used for presentations. The presentation computers use PowerPoint with **Windows** operating system.

Poster presentations

The preferred poster size is B1: 100 cm height, 70 cm width (max 100x90cm).

Each poster will be **assigned a number** (published in the final program and on the web site) that will lead to the poster panel provided by organisers. Posters should be mounted after the on-site registration (Sunday evening) and during Monday, but no later than 1 hour before the start of the Poster session. Poster **mounting tools** will be provided on the site.

All posters will be exhibited in the **Lecture Hall foyer** during the whole symposium and presented during the **Poster session (Tuesday afternoon)**. All poster presenters are expected to be available on Poster session for questions and discussion. Posters should remain on panels at least until poster session is officially over.

Awards

"Best student speaker prize"

Oral presentations held by undergraduate students and PhD students will be judged by the members of the Scientific Committee. Main criteria: preparedness of the presenter, clarity, design. Based on the proposal of the jury one speaker will be awarded.

"Best student poster prize"

All posters presented by undergraduate students and PhD students will be judged by the members of the Scientific Committee. Main criteria: presentation of the scientific content, clarity, structure, design. Based on the proposal of the jury one poster will be awarded.

Presentation & Workshop

All participants are welcome in the **Special presentation & Workshop** at **Monday evening**. Both the presentation and the workshop will be provided by the conference partner, the **NGS GEEKS Division of I.T.A.-Intertact, s.r.o.**, which states that *"Every month there are many innovations in NGS. Some take our breath away, others make us laugh out loud."*

Presentation: Technologies for Advanced Biology Research

The development of various approaches to studying biology using specific methods for NGS library preparation is so rapid that not many can follow up. Therefore, we offer a pre-selected overview of technologies that are, in our opinion, of high interest to experimental field biologists, solving the most burning issues such as (i) insufficient amount of sample, (ii) detailed analysis of genome and transcriptome, (iii) low quality of nucleic acid, (iv) portable instrumentation for qPCR and sequencing, or (v) user-friendly sample storage management.

Workshop: Nanopore Sequencing for Biologists

The workshop's scope is to educate participants on Oxford Nanopore sequencing technology and the manual work necessary to create a sequencing library and launch sequencing experiments. Short part will be dedicated to sequencing experiment QC data analysis in EPI2ME analysis software.

Plenary Lectures

Prof. Núria Bonada (Spain) is Full Professor in Biology and Ecology at the University of Barcelona, Serra Húnter Fellow, ICREA-Academia since 2022, member of the Research Institute of Biodiversity (IRBio), and head of the Freshwater Ecology, Hydrology and Management (FEHM) research group. She has focused her research on the ecology of river ecosystems, combining fundamental, applied, and social aspects. In particular, she has been interested in understanding the effects of natural and anthropogenic disturbances on freshwater biodiversity (mainly macroinvertebrates) to improve river conservation and management worldwide, and how citizens can contribute to this research and benefit from it. Her studies on macroinvertebrates have been conducted in different river ecosystems of the world and at different scales (continental, regional, local) but, in particular, on intermittent rivers and in Mediterranean regions. Despite intermittent rivers occupy a large portion of the global river network, are ecologically unique, and are expected to be more common in the future, she has shown there are still many research gaps and are poorly recognised by managers and the general public to propose successful adaptive management plans. In particular for Mediterranean rivers, where drying has triggered many evolutionary adaptations and are especially biodiverse, she has shed some light on (1) how this biodiversity is organised in space and time to cope with drying, (2) how human activities are jeopardising Mediterranean rivers, (3) how these rivers should be managed to halt biodiversity loss and ensure healthy ecosystems, and (4) how we can effectively engage citizens in research and management. Her research has resulted in more than 150 scientific publications as papers, books and book chapters, and has participated in more than 40 national and international research projects and contracts. She has been Associate Editor of *Hydrobiologia*, *Aquatic Sciences* and *Biology Letters*, and she is currently a member of the editorial board of *Freshwater Science*, *Knowledge and Management of Aquatic Ecosystems*, and the *Boletín de la Asociación Española de Entomología*. She has a collaboration network of 10+ national and 30+ international researchers. She is now the President of the Iberian Association of Limnology (AIL) and has been member of the executive committee of Freshwater Biology Association (FBA).



Dr. Astrid Schmidt-Kloiber (Austria) is senior scientist at the Institute of Hydrobiology and Aquatic Ecosystem Management at the University of Natural Resources and Life Sciences in Vienna (BOKU University). She is a freshwater ecologist by training and member of the working group RHEOPHYLAX, dealing with topics related to benthic invertebrates in a changing environment. In her research she mainly focuses on freshwater biodiversity including data management and processes of scientific data publishing. She is the main driving force behind the *Freshwater Information Platform*, a knowledge base offering all kind of resources for freshwater scientists, managers and policy makers. Additionally, she has established and maintains *freshwaterecology.info*, a Europe-wide database for ecological preferences and biological traits of macro-invertebrates, fishes, macrophytes, diatoms and phytoplankton. Astrid is European co-chair of the *Freshwater Biodiversity Observation Network* (FWBON) and co-leads the data & synthesis pillar in the *Alliance for Freshwater Life*. Together with colleagues from the UK and Austria she has founded an *IUCN Species Specialist Group for Ephemeroptera, Plecoptera and Trichoptera* where she takes care about the conservation of these organism groups.



Dr. Amrita Srivathsan (Germany) is a research scientist at the Museum für Naturkunde – Leibniz-Institut für Evolutions- und Biodiversitätsforschung in Berlin. She is a member of the Center for Integrative Biodiscovery and her work is focussed on the field of species interactions and developing tools for biodiversity research, with a focus on bioinformatics. She is the developer of ONTbarcode, a user-friendly software facilitating large-scale barcoding using Nanopore sequencing technology and has been developing large-scale barcoding pipelines for over a decade. She is focussed on developing techniques and providing easy-to-use solutions for large-scale species discovery and integrative taxonomy efforts and these pipelines are being routinely used for handling thousands of insect specimens. Amrita obtained her PhD in a joint programme between National University of Singapore and Imperial College London during which she specialised in developing metagenomic techniques for diet analysis, laying the groundwork for her



subsequent research. More recently she has been focused on developing cost-effective methods for studying species interactions at scale notably through metabarcoding of fly fecal samples. This approach offers scalability and affordability, crucial for widespread species interaction studies and monitoring of vertebrate populations. She is an Associate Editor of *Cladistics*.

Dr. Arne J. Beermann (Germany) is a scientist at the department of Aquatic Ecosystem Research, led by Florian Leese, at the University of Duisburg-Essen. While being a morphotaxonomist and marine biologist by training, his main research focuses on employing DNA-based identification approaches to characterise freshwater macroinvertebrate communities. As a member of the former German Barcode of Life I (GBOL I) initiative, he used integrative taxonomic approaches to identify single invertebrate species and complement reference sequence databases. Curating reference databases and developing standards



for reliable barcoding and metabarcoding identification results is the objective of one of his current projects, the “dbDNA project”. Within his PhD studies, Arne started exploring the potential of using DNA metabarcoding to analyse the effects of multiple stressors on macroinvertebrate communities, which includes the benefits of high taxonomic resolution but at the cost of a lack of abundance data. He continues his interest in multiple stressor research as part of the Collaborative Research Centre (CRC) 1439 “RESIST” (<https://sfb-resist.de/>), in which, among a multitude of other aims, the combination of DNA- and image-based methods are tested to obtain both high taxonomic resolution and abundance/biomass data. Beyond that, Arne is involved in the GeDNA project testing the potential of DNA metabarcoding for routine freshwater monitoring in Germany as well as the TrendDNA project exploring long-term biodiversity trends using suspended particulate matter from the German environmental specimen bank. Arne was responsible for DNA metabarcoding of macrozoobenthos in the Joint Danube Survey 4 and will continue his efforts in the upcoming JDS5 sampling campaign. He was part of the EU Cost Action CA15219 “DNAqua-Net” and is a current member of the ongoing eDNAqua-Plan project.

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Abstracts

Plenary lectures

It takes two to tango: on the benefits and limits of molecular approaches for assessing freshwater biodiversity

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The advent and ongoing advancements of molecular approaches revolutionised how we assess biodiversity. DNA barcoding and DNA metabarcoding are useful tools for identifying specimens individually or from bulk, and eDNA metabarcoding utilises analysing DNA traces from environmental samples such as water or sediment to assess biodiversity less invasively. In addition, analysing RNA opens up the possibility to go beyond identification and towards analysing gene expression patterns under different environmental conditions. And while molecular approaches are generally faster and achieve on average a higher taxonomic resolution, they often come at the cost of lacking information such as on abundance or biomass. This presentation will give an overview of recent applications of molecular approaches for assessing freshwater biodiversity using many examples from our own working group. We will emphasise the benefits of the respective methods for the distinct study foci, but also address existing limits and where options of combinations with other methods are particularly beneficial or even mandatory. In particular, we will address the dependency of DNA-based identification methods on reference sequence libraries and how we can achieve robust taxonomic assignments, which are a basis for molecular biodiversity assessment. We will present test cases on how DNA metabarcoding can be used to assess stream macroinvertebrate communities in biomonitoring programs (Joint Danube Survey 4 and GeDNA project) and when analysing effects of multiple stressors (CRC 1439 "RESIST"). In the latter context we will specifically address the lack of abundance and biomass data, how imaged-based approaches can be used complementary and how RNA can be used to analyse stressor effects beyond traditionally measured responses such as mortality or abundance shifts. We will conclude by highlighting how eDNA can be analysed for both aquatic and terrestrial organisms, and even be used for non-invasive ecological status assessments.

The versatility of molecular approaches in biodiversity assessment cannot be overstated. Their ability to identify species rapidly and accurately, even from complex environmental samples, has transformed our understanding of ecosystems. However, as with any tool, there are limitations and challenges that must be addressed. To fully realise their potential, researchers from different disciplines (taxonomy, ecology, molecular biology) – but also funders, policy makers and regulators that benefit from the data as end users – must collaborate to address challenges such as incomplete and uncurated reference libraries, quality assurance and quality control of the workflow to avoid false-negative and false-positive results and tackle the lack of abundance and biomass data. Thus, the strongest added value will unfold if molecular methods are used in combination with existing methods from different disciplines. By doing so, we can continue to explore, assess, and monitor biodiversity and inform more effective conservation and management strategies.

Freshwater biodiversity in Mediterranean climate regions: current status and future trends

Núria BONADA

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Freshwater ecosystems account for 0.3% of the planet's freshwater but they are the habitat for 9% of all described species and 35% of vertebrate species. The levels of freshwater biodiversity loss are alarming, doubling those found in terrestrial or marine ecosystems. Mediterranean climate regions are considered global hotspots of biodiversity, also for freshwater organisms. Rivers in these regions (med-rivers) are unique ecosystems because of their predictable winter flooding and summer drought regimes. They support many species adapted to both floods and droughts, and their high levels of freshwater biodiversity are explained by past historical events and current environmental heterogeneity. At the same time, Med-rivers have been affected for centuries, in some cases millennia, by multiple human activities that increasingly threaten their biodiversity. These threats include changes in land use, nutrient loads, heavy metal concentrations, salinity, water withdrawals, invasive species and, more recently, xenobiotics or emerging organic pollutants. In addition, future climate change scenarios predict increases in drought conditions and in the occurrence of extreme events, such as floods, heat waves, and wildfires. The diversity of freshwater organisms is declining more rapidly in med-rivers than in rivers anywhere else in the world and, for some taxonomic groups, Mediterranean regions have more introduced than native species. Freshwater biodiversity conservation in med-rivers requires innovative approaches to account for both natural and human disturbances. Current protection figures, including the Natura 2000 network, do not appear to be very efficient in protecting freshwater biodiversity, and current methods to assess the ecological status fail when applied to characteristic med-rivers. To ensure the protection of freshwater biodiversity and ecosystems in Mediterranean climate regions, the adaptation and development of specific management protocols and actions to the characteristics of med-rivers is needed, specially under future climate scenarios.

Walking the line from single species to information infrastructures

Astrid SCHMIDT-KLOIBER

Institute of Hydrobiology and Aquatic Ecosystem Management, BOKU University, Vienna, Austria

Alongside the climate crisis, the biodiversity crisis stands as the most significant environmental challenge of our time. Freshwaters and wetlands, although covering only a small fraction of the earth's surface, are among the richest habitats in terms of species diversity. This incredible diversity often goes unnoticed, hidden beneath the water's surface and thus more difficult to access and assess than plants or animals on land. Unlike terrestrial habitats, the biodiversity of freshwater ecosystems remains insufficiently documented.

In the spirit of "We can only appreciate and protect what we know" (loosely based on Konrad Lorenz), the journey to biodiversity protection and recovery begins with species identification and taxonomy. Unfortunately, we are witnessing a dramatic decline in taxonomic expertise. Equally crucial is the collection of data on species occurrences, which forms the foundation for analysing biodiversity patterns. However, these valuable data often remain tucked away in scientists' drawers and computers. Only by openly publishing distribution data we can facilitate analyses and assessments across different spatial scales, which are essential for informed policy decisions.

This talk will guide you through the various stages of the data pipeline, from species collection to data publication, shedding light on the current status, existing challenges, and policy needs.

Filling the reference databases: rapid, large-scale barcoding using Nanopore sequencing

Amrita SRIVATHSAN

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Despite centuries of exploration, a significant portion of the Earth's biodiversity remains undiscovered and poorly understood, particularly among hyperdiverse taxa and in species-rich regions of the world. Traditional methods of species discovery and description are often slow and resource-intensive, highlighting the need for innovative, cost-effective, and decentralised approaches. Moreover, little do we know about how most species interact with each other. It is essential therefore that we enable large-scale species discovery, integrative taxonomy, and interaction research. Large-scale DNA barcoding is a critical part of the toolkit that can help us in addressing these by filling the reference databases, enabling the rapid identification, and pre-sorting of unidentifiable specimens. This approach is particularly effective for taxa like insects, where specimens can be processed using a “reverse workflow”. In this workflow, all collected specimens are sequenced first, and then grouped into putative species units for further examination by expert taxonomists. A key challenge in this process is obtaining DNA barcodes from thousands of specimens efficiently and affordably. The advent of the MinION, a portable and real-time sequencing device, addresses this challenge. The MinION is not only affordable, with an initial setup cost of less than \$5,000, but also facilitates decentralised barcoding efforts globally. These features, along with low-cost and simple molecular methods are ideal for large-scale barcoding projects.

I will present a user-friendly workflow designed to streamline large-scale barcoding using the MinION, along with the software ONTbarcoder2, which is equipped with a GUI that processes raw data from the MinION sequencer. ONTbarcoder2 generates consensus DNA barcodes, corrects errors, and ensures quality control. One of the significant advancements in ONTbarcoder2 is its real-time barcoding capability, which allows for the rapid identification of unknown specimens as the sequencing data is generated. This feature greatly enhances the speed and allows users to stop the run when desired amount of data is collected. Using this workflow, we can now routinely generate approximately 10,000 barcodes per MinION flow cell at a cost of less than \$0.10 per barcode. Additionally, for smaller-scale projects, the “Flongle” flow cell offers the ability to barcode several hundred specimens.

In addition to reference barcodes, new sequencing technologies are allowing for the study of species interactions at scale. As an example, I will discuss a study on insect-vertebrate interactions using fly fecal samples. By removing the need for DNA extractions, a large number of insects can be processed. Combined with MinION sequencing, we can now begin to unravel ecological interactions for a broad range of taxa and in various environments. These developments give us the capacity to study complex ecological interactions and obtaining natural history data for a number of species for which we lack such data.

Abstracts

Oral presentations

Habitat restoration in the Netherlands: does reintroduction of large wood facilitate macroinvertebrates?

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Before the Rhine regulation, riparian forests used to grow on the point bars and riverbanks in the Netherlands. Dead trees that fell into the river provided a very important substrate for riverine macroinvertebrates, as these lowland rivers have otherwise only soft sediments. Unfortunately, in the 19th century the forests were removed and the large wood in the rivers was pulled out for safety reasons. Now water quality has recovered, the lack of wood as a habitat may be one of the factors that impede the return of many typical riverine macroinvertebrates in the lowland rivers.

Therefore, since 2013, large wood has been introduced in various experimental sites. The goal was to gain insight and to enhance typical riverine macroinvertebrates, create a higher overall biodiversity and a better score for the Water Framework Directive. Whole tree trunks with branches and roots were safely anchored in different river stretches, in the main channel as well as side channels. In recent years large wood has also been applied in restoration projects in lakes.

At the start of the monitoring, we provided the trees with removable parts that were brought to the surface by divers. Now we use an airlift dredge (underwater vacuum cleaner) to sample macroinvertebrates. This method is faster, more effective on rough structures than netting, and leaves the wood intact.

Within one year the introduced wood offered habitat for a large number of macroinvertebrates. Some species were found that are very rare in our rivers, for example *Diamesa insignipes* or *Brachycentrus subnubilus*. The fauna on wood was compared with the fauna before the introduction and with the fauna of control sites. In another project the fauna on introduced wood is compared with the fauna on the omnipresent basalt stones (used for riparian protection) nearby. Factors that determine the success of these habitat restoration projects will be discussed.

An overview of the lateral line organ in amphipods with emphasis on the *Gammarus* species flock from the ancient Lake Ohrid

Luiz Felipe ANDRADE, Tomasz MAMOS & Michał GRABOWSKI

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The lateral line organ is an intriguing sensory structure of gammarids, presumed to function as chemo-, mechano-, and/or electro-receptors. This organ is composed of two rows of dorsal and lateral units that are spread along the body. These structures are often found on each somite and telson, and each unit is characterised by a nearly straight or curved excavation on the cuticle with an arrangement of flattened microtrichs inserted within a rounded concentric “ring”. Despite some previous studies shedding light on the lateral line organ, many aspects of its composition and function are still unclear. As such, our study aims to investigate the structure and variation of this organ in amphipod crustaceans from diverse habitats and modes of life, with emphasis on the *Gammarus* species flock from the ancient Lake Ohrid. Preliminary analyses of species of 20 amphipod families from a broad geographic range show remarkable differentiation of the lateral line structure, making it possible to assume it as a potential morphological marker of adaptive radiation. As a study case, specimens collected from Lake Ohrid across a depth gradient from 5 to 240 meters were compared with sister species living in local springs and streams, all exposed to varied water current intensities, predation pressure and chemical composition. For the initial analysis, 10 males and 10 females of similar sizes from five species (*Gammarus paretiniiformis*, *G. roeselii*, *G. sketi*, *G. solidus* and *G. stankokaramani*) were selected, coated with gold, and analysed through Scanning Electron Microscopy. The results show clear interspecific differences in the morphology of the lateral lines, including the degree of curvature of the excavations and the number of microtrichs of species from the same sampling site. Additionally, size- and sex-specific changes were observed. Based on these observations, we hypothesize that, in different environmental conditions, even the conspecific individuals might show divergent patterns in the structure of the lateral line. This could suggest that the organ is a specialised and adaptable structure, helping the gammarids in spatial orientation.

Spatial-temporal dynamic of syntopic cryptic species of the *Gammarus fossarum* complex (Amphipoda) and their ecological differentiation

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Gammarus fossarum, a wide-ranging cryptic species complex, has exceptional diversity in the Western Carpathians, whereat local syntopy of distinct lineages is common. Previous research from Western Europe, as well as the Carpathians, has indicated that at least some of *G. fossarum* lineages differ ecologically (e.g., in resistance to anthropogenic pollution, or interactions with parasites). Within the Vsetín region, Czechia, three highly divergent, reproductively isolated lineages of the complex, i.e., biological species, commonly co-occur. In this project, we explore the puzzling nature of this coexistence, specifically to what extent the lineage composition is influenced by the local conditions. Conveniently, many streams of the area exhibit a downstream environmental gradient, leading to a gradual change in conditions from forested headwaters to an urbanised stream mouth. We expect that local conditions promote dominance of certain lineages, and thus the compositions of the *Gammarus* communities change gradually along the stream path. We hypothesise that this pattern should be consistent between streams, and temporally stable. Three streams were selected for their moderate length, downstream environmental gradient, and the known presence of all three studied lineages. We sampled ten sites along the entirety of each stream during the spring, summer, and fall over two years. Mitochondrial 16S rDNA sequencing of 30 individuals per site was used to determine lineage composition. Preliminary results show a gradual change in dominance along and temporal stability between the spring season of two years in one of the streams. On the contrary, two other streams exhibited an irregular pattern, though this pattern was similar between them. So far, it seems that the environmental gradient of anthropogenic pressure is not the key driver of *Gammarus* lineage distribution in the studied area, but more data is needed to support or reject this conclusion. As our research progresses, it will enable us to compare the dynamic environment of streams with more stable habitat of spring fens, where alternative food source use and body size differences seem to promote the syntopy of *Gammarus* lineages. Interestingly, we did not detect body size differentiation among syntopic lineages in the streams. This may indicate that the lineage syntopy is driven by alternative mechanisms in different environments.

Introduction of Ponto Caspian crustaceans into a central European lake: consequences for crustacean assemblage and food web

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To improve fish feeding and production, many lakes in the Baltic Sea basin were stocked with Ponto-Caspian peracarid crustaceans during the mid-20th century. Lake Dusia, the third largest Lithuanian lake, has been inhabited by five native peracarid crustacean species, including “glacial relict species”. After the deliberate introduction of Ponto-Caspian species this lake during the early 1980s sheltered ten peracarid crustacean species and was declared unique among the temperate and boreal lakes of Europe in richness of this assemblage. Since then, a drastic shift in the peracarid assemblage took place. All but one native peracarid species went extinct. Fish food composition data and application of stable isotopes for food web analysis clearly showed that introduced peracarids are readily assimilated by fish. However, the evidence of improvement of fish feeding and production has not been documented clearly. The outcomes and patterns of introduction of alien crustaceans on lake Dusia ecosystem will be presented in more detail and discussed.

Altitude and human-induced landscape transformations as main drivers of midge communities at Balkan springs

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The western Balkan Peninsula is a karst upland and mountainous region where springs play a crucial role as habitats for benthic invertebrates. They harbour a significant portion of freshwater biodiversity, especially in areas lacking large rivers and lakes. Chironomidae and other midges are major components of spring benthos due to their short life cycles and dispersal abilities. Research conducted on the 37 rheocrenes of the Bjeshkët e Nemuna mountains in Kosovo and 19 springs in the Cvrcka River system in Bosnia suggests that species composition and biodiversity in the region are influenced by altitudinal hydroclimatic gradients and microregional landscape conditions. The midge communities reveal four altitudinal zones in the Bjeshkët e Nemuna mountains and three groups of springs in the Cvrcka River valley based on human impact. Springs utilised by humans on a moderate scale exhibit the highest midge biodiversity. This is because the efficient discharge and bottom substrate that favour midges coincide with suitability for human use. Higher mountain springs stand out from those in other altitudinal zones due to their perennial character, supported by the humid weather at 1400–1500 m a.s.l. The spring fauna displays a patchy distribution, and even rheocrenes located near each other may be inhabited by different species. These habitats should be recognised as supporting biodiversity resources, particularly in protected areas such as national parks, and should be sustainably utilised in rural regions.

Unveiling mitogenome of diving beetle *Agabus bipustulatus* (Linnaeus, 1767) using long-read nanopore sequencing

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Recent advances in DNA sequencing techniques have greatly accelerated the acquisition of large-scale data. Among others, access to the information on complete mitogenomes has been greatly facilitated, improving the resolution of phylogenetic or other analyses requiring robust and reliable DNA data. Among the most promising technology in this regard is long-read nanopore sequencing, developed by Oxford Nanopore Technologies. This study aims to elucidate the composition features of the mitogenome of *A. bipustulatus*, achieved through long-read nanopore sequencing.

Samples were collected from the Tatra Mountain glacial lakes (Western Carpathians, Slovakia), and High-Molecular-Weight DNA was utilised for library preparation. Sequencing was performed using the Ligation sequencing gDNA – Native Barcoding Kit 96 V14 (SQK-NBD114.96). Bioinformatic processing of reads for mitogenome generation involved several programs, including Flye, MitoFinder, Minimap2, Medaka and MitoS2. Nucleotide composition was analysed using MegaX, and skewness was calculated utilising standard formulae. Start and stop codons were determined using exPasy. The resulting mitogenome was 17,876 base pairs (bp) long, with sequencing data achieving 139× coverage. Among the 13 protein-coding genes (PCGs), three (*nad6*, *nad3*, *nad1*) initiated with an ATA start codon, five (*cox2*, *atp6*, *cox3*, *cob*, *nad4*) with an ATG start codon, four (*nad2*, *atp8*, *nad5*, *nad4l*) with an ATT start codon, while the *cox1* gene commenced with a CCG. Codon usage analysis revealed *Leu* and *Asn* as the most frequent, while *Cys* and *Arg* were the least common. The stop codon TAA predominated among PCGs, with only 3 (*nad3*, *nad1*, *cob*) ending with TAG. The overall base composition of the 13 PCGs, which had a total length of 11,181 bp, was 34.40% for A, 43.56% for T, 11.58% for G, and 10.48% for C. The total length of tRNAs was 1,460 bp, with individual lengths ranging from 63 to 71 bp (the overall base composition of tRNAs was A: 39.66%, T: 38.91%, G: 11.58%, C: 9.74%). The total length of the 2 rRNAs was 2049 bp, with an overall base composition of 38.54% A, 43.72% T, 11.47% G, and 6.31% C. The length of the control region was 2,955 bp (A: 49.60%, C: 3.90%, T: 42.80%, G: 3.70%). Skewness analysis revealed a positive AT skew in the control region (0.074) and tRNAs (0.010), while PCGs (-0.118) and rRNAs (-0.063) exhibited a negative AT skew. The GC skewness was negative in the control region (-0.026) and positive for PCGs (0.050), tRNAs (0.092), and rRNAs (0.290). Compared to previously published diving beetle mitogenomes, the sequence obtained by nanopore-based long-read sequencing is significantly more reliable concerning

problematic regions of mtDNA. Data obtained in this way can be a reliable basis for subsequent comparative molecular studies, examining gene arrangement or differences in mitogenome size and composition. The study was supported by the European Union NextGenerationEU project No 09I03-03-V01-00075, titled *“Scholarships for excellent researchers threatened by the conflict in Ukraine”* under the Recovery and Resilience Plan of the Slovak Republic, and by the Slovak National Grant Agency (VEGA), project no. 2/0084/21.

Variability of isotopic niche in invasive spiny cheek crayfish *Faxonius limosus* in natural and anthropogenically altered riverine ecosystems

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Man-made alterations of natural habitats are a major issue in ecology and conservation of riverine ecosystems. This applies explicitly to construction of dam reservoirs which result in fragmentation of river habitat and transformation of lotic to lentic ecosystem. The latter has an impact on the biota of altered river reach by changing species composition and/or by altering life histories of species remaining in new habitat. In this study we tested if freshwater, alien spiny-cheek crayfish *Faxonius limosus* shows variability in values of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) related to the individuals' origin (dam reservoir or river downstream). Presumably, stable isotopes should outline trophic aspects of crayfish life histories from different habitat types. We hypothesised that 1) overlap of isotopic niches (in $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ isospace) between two distinct habitats would be lower than between the same habitat types within locality; 2) niche width of crayfish from rivers is wider than from reservoirs; 3) trophic position (based on $\delta^{15}\text{N}$) of crayfish from rivers would be higher than from reservoirs; 4) crayfish with higher body condition index would show higher trophic position.

We analysed 379 crayfish from tree reservoir-river downstream pairs in South-Central Poland collected in 2019 due to body condition index analysis. Crayfish were weighted and measured to conduct further body condition analysis. Further, dried, and homogenised abdominal muscles were sampled for stable isotopes analysis. Samples included gradient of age (reflected in individuals' sizes) from the youngest to oldest individuals available in the Autumn.

The following results were obtained (in order of hypothesis above): 1) overlap in isotopic niches were observed, but were not related to habitat type; 2) niche widths were not much variable with one exception of crayfish from the largest studied reservoir revealing the largest niche area; 3) trophic position, although variable, was not related to habitat type but more related to locality from where the crayfish were; 4) body condition index in general was not correlated with $\delta^{15}\text{N}$ values, which suggest that resources allocation is not dependent on the type of food utilised.

The results show that in general there are differences in feeding strategies among crayfish from different localities, but they seem to be more related to very local food conditions than to habitat differences between dam reservoirs and rivers. Further studies, including more replications of river-reservoir pairs and/or variable riverine habitats are needed for better understanding potential impact of such ecosystem alteration on crayfish.

Flow intermittence changes the occupancy frequency distribution of stream chironomid assemblages

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In community ecology research, the characterisation of species occupancy frequency distribution (OFD) patterns has been proven to be a suitable method to understand the mechanisms underlying the organisation of the communities on a regional scale. OFDs provide information on the relative dominance of rare and common species in the communities by multiple shapes of histograms based on the occurrence frequencies of the species. Among them, unimodal (characterised by rare species) and bimodal (characterised by additional common species) patterns might indicate well-structured communities, organised by various local and regional filters. In the case of passive dispersers, like Chironomidae, a right-skewed unimodal OFD pattern is expected in undisturbed conditions at a regional scale. We studied the OFD pattern of stream-dwelling chironomid assemblages in drying river networks (DRNs) suffering from various degrees of intermittency. We assumed that flow intermittency, as an environmental disturbance, can cause changes in the organisation of the assemblages. Quantitative samples were taken bimonthly within the framework of the DRYvER project (Horizon2020 #869226) through six sampling campaigns in 2021. Chironomid larvae were identified morphologically to the lowest possible taxonomic level (targeting species level) from Hungarian, Croatian and Czech DRNs. In contrast with our expectations, in the permanent sites, the chironomid assemblages showed bimodal OFD pattern characterised by many rare, relatively few moderately frequent and several common species. On the contrary, the assemblages in intermittent streams were characterised by a rather right-skewed unimodal OFD pattern, with a high number of rare species but few common species, and relatively high proportion of moderately frequent species. Species that were common in permanent streams become moderately frequent or rare in intermittent streams. It suggests that previously suitable habitat decreased in the area due to flow intermittence. Our results demonstrate that drying events can have a remarkable influence on the processes that shape the chironomid assemblages at a regional scale.

Insights into the trophic ecology of invasive Ponto-Caspian mysids (Crustacea: Mysida)

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Four euryhaline mysid species from the Ponto-Caspian region have become invasive in Europe in recent decades causing considerable changes in the ecosystems. In this presentation, I would like to summarise our recent results on the trophic ecology of these omnivorous species. In the first study, we aimed to clarify the formerly controversial filtering mechanism in mysids using *Limnomysis benedeni* as a model species and provided evidence for the involvement of the maxillae and the existence of the ventral filtration current. We then compared the filter areas and mesh sizes of the four invasive Ponto-Caspian species and quantified their filtering capacity in an aquarium experiment. The result showed that *Limnomysis benedeni* – featuring an additional pair of filtering screens – is the most effective filter feeder of the four species, but all of them can utilise suspended particles as a food source. In another experiment, we compared the predatory functional responses of the species using *Daphnia pulex* as prey. The attack rates indicated that the pelagic *Hemimysis anomala* is the most effective predator of the four species, while *Katamysis warpachowskyi* had the lowest consumption. *Limnomysis benedeni* and *Paramysis lacustris* showed intermediate predatory efficiencies similar to each other. These differences were also reflected in the stable isotope-based trophic positions of the species.

Resistance, not resilience traits, structure macroinvertebrate communities in newly drying stream sections

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Transitioning from perennial to non-perennial flow regimes causes ecological shifts in aquatic communities. Aquatic macroinvertebrates deploy resistance and resilience strategies to cope with flow intermittency, crucial in rivers with long-term seasonal dry episodes. Less is known, about how these strategies support community persistence in streams that only recently have experienced drying, and where local assemblages lack such adaptations. Our study conducted two four-season campaigns, separated by a one-year break, to assess macroinvertebrate responses in newly drying intermittent streams by comparing intermittent and perennial stream sections. We characterise communities from structural and functional perspectives, and then evaluate the response at the trait state level. We observed a decline in taxa richness and abundance, but not structural diversity, in response to flow intermittency. Resistance traits are more important than resilience traits in structuring macroinvertebrate communities in newly intermittent stream sections. Taxa in intermittent sections exhibit a smaller trait space, indicating lower functional redundancy. The macroinvertebrate response to intermittency lacks a predictable pattern, suggesting time-dependent and trait-state-specific colonisation by adapted taxa and community assembly with resistance and resilience strategies. As river drought increases due to climate change, recognising the temporal dimension becomes crucial for understanding ecological responses to intermittency.

Aquatic invertebrates inhabiting specific anthropogenic microhabitats created in the underground river channel of Brzoza River (Lodz, central Poland)

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The river network of central Poland's largest agglomeration – Łódź Agglomeration consists of dozens of small watercourses due to its location on a first-order watershed. In the 19th and 20th centuries, the river system was significantly transformed as a result of the city's rapid growth, and many rivers were turned into sewers. For these reasons, many of the rivers within the city of Łódź are located in covered channels beneath the surface of the city. These canals create specific microhabitats and microclimates similar to those found in caves. To study these specific habitats, research was carried out in one of the underground river channels in the Łódź area. The chosen channel is 200-metre built from ferroconcrete prefabricate and is located under the former national road no. 1.

The main objective of the project was to assess the species diversity of the invertebrate fauna of the underground river channel and compare it to the fauna surrounding its inlet and outlet. To carry out the study, organisms inhabiting the channel were collected monthly for one year (04/2023–03/2024) from three microhabitats within the channel: dry walls, wet walls/groundwater seeps and river sediment. The collected material was determined to the lowest possible taxonomic level using morphological and molecular methods.

The results indicate a strong influence of the covered channel, and the thermal conditions present on the phenology and development cycles of insects. The groundwater outflows on the canal walls provide an important microhabitat for organisms requiring water of high purity. Only within this microhabitat larvae of *Chaetocladius perennis* (Diptera: Chironomidae) were detected. Also, the groundwater outflows were the only microhabitats where larvae of *Eubria palustris* (Coleoptera: Psephenidae) were observed. It was the first observation of that species in central Poland and one of the few known locations in such a highly urbanised area. Preliminary results show that the so far unstudied underground channels of rivers in Poland can be a habitat for rare and vulnerable species and exhibit greater biodiversity than was expected.

Bioaccumulation of chemical elements at post-industrial freshwater sites varies predictably between habitats, elements, and taxa

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Man-made standing waters can provide important secondary habitats for freshwater invertebrates and other biota, but they also pose risks to the local biota through increased pollution levels. We studied the bioaccumulation of chemical elements, with emphasis on heavy metals, in common invertebrate and fish species in post-mining habitats in the Czech Republic. We developed a systematic approach to compare the bioaccumulation of 11 elements in a mollusc (*Physella*), four predatory insects (diving beetle *Rhantus*, heteropteran *Nepa* and larvae of *Anax* and *Orthetrum* dragonflies) and two fish species (*Perca* and *Rutilus*) in less polluted mining ponds and highly polluted fly ash lagoons.

The relationships between environmental and tissue concentrations were described by element-, taxon- and habitat-specific power law relationships. We showed that environmental concentrations determined the tissue concentration of non-essential elements (Al, As, Co, Cr, Ni, Pb and V) across all taxa. Bioaccumulation of all elements varied among taxa. We detected higher bioaccumulation of Al and Pb in mining ponds and of As, Cu, Mn, Pb, Se, V and Zn in fly ash lagoons, but the differences were often minor. Bioaccumulation of some elements further increased in mineral-rich localities. Tissue concentrations of most elements were more closely related to substrate than to water concentrations and the proximity to substrate, rather than trophic level, drove increased bioaccumulation levels across taxa. This highlights the importance of substrate as a pollutant reservoir in standing freshwaters and suggests that benthic taxa (*Physella*, *Nepa*) are good bioindicators of pollution levels. Despite the higher environmental risks in fly ash lagoons than in mining ponds, the observed ability of freshwater biota to sustain high pollution levels supports the role of post-industrial sites as refuge habitats in Central European landscape.

Invasive Ponto-Caspian amphipods experience a significant trophic niche contraction outside the native range

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Range expansion of invasive species is often assumed to be associated with an increased trophic niche breadth. However, evidence remains inconclusive due to a scarcity of studies comparing both the native and invaded ranges. Here, we test the trophic niche expansion hypothesis by examining stable isotopes and diet-related functional morphological traits across native (NW Black Sea) and invaded (SE Baltic Sea) ranges of amphipods *Dikerogammarus villosus* and *Pontogammarus robustoides*, originating from the Ponto-Caspian region – a major source of invaders in Holarctic inland waters. Stable isotopes revealed that both species underwent a twofold dietary niche contraction with a shift towards decreased carnivory in the invaded range. This dietary shift was morphologically mirrored by an overall attenuation of prey grasping appendages, antennae, and mouthpart palps. The magnitude of dietary and morphological change was greater in *D. villosus*, indicating a greater adaptive potential. Our findings indicate that previous experimental reports of aggressive predation in *D. villosus* reflect opportunistic foraging and align with local stable isotope studies which generally indicate a low trophic position. We conclude that Ponto-Caspian species can undergo rapid dietary changes outside the native range, and that a broad dietary niche is not a prerequisite for successful non-native establishment.

First insight into the aquatic beetle genetic diversity of the Pannonian Ecoregion

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The Pannonian Basin, nestled in the heart of Europe, boasts characteristic habitats that harbour diverse wildlife shaped by its distinctive geological history and unique geographical and climatic features. From a phylogeographical perspective, this region stands out due to its abundance of endemic species and its role as a refugium and a gateway to the Balkan and eastern steppe fauna. Encompassing 133,000 km², the Pannonian ecoregion includes the entire Hungary and extends also across a part of Serbia, Romania, Slovakia, Croatia, Czechia, and Ukraine, and is classified by some to include minor portions of Austria and Slovenia.

The water beetle fauna of the Pannonian Basin is relatively well-documented, with records of approximately 350 species in 14 families and over two hundred thousand occurrence data entries in databases. Over the past two decades, DNA barcoding has been crucial in studying European water beetles, with more than half of the species barcoded and possessing at least one reference barcode. However, systematic barcoding remains essential at local levels to uncover hidden molecular diversity, spatial distribution patterns and elucidate phylogenetic relationships.

Compared to other regions, the Pannonian ecoregion lags behind in public reference barcoding data. It can be described as a large white spot, with only Hungary and Slovakia contributing sequences for less than 4% of known species (14 and 7 sequences for 6 and 7 species, respectively). These sequences originated from only 7 sites; most of the species only possess a single sequence. We initiated a project last year to address this deficiency by providing numerous and comprehensive barcode records. As a start, ~200 sequences representing ~70 species collected from ~30 sites across Hungary were provided. In this talk, we highlight key aspects of our dataset, showcasing unique BINs and intriguing case studies that contribute to a deeper understanding of aquatic beetle diversity within the Pannonian ecoregion.

Genetic diversity of Diptera – DNA barcode analysis in the assessment of the ecological integrity of freshwaters from the Romanian Carpathians

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Dipterans are key players in the assessment of the ecological integrity of freshwaters. However, species-level identification of most taxa remains challenging, especially in morphologically cryptic larvae. To improve these shortcomings, 1,149 mtCOI barcode sequences were generated from a bulk sample of macroinvertebrates, collected in different mountainous headwaters and large lowland rivers in the Romanian Carpathians. These corresponded to 177 morphologically identified species and 77 undetermined taxonomic units, represented mostly by larvae. Analysis of the Barcode Index Number (BIN) System revealed 287 BINs, of which 82.5% coincide strongly with known species boundaries. However, DNA gap analyses yielded significant intraspecific differences, whereby K2P distances at this level ranged from 0% to 16.22%, with an average of 2.81%, whereas interspecific distances within a genus ranged from 0% to 26.97%, with an average of 13.15%, revealing the likely occurrence of overlooked cryptic or pseudo-cryptic species, especially in mountainous headwaters. 95 BINs were assigned for the first time in BOLD, with 31 representing unknown operational taxonomic units. Species-level identification was successful or confirmed for 531 larvae, based on genetic similarity to corresponding adults, from which 48 larvae (28 taxa) were assigned to species-level exclusively using their genetic similarity in the BOLD database. Our data considerably improved the species-level identification effort of macroinvertebrates in a region with exceptionally high aquatic biodiversity and a considerable number of regionally restricted or endemic taxa. Our results support the utility of DNA barcodes to delimit species boundaries for the majority of Diptera groups, especially in morphologically cryptic larvae, and provide an effective taxonomic tool to improve routine bio-assessment protocols with high-quality data.

Fantastic beetles and where to find them? Do spring floods govern the occurrence of *Graphoderus bilineatus* in Hungary?

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The *Graphoderus bilineatus*, a highly protected species listed as Vulnerable (VU) by the IUCN Red List and designated under Natura 2000 in Hungary, holds significant conservation importance at both national and European levels. Despite increasing literature on its ecology, knowledge gaps persist, as the life history, development, and ecological preferences of the *G. bilineatus* exhibit considerable variability across regions and over time. Understanding these variations is crucial for developing effective long-term conservation strategies targeting both the species and its habitats. Decades of field observations in Hungary have revealed a consistent pattern: the abundance and presence of *G. bilineatus* are closely linked to spring floods, particularly within floodplain habitats, a phenomenon less observed in other European countries to the north. We quantified the time intervals between the occurrence of spring floods and the presence or absence of the species in known habitats along the Drava, Danube, Bodrog, and Tisza rivers. Our findings demonstrate a significant correlation between the occurrence and appearance of the species and spring floods. Furthermore, our data suggest that these floods may act as a triggering mechanism, prompting the *G. bilineatus* to initiate flight and potentially colonise new water bodies within its range. Understanding the interplay between spring flood dynamics and the ecology of *G. bilineatus* is essential for informing targeted conservation efforts aimed at preserving this species and its associated habitats.

Stage-specific effects of warming on the larval development and temperature-size rule in *Sympetrum striolatum*

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Warming, predicted to be up to +8°C by 2100, can impact freshwater biota through a range of mechanisms such as individual growth rate modifications and altered nutrient cycling. Among the most affected are ectotherms, which constitute 95% of aquatic life. Body size reduction of ectotherms has been proposed as one of the universal responses to global warming. Observed body size shifts in ectotherms can be explained by the “temperature-size rule” (TSR) stating that organisms grow faster but reach a smaller size at a given stage of development (e.g., size at maturity or adult size) at higher temperatures. However, it remains unclear what physiological mechanisms might regulate TSR and whether the same pattern is always shown during ontogenetic development.

In this context, we examined the effects of warming on individual growth and development of the larvae of the dragonfly *Sympetrum striolatum* in a laboratory experiment. Eggs of *S. striolatum* were exposed to six constant temperatures (9, 13, 17, 21, 25 and 29°C), and development of larvae was followed from hatching to adult emergence. Moulting, mortality, and emergence success were recorded throughout the experiment. The first, third and last instar exuviae, as well as dead larvae were collected for body measurements.

Temperature had a strong effect on growth, development, and survival rate of *S. striolatum* larvae. At 9°C, the eggs did not hatch after 5 months of exposure, while all the larvae died within two weeks at 29°C. Furthermore, the larvae successfully hatched and continued to develop at 21 and 25°C, while contrasting survival and larval development were observed at 13 and 17°C. As expected, larvae grew faster at higher temperatures, but we did not detect a decrease in growth rates at temperatures near the upper thermal limit. We also observed that during the first stages, body size declined at higher temperatures within this range of temperatures. However, this relationship was reversed during development, such that body size of late larval stages (F0) and adults increased with temperature, which contrasts with the expected TSR found in many ectotherms. Our results challenge the generality of TSR and suggest that body size responses to warming can vary through ontogeny, possibly in relation to adaptive constraints.

Small hydropower plants' impact on the ecological state of the benthic macroinvertebrate communities

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Categorised as renewable or green energy sources, small hydropower plants (SHPs) are sometimes generalised as ecologically safe. However, in some cases, they are directly altering the natural hydromorphology of small rivers and indirectly the physical, chemical, and biotic parameters of these ecological systems at both local and water body-scale. Habitat deterioration may affect communities like fish, periphytic algae or benthic macroinvertebrates (BMIV) through mechanisms like increasing water transparency, flow and velocity, sediment types and granulometry changes and uniformization of microhabitats. BMIV, for example, are affected by rapid changes of water flow or depositional microhabitats for leaf packs.

European legislation does not require a unitary regulation of legal requirements, yet there are some recommendations to implement at a national level, based on the Water Framework Directive, like hydropеaking mitigation, minimum ecological flow, or sediment transport.

This study investigated, during spring and summer 2019, the ecological effects of 17 SHPs built on 22 headwater streams on macroinvertebrate communities in Romania. Sampling sites were located both upstream and downstream of the intake points of SHPs, aiming to quantify changes in the taxonomic composition of BMIV communities, assess the species-specific response of different BMIV species and identify the abiotic variables explaining the community structure and its spatial-temporal dynamics. Generally, a lower number of BMIV taxonomic families was recorded downstream of SHPs intakes. As expected, species explaining community structure belong to Ephemeroptera, Plecoptera and Trichoptera taxa. Among the stream bed hydromorphology and physical-chemical parameters, mean water depth, velocity, and flow are the most influential. Although, according to the Water Framework Directive, the ecological status based on BMIV community structure and diversity scored as “high quality” which does meet the European agreed goals, there are temporal variations of some indices which suggest potential future modifications and therefore a need for extended monitoring programs.

***Hydropsyche* in the multiverse of rivers – a multi-scale, multi-model ensemble approach to explain the longitudinal distribution of larval net-spinning caddisfly species-groups**

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Riverine ecosystems are dendritic, linear networks that are connected longitudinally, laterally, and vertically. Environmental gradients, as a result of uni-directional flow, create a great number of diverse but still interconnected microhabitats, making stream ecosystems the most heterogeneous, most complex, hierarchically nested systems where ecological processes both in the upstream reaches and the adjacent catchments govern local conditions. Examining, explaining, and especially modelling the relationships between riverine species and their environment are therefore particularly challenging and require a multi-scale and multi-model approach.

In the present study, our aims were to build an ensemble of base learner machine learning (ML) models based on selected multi-scale environmental parameters shaping the sequential distribution of ten Central European species of the genus *Hydropsyche* that could explain and effectively predict the occurrence of species and/or groups of them with similar ecological preferences.

Our results show that several gradient-like and pollution related factors that are usually measured as part of monitoring programmes (such as those of Water Framework Directive) at finer spatial scales and in higher detail could be considered suitable to complement or substitute those (e.g., topographic, geographic or precipitation data) that are routinely included in species distribution models (SDMs). It is also revealed that an ensemble of multi-scale models can outperform single modelling algorithms in mapping the longitudinal distribution of species and species groups.

Biodiversity of Carpathian springs – an overview of the results of the Polish-Slovak-Hungarian project

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Springs possess several unique environmental characteristics, including thermal and chemical stability, typically low oxygen content, and isolation by geographical or physicochemical barriers. This can result in low gene flow between springs and local episodes of adaptive radiation, potentially leading to significant differences in biodiversity between different springs and high levels of endemism. Moreover, strong directional selection in isolated habitats greatly reduces the range of morphological variation, potentially favouring speciation, including cryptic species. The study of cryptic diversity is crucial for a full understanding of species distribution, ecology, assessment of endemism patterns, as well as the development of appropriate biomonitoring and conservation strategies. All these characteristics make springs hotspots of biodiversity and endemism. Paradoxically, although springs are among the key ecosystems for the functioning of the biosphere, they remain understudied. Our international project aims to provide original and detailed information on the location, environmental properties and biodiversity of different types of springs in five model Carpathian ranges with different geographical locations and geological structures in Poland and Slovakia: 1. the Orava Beskid (northern Slovakia, flysch rocks) and the Żywiec Beskid (southern Poland, flysch rocks); 2. the Považský Inovec (western Slovakia, carbonate rocks); 3. Bukovec Mountains (north-eastern Slovakia, flysch rocks), Bieszczady (south-eastern Poland, flysch rocks); 4. Veporské Mountains (central Slovakia, crystalline rocks); 5. Beskid Wyspowy (southern Poland, flysch, volcanic rocks). During the two-year study, we collected samples of macroinvertebrates from 52 sites located in the above mountain ranges. The dominant groups were Oligochaeta, Bivalvia, Gastropoda, Amphipoda, Isopoda, Ephemeroptera, Odonata, Plecoptera, Trichoptera, Coleoptera, and Diptera. DNA was extracted from more than 1,500 individuals. Approximately 900 DNA barcodes, representing 14 orders and four classes of invertebrates, have been obtained so far. They have been deposited in the Barcode of Life Data System (BOLD) public repository as the basis for a reference DNA barcode library for Carpathian springs. So far, BOLD algorithms grouped the DNA sequences into 74 molecular operational taxonomic units (BINs – Barcode Index Numbers), which are rough equivalents of species, of which 23 BINs are new to BOLD. We will provide

an overview of the project as an example of a successful bottom-up international initiative focusing on poorly studied Carpathian aquatic habitats and funded by both national and international channels, as well as the first results on faunal and genetic connectivity between springs.

Funding provided by APVV SK-PL-21-0023, NAWA BPN/BSK/2021/1/00084/U/00001, UniLodz IDUB NR4/ML/2022, Erasmus+ BIP: Biodiversity of the Carpathian Springs.

Massive DNA barcoding of aquatic insects in Lake Skadar basin points to high diversity but surprisingly low faunal connectivity between waterbodies

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Lake Skadar (LS) is the largest and the youngest Balkan lake shared by Montenegro and Albania. A characteristic feature of its basin is the presence of numerous geologically old karstic spring systems. The lake and its basin are known for rich biodiversity and endemism. Recent studies show that local aquatic invertebrates, particularly crenic fauna, are far from being well-documented. The lake is protected and included in the Ramsar list of wetlands of international importance. However, the ecosystem of the LS basin is highly endangered due to recent development of tourism industry and associated anthropogenic pressure. Thus, a rapid survey of the local aquatic biodiversity is of utter importance. Our study, done in May/June 2018, encompassed 58 sites in springs, streams and lake coastal waters, scattered throughout the LS basin. Using the PacBio Sequel II System in CBG (Guelph), we obtained over 4,500 COI barcodes for aquatic insects, mostly Diptera, Ephemeroptera, Plecoptera, Trichoptera, Odonata and Coleoptera, belonging to 60 families and, based on the Barcode Index Number algorithm, clustered to over 500 BINs that can be treated roughly as imperfect yet handy species-equivalents. In numerous cases, particularly aquatic insects (e.g., Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, and Diptera), the survey provided records of species new to LS basin, Montenegro, Albania and also some potentially new to science. Regarding spatial distribution of BIN diversity, our results not only showed high BIN-level diversity in the relatively small area, but also high distinctiveness of BIN composition and little genetic connectivity, hence isolation, also between localities close to each other. It points to the importance of preserving the regional waterbodies at a very local scale, as even degradation of single waterbodies can potentially lead to irreversible biodiversity loss. The barcodes will provide a possibility for employing cost-effective methods such as DNA metabarcoding and, in perspective, ecologically harmless eDNA sampling in bioassessment and biomonitoring of local freshwater ecosystems. The work was supported by the Montenegrin Ministry of Sciences ("DNA-Eco" project), by National Agency for Academic Exchange (NAWA, PL) and National Science Centre (NCN, PL).

Navigating through drying histories: switching roles of connectivity in Trichoptera metacommunity organisation

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Climate change induces unprecedented dry periods in previously perennial streams, significantly impacting aquatic macroinvertebrate metacommunities. We assumed that the historical (i.e., several dozens of years) patterns of presence or absence of drying in dry river networks (DRNs) modifies the relative importance of local and regional, spatial, and niche-related environmental factors in metacommunity organisation. In this study, we sampled Trichoptera metacommunities in three Drying River Networks (DRNs) along a latitudinal gradient across three countries, also representing a drying gradient. In Croatia, drying has been present in the DRN for a very long time, with an almost constant annual occurrence. At the other end of the scale is the Hungarian DRN, where the drying has appeared in recent years and with varying spatial and temporal annual intensity, but is becoming more and more intense, while the drying history of the Czech DRN lies between these two with highly unpredictable occurrences. Here, we hypothesised that the relevance of spatio-temporal connectivity differs according to the historical drying patterns in each catchment. In 2021, the Trichoptera metacommunities were sampled six times bimonthly at 20–25 sites per DRN, and a comprehensive set of local and regional environmental parameters were recorded. Spatial variables were generated through Moran's eigenvector maps (MEM) and asymmetric eigenvector maps (AEM) from watercourse distances among sites in each river network. Spatio-temporal connectivity was assessed using the STcon framework, and values were calculated for different time frames (for 30, 200, 365, 730, 1825, and 3650 days before sampling). We analysed the relative relevance of spatial (MEM and AEM), niche, and spatio-temporal (STcon) drivers of these communities by means of hierarchical variance partitioning. The explanatory power of the STcon was the highest in the Hungarian DRN and the lowest in Croatia, with significant differences observed across different time frames. In the Hungarian DRN, the connectivity calculated for time frames shorter than one year proved to be the most relevant factor, while in the case of the Croatian DRN, the five- and ten-year-long connectivity hold the greatest explanatory power. These findings underscore the pivotal role of spatio-temporal connectivity in shaping metacommunities, albeit with varying impacts across river basins characterised by different drying histories. Such insights are critical for understanding and managing the ecological ramifications of climate-induced alterations in aquatic ecosystems. This work was supported by the EU-funded DRYVER project (869226).

First comprehensive overview of invasive aquatic macroinvertebrates in Israel

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Freshwater ecosystems across the globe confront mounting challenges due to invasive, non-native species. Streams, in particular, are susceptible to the establishment of non-native species, leading to significant alterations in community dynamics, nutrient cycling, and overall ecosystem functioning.

During the last decade we have been monitoring aquatic macroinvertebrates across Israel as part of an ongoing effort to assess the ecological state of these ecosystems. The database contains >1,000 biological and ecological samples collected from ca. 400 reaches in 105 streams across the country.

Overall, we documented 22 alien taxa (16 families, 5 orders): two polychaetes (*Namalycastis hawaiiensis*, *Nereis persica*), one leech (*Barbronia* sp.), 10 snails (*Pyrgophorus coronatus*, *Potamopyrgus antipodarum*, *Pseudosuccinea columella*, *Radix* sp., *Physa fontinalis*, *Physella acuta*, *Ferrissia clessiniana*, *Planorbella duryi*, *Mieniplotia scabra*, *Tarebia granifera*), one marine bivalve (*Mytilopsis sallei*) and 8 decapods (*Neocaridina denticulate*, *Monocorophium insidiosum*, *Belzebub hansenii*, *Cherax quadricarinatus*, *Procambarus clarkii*, *Procambarus virginalis*, *Penaeus aztecus*, *Callinectes sapidus*).

Non-native species are highly widespread across the country, with at least one of these species in 70% of the sampled streams. In some cases, up to 6 alien species were recorded from the same reach. The most abundant invasive taxon is *P. acuta* which was found in all 10 basins and at >50% of the monitored sites; followed by *P. coronatus* occurring in 70% of the catchments and 20% of the sampled streams. Nevertheless, in some sites the latter species was found to be extremely abundant with >10,000 specimens per sq. meter.

The pathways of alien introduction are not always clear, although some freshwater species (e.g., *M. scabra*, Crayfish) were probably introduced into the wild through aquarium content releases, whereas others (e.g., *P. antipodarum*) could have been unintentionally spread as “stowaways” on aquatic and semi-aquatic plants. Several marine species (e.g., *M. sallei*, *M. insidiosum*, *P. aztecus*) have been found to reside in the estuaries of coastal streams, probably through discharged ballast waters.

The ecological impacts of an extensive alien species occurrence have not been fully investigated, and currently there are no strategies to eradicate these alien species in Israel. An attempt to exterminate *P. clarkii* by drying a short stream section was so far considered unsuccessful. This situation calls for a joint national effort to test and effective legislative prevention measures and apply eradication techniques.

Spatio-temporal niche partitioning drives a unique case of stable coexistence of two freshwater amphipods in Sicily

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Species coexistence has been one of the focal points in ecology. Particularly, the coexistence of closely related taxa occupying similar niches provides an interesting research topic. According to ecological theory, such an event is unlikely. However, recent evidence indicates that this phenomenon emerges across a wide range of phylogenetically distinct taxa and from all types of habitats. Nevertheless, the stability and the mechanisms facilitating such coexistence remain unknown. Therefore, a multitude of evidence is needed to better understand the dynamics and eco-evolutionary drivers underlying this ecological paradox. Here, we focused on a unique case on the island of Sicily where two local amphipod species, *Homeogammarus sicilianus* and *Homeogammarus adipatus*, co-occur in a single river system, Fosso del Tempio. Despite being widespread across the island, they solely co-occur in the studied system. This raises the question of whether this coexistence is stable and supported by niche differentiation or reflects an unstable, possibly seasonal, co-occurrence. By applying an array of DNA-based methods and seasonal fine-scale sampling, we aimed to investigate potential niche differentiation by studying the microhabitat preferences, dietary habits, reproductive activity, and potential influence of parasites on the co-occurring amphipod species. Our results indicate that both species co-occur at nearly all studied sampling sites across the entire river system, regardless of the season. We also found significant differences in microhabitat distribution amongst both species, with *H. sicilianus* occurring more in biotic and *H. adipatus* found more in abiotic microhabitats. DNA metabarcoding results revealed different feeding strategies between the two species, with *H. adipatus* being a more specialised feeder and *H. sicilianus* having a more generalistic diet. We also found differences in species' reproductive activity over the year. Finally, even though both species shared the same parasite lineages, the parasite composition and prevalence patterns significantly differed between the two host species. The obtained results support a stable coexistence between the two studied species. However, based on the observed niche differences, *H. sicilianus* seems better suited to outcompete *H. adipatus* in a longer evolutionary timescale, challenging the stability of observed coexistence. Our results indicate that a highly integrative framework is needed to disentangle drivers of species coexistence, supporting its use for studying similar cases.

Diptera species turnover and dominance shifts triggered by climate-driven changes

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The diverse pressures of climate change have influenced many habitats, especially freshwater ones due to their greater sensitivity to stressors. Aquatic Diptera make up more than 50% of all aquatic insect species described, which makes them an ideal group to monitor the changing climate as their diverse assemblages can reflect functions within the entire community. The aim of this research was to identify the variations in an aquatic dipteran community during a 15-year period at a tufa barrier in a karst barrage lake system and the environmental factors that have the highest influence on this community. We analysed monthly data collected between 2007 and 2021, when we collected adult specimens using 6 pyramid-type emergence traps. In total 167 taxa from 13 different families were gathered. NMDS based on Bray-Curtis similarity analysis among assemblages revealed the segregation of samples based on different current velocity and substrate, indicating the importance of microhabitats in dipteran community structuring. Dipteran taxa indicative of specific five-year time periods within the research were identified and were associated with changes in the environmental conditions, especially discharge. The threshold indicator taxa analysis presented specific species' responses to changing discharge rates. The study shows that discharge rate, not water temperature, is the critical factor shaping dipteran composition, whether by removing or adding taxa to the community. Species turnover showed an overall decrease in species number, i.e., in species richness throughout the research period. We conclude that changes in the dipteran community are not visible when analysing just the diversity indices, because of the huge functional traits, niches, adaptations, and species diversity of the group, but when determining environmental influence on the community in long term research these should be combined with other data such as the overall abundance, the total number of species, as well the species turnover.

Long-term recovery of aquatic macroinvertebrates determined by the degree of the disturbance after an industrial disaster (Red Sludge Disaster, Hungary)

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A ten-year-long examination of macroinvertebrate community recovery was conducted following a catastrophic spill of highly alkaline red sludge (pH>13) into lowland streams. Our primary objective was to compare recovery patterns after coarse and fine-grain disturbances, focusing on two aspects: i) trend analysis to reveal mean changes of six community parameters, and ii) variation analyses to assess parameter changes over time. We conducted statistical analysis on long-term data series of macroinvertebrates obtained from quantitative samples collected at four sections with varying degrees of disturbance along the impacted stream sections. We developed a comprehensive theoretical framework comprising a series of sequential phases: Ramp-up, Overshoot, and Oscillation Phases. i) A trend analysis revealed that disturbances show a gradual recovery pattern, while variance analyses showed an asymptotic convergence to an equilibrium. ii) Evaluating these trends across phases unveiled that the initial recovery phase exhibited a steep trajectory, lasting 4–9 months, irrespective of disturbance severity. Coarse-grain disturbances induced a remarkable Overshoot phenomenon across all community metrics. The more severe the disturbance, the greater the height and duration of the Overshoot. Our results suggest that the presence or absence of Overshoot can serve as an indicator for coarse-grain disturbances in the context of large and infrequent disturbances (LID). The entire recovery process lasts 2.5–3 years irrespective of the severity of the LID. In conclusion, a minimum survey duration of two and half years is deemed imperative to capture the phases of recovery, and changes associated with LID are not expected to extend beyond the three-year threshold. The theoretical framework, including Overshoot parameters, may assist future studies in comparing recovery patterns of different LID types. Furthermore, our theoretical framework is likely to be applicable to other groups of organisms given a sufficiently long monitoring of recovery, influenced also by the length of reproductive cycles.

Unfolding the wings: predicting wing morphology from body size in aquatic beetles

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Flying is one of the most significant dispersal strategies among insects, enabling them to colonise new habitats. Among these adept flyers, beetles (Coleoptera) stand out with their perfected wing structures, featuring elytra and intricate folding mechanisms that shield their hindwings from external influences. As the beetles colonised aquatic habitats, they had to adapt to very special environments. The majority of the aquatic beetle species are able to fly and in many cases the ability to fly is essential, especially in sensitive habitats. Newly emerged aquatic habitats are firstly colonised mostly by beetles. When faced with adverse conditions, water beetles often resort to aerial dispersal in search of more suitable environments. Despite the critical role of flight, considerable variability exists in wing morphology among species and even within populations, suggesting potential adaptation to diverse habitats. Body length is not necessarily determining the ability of flight, but indeed it is a significant factor. Our main question was if it is possible to find a significant correlation between the body length of different aquatic beetle species and the morphology of their wings, and which parameters of the wing can be reliably predictable by using the body length of the species. Utilising digital landmark measurements, we examined 15 parameters of wing morphology in 100–50 specimens of *Hydrobius fuscipes* (Hydrophilidae) and *Rhantus suturalis* (Dytiscidae). After preselection of variables, linear regression and generalised additive models as well as PCA and CVA methods were used to test and visualise the complex relationships in the dataset. Both species were characterised by high variance, but our findings reveal a significant positive correlation between body size and wing length, indicating that larger beetles tend to possess longer wings. Moreover, our analysis suggests that additional wing parameters can be predicted based on body size, highlighting the potential for body length as a reliable predictor of beetle wing morphology. Our study also uncovered significant differences in body and wing parameters between male and female specimens within these species, underscoring the importance of considering intra-specific variations.

Unravelling the complexity: exploring the combined effects of emerging contaminants and elevated temperature on caddisflies

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Urbanisation and inadequate wastewater treatment contaminate freshwater ecosystems with various chemicals (e.g., pharmaceuticals, personal care products, surfactants). Due to climate change, extreme weather events (e.g., heatwaves, floods, droughts) are becoming more common. These stressors interact leading to either additive or non-additive (synergistic or antagonistic) effects that threaten aquatic insects. Aquatic insects perform various important ecological functions in freshwater and terrestrial ecosystems and have been recognised as effective and reliable accumulation- and response bioindicators to pollution as well as indicators of ecosystem health. Accordingly, the current study aimed to investigate the response of two ecologically different species of caddisflies to individual and combined effects of emerging contaminants (ECs) and elevated water temperature. To that end, we conducted a 21-day microcosm experiment using a simplified freshwater food web containing moss and caddisfly larvae of *Drusus croaticus* and *Allogamus uncatus*. The experiment included control (C), elevated water temperature (T2 = C + 4°C), emerging contaminants mixture (EC), and multiple stressor treatment (MS = EC + T2). We analysed caddisfly mortality, body weight, locomotor activity (average distance per unit mass) as a proxy for behavioural changes, and metabolomic profiles of each species. Furthermore, we compared species-specific effects of selected stressors. ECs exposure increased average body weight in both species. Elevated water temperature had contrasting effects on locomotor activity, increasing larval average distance in *D. croaticus* but reducing it in *A. uncatus*. Elevated temperature and exposure to multiple stressors had comparable effects on the metabolomic profile of *A. uncatus*. Elevated temperature predominantly impacted the metabolome of *D. croaticus* following one week of exposure, while multiple stressor treatment demonstrated a more pronounced effect after three weeks. Additionally, elevated temperature led to increased mortality rate of *D. croaticus* larvae. The positive correlation between locomotor activity and metabolome indicates that molecular-level effects translate into behavioural changes. These findings emphasise the importance of thoroughly assessing the susceptibility of freshwater organisms to adverse effects of single and multiple stressors, offering crucial insights for conservation strategies, and understanding the ecological consequences in freshwater ecosystems in the context of global change.

Biodiversity and conservation potential of freshwater habitats in fly ash sedimentation lagoons

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Fly ash sedimentation lagoons, remnants of the energy industry, pose environmental risks due to heavy metal contamination but they have also been revealed as refuges for threatened terrestrial biodiversity. Surprisingly, the freshwater biodiversity of these lagoons remains unknown despite such a lack of knowledge strongly limits the efficient restoration of fly ash deposits. We conducted the first comprehensive survey of freshwater biodiversity, including nekton, benthos, zooplankton, phytoplankton, and macrophytes, in fly ash lagoons across industrial regions of the Czech Republic. To assess their conservation potential, we compared their biodiversity with that of abandoned post-mining ponds, known strongholds of endangered aquatic species in the region. Of the 28 recorded threatened species, 15 occurred in the studied fly ash lagoons, some of which were less abundant or even absent in the post-mining ponds. These species typically inhabit nutrient-poor, fishless waters with rich vegetation, including specialised extremophiles. While species richness and conservation value of most groups in fly ash lagoons did not significantly differ from post-mining ponds, benthos, zooplankton, and macrophytes exhibited slightly lower richness. Although concentrations of certain heavy metals (mainly Se, V, and As) were significantly higher in the fly ash lagoons, they did not significantly affect species richness or conservation value of the local communities. Thus, differences in species composition appear unrelated to water chemistry. Our findings highlight the importance of fly ash lagoons, as well as other post-mining ponds as refuges for threatened aquatic species and suggest their preservation during site restoration.

Age-dependent variation of aedeagal morphology in *Agabus uliginosus* and the status of *A. lotti* (Coleoptera, Dytiscidae)

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The taxonomic status of *Agabus lotti* within the *Agabus uliginosus* species group has been a subject of debate due to morphological similarities and lack of molecular data. In this study, we conducted a comprehensive morphological and molecular analysis of specimens from Central Europe, focusing on the Hungarian population. Morphological comparisons of genital structures revealed age-dependent variations, suggesting a gradual transition from *A. lotti* to *A. uliginosus*. Molecular analysis of COI sequences further supported this hypothesis, showing minimal genetic differences among most specimens, with only one individual exhibiting distinctiveness. Therefore, *A. lotti* should be regarded as a junior synonym of *A. uliginosus*. Our findings highlight the need for additional multi-marker studies covering a broader geographic range and including both molecular and morphological approaches to elucidate the taxonomic and phylogenetic relationship within this species group. The inclusion of Hungarian samples notably enriched the diversity of haplotypes, emphasising the importance of expanding sampling efforts in future research.

First COI barcode reference library for the ancient Lake Ohrid basin – how much have we progressed?

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Lake Ohrid, located at the border of North Macedonia and Albania, is considered the oldest lake in Europe (1.5 My). It has been designated a UNESCO World Heritage Site for its high level of endemism. Its biodiversity is still not fully known, and only few taxonomic groups have been analysed using an integrative approach that includes DNA methods (e.g., freshwater snails, leeches from the genus *Dina*, asselids and gammarids). What is more, some taxa are particularly challenging in identification, contain undescribed species or represent complexes of cryptic species so the DNA barcoding comes in handy for such.

The main aim of our study is to create a reliable publicly accessible reference library of macroinvertebrates inhabiting Lake Ohrid basin and its surrounding springs. The samples were collected in 2019 and 2022 by dredging or kick-sampling and preserved in 96% ethanol. The material was sorted into higher taxonomic groups. The animals belonging to Diptera, Gastropoda, Bivalvia, Turbellaria, Trichoptera, Hemiptera, Plecoptera, Oligochaeta, Coleoptera, Hydracarina, Ephemeroptera, Isopoda and Odonata were divided into morphotypes. From one to five individuals (1920 in total) of each morphotype the cytochrome c oxidase subunit I (COI) DNA barcode was obtained. The PCR products were sequenced using the Sanger and Oxford Nanopore platform. All obtained sequences were deposited in the Barcode of Life Database (BOLD). The final dataset contained 1460 barcode sequences belonging to 322 Barcode Index Numbers (BINs) of which 47 were new for BOLD. Additionally, to provide information about distribution of microorganisms in the Lake, substrate from different habitat zones was processed using a metabarcoding approach with 7 markers. Our study suggests that there are still some undescribed invertebrate species inhabiting Lake Ohrid basin and its springs. There is a need for further investigation of Lake Ohrid fauna to fully understand this unique ecosystem and protect it from human impacts and degradation.

This work is funded by the Polish National Science Centre (OPUS16 grant Nb. UMO-2018/31/B/NZ8/03103).

Role of invertebrate dispersal pathways in intermittent streams recolonisation after rewating – an in situ experimental study

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The dispersal abilities of aquatic organisms and their recolonisation abilities are generally underexplored in major biotic groups. This deficiency is a principal obstacle to understanding dispersal as a regional process structuring ecological communities, especially in spatiotemporally dynamic intermittent streams. Freshwater invertebrates are commonly spread inhabitants of inland aquatic systems, characterised as frequent, widespread dispersers able to reflect environmental conditions and their changes with a fast response at the species, population, or community level. Stream intermittency represents a hydrological disturbance acting as an environmental filter providing intense selection pressure on instream biota – it significantly regulates population and community structure and dynamics, particularly in the current period of accelerated climate change.

We tried to experimentally examine different recolonisation pathways used by benthic macroinvertebrates in terms of both taxonomic and functional community composition and evaluate their potential for successful community recovery after the drying period, expressed in total abundance and biomass. We considered four possible recolonisation pathways (i.e., downstream, upstream, hyporheic and aerial) with corresponding experimental units installed in situ, where only one of the mentioned recolonisation ways was allowed. Each treatment was represented by three replicates composed of wired boxes filled with an artificial gravel substrate exposed in a stream for two weeks.

Significant differences among treatments were evident in the alpha-diversity indices; Shannon's diversity index was lowest in the hyporheic treatment, whereas the taxa richness and total abundance were lowest in the aerial treatment. Total biomass was highest in the hyporheic treatment, despite its lowest biodiversity. Two invertebrate groups, chironomids, and crustaceans dominated all the treatment types. The analysis of species traits showed different metacommunity structures in each treatment. Hyporheic treatments were frequently populated by inhabitants preferring sandy and gravel substrates, or species typical for a hyporheic zone. Species with flexible life cycles predominantly colonised aerial treatments. In upstream treatments dominated species from lower stream zones or species typical for standing waters, whereas species from upper stream sections were less frequent.

While downstream drift was the most favourable pathway regarding biodiversity, the hyporheic pathway was the most important recolonisation way considering biomass. According to our study, the aerial and upstream recolonisation pathways have only a marginal contribution to community recovery after rewating in a short-term horizon. The study was supported by the H2020 project DRYVER (869226).

Integrative taxonomy of Ephemeroptera, Plecoptera and Trichoptera of Przemyskie Foothills and Bieszczady Mountains streams

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The Bieszczady Mountains and Przemyskie Foothills are characterised by the occurrence of remnants of Carpathian wildernesses recognised as one of the most important pristine mountain landscapes in Poland. The biodiversity of aquatic insects in the streams of the Bieszczady Mountains and Przemyskie Foothills has not been studied extensively yet. These regions are an attractive tourist destination, and therefore the fauna of their streams is potentially exposed to habitat loss and water pollution. Learning more about the biodiversity of both regions can be crucial in implementing plans for their protection. This project focuses on aquatic insects from EPT index groups (Ephemeroptera, Plecoptera, and Trichoptera) as an indicator of the potential biodiversity of streams and their water quality. The DNA barcoding approach followed by morphological identification was used for estimating cryptic diversity and to extend the Polish barcode library (PolBOL) in the Carpathians. The invertebrates were collected from different dominating mesohabitats, namely stones, gravel, wood, and detritus, to ensure the biodiversity of the streams was fully captured. The results will give an insight into the level of diversity of aquatic insects in both regions, as well as help dispel the discussion on the desirability of expanding the buffer zone of Bieszczadzki National Park and the establishment of the Turnicki National Park, which could offer additional protection for the streams of the Przemyskie Foothills.

Research is financed by the University of Lodz Students Grant "Integrative taxonomy of mayflies and stoneflies of Przemyskie Foothills and Bieszczady Mountains" no. SGB_647 and FAN(B) project "Benthos of streams in Pogórze Przemyskie – issues for planned Turnicki National Park".

A new tool in the toolbox: evaluating the potential of DNA metabarcoding for the monitoring of macroinvertebrates in regulatory context

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With the establishment of the Water Framework Directive (WFD), the European Union has set the goal to achieve good ecological status for all water bodies in Europe. To assess the biological quality, biodiversity assessments are carried out with established morphology-based methods. However, traditional biomonitoring remains cost- and time-consuming. Here, DNA-based methods, such as DNA metabarcoding, have the potential as new complementary and efficient tools for regulatory biomonitoring. However, while DNA-based methods have been long established in the scientific community, a validation as a method for regulatory biomonitoring has to be conducted yet.

Therefore, the GeDNA project was initiated to leap the gap between the scientific community and the applied sector. One aim of the project is to evaluate DNA metabarcoding for the assessment of macroinvertebrates and the comparison to traditional methods. Therefore, macroinvertebrate bulk samples were collected in two WFD sampling campaigns (2020 and 2021) across Germany. All samples were morphologically identified by taxonomic experts and subsequently processed with DNA metabarcoding.

Results revealed high concordance in species composition for benthic invertebrates. DNA metabarcoding identified significantly more non-biting midge taxa and exhibited similar proportions of the important bioindicator 'EPT' taxa (mayflies, stoneflies, caddisflies: Ephemeroptera, Plecoptera, Trichoptera). Ecological status classes derived from invertebrate assessments showed high congruence between the two methods (Spearman rho = 0.86). The choice of abundance or presence/absence data had minimal influence on the assigned status classes for both BQEs.

In conclusion, this pilot study coordinated via federal, and state environmental agencies demonstrated high similarity between the methods for the stream types analysed, suggesting suitability of DNA metabarcoding of benthic invertebrates for WFD monitoring without major modifications.

Aquatic beetles in Tatra lakes – when globally small players mean a lot in terms of genetic diversity

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Alpine regions cover less than 3% of the Earth's land surface, excluding Antarctica, but they represent a particularly unique ecosystem with a high degree of endemism and many endangered species. During the last glacial maximum, most of the alpine areas were covered by glaciers and firms, the gradual melting of which created ~50,000 alpine lakes and ponds only within Europe. At a time of increasing global warming, these unique freshwater habitats are an important refuge for many freshwater cold-tolerant species but also for those that are not adapted to rising temperatures. However, their remoteness, short ice-free period, and frequently difficult accessibility make research on these ecosystems difficult. The aim of this study, as a part of a broader research initiative on barcoding Tatra Mts freshwater fauna, was to analyse in detail the genetic diversity of common inhabitants of alpine lakes and ponds, the water beetles.

Within 387 sequences from 105 localities sampled between 2007–2018, 68 morphologically identified species in 27 genera and eight families were recorded, of which 1/3 was found for the first time in this area. To evaluate the genetic uniqueness of the Tatra beetle fauna, we compared our data with those available in the BOLD database (>1,700 sequences from 26 countries). The total alignment consisted of 657 haplotypes representing 106 BINs. Additional delimitation approaches resulted in 81 (ASAP) and 92 (mPTP) MOTUs. On average, ~75% of the globally captured putative species/BINs were also recorded within the Tatra Mountains. The recorded species consisted of 179 haplotypes, of which 30% were found exclusively here, confirming the important role of the Tatra Mt lakes in maintaining unique genetic information. Relatively high intraspecific genetic diversity was also confirmed, especially in three recorded species, each consisting of two phylogenetically distant, likely Pleistocene lineages.

Our study confirmed that even relatively small but richly diversified alpine lakes can accumulate high genetic diversity and be an essential source of unique genetic information. Our data also significantly enriched the regional DNA barcode database, enabling more effective future monitoring of valuable alpine freshwater habitats. The study was supported by the project VEGA 2/0084/21.

Distribution, diversity, and diversification of *Gammarus* species flock in the ancient Lake Ohrid, overview of the project progress

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Lake Ohrid, located in the Balkan Peninsula at the Albanian/Macedonian border, is the oldest European lake (ca. 1.3 My old), one of the world's smallest ancient lakes and an important biodiversity hotspot. At the same time, considering the lake surface, it harbours the highest level of endemism, especially in the case of amphipod crustaceans (ca. 90%). Most of Lake Ohrid amphipods belong to the local endemic *Gammarus* (Amphipoda, Crustacea) species flock. Still, the evolutionary processes underlying speciation within this group in ancient lakes are weakly understood. Therefore, the Ohridian *Gammarus* species flock, encompassing about a dozen species, is an exceptional model for testing adaptive radiation compared to other such systems. The current study aims to unravel the evolutionary processes behind the origins of the endemic *Gammarus* species flock in this ancient lake using an integrative approach. We incorporate molecular study through extensive COI barcoding and transcriptome sequencing as well as a detailed examination of the functional morphology of the species flock and relatives.

In a result, we see that the distribution of the *Gammarus* species flock is vertically structured, reflecting habitat zonation and suggesting parapatric speciation as one of the possible mechanisms behind the flock's diversification. Two new molecular units representing putatively new species are revealed. The onset of flock radiation overlaps with the time of lake formation, while the subsequent speciation events and demographic changes relate presumably to glacial and postglacial water level changes and to the colonisation of new depth ranges and the associated springs. The transcriptomic data show complex patterns of diversification, probably resulting from hybridisation during the flock radiation, while morphological variability shows relatively high diversity and divergence between species. All this suggests that adaptive radiation was the main force behind origins of the *Gammarus* species flock in ancient Lake Ohrid. This work is funded by the Polish National Science Centre (OPUS16 grant Nb. UMO-2018/31/B/NZ8/03103).

The benthic communities in streams of Przemyskie Foothills – issues for the planned Turnicki National Park

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The Bieszczady Mountains have been protected under Bieszczady National Park since 1973. Przemyskie Foothills that border the Eastern Carpathians from the north are also to be protected under Turnicki National Park, which has been in the plans for the last 40 years. The Przemyskie Foothills terrestrial biodiversity has been well documented, while the region's freshwaters have not been studied extensively in recent years. The necessity to explore and ascertain the disparities between the two regions prompted the initiation of comprehensive studies on stream-dwelling invertebrates. Our research is focused on mite midges, caddisflies, mayflies, stoneflies, bugs, and other insect larvae, along with amphipods, molluscs, and annelids.

A survey of the fauna in the Przemyskie Foothills and the Bieszczady National Park indicates that these regions are predominantly inhabited by rheophilic species of Chironomidae associated with clean upland and mountain streams, such as: *Polypedilum convictum*, *Tvetenia verralli* and *Rheocricotopus chalybeatus*. Rare mountainous caddisfly and stonefly species have also been recorded. Among amphipods, endemic East Carpathian species are dominant. The benthic communities of Przemyskie Foothills are generally diverse and represent rare taxa that prefer mountainous, pristine streams, which are also present in Bieszczady. The presented research clearly proves that freshwater streams should be protected under the planned Turnicki National Park.

The project is financed by the FAN(B) project "Benthos of streams in Pogórze Przemyskie – issues for planned Turnicki National Park".

No way back: strong negative effects of stream intermittency on dragonfly (Odonata) assemblages

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In the last decades the proportion of intermittent rivers and streams has significantly increased as a consequence of climate change, affecting macroinvertebrate assemblages. Among them, dragonflies are usually top predators in stream ecosystems and any change in their assemblages might influence the whole community. Although they are considered potentially less affected by flow intermittence due to their strong dispersal abilities, high fecundity and high temperature tolerances, our knowledge on the effect of intermittency on Odonata is still limited. In the framework of the DRYvER project (Horizon2020 #869226) stream-dwelling Odonata were studied in three ecoregions of Europe differing in climate and intensity of intermittency: in the Boreal ecoregion, with a cold temperate climate and in general no flow intermittence (Finland); in the Pannonian ecoregion, with continental climate, and where the flow intermittence is a relatively new phenomenon (Hungary), and in the Mediterranean ecoregion, with Mediterranean climate and seasonal flow intermittence (Spain). Quantitative samples were taken bimonthly through six sampling campaigns in 2021/2022. Odonata larvae were identified at species level and their body length was measured. Dragonfly larvae were almost absent from intermittent sections, even if surviving might have been possible in remaining pools. In cases when Odonata larvae occurred in intermittent sections, the number of species and number of individuals, and therefore their diversity were significantly lower than in permanent sections. Differences were detected in the size distribution of larvae between permanent and intermittent sections, but it was strongly species-specific depending on the life history of the species. The changes in size distribution showed temporal variability, i.e., the size of the larvae was significantly smaller after drying up in intermittent streams, indicating the occurrence of only newly hatched larvae. Our results demonstrate a remarkable negative effect of intermittency on dragonfly assemblages. Although some species would be able to re-colonise the rewetted sections, their long larval development prevents them from establishing a viable population in these sections.

Exploring the osmoregulation of two Ponto-Caspian invaders through the expression of membrane proteins

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The voluntary or accidental introduction of non-indigenous species (NIS) is one of the major challenges affecting the environment, leading to a homogenisation of biodiversity worldwide. One of the main sources of freshwater NIS in Europe is the Ponto-Caspian region, which has a turbulent geological history characterised by several sea-level changes and fluctuating environmental stressors, such as salinity, temperature, and oxygen. Two remarkable invasive species from Ponto-Caspian region are the gammaroid amphipod *Dikerogammarus villosus* (Sowinsky, 1894) and the annelid *Hypania invalida* (Grube, 1860), which underwent a rapid geographic range expansion in central and western Europe. Salinity regime tolerance is considered one of the most significant factors limiting the distribution of the species in aquatic environments and indeed, among the traits invasive species display, a higher euryhalinity seems to facilitate the establishment and the invasion success of species in new habitats. The invasion of freshwater habitats by brackish or marine species has been reported frequently for those coming from the Ponto-Caspian region, notwithstanding the high energetic costs required to retain osmotic levels in body fluids when inhabiting freshwater environments. The work aims to assess the expression of some proteins involved in osmoregulation, such as Na-K-2Cl co-transporter (NKCC) and aquaporins (AQPs). Data about these proteins are scarce and scattered; however, we recently recovered *in silico* the presence of several paralogs of AQPs in both amphipods and annelids, and we experimentally confirmed their presence in the latter. We searched and annotated putative AQP genes in public genomes and transcriptomes, inferring their evolutionary relationships through phylogenetic analyses and discussing their putative physiological relevance. Our results showed how most of the AQPs of amphipods are similar to those of other crustaceans, despite the Prip-like displaying different paralogs, and report for the first time a putative Aqp8-like for arthropods. We also found that the candidate genes of Prip-like, Bib-like, Aqp12-like, and Glp-like may help solve deeper relationships in phylogenies of amphipods while leaving uncertainties in shallower parts. Concerning the annelids, we identified a total of 401 Aqp sequences in 27 annelid species, including 367 sequences previously unrecognised as Aqps. Similar to vertebrates, phylogenetic tree reconstructions clustered these annelid Aqps in four clades: AQP1-like, AQP3-like, AQP8-like and AQP11-like. We found no clear indication of the existence of paralogs exclusive to annelids; however, several gene duplications seem to have occurred in the ancestors of some Sedentaria annelid families, mainly in the AQP1-like clade. Our results suggest a diversification of the structures and functions of AQPs in Annelida comparable to that observed in other taxa.

Macroinvertebrates of the Prut River basin, Republic of Moldova

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The Prut River is a transboundary river, the second largest river in the Republic of Moldova, 695 km long in our country and the last left tributary of the Danube. Macroinvertebrates have been collected at 19 sampling stations from riverbed and adjacent lakes. The standard methods were applied: dredge, Petersen and Ekman grabs and hand sampling. Sampling was carried out seasonally during 2014–2023. Adults of some Ephemeroptera, Trichoptera and Odonata were also sampled.

High species diversity and many rare species were recorded at some stations, including: *Theodoxus transversalis* (Pfeiffer, 1828), a rare mollusc listed on the International Union for Conservation of Nature (IUCN) Red List and *Unio crassus* Philipsson in Retzius 1788 (IUCN Red List). At the same time, *Gomphus* (= *Stylurus*) *flavipes* (Carpentier, 1825) and *Palingenia longicauda* (Olivier, 1791), a threatened European species listed in Appendix II of the 1998 Berne Convention, were also recorded. Such rare and protected species for Republic of Moldova as: *Chalcolestes parvidens* (Artobolevsky, 1929), *Lestes macrostigma* (Eversmann, 1836), *Anax imperator* Leach, 1815 and *Sympetrum fonscolombii* (Selys, 1840) have been sampled and identified as imago.

The highest number of EPT taxa, groups of benthic invertebrates most sensitive to pollution of water bodies and general degradation of aquatic ecosystems, was recorded in Braniște and Tețcani sampling stations. A total of 258 benthic invertebrate taxa were identified, including: Gastropoda – 27, Bivalvia – 20, Polychaeta – 1, Oligochaeta – 20, Crustacea – 24, Ephemeroptera – 24, Trichoptera – 25, Odonata – 28, Chironomidae – 54 and other groups – 35. The most numerous group was Chironomidae, numbering 54 taxa. Invasive species such as *Corbicula fluminea* (Müller, 1774), *Corbicula fluminalis* (Müller, 1774), *Sinanodonta woodiana* (Lea, 1834), *Dreissena rostriformis bugensis* Andrusov, 1897, *Ferrissia fragilis* (Tryon, 1863) and *Branchiura sowerbyi* Beddard, 1892 were also found. Here we should note the appearance of *Macrobrachium nipponense* (De Haan, 1849) (Decapoda, Palemonidae) in the Lower Prut, in 2022. The main negative factors affecting macroinvertebrate communities in the Prut River are wastewater runoff from households and appearance and dispersal of invasive species.

This study was carried out within the following projects: 09.832.08.07A, 11.817.08.13F, 11.817.08.15A, MIS ETC 1150, MIS ETC 1676 and 15.817.02.27A.

The development of river-type-specific indices for assessing the ecological status of Lithuanian rivers using macroinvertebrates

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Macroinvertebrates are pivotal in assessing the ecological status and integrity of aquatic ecosystems, as they reflect the complex interactions between environmental conditions and anthropogenic pressures. This study aims to refine the assessment of Lithuanian rivers by developing specific multimetric indices tailored to different river types, moving beyond the current use of a uniform index (LRMI) across various river types in Lithuania. Utilising macroinvertebrate data collected from Lithuania's national river monitoring between 2010 and 2022, we first evaluated the impact of sorting and identification errors in macroinvertebrate samples. This involved comparing taxonomic lists generated from standardised sampling and processing protocols against our newly established Lithuanian operational taxa list. The adoption of operational taxonomy in Lithuania represents a crucial advancement in enhancing the methodologies for ecological status assessment. It proposes standardised taxonomic frameworks that bolster the accuracy and reliability of species identification. Subsequently, we developed several river-type-specific metrics sensitive to anthropogenic stressors. These metrics were then integrated into novel river-type-specific multimetric indices for evaluating the ecological status of Lithuanian rivers. The results and implications of our analysis will be presented and discussed.

Dry stream channels as attractive habitats for multiple biotic groups

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Current trajectories of climate change are manifested in the river networks of Central Europe by dramatic shifts from a perennial to an intermittent flow regime. Decrease of annual discharge is also associated with remarkable seasonal shifts in the flow duration, when first dry episodes can occur even in early spring (April-May) and could last until the cold season in winter (December-January) with the flow absence over half a year. Such prolonged dry episodes offer an extended time-window for stream colonisation by many terrestrial groups, which originally use habitats surrounding river channels. Newly occurred biotope of dry riverbed could provide an attractive environment (e.g., exposed sandy and gravel bars), that is not available in regulated rivers and anthropogenically modified floodplains.

Effects of stream drying on aquatic biota were more intensively studied in the last decade also in temperate Central Europe; however, interactions between terrestrial and aquatic groups are still poorly examined. Nevertheless, we can expect multiple impacts of invading terrestrial fauna and flora on aquatic biota during the dry phase (e.g., predation in pools and dry bottom, streambed ploughing, colonisation by terrestrial plants, etc.). Additionally, some of these effects could persist and consequently affect aquatic communities after the flow resumption. We show some examples of exploitation of dry channels by terrestrial groups, which could affect aquatic biota such as macroinvertebrates and fish (e.g., predation by terrestrial invertebrates and large vertebrates as wild boars or piscivorous birds). Moreover, some terrestrial invertebrates and plants including endangered taxa could recognise dry riverbed as attractive habitat, which enable them successful reproduction and expansion of populations. Such long-time dry reaches with sufficient light exposition are also rapidly colonised by terrestrial vascular plants, which could later offer food resources and shelter for aquatic macroinvertebrates in the early stages of recolonisation after rewetting.

Contrary to our expectations, we also recorded a few examples of an active movement of strictly aquatic stages or macroinvertebrates, which migrate not only within dry channels, but also actively immigrate to terrestrial riparian habitats several meters outside the channel. These findings shed a new light on the role of currently spreading habitats of intermittent streams, which emphasise an urgent need of their examination during progressing climate changes in the temperate zone of Central Europe. Research was supported by the projects H2020 DRYVER (869226) and TACR project SS06010258.

Environment, phylogeographic lineages or ranges – what drives the high morphological variation of the successful invader, *Dikerogammarus villosus* (Sovinsky, 1894) in European aquatic ecosystems?

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Phenotypic diversity plays a crucial role in facilitating the establishment and expansion of invasive populations in new environments. Particularly high levels of plasticity can be observed among the diverse Ponto-Caspian fauna that invaded inland European waters. However, the precise influence of the environment on shaping the phenotypic variation of these successful invaders remains inadequately understood. A good model species for such studies is *Dikerogammarus villosus* (Sovinsky, 1894) (Gammaridae, Amphipoda). This highly predatory and voracious species invaded a notable part of Europe within a few decades. It originated in the Ponto-Caspian region and the two isolated populations from the Danube and the Dnieper deltas colonised Europe through two phylogenetic lineages – the Western (via Danube) and the Eastern (via Dnieper). Although this species has a broad distribution and is currently expanding in Europe, its phenotypic diversity related to traits that determine invasive success was not studied. Therefore, we aimed to test morphological variation of functional traits that directly or indirectly reflect the diet of *D. villosus* across the European continent. Our results show that *D. villosus* exhibits a high morphological variability across Europe and that it is mainly shaped by the type of environment. We discovered an enhancement of predation-related traits in brackish waters compared to freshwaters. These results suggest that *D. villosus* could exert a greater threat on macroinvertebrate communities in brackish environments. Morphological differentiation among phylogenetic lineages and native vs. invaded ranges is weaker and mainly concerns locomotion and food processing traits (i.e., mouthparts and stomachs). Both lineages display a unique pattern of morphospace change between native and invaded ranges. A strong morphospace expansion in the invaded range of the Eastern Lineage suggests a higher plasticity of this lineage and possibly higher success in establishment in new habitats. Our results highlight the importance of accounting for ecological, evolutionary, and biogeographical factors when studying the phenotypic diversity of invasive species at broad continental scales. The results will be an important input to understanding the invasion dynamics of Ponto-Caspian amphipods in Europe.

Comprehensive database of drying resistance and resilience traits from drying rivers across Europe

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Drying River Networks (DRNs) are highly dependent on the dynamics of local climate conditions, which greatly influence the flow regime, the duration of dry and wet periods and the frequency of drying events. As such, DRNs are one of the most vulnerable ecosystems to climate change and anthropogenic exploitation. In order to secure biodiversity, functional integrity and ecosystem services in drying river networks, intricate ecological dynamics governing these ecosystems need to be unravelled. In this effort, the trait-based approach is utilised to emphasise the significance of functional biodiversity in sustaining ecosystem processes under environmental stressors and to identify in which measure each functional trait exhibits resistance or vulnerability to drying events.

A part of that effort is building a comprehensive database containing information on drying resistance (ability to endure) and resilience (ability to recover) traits for freshwater bacteria, fungi, diatoms, macroinvertebrates, and fishes. Through collaborating with experts and studying literature, a list of traits relevant to drying event survival was compiled for each of the five biotas. Each trait has been categorised, coded and its function described, and freshwater community information was obtained during the sampling process for project DRYVER. Subsequently, the database was compiled assigning trait values to listed taxa using readily available data from existing databases and published literature. The database's development involved rigorous collection, cleaning, standardisation, and harmonisation of trait data to ensure the thorough representation and precision of trait variability across biota. Using the database, trade-off analysis was conducted.

Construction of the database is a continuous process as newly described trait data is appearing and newly developed technologies offer better solutions for higher levels of precision. Through collation and further analysis of trait data, the trait database enhances our understanding of the ecological mechanisms underpinning DRN resilience which contributes to better understanding of the effects of climate change on freshwater biodiversity across Europe.

***Neocaridina davidi*, a feral shrimp in central Europe: range expansion and hidden parasites**

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The release of ornamental pets and associated pathogens outside their native range might directly or indirectly impact the recipient community. In central Europe, feral freshwater species originating in tropical and sub-tropical regions are typically restricted to thermally altered environments such as heated waters and thermal springs. However, species like the shrimp *Neocaridina davidi* have high environmental plasticity and may acclimate to cooler temperatures. A widening thermal niche may eventually overcome thermal barriers, further expanding the range, and enhancing transmission opportunities for host generalist parasites. This study investigates the observed (field observations) and theoretical (species distribution models) range expansion of *N. davidi* and associated parasites across Europe. We report the establishment of three new *N. davidi* populations in thermally altered waters in Germany, Hungary, and Slovakia, and a range expansion into colder environments. Species distribution models predict suitable thermal habitats in Mediterranean regions, with a foreseeable expansion towards Western Europe and the Balkans by 2050.

Furthermore, we confirm the presence of the microsporidian parasite *Ecytonucleospora hepatopenaei* in feral *N. davidi* populations throughout Europe and expand the list of microsporidians found in this host from two to four. We present the first evidence of parasite transmission between *N. davidi* and the invasive crayfish *Procambarus clarkii*, indicating a potential for parasite exchange with native fauna. Such possibility, coupled with an ongoing range expansion of *N. davidi* bolstered by human-mediated introductions and climate change, will likely exacerbate the impact on native biota.

Exploring the hidden world: first insight into diversity of epikarst invertebrates in the Western Carpathians

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Epikarst, the uppermost zone of the karst landscape, functions as a critical interface between soil and carbonate rocks, forming a perched aquifer that stores and gradually releases water infiltrated from precipitation. This unique environment serves as both a water reservoir and a subterranean aquatic habitat for minute invertebrates, yet its biological and ecological processes remain significantly understudied. Between 2019 and 2020, we conducted extensive sampling of epikarst invertebrates using 27 filtering devices in the Demänová Cave System, northern Slovakia. Our integrative approach employed both conventional morphological techniques and molecular tools, specifically DNA barcoding, to evaluate the invertebrate diversity of this understudied subterranean ecosystem. Utilising the mitochondrial cytochrome oxidase subunit I (COI) gene as the standard barcoding marker, we successfully generated 784 DNA barcodes representing 36 morphospecies from 17 orders and 6 animal classes, with a predominant representation of Arthropoda (98.3%). Morphological identification revealed 10 obligatory aquatic species, including four stygobionts limited to groundwaters and nine amphibiotic species, as well as 17 terrestrial species, including five troglobionts associated with the cave environment. Our study provides a unique documentation of the genetic and species diversity of Metazoa within the epikarst, with more than 70% of Barcode Index Numbers (BINs) being new to the Barcode of Life Data Systems (BOLD). Importantly, 24 out of 28 morphologically identified subterranean species lacked sequence coverage in BOLD, emphasising the novelty of our findings. Notable cases of intraspecific divergence were observed, particularly in subterranean taxa, showcasing the existence of morphologically indistinguishable cryptic species within the fragmented karst system. Our data provide evidence for undescribed species of harpacticoid copepod *Elaphoidella* and suggest the potential presence of a new-to-science family within the order Amphipoda. In the case study of the Demänovský cave system, we demonstrate that the genetic and species diversity of invertebrates associated with seeping epikarst waters is vastly understudied. Our findings shed light on the overlooked realm of subterranean biodiversity, emphasising the need for future extensive investigations into groundwater life diversity in the Western Carpathians and adjacent regions. This study serves as a crucial foundation for understanding and conserving the unique ecosystems thriving in the depths of the epikarst.

Diversity and distribution of epigean amphipods in Poland through DNA barcodes

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Amphipods are among the most abundant and diverse crustaceans in Palearctic freshwaters. This group is extensively used in applied and basic research, including biodiversity assessments, ecotoxicology, and evolutionary ecology. Nevertheless, even well-studied model organisms may exhibit overlooked cryptic diversity. DNA barcoding, based on partial cytochrome c oxidase subunit 1 (COI) sequences, has been proven to be a valuable tool for species determination and revealing the cryptic diversity in many taxa, including amphipods.

Here, we present studies on epigean freshwater and brackishwater amphipods from Poland. Recent findings and descriptions of new species enhanced the Polish checklist to at least 27 species. Nine species (33%) of Polish Amphipoda fauna are invasive species, seven of which have Ponto-Caspian origin. We can also find five brackish species (*G. zaddachi*, *G. salinus*, *G. locusta*, *G. oceanicus*, *Apocorophium lacustre*) and one semi-terrestrial talitrid – *Cryptorchestia garbinii*.

Here, DNA barcodes from 993 individuals and 26 species were analysed, including sequences from previous studies and more than 400 newly generated DNA barcodes. We studied representatives of the six families: Gammaridae (831 ind., 19 species); Pontogammaridae (118 ind., 2 species), Corophiidae (20 ind., 2 species), Talitridae (12 ind., 1 species), Pallaseidae (3 ind., 1 species), Crangonyctidae (1 ind., 1 species). We found 38 BINs and exact correspondence between BINs and traditionally recognised species matched for 21 species (78%). Deep intraspecific sequence divergences with distinct lineages were revealed within six species, with two (*Gammarus pulex*, *G. salinus*, *G. tigrinus*), three (*Echinogammarus ischnus*, *G. fossarum*, *G. tatrensis*), and four (*G. jazdzewskii*) BINS, respectively.

Our findings are the first, almost complete, country-scaled attempt to provide a DNA barcode reference library of Amphipoda.

Lake Prespa, a hotspot of freshwater biodiversity – assessment of water quality using benthic macroinvertebrates as a Biological Quality Element

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Most European countries use national assessment systems for freshwater bodies with the main objective of assessing the ecological status of water quality based on living organisms, including benthic macroinvertebrates as a biological quality element (BQE). As aquatic macroinvertebrate assemblages are typical of the different types of water bodies, they are used as important indicators of the prevailing aquatic ecosystem and directly describe the condition and quality of the water. In this study, we present data on the taxonomic composition, diversity, and community structure of bottom macroinvertebrates from Lake Prespa and the assessment of water quality based on the BQE benthic macroinvertebrates. The sampling was carried out in the period 2021-2022 at a total of seven sites across the shoreline of Lake Prespa in North Macedonia. Water quality of the littoral zone was assessed from moderate to bad (III-V class). Concerning the samples from the profundal zone, the collected specimens belonged to the Oligochaeta and Chironomidae groups, which indicates a higher degree of eutrophication of the lake's ecosystem. The decline in water quality was primarily expected as a result of the lowering of the water level in the comparison of the two study years. Among the species endemic to Lake Prespa, *Pyrgohydrobia prespaensis* (Urbanski, 1939), *Gammarus triacanthus prespensis* (Karaman S. & G. 1959), *Dreissena carinata* (Dunker, 1853) and *Atyaephyra thyamisensis* Christodoulou, Antoniou, Magoulas & Koukouras, 2012 were recorded. Nevertheless, within this survey we contribute to the specific lake's macroinvertebrates community representatives and further to the unique endemic assemblages from Prespa Lake, that characterised the lake as a "unique hotspot".

The study was made possible thanks to the Prespa Ohrid Fond Trust (PONT) grant "Lake Prespa Monument of Nature and Ezerani Nature Park: Operational Plans 2021-2023".

Are semi-mountainous and mountainous benthic communities suitable for the delineation of the 6th (Eastern Balkan) and 7th (Hellenic Western Balkan) hydrofaunistic ecoregions?

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According to the original concept of Illies (1978), adopted by the EU Water Framework Directive (WFD 2000), the areas of North Macedonia and Greece are divided longitudinally along the Vardar/Axios River as the border between two ecoregions: Ecoregion 6 – Greek Western Balkans (right tributaries) and Ecoregion 7 – Eastern Balkans (left tributaries). To date, there are no macroinvertebrate data that would support an adjustment of the boundaries between the two hydrofaunal ecoregions for the area of North Macedonia. The aim of this study is an approach to a more realistic definition of the hydrofaunistic boundaries in the territory of North Macedonia, with respect to aquatic macroinvertebrate communities, based on the semi-mountainous and mountainous river types. To achieve this goal, a comparative analysis (PCA, Principal Component Analysis) of the macroinvertebrate communities from the studied areas (94 sites from eight river basins belonging to the three ecoregions 5, 6 and 7) was performed to verify their affiliation to a specific ecoregion. The results showed that the strict delineation of hydrofaunistic boundaries between the studied ecoregions is complex, mainly due to the peculiarities in the geographical distribution of macroinvertebrates and the biotypic characteristics of the rivers themselves, which show greater similarities in the ecoregions' border zones. As the selected study sites from all three ecoregions were chosen based on their categorisation as semi-mountainous and mountainous river types, one would expect greater similarity between areas of similar elevation and substrate. Regarding the distribution of the studied watersheds of the selected semi-mountainous and mountainous rivers, the obtained results indicate the shift of the border to the west, where the entire Vardar watershed belongs to Ecoregion 6 – Greek Western Balkans. These results would be a good basis for future research in relation to the other biological quality elements in order to find the best approach to support the proposed delineation between ecoregions 6 and 7. This study was funded by "Programme for Support of Young Researchers and PhD Students at the Bulgarian Academy of Sciences" (Grant No. DFP-17-108/28.07.2017 – "Implementation of biotic indices BMWP and ASPT in order to evaluate the ecological status of mountain and semi-mountain rivers in the 7th Ecoregion (Eastern Balkans)") and by the "Japan Funds-in-Trust Project" (REF.: ERI/MSP/PPF/LZF/15.037) – "Management of freshwater bodies with implementation of EU-Water Framework Directives using aquatic macroinvertebrates as the necessary measure for sustainable development of freshwater ecosystems in less developed countries".

Long-term research of aquatic biting midges (Diptera: Ceratopogonidae) in the Plitvice Lakes National Park

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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 (6 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 Jackknife 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 emphasised once more with the finding of *Dasyhelea gothlandica* Strandberg & Johanson 2015, recorded previously only from Sweden (more than 2,000 kilometres away), which is fascinating given the relatively modest flight capabilities of ceratopogonids. This finding sheds the light on the vast gaps in knowledge on the ecology of this fascinating insect group.

From darkness to light: molecular phylogenetic analysis of the amphipod *Niphargus elegans* reveals repeated colonization of epigean environments from groundwaters

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Niphargus elegans Garbini, 1894 is one of the few surface species in the predominantly groundwater genus *Niphargus*. Described on material from the surroundings of Verona, Italy, it shows a wide distribution in the Padanian Plain and along the Adriatic coast where it inhabits surface streams, rivers, and ponds. As for many niphargid species, the taxonomy of *N. elegans* is intricate. Considered by some authors as a subspecies of the central-European *N. puteanus* (Koch, 1836), it was later enlarged to include spring-dwelling subspecies in Slovenia (*N. illidzensis slovenicus* S. Karaman, 1932, subsequently transferred to *N. elegans* by Gordan Karaman) and Croatia (*N. elegans zagrebensis* S. Karaman, 1950); furthermore, it was reported from springs and caves in Romania.

We conducted a DNA-based species delimitation using four molecular markers (two nuclear rRNA gene fragments – ITS and 28S, the nuclear histone H3 gene and a fragment of the mitochondrial COI) to investigate the relationships among the alleged *N. elegans* subspecies as well with Italian and Romanian populations attributed to *N. elegans*. A phylogenetic analysis based on a concatenated dataset of four markers was also performed to investigate the relationships between *N. elegans* and two other surface niphargids, *N. valachicus* Doboreanu & Manolache, 1933 and *N. hrabei* Karaman, 1932, both widely distributed in the Danubian basin in western Europe. We tested (i) whether *Niphargus elegans* is monophyletic and whether its alleged subspecies are actually distinct species, (ii) whether its phylogenetic position suggests a secondary colonisation of the epigean environment and, in that case, (iii) whether *N. elegans*, *N. hrabei* and *N. valachicus* independently colonised surface waters from groundwaters.

Results show that despite their morphological similarities, the three *N. elegans* populations from Italy, Slovenia, and Croatia are three distinct, closely related species. By contrast, Romanian populations previously called *N. elegans* belong to a different clade (close to *N. valachicus*) that is a complex of new or poorly described species, including *N. pater* Mehely, 1941. Italian *N. elegans*, *N. hrabei* and *N. valachicus*, despite their ecological similarity, are not closely related and independently colonised surface waters from three different groundwater ancestors. These results highlight that although the genus *Niphargus* shows major adaptations to groundwater life (such as eye and pigment loss), it repeatedly colonised surface waters in different geographical areas in Europe.

Downsizing plastics, upsizing impact: how microplastic particle size interplays with *Chironomus riparius* bioturbation activity

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Understanding the impacts of microplastic pollution on freshwater ecosystems is crucial due to its far-reaching environmental consequences. However, while the general understanding of microplastic pollution's impacts is growing, there remains a critical gap in knowledge regarding the specific effects of different microplastic particle sizes. This study aims to address this gap by focusing on the distinct effects exerted by varying sizes of microplastic particles on the bioturbation activity of *Chironomus riparius*. Bioturbation, the process by which organisms disturb sediment layers through their activities, plays a significant role in sediment dynamics and nutrient cycling in aquatic environments. By investigating how microplastic particle size influences bioturbation patterns, this research seeks to provide insights into the mechanisms driving the fate and behaviour of microplastics in freshwater ecosystems. Methodologically, luminophore sediment profile imaging (LSPI) was utilised to follow the movement of microplastic particles within sediment layers. LSPI is a non-destructive technique that allows for the visualisation of particle distribution and transport dynamics in the sediment. Importantly, the study comprised two different size ranges of polyethylene particles: blue spheric particles (53–63 µm) and larger red spheric particles (250–300 µm), mimicking the luminophores. The particles were mixed in the first layer of sediment in equal proportions at a ratio of 0.076% (particles to sediment). Preliminary findings indicate that both large and small microplastic particles exhibit downward transport within sediment layers. However, there are notable differences in their interactions with *C. riparius* larvae and their impact on bioturbation patterns. Specifically, smaller microplastic particles appear to have a more pronounced effect on larval locomotive activity and are prevalent within the digestive tracts of the organisms. This suggests that particle size plays a crucial role in determining the bioavailability and uptake of microplastics by aquatic organisms, with smaller particles potentially posing a higher risk to ecosystem health. Furthermore, the temporal pattern of the reworking associated with the two different particle sizes shows distinct dynamics,

highlighting the complexity of microplastic interactions in freshwater environments. Additionally, we observed that larger particles, intended to mimic the characteristics of luminophores, exhibit a different transport behaviour compared to it, suggesting the importance of particle shape in influencing their fate and behaviour in aquatic systems. Overall, this study contributes to our understanding of how microplastic particle size influences their fate and interactions within freshwater ecosystems, highlighting the need for targeted mitigation strategies to address the escalating threat of microplastic pollution.

TrendDNA: studying long-term biodiversity change using environmental DNA contained in the German Specimen Bank

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In the current era of rapid ecosystem change, long-term biodiversity data pose the basis for quantifying trends, predicting consequences, and supporting management actions. However, long-term biodiversity data are scarcely available. A long-term sample collection that has been little explored so far is the German Environmental Specimen Bank (ESB), which contains terrestrial, freshwater, and marine samples collected for decades in a highly standardised manner. Samples are collected monthly or annually using standard operating procedures and are then stored in aliquots in cryotanks at -150°C. The ultra-cold storage condition preserves environmental DNA (eDNA) present in the samples, which is an ideal source for holistic biodiversity assessments. The TrendDNA project aims to test, if eDNA-based methods such as DNA metabarcoding and metagenomics are suitable for studying biodiversity change using the ESB samples. One of the ESB sample types is suspended particulate matter (SPM), collected from 13 different sites in the Rhine, Saar, Danube, Elbe, Mulde, and Saale rivers, dating back to 2005. SPM is collected monthly using sedimentation traps, which are then pooled into yearly homogenates. In the project, we are analysing 211 SPM samples from 6 rivers and 17 years using eDNA metabarcoding targeting fish and invertebrates. We highlight that eDNA data from the TrendDNA project provides comprehensive and plausible taxa lists, shows evidence of the introduction of invasive species, and unveils significant patterns of faunal community changes across years and sites. These results can aid in understanding biodiversity patterns, species turnover, tracking the invasion of species, assessing the progress of endangered species protection, and predicting future change. We provide an outlook on further project results and highlight the potential that the ESB can play in complementing long-term biodiversity monitoring in Germany.

Diversity of non-biting midges (Chironomidae) inhabiting common water moss (*Fontinalis antipyretica* Hedw.) microhabitats – a case study from river Rawka (central Poland)

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Vegetation in the riverine ecosystems plays a crucial role in providing food and shelter for the invertebrates. Not only the vascular plants but also the aquatic mosses are important habitats for the benthos. The common water moss (*Fontinalis antipyretica* Hedw.) occurrence is limited by a presence of solid substrates, e.g., stones or submerged wood to which plant can attach. *F. antipyretica* occurs in highly oxygenated and clear rivers. Therefore, the common water moss as the microhabitats for Chironomidae in the lowland river Rawka (central Poland) was studied. Rawka is protected as a nature reserve, being one of the few natural rivers of the Polish Lowlands. Its benthic assemblages have not been studied extensively so far, only preliminary research conducted indicates that Chironomidae are the least-known and most underestimated group. As the riverbed of the Rawka is not regulated or dredged, it exhibits a variety of moss microhabitats. The material was collected from 22 sites, including samples of Chironomidae larvae and algal periphyton, and a control sample from the bottom. The physicochemical conditions have been examined. As the diatoms are the main component of periphytic invertebrates' diet, their species composition and richness were analysed. The preliminary results indicate 20 species of Chironomidae, including 3 new species to the Polish fauna. Most of the recorded species are rheobiontic and small-sized, i.e., *Rheotanytarsus photophilus*, *Eukiefferiella claripennis* and *Thienemanniella majuscula*. Eighteen Chironomidae species have been recorded for the first time from this river which leads to the hypothesis that aquatic moss microhabitats hide a substantial diversity of Chironomidae. The new data contribute to the knowledge concerning the aquatic fauna of Rawka Nature Reserve and shed new light on aquatic moss as an important habitat for macroinvertebrates.

Disentangling environmental drivers of macroinvertebrate community structure: the role of stream drying and wastewater pollution

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Due to the ongoing climate change bringing long-term deficits of precipitation together with rising evapotranspiration related to higher temperatures, there is an increasing proportion of intermittent streams in continental temperate zone. Drying streams are exposed to diverse anthropogenic impacts including the input of urban pollution. The outflow of wastewater from sewage treatment plants are one of the most common forms of organic pollution in stream ecosystems and has adverse effect on benthic invertebrates. The response of stream biota to increased saprobity is well described in perennial systems. However, the evidence of structural and functional changes in benthic invertebrate assemblages in polluted intermittent streams is scarce.

Using the “real world experiment” with sampling sites up- and downstream of sources of wastewater pollution in intermittent/perennial streams we studied how both factors affect benthic invertebrate community. We analysed water chemistry, duration of dry period and benthic invertebrate communities within the dataset of 16 sites from the Czech Republic. Benthic invertebrate community clearly differed among the four studied groups (perennial non-polluted, perennial polluted, intermittent non-polluted, intermittent polluted) and was organised along two gradients. These gradients represent both studied factors (pollution, intermittence) and our results suggest that both factors have distinct but not the same effect on benthic invertebrates. Project was supported by the Czech Science Foundation (GA23-05268S) and H2020 project DRYVER (869226).

Research of aquatic invertebrate communities in ephemeral wetlands on arable land

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Temporary wetlands belong to the most threatened ecosystems within Europe inhabited by a unique fauna of aquatic invertebrates. However, we still do not have much information about the main determinants of the composition and diversity of their communities. We have sampled altogether 36 ephemeral field wetlands in southern Moravia (SE part of the Czech Republic), which periodically appear on arable land after heavy rains or snow melting. To evaluate the seasonal aspect, sites were sampled after flooding in spring and summer. Non-biting midges (Chironomidae), aquatic beetles (Coleoptera), water bugs (Heteroptera), and true flies (Diptera) were the most dominant taxa. Field wetlands varied significantly between seasons in algal cover, water temperature, and some chemical characteristics of water. Both higher numbers of species and higher abundances of invertebrates were found during the summer season. Large branchiopods (Branchiopoda: Anostraca, Notostraca, Spinicaudata) were the main drivers of variability in community composition during the summer season, with increasing water turbidity, and decreasing numbers of species and abundance of other invertebrates as their numbers increased. Among the environmental variables, seasonality, turbidity, and water temperature contributed most to the variability in the community composition. Another important factor influencing the colonisation of these temporary habitats is the presence of seed banks. Based on zooplankton sampling, we found a significant positive influence of the continuity and history of sites on species diversity and the occurrence of regionally rare species. Compared to other standing water habitats, ephemeral field wetlands host unique communities of aquatic invertebrates, including several protected and endangered species, and serve as the last remnants of the landscape suitable for the occurrence of these unique communities. This is also why they deserve attention and protection, especially against targeted drainages or conversion into permanent water bodies with the subsequent appearance of fish. Our results represent one of the first insights into the diversity and functioning of aquatic invertebrate communities in the unique habitats of ephemeral field wetlands in central Europe.

The effects of drought and habitat degradation on environmental filtering and limiting similarity

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Drought and the deterioration of habitats can happen independently or at the same time, impacting the presence of specific characteristics in groups of stream-dwelling invertebrates. Within the context of limiting similarity theory, we explore whether environmental filtering or limiting similarity functions as the main influencing factor under three different environmental conditions: unstressed (where there is constant water flow and a good ecological potential), single-stressed (due to drought or poor ecological potential), and multi-stressed environments (affected by both drought and poor ecological potential). The traits of macroinvertebrates were monitored monthly, from March to December 2020, two trait databases of macroinvertebrates were used (DISPERSE and Freshwater Ecology) for trait analyses. Our results strongly support the idea that in both unstressed and single-stressed environments, limiting similarity is the primary factor shaping the structure of the macroinvertebrate community. It's not just the quantity of stress, but rather its quality, that amplifies the impact of environmental filtering. The drought has a greater impact on the aquatic ecosystem compared to habitat degradation as it eradicates living conditions when water dries up, whereas poor habitat conditions only limit them. Our research identifies universally beneficial trait states that remain consistent across different stressors, alongside specific trait states that exhibit unique responses to each type of stress. This study offers valuable perspectives for the management of freshwater habitats and the development of adaptive conservation strategies in light of the rising global frequency of droughts.

The resilience of stream food webs to drying: insights from European drying river networks

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While intermittent rivers have long been common in southern regions of Europe, global change is currently prolonging dry periods and causing intermittent flow in formerly perennial rivers, even in northern regions of Europe. The consequences of drying for the structure and productivity of the food webs in these systems are still unknown. Here, we aimed to investigate the stability of river food webs in the face of drying. We sampled six Drying River Networks (DRNs) across six European countries (Croatia, Czechia, France, Finland, Hungary, and Spain) within the EU-funded DRYvER project (869226). These DRNs varied along a latitudinal gradient and in terms of their historical exposure to intermittent flow. We sampled aquatic macroinvertebrate communities and assessed hydrological conditions every two months for one year. We studied taxon-specific biomass using image analysis and estimated secondary productivity from biomass and water temperature data. We found that (1) intermittent branches of river systems were less productive than perennial branches, and (2) DRNs in more southern regions exhibited lower declines in secondary productivity compared to DRNs experiencing drying recently. Crustacea and Diptera were identified as the primary contributors to secondary productivity, but different orders of invertebrates contributed to productivity in different DRNs and within intermittent and perennial sections. We discuss our findings in light of the future of river networks in Europe under expected climate change.

One step closer to Biomonitoring 2.0: a case study reinforcing the perspective of routine use of metabarcoding data in the multimetric assessment of the ecological quality of freshwater ecosystems

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Routine biomonitoring of freshwater ecosystems still follows a conventional methodology based on the primary morphological identification of the five biological quality elements (phytoplankton, macrophytes, phytobenthos, benthic invertebrate fauna and fish). In addition to the time and financial demands or often complicated morphological determination especially in megadiverse benthic invertebrates, this approach can also be problematic due to the methodical processing of the collected bulk samples. For example, the subsampling method causes the final analysis to be based on only a limited sample volume. Consequently, important bioindicator taxa may be overlooked, and the face of true biodiversity may be distorted, leading to an inaccurate ecological status assessment. Therefore, this study aimed to test the applicability of DNA metabarcoding at long-term monitored sites, aiming to streamline the process, remove the aforementioned shortcomings and thus increase the efficiency and scale of freshwater biomonitoring. Samples of benthic invertebrates were collected at 12 sites from different categories of water bodies. Without time-consuming bulk processing and morphological determination, the obtained final taxa lists based on metabarcoding data confirmed a 14% increase in identified species within the five target benthic classes (Insecta, Clitellata, Malacostraca, Bivalvia and Turbellaria) compared to previous conventional (WFD) sampling. Biological indices included in the multimetric evaluation of the ecological status were calculated using the program ASTERICS, and the same or higher ecological quality class was identified compared to previous assessments. More detailed indices analysis showed that the ecological quality class changed more significantly in 5 localities (from 2 to 1), mainly due to the influence of the BMWP and EPT taxa indices, which indicates a higher sensitivity of metabarcoding approach in detecting the present macroinvertebrates species spectrum. The results of this study provide useful data on the effectiveness of DNA metabarcoding and have a great potential to contribute to the implementation of DNA approaches in routine biomonitoring of freshwater ecosystems.

Faster aerobic metabolism in invasive Ponto-Caspian vs. native amphipods

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Ponto-Caspian amphipods are among the most successful aquatic invaders. In the last century, their rapid spread across the Holarctic has been marked by transformations of native macroinvertebrate communities, local species extirpations, and potential food-web changes. Often the success of these species is attributed to enhanced environmental tolerance, reproductive success, aggressiveness, and/or efficient assimilation of food resources. We propose that elevated metabolism may underlie all these notorious traits ascribed to their invasiveness. To that end here we tested the hypothesis that across the freshwaters of the Southeastern Baltic region, the Ponto-Caspian amphipods have a higher aerobic metabolism than the natives. We conducted closed-chamber respirometry across the range of dissolved oxygen (DO; normoxia to anoxia) in 11 gammaroid species after 2–6 weeks of laboratory acclimation to the measurement temperature (~17°C). Six included species were native (*Gammarus jazdzewski*, *G. lacustris*, *G. pulex*, *G. varsoviensis*, *Pallaseopsis quadrispinosa*, and *Synurella ambulans*) and five were of the Ponto-Caspian origin (*Chaetogammarus warpachowskyi*, *Dikerogammarus haemobaphes*, *D. villosus*, *Obesogammarus crassus*, *Pontogammarus robustoides*). To standardise the oxygen consumption (OC), we first established the species allometries in normoxia. Then we analysed the 808 curves of OC vs. DO using single-breakpoint piecewise linear regressions. Using the resulting parameters that describe the curve shape, we classified the respiratory responses to oxyconformation (rising increasingly), oxyregulation (rising decreasingly), or oxystress (rising and then falling OC with increasing DO). While the allometry steepness varied more by species than by the group of origin, the offset was consistently higher in the Ponto-Caspian species, translating into ~60% higher oxygen consumption. Oxyregulation response was the most common in both groups; however, among the newcomers, oxyregulation was less common and not as efficient, while oxyconformation and oxystress emerged more often and more articulately. Confirming the hypothesis, the results support our idea that the invasive Ponto-Caspian gammaroids primarily share elevated metabolism. However, superiority in well-oxygenated environments may come at the cost of decreased competitiveness in impoverished conditions due to unmet oxygen needs. The differentiation in oxygen demand may allow for coexistence between the native and invasive amphipods by habitat partitioning at the meso- or macrohabitat scales, with smaller lakes, closed bays and/or river pools serving as refuges for the aboriginal species. On the other hand, the more even distribution of response shapes within the newcomer species may also indicate adaptation potential even to such environments. This study was financed by the Research Council of Lithuania (Contract No. S-MIP-20-26).

Species level identification of Simuliidae (Insecta, Diptera) from Romania using DNA barcode data

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A total of 275 larvae, pupae, and adults of Simuliidae from different watercourses in Romania were examined to test the utility of the standard DNA barcode region of the mtCOI gene as a species-level identification tool for all life stages. 29 morphospecies from 3 genera were analysed: genus *Prosimulium* Roubaud, 1906 with one subgenus and 4 species, genus *Twinnia* Stone et Jamnback, 1955 with a genus *Simulium* Latreille, 1802 with 4 subgenera and 24 species. The Neighbor-Joining tree, inferred based on the DNA barcode sequences, grouped most specimens according to species or species-groups recognised by morphometric studies. Intraspecific sequence differences within morphospecies ranged from 0.00% to 5.96%. In some cases, however, the larger differences (2.33–5.96%) indicated the presence of important cryptic diversity. In total, we generated 233 DNA barcode sequences during our study, thus obtaining a total of 30 different BINs and 26 species. During the present work, 8 species are recorded for the first time from Romania: *Twinnia hydroides* Novák, 1956, *Simulium petricolum* (Rivosecchi, 1963), *S. beltukovae* (Rubtsov, 1956), *S. cryophilum* (Rubtsov, 1959), *S. craigi* Adler & Currie, 1986, *S. trifasciatum* Curtis, 1839, *S. reptantoides* Carlsson, 1962 and *S. aff. monticola*. The Simuliidae fauna from Romania is now represented by 72 different species. The DNA barcode data combined with morphology are a useful tool for the identification of Simuliidae species in Romania, however, we noticed large gaps in the case of species that are range restricted or regionally distributed, including some endemic species. In the case of the latter category, the lack of molecular data makes species-level identification difficult, which is further complicated by the high cryptic diversity characteristic for the region.

The ecology of benthic invertebrates as a contribution to ecosystem-based management of large rivers

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Large rivers are important because they provide various ecosystem services to humans, but they are still subject to human-induced changes. The multiple transformations of large rivers have resulted in various stressors to the aquatic ecosystem, reflecting both structural changes and a shift in ecosystem functions. Ecosystem-based management is necessary to maintain the diverse ecosystem services. However, ecological information must be integrated to implement meaningful ecosystem-based management. Benthic invertebrates are an important ecosystem component in large rivers and respond to multiple stressors. However, the observed responses depend on the length of the stressor gradient. Ecological studies of large rivers rarely use data collected along the entire stressor gradient from near-natural to highly disturbed conditions. We conducted the study on large rivers in two neighbouring countries, Slovenia, and Croatia. Data on benthic invertebrates and stressors were obtained from five large rivers: Sava, Drava, Mura, Kupa and Una. The selected sampling sites reflected the different levels of disturbance caused by multiple stressors, e.g., hydromorphological alteration, catchment land use and water quality. Twenty-four environmental variables were measured or calculated: five hydromorphological, fourteen water quality and five catchment land cover/land use variables. The biological data were collected as part of the WFD monitoring and assessment system development programs in Slovenia and Croatia. We detected differences in responses along six environmental gradients, suggesting that large river benthic invertebrates can be used to independently assess the effects of multiple stressors (hydromorphological alterations, eutrophication, land use impact due to arable land, and permanent crops). In addition, the effects of hydromorphological alterations dominated water quality and land use change issues, but the latter two also need to be considered in management. Quantifying the specific impacts of hydromorphology, water quality and land use allows water managers to better understand how large rivers have changed and to make predictions for future ecosystem conditions. We argue that an ecological understanding of multiple stressor environments will have a profound impact on management performance, not only by assessing the impacts of stressors separately but also by reducing costs through the establishment of a hierarchy of actions that will prevent the decline in ecological quality of large rivers.

Invertebrate DNA metabarcoding sheds light on the environmental gradients of the Tatra Mountain lakes

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The glacial lakes in the Tatra Mountains (Western Carpathians, Slovakia) and their invertebrate fauna have been attracting scientific attention for over a century. This relatively small area with numerous steep valleys, stretching across vegetation zones over an altitudinal gradient, offers a promising basis to study the impact of changing environmental conditions on species composition and distribution. A typical example documented throughout the last decades is acidification and subsequent recovery of the lakes upon switching to less polluting industrial practices. The ongoing climate change poses yet another challenge to the unique biota inhabiting these pristine alpine ecosystems. Thus, their biodiversity requires further monitoring, which can take advantage of DNA metabarcoding as a means to circumvent the drawbacks of morphological identification and manual sorting of samples. Here, we present outcomes of such study, focusing on Tatra Mountain valleys where lakes are dispersed throughout the altitudinal gradient. Littoral benthos was sampled from 17 lakes by the kicking technique in September 2017, 2019–2022. Organisms were picked out from each sample fixed with ethanol, dried, homogenised in a bead mill, subjected to spin-column DNA extraction, the COI marker amplified using BF3/BR2 PCR primers, and sequenced on the Illumina MiSeq platform. The sequences were processed using the Cutadapt, PEAR, VSEARCH, LULU and BOLDigger algorithms, obtaining taxonomic identification for the resulting OTUs (operational taxonomic units, clustered at the species-level 97% sequence identity). The patterns in community composition were analysed through TaxonTableTools, R packages Vegan and indicpecies, also relying on a rich dataset of the lakes' chemical and physical parameters. Upon revision and abundance filtering, the dataset contained 537 invertebrate OTUs, where 199 aquatic species were identified. Altitude was reflected considerably well in the metabarcoding ordination diagrams, whose first two axes explained 17.5% of variability in the overall distance matrix, or up to 55% of variability per individual valleys. However, several lakes broke the altitudinal order, rather reflecting the importance of overall lake conditions and nutrient content (P, organic C and N). Possible indicator species were highlighted both for the alpine cluster (OTUs identified as *Pseudodiamesa nivosa*, *Micropsectra radialis*, *Cernosvitoviella pusilla*, *Daphnia pulex*) and for sub-alpine lakes (*M. notescens*, *Prodiamesa olivacea*, *Cricotopus* sp., *Agabus bipustulatus*, *Nemoura cinerea*). The approach proved to be remarkably efficient for ecosystem assessment through simultaneous detection of a broad range of taxa including benthic macroinvertebrates, planktonic crustaceans, meiofauna, and numerous terrestrial groups. This study was supported by the project VEGA 2/0084/21.

Drought driven directional changes in presence-absence macroinvertebrates community metrics

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Understanding the influence of environmental stressors on community dynamics is essential for evaluating ecosystem resilience and conserving biodiversity. In this investigation, we focus on aquatic macroinvertebrates as indicators of freshwater ecosystem health in order to assess the effects of drought conditions on presence-absence community metrics. Beta diversity encompasses both community composition and species turnover, reflecting non-directional and directional changes, respectively. Despite various methods for partitioning variation and assessing community-level phenomena, approaches to evaluating species turnover along temporal gradients based on a unified conceptual framework are currently limited. In our study, we explore the possibility of examining different aspects of directional changes along a temporal gradient using presence-absence community data. We define a spectrum of turnover and nestedness concepts by integrating measures of community overlap, species loss, and gain across sampling units while developing functions for their quantification. These concepts function as indicators of ecological phenomena with measures derived from raw species counts as well as relativized forms such as the Jaccard and Sørensen indices. The results highlight that actual communities display directional responses to specific ecological gradients, thus shedding light on previously overlooked aspects within the field of ecology.

By elucidating how presence-absence community metrics respond under drought conditions, our study contributes towards gaining greater insights into how environmental stressors shape freshwater ecosystems, crucial knowledge necessary for formulating conservation strategies aimed at mitigating climate change impacts on aquatic biodiversity management practices.

Bridging boundaries: exploring aquatic-terrestrial contaminant transport via emerging aquatic insects

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Aquatic insects serve as vital inter-habitat linkages between aquatic and terrestrial ecosystems due to their life cycle encompassing both environments. They facilitate the flow of energy and nutrients and play a crucial role in transporting waterborne contaminants to terrestrial environments. Our project focused on investigating the environmental fate of emerging contaminants (ECs), particularly pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs), originating from wastewater effluents and accumulating in aquatic insects. Conducting in situ studies in NW Croatia, we demonstrated the bioaccumulation of multiple PhACs and EDCs in the aquatic stages of Odonata and Trichoptera. Subsequently, we observed their transfer across the aquatic-terrestrial ecosystem border via emerging adults. Comparison of EC concentrations in aquatic and terrestrial life stages indicated bioamplification, or increased body burden of many compounds, particularly in Trichoptera emerging adults. Furthermore, our results suggest that differences in biological traits between taxa, such as type of metamorphosis and feeding behaviour, play a defining role in bioaccumulation and bioamplification patterns of PhACs and EDCs in aquatic insects. Additionally, our results revealed the presence of waterborne PhACs and EDCs in terrestrial invertebrates in the riparian zone (e.g., Aranea, Isopoda, Coleoptera), shedding light on contaminant transfer in aquatic-terrestrial food webs across different trophic levels. Notably, trophic transfer of PhACs and EDCs was observed extending beyond a 3-meter distance from the river, underscoring the necessity for further research into the extent of waterborne contaminants in riparian zones. In summary, our findings contribute significant insights into contaminant transport mechanisms dependent on ecological processes linking aquatic and terrestrial ecosystems.

First genetically confirmed discovery of *Niphargus* (Malacostraca: Niphargidae) in Germany north of the Ice Age glaciation

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Niphargids (Malacostraca: Niphargidae: *Niphargus* sp.) constitute a family of crustaceans, with over 450 species described to date, predominantly inhabiting subterranean environments. As a result, they exhibit cavernicolous adaptations, including depigmentation and eyelessness. These crustaceans are widespread, ranging from Spain to Iran, with approximately 30 species identified in Germany. However, evidence of their occurrence north of the glacial boundary from the last ice age glaciations is scarce. Previous records from the United Kingdom, the Netherlands, and Poland either turned out to be misidentified or lacked sufficient recollection.

This study presents, for the first time, DNA barcoding confirmed records of *Niphargus* from three German springs, specifically rheocrenes, north of the former glaciation, situated approximately 70 km north of the glacier edge associated with the Elster and Saale glaciations. These springs are approximately 60 kilometres apart from each other.

All three springs harbour *Niphargus aquilex*, a species widely distributed from England to northern France, Belgium, Luxembourg, and Germany, albeit not commonly found. The Folmer fragment of the Cytochrome c Oxidase Subunit 1 (COI) gene was sequenced for 19 individuals, revealing two haplotypes. The more common haplotype is also present in the southern distribution area of the species, providing evidence of post-glacial migration to the north. Contrary to initial assumptions, the species did not survive the ice age in nunataks or under the ice sheet.

The new findings, however, prompt several questions. Although 18 of the 19 sequences are identical and match sequences found in the south, one sequence is missing in the south. Could this indicate a population that survived under the ice? Alternatively, is it a recent mutation involving at least two base pairs within the presumed 100,000 years? The *N. aquilex* group is a species complex comprising 10 to 20 species, the majority of which are cryptic, while the rest are pseudocryptic. Why, then, has only one species from this complex migrated northward? Unlike other species (e.g., *Niphargus puteanus*, *Niphargus fontanus*), which have migrated northwards parallel to the rivers, *N. aquilex*'s colonisation of the north poses unique questions about its adaptive mechanisms.

Revised overview of the subterranean aquatic fauna of Luxembourg

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The Grand Duchy of Luxembourg is located in the transition area between Western and Central Europe. A team has been established at the National Museum of Natural History Luxembourg that focuses on recording subterranean life. The project “Scientific findings, basic data collection and monitoring of subterranean habitats in the Grand Duchy of Luxembourg” focuses on recording all plant, fungal and animal species living in underground environments. Luxembourg is rich in natural caves but also artificial cavities, e.g., mines for iron ore, copper, antimony, slate, gypsum, dolomite. Also, subterranean military installations such as the casemates of the City of Luxembourg comprise prominent objects. However, running or standing waters in underground environments being directly human-accessible are scarce. Therefore, groundwater measuring points and shaft wells are sampled, in addition to springs as outflows of groundwater. Interesting results were also obtained from the investigation of interstitials close to riverine shorelines.

Specimens were collected mainly by sieving with four sieves with mesh sizes of 5,000 µm, 1,000 µm, 600 µm and 200 µm. Meat-baited tin cans were placed in mines. Numerous experts carry out the morphological identification of various animal groups. Questionable results and species thought to be new to science are genetically analysed in the museum's own laboratories.

An overview of the proven classes and orders is given. As can be expected, the focus of subterranean aquatic fauna is on crustaceans. Ostracods (12 species) and copepods (27 species) are frequently found. The malacostracans are represented by the orders of isopods (two species) and amphipods, the latter by the families Crangonyctidae (one species), Pseudoniphargidae (one species), and Niphargidae (eight species). Two *Niphargus* species are new to science. They will be described in 2024 and one even bears the name of the Grand Duchy.

The faunistic outcomes from caves are used for the Habitats Directive assessment of the Habitat type 8310. In addition, artificial cavities with an unusual species inventory are protected, such as the slate mine Laura in Haut-Martelange in which *Niphargus boulangei* was found has already been categorised as being of high conservation value. So far, only one locality of this species (northern France), which has probably been destroyed in the interim, has been published. In the meanwhile, there are sufficient records to analyse them biogeographically, then in connection with neighbouring regions. Furthermore, previously neglected biotope groups, such as castle wells or groundwater measuring points, are to be intensively investigated. We also hope that new collection techniques such as downpipes, net collectors and wood washing will yield previously unknown results.

Preliminary results on macroinvertebrate communities in freshwater outflows of a cliff coastal zone in the Baltic Sea

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Macroinvertebrates are capable of surviving in different unstable habitats that might be temporal, undergo severe disturbances or suffer from human pressure. We investigated freshwater water outflows from the cliff shore of the Baltic Sea in the Polish Pomerania. These specific spring types suffer mainly from flooding by sea water during the storms, accompanied by cliff erosion, but also constant human impact, such as littering or trampling. Some of them have low volume of water discharge and may disappear during severe droughts; others are more stable, with higher discharge. The main objectives of the study were to describe the macroinvertebrate taxa composition in these habitats, to estimate their abundance, and to decipher the environmental factors shaping macroinvertebrate communities. Sampling took place twice, in late spring and early autumn of 2023. Three samples of macroinvertebrates were collected from each of the 14 investigated water outflows using a small net (modification of 'kick-sampling' method) and equivalent sampling effort across sites (ca. 500 cm² area covered per sample). Larger specimens were sorted in the field using a tray, and a 50 ml volume of substrate was taken from each tray to search for smaller or less conspicuous macroinvertebrates in the laboratory. We measured water temperature, oxygen saturation, water hardness, pH, conductivity, N and P concentration (total, organic, and mineral forms), chlorophyll-a concentration, organic matter content, and substrate granulometric variables. The springs represented very different types – some of them were mere water outlets straight onto the beach with no vegetation; others were situated higher on the cliff and accompanied by thick vegetation. There was also one spring within the forest overgrowing the cliff verge.

We observed high variability in some water chemistry variables at the study sites, particularly nutrients, and a generally low oxygen concentration. We also found a remarkable diversity of macroinvertebrates in the investigated water outflows, with dominance of dipteran larvae of Ceratopogonidae, Chironomidae, Limoniidae, Muscidae, Psychodidae, Ptychopteridae, and Tipulidae. Other macroinvertebrate groups, such as Coleoptera, Hydrachnidia, Hemiptera, Isopoda and Trichoptera were represented by singular or a few taxa. The abundance of different groups was extremely variable, depending mostly on substrate types or plant presence, rather than water chemistry variables. Our results also suggest considerable resistance of these assemblages to sea water flooding. Overall, our study contributes to the understanding of ecological communities of freshwater outflows in coastal areas. These communities might serve as assembly models in isolated environments prone to disturbance, where resistance strategies are of utmost relevance for the ecological integrity of these ecosystems.

Can macrophytes affect the structure of Chironomidae larvae also in nearby unvegetated zones?

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It is well known that in zones covered with macrophytes, the diversity and abundance of Chironomidae larvae is generally greater than in unvegetated zones. The results of the first author's research indicate that at a short distance from macrophytes, the diversity and abundance of Chironomidae larvae are still relatively high (despite the lack of plants) and gradually decrease as we move away from the macrophytes. This suggests that macrophytes affect the Chironomidae larvae structure also in nearby unvegetated areas. It was assumed that this factor may be the presence of coarse detritus (particles with a diameter $>500\text{ }\mu\text{m}$) in bottom sediments, originating from the decomposition of macrophytes. The research was carried out in two different types of water bodies, a lake, and a lowland dam reservoir. Two sampling sites were located in each of them, both in places without macrophytes. One of them was located approx. 20 m from the macrophytes, so-called site D "detritus" (more coarse detritus in the sediments) and the second one approx. 80 m away, site WD "without detritus" (less coarse detritus in the sediments). The depth, oxygenation of the over bottom layer of water and the sediment type at both sites were very similar. Several series of samples were taken during one year. It was found that the number of taxa and diversity (Shannon index) of Chironomidae larvae were higher at site D, both in the lake and in the dam reservoirs. However, their density in the lake was also higher on site D, but in the dam reservoirs on site WD. The dominance structure of Chironomidae larvae also differed between sites D and WD. The obtained results confirm our hypothesis that the presence of coarse detritus in bottom sediments may be an important factor shaping the Chironomidae larvae structure. The presentation will present potential mechanisms of the impact of coarse detritus on Chironomidae larvae.

Abstracts

Poster presentations

Three new *Gammarus* species of the *G. balcanicus* complex from Romania defined via integrative methods

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Gammarus is a very diverse genus of amphipod crustaceans found throughout freshwater habitats in Europe. It comprises ca. 280 species and most of its diversity worldwide was described relying on morphological studies that have been ongoing for many decades. However, recent advances in molecular techniques have revealed a surprising amount of cryptic diversity within this group. In our study, we use an integrative approach to describe three new species from Romania, which represent divergent lineages within the *Gammarus balcanicus* complex. The examined material for these three lineages was collected at three distinct locations, Varciorog, Piatra Craiului Mountains and Dobrogea plateau, and is deposited at the Department of Invertebrate Zoology and Hydrobiology of the University of Lodz. For molecular species delineation and phylogeographic analyses, we used mitochondrial (COI, 16S) and nuclear (28S, EF1a, H3) markers. For the morphological study, appendages of selected individuals were dissected, mounted in glycerine gel slides, photographed, and digitally drawn using CorelDRAW 2018. Our results show that the three new species are divergent both in the case of mitochondrial and nuclear markers. These lineages originated probably in the Southern Carpathians, already in the Miocene. While two lineages are limited to singular locations, the third one colonised the whole Dobrogea plateau and went through a period of demographic expansion at ca. 100 kya. Morphological analysis shows that the three new species can be distinguished from the neotype of *G. balcanicus* and other closely related species by a set of characters encompassing: the amount of posteromarginal setal groups and the length of the gland cone on the antenna 2; presence/absence of calceoli on antenna 2; setal placement on maxilla 1; shape and armature on the palms of gnathopods 1-2; setal arrangement on pereopods 3-4; the shape of the posteroventral corner of epimeron 3; dorsal armature on pleonites and urosomites 1-3; and armature on telson.

Influence of wastewater-treatment effluent on water quality and benthic invertebrates during drought conditions

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The release of treated effluents from wastewater-treatment plants (WWTPs) affects not only chemical composition of water but also the benthic invertebrate community. We examined the impact of WWTPs and other smaller effluent on water quality and benthic invertebrate community composition. For 26 selected effluents, there were available benthic invertebrate data from monitoring sites upstream and downstream the WWTPs. Concentrations of selected water quality parameters were studied on 18 pairs of monitoring sites. The distance of the monitoring sites from the effluent was usually up to 5 km. An important selection criterion was the absence of a significant tributary with possible other pollution sources between the monitoring sites. Benthic invertebrate sampling was carried out in the period from 2007 to 2019; chemical data were available from 2013 to 2022 and included both dry and wet years. WWTPs discharge into the recipients accounted for 1 to 88% of the recipient flow during drought conditions (flow Q355d was considered as the limit of hydrological drought).

To illustrate the water quality situation upstream and downstream the WWTPs, the total phosphorus parameter was chosen as the average of the values measured in the summer months. The results showed a worse situation in the summer months and in dry years, which means a very possible acceleration of the deterioration in relation to the increasing trend of air temperatures. Only data from operational monitoring was used, rapid rainfall-runoff events were likely not captured which means that the situation may actually be even worse. Implemented measures are not sufficient even if effective measures exist. A change in legislation, which would bring the limits specified in Government Regulation No. 401/2015 into line with the objectives of good status of water bodies, is absolutely necessary as well as stricter limits for best available technologies in wastewater disposal. In the case of phosphorus, only respecting the goals of good ecological status will lead to an effective improvement of the eutrophication in our rivers and reservoirs.

Benthic invertebrates were studied in terms of community composition and representation of functional groups in monitoring sites upstream and downstream the WWTPs. When comparing monitoring sites where the share of discharged wastewater was higher than 5%, the ecological status of benthic invertebrates evaluated according to the WFD was strongly affected, especially in terms of the representation of sensitive species of taxa. The change in the representation of food strategies of benthic invertebrates was also significant.

This research has been supported by the PERUN Competence Centre (TAČR project SS02030040 Prediction, Evaluation and Research for Understanding National sensitivity and impacts of drought and climate change for Czechia). Water quality and benthic invertebrate data comes from the operational monitoring of the state enterprises Povodí.

Can Chironomidae assemblages indicate water scarcity in temperate streams?

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The impacts of climate change are increasingly evident worldwide, including Hungary, where observable shifts in both the average precipitation and their distribution have occurred in recent decades. Consequently, numerous once-permanent small streams have transitioned to intermittent flow patterns, necessitating adaptation among resident organisms. Among these, the Chironomidae family (non-biting midges) stands out as a prevalent group found across various aquatic and wetland habitats, owing to their abundance and short life cycle, rendering them suitable for assessing the ecological status of the watercourses. Our aim was to determine whether Chironomidae assemblages can indicate the difference between permanent and intermittent streams in temperate zones. Quantitative sampling was conducted seasonally over a one-year period in the Bükkösd-víz catchment area in Hungary. Stream sections were categorised into five types based on their flow history: 1) permanent and stable; 2) vulnerable (permanent but with recognised low flow); 3) dried more than five years before sampling; 4) dried more than one year but less than five years before sampling; and 5) dried within the year preceding sampling. We evaluated both the structural (i.e., taxonomic) and functional (i.e., traits associated with drying) composition of chironomid assemblages. Multivariate analyses revealed significant differences in assemblages between permanent streams and other types. Taxonomic composition displayed a gradient from streams that dried up within a year prior to sampling to vulnerable ones. Conversely, functional composition showed significant differentiation between vulnerable streams and those that experienced drying within the year preceding sampling. Our findings demonstrate that drying events appearing in a previously permanent river network significantly alter the taxonomic and functional composition of chironomid assemblages. Furthermore, taxonomic composition may provide insights into the timing of preceding drying events.

The Chironomidae stratigraphy in the Holocene sediments of Lake Kuutsjärvi (Northern Finland) with comparison to the Late Glacial midge communities in central Europe

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The Chironomidae communities in the Arctic predominantly developed during the Holocene, later than in central Europe where the midge fauna was established during the Late Weichselian. The mean July air temperature, a key factor influencing midge development, maintained a similar level during the Holocene in northern Scandinavia as it did during the Late Glacial in central Europe. The comparison between the Late Glacial midge fauna in central Europe and the Interglacial midge fauna in Scandinavia is thus justified. This study presents the Holocene stratigraphy of Chironomidae communities at Lake Kuutsjärvi in northeastern Finland. The cold climatic conditions and oligo/mesotrophic status of the lake contribute to the low abundance of chironomids. Additionally, the specific bathymetry, with a broad deep-water zone and a very narrow littoral zone, exacerbates the impact of climate. Nevertheless, as many as 91 Chironomidae morphotypes were identified in the Holocene lake sequence. The dominant taxa are adapted to low temperatures, thriving in the profundal zone during Holocene Thermal Maximum. Some species, which are typically restricted to flowing waters in central Europe, appear in stagnant waters in the Arctic due to the better oxygen saturation in oligotrophic, cold waters. The seepages, small brooks, and moss blankets along the lake shore host taxa that in temperate latitudes are typical of mountain springs. While it might be expected that North Scandinavian waters would resemble lakes in the periglacial landscape of central Europe during the ice age, midge assemblages in Lithuania, Poland, and the Carpathians during the MIS2 reveal a different taxonomic composition. In middle Europe there is a much higher domination of common, warm-adapted taxa and a lack of very cold stenotherms like *Paraphaenocladus pseudirritus*, *Abiskomyia*, and *Parochlus*.

Changes in diversity metrics of aquatic macroinvertebrate assemblages along the intermittency gradient

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Drying up has a major role in shaping communities and significantly influences the composition of the aquatic macroinvertebrate community and their diversity metrics (e.g., taxa number, number of individuals, Shannon diversity). Furthermore, the macroinvertebrate taxa groups (e.g., Odonata, Heteroptera, Coleoptera, etc.) may have different functional trait-complex, which determine how they respond to stress at individual level. However, the trait-complex appears coupled in the individual, so it is assumed that phylogenetically and taxonomically closely related species (that belong to the same taxa group) have more similar trait-complexes than more distantly related species. Based on this assumption, different taxa groups within aquatic macroinvertebrate communities may respond differently to changes in the intermittency of water flow. In our research, we performed a stressor-impact study along an intermittency gradient in the context of the intermediate disturbance hypothesis (IDH) framework, which posits that moderate level of disturbances can have beneficial effects on ecosystem structure and function. Sampling was carried out bimonthly at a total of 15 sampling sites in four intermittent streams from May 2021 to March 2022 in the Hungarian lowland region. Hydrological condition was measured by in-stream data loggers, and intermittency gradient was characterised by the number of drying days during the sampling period. Our results suggest that the moderate intensity of drought is beneficial for less competitive communities, but detrimental for communities with high dispersal ability. Revealing changes in diversity metrics and determining the unique response by taxa groups of aquatic macroinvertebrate communities along the intermittency gradient are crucial in index development, bioassessment and water management.

Analysis of genetic diversity and geographic distribution of the freshwater Amphipoda in the Lake Skadar basin

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Lake Skadar basin, located on the border between Montenegro and Albania, is one of the key hotspots of biodiversity and endemism on a European scale. The area is characterised by high habitat heterogeneity and temporary isolation limiting species migration. It covers an area of diverse geomorphological, tectonic, and hydrogeological structures and is an important point in the context of analysing the evolutionary history of the Balkan Peninsula's fauna. It includes an extensive and ancient system of karst springs and a relatively young lake. However, the area is at risk of biodiversity loss due to climate change and human interference associated, for example, with increasing tourism development. The aim of our study was to assess the genetic diversity and geographic distribution patterns of freshwater gammarid amphipods in the Lake Skadar basin, Balkan Peninsula. Based on morphological observations, four morphospecies were identified: *Gammarus balcanicus* Schäferna, 1922, *G. roeselii* Gervais, 1835, *Pectenogammarus thoni* (Schäferna, 1923) and *Pectenogammarus scutarensis* (Schäferna, 1923). The morphological identification was confronted with molecular data. The study was based on DNA barcoding with third-generation sequencing based on Oxford Nanopore Technologies (ONT). The obtained sequences were used to create a reference DNA barcode library in The Barcode of Life Data System (BOLD). Currently, 326 sequences have been obtained from the 32 sites studied. The analyses conducted indicate that the cryptic diversity within the conventionally described species of amphipods is high. Currently, 11 BINs (Barcode Index Number) have been assigned to the deposited sequences. Seven of the assigned BINs are unique and new for BOLD. However, preliminary species delimitation using the ASAP method indicated that 10 MOTUs (molecular operational taxonomic units) could be distinguished among the sequences obtained. Sequence analysis of *G. balcanicus* also indicates high genetic variability of the indicated species relative to the data present in the BOLD database. Among the analysed individuals of *P. scutarensis*, we also observed introgression of mtDNA from *P. thoni*, confirming previous studies (Jabłońska *et al.* 2019). Our results underscore the need to intensify research on the Balkan Peninsula to assess the diversity of local hydrobionts.

Effects of urbanisation on the diversity and assemblage structure of macroinvertebrates in low-order streams of Balaton Uplands, Hungary

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Urbanisation is one of the most serious threats to stream ecosystems worldwide. Our aim was to investigate the general effects of urbanisation in a moderately urbanised landscape and to assess the relationship between local environmental variables and biotic attributes of macroinvertebrate assemblages. We found that local habitat properties presented degraded conditions at urban sites. Urbanisation had a negative effect on the richness and Shannon diversity of macroinvertebrate assemblages and altered assemblage composition. Individual streams showed considerable variability under natural conditions, as well as in their response to urban effects. Subsequently, the mitigation of the effects of urbanisation might also need considerable variability in the type of actions required.

Benthic macroinvertebrates in the upper and middle section of the Oder River after the ecological disaster in 2022

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Benthic invertebrates belong to the biological elements utilised in the assessment of the ecological status/ecological potential of waters in accordance with the European Water Framework Directive (WFD) due to their response to various stressors, including alteration in hydro-morphological conditions, physicochemical parameters of the water, including oxygen, trophic conditions, and salinity. They are widely acknowledged as excellent indicators for biological assessment. Therefore, the aim of the research initiated in the autumn of 2022 was to assess the diversity of bottom fauna communities in the Oder River after the ecological disaster. The research was carried out at 5 sites located between 430 km and 670 km of the river course. Additionally, sampling was also done in the Gliwice Canal, which is connected to the upper Oder, and in Kłodnica River (a right-side tributary in the upper course of the Oder River).

The results of the research indicate high variability of the bottom fauna. In the Oder River, Oligochaeta, Chironomidae and *Potamopyrgus antipodarum* constituted the constant elements of the macrobenthos at most sites. In the Gliwice Canal, also Coenagrionidae and *Physa acuta* were constantly present. However, in Kłodnica, Amphipoda (*Apocorophium lacustre*, *Gammarus tigrinus*, *Dikerogammarus villosus*) was also a permanent element of the zoobenthos. During the research period, the percentage of particular taxa in the structure of benthic assemblages changed at all sections, both in the Oder River, the Gliwice Canal, and Kłodnica. At most sites, a high proportion of alien species, including invasive species, was found. In total, 9 alien invertebrate species were indicated – 2 species of polychaetes (*Hypania invalida*, *Laonome xeprovala*), 3 species of amphipod crustaceans (*Chelicorophium curvispinum*, *Gammarus tigrinus*, *Dikerogammarus villosus*), 2 species of snails (*Potamopyrgus antipodarum*, *Physa acuta*) and 2 species of bivalves (*Dreissena polymorpha*, *Corbicula* sp.). The research undertaken in 2022 is being continued, which will allow for a more complete analysis of the bottom fauna alteration over a long period of time after the ecological disaster on the Oder.

The thick-shelled river mussel *Unio crassus* in the Netherlands: a feasibility study for population recovery of an iconic species

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The freshwater mussel *Unio crassus* (thick-shelled river mussel) has not been found in the Netherlands for decades, due to its sensitivity to poor water quality (pollution, low oxygen, salinisation, and high nutrient concentrations). The mussel is an ‘ecosystem engineer’ as it alters its environment by filtering water, fixating sediment, and providing hard substrate for other organisms. The mussel extinction has therefore presumably been a drawback for other riverine species in the Netherlands as well. In recent years the abiotic conditions in Dutch rivers have significantly improved, offering opportunity for recovery of the mussel. The goal of this study was to find out if habitat conditions are suitable enough for population recovery of *U. crassus* in the Netherlands. The hypothesis was that the physical and chemical water quality is sufficient, but that other habitat requirements and lack of a source population pose a bottleneck for recovery.

A feasibility study was carried out, based on literature research and available data on fish populations (host for mussel larvae). The conditions in Dutch rivers seem to fit for population recovery of *U. crassus*: chemical water quality (oxygen, ammonium, pH, nitrite, nitrate and, in most waters, phosphate) is sufficient, the physical habitat (substrate and flow velocity) is mostly in order and there are 21 fish species that can function as a host for the larvae of *U. crassus*, six of which are considered good hosts (carrying high densities of mussel larvae in their gills). Based on the analysis of the chemical, physical and biological (host) conditions of Dutch rivers, a map was created, indicating promising locations for population recovery. Permanently flowing floodplain channels that are two-sided connected to the main river, containing high densities of hosts, seem the most suitable. Because the mussel is extinct in the Netherlands, natural population recovery will have to be catalysed by colonisation from upstream waters. However, the species is rare in the entire catchment area, making it unlikely the species will reach the Netherlands on its own. In the footsteps of neighbouring countries, a reintroduction program might be considered, including detailed habitat suitability measurements on the intended locations.

New fossils from Romania illuminate the evolution and paleobiogeography of Ponto-Caspian gammaroid amphipods

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The Ponto-Caspian radiation of gammaroid amphipods is one of the world's most diverse lacustrine crustacean radiations as well as the only lacustrine amphipod radiation with a fossil record (dating back 9 Ma). Up to 40% of these species have now spread outside their native range due to human intervention. However, our evolutionary understanding of this diverse clade is fragmentary and incomplete because the relationship between fossils and extant species remains unknown. Here, we present the first morphology-based phylogeny of Ponto-Caspian gammaroids that includes 99% of extant taxa, all previously known fossils, as well as newly discovered fossil specimens from the Late Miocene (10.5 Ma) of eastern Romania. We find that all fossil species belong to a single lineage that is an evolutionary dead-end, having no living descendants despite being widespread throughout the Paratethys. This clade has an ambiguous phylogenetic relationship with extant taxa, though it is nested within the radiation's crown group. Our analyses also reveal that the new Romanian fossils belong to a new genus with two new species. Overall, our study reveals that the complexities of Ponto-Caspian amphipod evolution cannot be fully understood only by looking at living species, highlighting the importance of including fossils in evolutionary studies.

Changes of macrozoobenthos community structure in Slaná river after the mine-water discharge

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One of the primary applications of macrozoobenthos research in freshwater habitats is water quality assessment. Macroinvertebrate community changes its structure as a response to changes in the environment, including negative anthropological influence, e.g., pollution and hydromorphological degradation. In the middle reaches of Slaná river, in February 2022 there was a huge (lasting) discharge of mine-waters, containing high amounts of iron, manganese, and sulphates, what was evident as long stretches of river coloured orange-red, and iron crust on stones and aquatic animals. To identify the extent of this negative impact on aquatic communities, macrozoobenthos was sampled in the autumn that year, and again in spring and autumn 2023. Samples were collected using benthos hand net (500 µm mash) according to AQEM on four sites, in the length of cca 20 rkm. Gočovo site (GČ) was located above the point source of mine-water, Henckovce site (HEN) just below, representing most impacted site, and further below were Rožňava-Nadabula (RVN) and Rožňava-Brzotín (RVP). Twelve invertebrate taxa groups were found in the monitored section, of which 5 belonged to permanent fauna, and 7 orders of Insecta represented temporary fauna, including in total 181 taxa. Diversity did not substantially differ among sites/years, but total abundance did. Highest total abundance in 2022 was recorded at GČ, followed by RVP with more than 3,000 ind.1.25 m⁻², and at the directly impacted HEN site only 1,018 ind.1.25 m⁻² were recorded. However, with time, the total abundance improved and in autumn 2023 1,884 ind.1.25 m⁻² were registered. Temporal changes in the community composition at HEN were evident as a very high abundance of *Hydropsyche* (Trichoptera) and Chironomidae representatives in 2022, decreasing in 2023. More importantly, there was an increase of more sensitive taxa – Plecoptera in general, particularly representatives of the *Leuctra* genus. Furthermore, in 2023 there was an increase in numbers of *Gammarus* individuals, sensitive to trace metal pollution, and appearance of environmentally more sensitive *Habroleptoides confusa* and *Rhithrogena semicolorata* (Ephemeroptera), and *Silo piceus* (Trichoptera). SIMPER analysis revealed that the difference between reference site GČ and most impacted site HEN was decreasing each succeeding sampling date, from 54.87 to 39.13%. Some differences in the community structure were undoubtedly the result of seasonal dynamics within macrozoobenthos that occur regularly and were observed at each site. Nevertheless, changes in the dominance between taxa more or less tolerant to degradation at the site closest to the mine outlet, indicates important influence of the polluted water on the invertebrate community, but also the ability of macrozoobenthos to recover over time. Further monitoring is vital for the protecting actions to be proposed and carried out, if necessary, to preserve this river from further degradation, but also to survey the applied measures.

Towards exhaustive DNA barcode reference library of life in Tatra lakes – massive MinION barcoding of gems in our mountains

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Lakes and ponds in the Tatra Mts, the highest part of the Carpathian Arch (Central Europe, Slovak-Polish border), were formed as remnants of the last glaciation. These unique but highly threatened aquatic biotopes contribute significantly to the preservation of ecosystem services in this region, thanks in part to the rich local biodiversity they contain. In recent years, innovative approaches such as DNA barcoding have been added to the traditional research methods that have been used to study lake biota for decades, allowing us to learn much more about their biodiversity.

Over the last 15 years, more than 200 aquatic habitats in the Tatras - of different types, at different altitudes (up to 2,200 m a.s.l.) and often remote - have been repeatedly sampled to study the genetic diversity of benthic macroinvertebrates and to create a high-quality and detailed reference library of their DNA barcodes. All groups of benthic macroinvertebrates from these glacial lakes were sequenced, especially the dominant oligochaetes and all orders of insects. In total, we obtained sequences of a standardised COI barcode fragment from almost 5,200 individuals, which are now stored in the BOLD database under the SKALP project. The first part of them was obtained by classical Sanger sequencing, but a significant part of the data (almost 4,000 barcodes, mainly from the most abundant groups Oligochaeta and Chironomidae) was produced thanks to third-generation sequencing. Massive DNA barcoding using MinION sequencer (Oxford Nanopore Technologies) has rapidly accelerated and enhanced our efforts to build the most comprehensive picture of aquatic life in these mountain lakes. The DNA barcodes represent more than 400 BINs, of which nearly 60 are unique to BOLD, highlighting the uniqueness of this area, which requires proper protection, as well as our still insufficient knowledge of its diversity. The dominant groups in terms of genetic diversity are oligochaetes (with 26 unique BINs), beetles, and dipterans, especially chironomids (19 unique BINs).

The newly created reference library of DNA barcodes is not only an exhaustive list of taxa living in Tatra lakes and ponds, but also an essential basis for correct analysis and interpretation of metabarcoding data from environmental DNA (eDNA) research, which is currently being carried out in the Tatra lakes and has a great potential for proper monitoring of their status. This study was supported by the project VEGA 2/0084/21.

Hydromorphological degradation modifies long-term macroinvertebrate responses to multiple stressors in Czech lowland rivers

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European lowland rivers have been exposed to extensive anthropogenic stress for almost a century. Despite substantial improvement in water quality, novel stressors, mainly climate change, continuously endanger them. Their long-term development has often been studied, yet the impact of hydromorphological degradation on these changes was rarely considered, even though its effect on riverine biota is known to be substantial. To elucidate how such a degradation modifies macroinvertebrate response in multiple-stressor environment, we evaluated water quality, hydroclimatic and biological changes in nine Czech lowland rivers over some 20 years, regarding their hydromorphological degradation degree. We divided our sites into highly degraded and less degraded sites and found that despite comparable development in all studied environmental variables, the two site groups exhibit distinct biotic changes. Highly degraded sites experienced decreased evenness, increased proportion of non-native taxa and functional reorganisation, changes congruent with continuous deterioration. In contrast, less degraded sites displayed several favourable changes, such as increased total and potamal indicator richness, as well as assemblage stabilisation with few functional changes. Increased abundance and upsurge in non-native taxa abundance and richness was found in both site groups. Despite these changes, no significant trends in well-established biotic indices were found. Our study shows that hydromorphological degradation modifies macroinvertebrate response to changing environmental conditions, such as reduced pollution, but also rising thermal and hydrological consequences of climate change, that are expected to amplify in the near future.

Biological control of mosquitoes as a tool for wetland protection?

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Wetlands in lowland river floodplains in Central Europe have been significantly reduced and degraded. This process did not avoid even the floodplains of the Morava and the Danube Rivers. Remnants of wetlands with an area of more than 5,000 ha in the Slovak part of the Morava floodplain are protected as a Ramsar site and the NATURA 2000 area. Some of them are located directly on the territory of the capital of Slovakia Bratislava, many other protected wetlands are located in the Danube floodplain. These wetlands are not only the home of rare species and a space for recreation, but also provide breeding sites for mosquitoes, which from spring to autumn reduce the quality of life of hundreds of thousands of inhabitants of the adjacent territories. Flooding during spring and summer creates conditions for the development of an extreme number of individuals of mosquitoes of the genus *Aedes*. In addition to reduced comfort for residents, they also pose health risks, as mosquitoes are vectors of various diseases. In addition, these species are very mobile, which is why they have an impact on a wide area. The public is therefore demanding a solution to the "mosquito problem", while rightly considering the protection of wetlands as one of the reasons why the mosquito calamity cannot be reduced. Is it possible to somehow combine the protection of wetlands with the reduction of mosquito plagues? We looked for an answer to this question in the recently completed Mosquito Bioregulation project, which we implemented together with Austrian partners within the Interreg SK-AT program, in the Slovakian-Austrian border area in the Moravian floodplain and in the Danube floodplain in the territory of Bratislava. In the poster, we will present the procedures for the biological control of mosquitoes, the results of mosquito monitoring in the project area, the results of the screening of mosquito-borne pathogens, the pyrethroid resistance among mosquitoes obtained during project implementation.

Simuliidae in Serbian small and medium size rivers

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Simuliidae (black flies) are very efficient and opportunistic colonisers of running waters, where they can be quite numerous and diverse. The study was conducted to increase the knowledge about the Simuliidae fauna in the small and medium size rivers of Serbia. These types of rivers in Serbia are very heterogeneous. They belong to 4 different hydroecoregions (ER5-Dinaric Western Balkans, ER7-Eastern Balkans, ER10-Carpathians, and ER11-Hungarian Plain), flows into three different drainage basins (Black Sea, Adriatic, and Aegean Basin) and are located in high altitude range. All of these provide different habitats that harbour a great diversity of black flies. In the period from 2013 to 2021, larvae and pupae of Simuliidae were collected from 118 locations throughout Serbia, covering all hydroecoregions, drainage basins and altitudes from 75 to 1,437 m. A total of 36 black fly taxa were identified. *Simulium ornatum* was proved to be the dominant and most abundant species, with 3,033 individuals detected at 57 sites. Other common species in small and medium size rivers in Serbia were *S. reptantoides*, *S. variegatum*, *S. equinum*, *S. argyreatum*, *S. reptans* and *S. balcanicum*. Species *Prosimulium tomosvaryi*, *S. ornatum* and *S. reptans* were present in all hydroecoregions of Serbia. Most species were found in high altitude range and could be considered euryvalent. The species *Prosimulium hirtipes* was common in wadable rivers and streams, while *S. brevidens* was found only at high altitudes. The highest Simuliidae diversity was found at altitudes from 500 to 800 m. All black flies communities were characterised by high beta diversity, which was reflected in high values of species turnover and low values of nestedness.

Macroinvertebrate communities in urban rivers and canals in the city of Belgrade

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With the fast development of urban areas, wastewater discharges are increasing and have a major impact on urban rivers and canals. These urban waterbodies become polluted, and the health of the ecosystem is seriously threatened. Macroinvertebrates are one of the biological quality elements used to assess the ecological status and health of rivers. The aim of this study was to investigate the macroinvertebrate communities and the present functional groups of the 16 rivers and canals in the Belgrade urban area: Danube, Sava, Kolubara, Beljanica, Pešten, Turija, Barajevska and Topčiderska rivers, Galovica, Progarska jarčina, Sibnica, Kalovita, Vizelj, Karaš, PKB, and Obrenovački canals. The samples were collected during high water levels using a multihabitat sampling approach in 2023. The ASTERIX software was used to calculate the ecological functional groups. All together 126 macroinvertebrate taxa were identified. The most abundant taxa were Insecta, Oligochaeta and Crustacea. The highest number of taxa (31) was found in the Beljanica canal while the lowest (5) was found in the Sibnica canal. All sites were characterised by the dominance of alpha- and beta-mesosaprobic organisms (tolerant of high and moderate organic matter loading), except for Turija where polysaprobic organisms dominated (species adapted to high organic load), which was also confirmed by the highest value of Zelinka & Marvan's saprobic index (3.42). In terms of zonation preferences, most of the species recorded were typical to potamal (lower stretches of a river). When analysing the communities according to flow preference, rheophilic species dominated in the streams, preferring zones with moderate to strong flow. The medium and large rivers were dominated by rheo-limnophilic taxa that prefer slow-flowing streams and lentic zones, while the canals were dominated by indifferent taxa that have no preference for a particular flow velocity.

Exploring cryptic diversity in *Niphargus* (Malacostraca, Amphipoda) in Western Carpathian spring habitats: a combined morphological and genetic approach

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Subterranean freshwater ecosystems remain poorly studied, despite their significance. They harbour a diverse array of invertebrates, occupying various groundwater habitats. Notably, crustaceans constitute a significant component of these ecosystems. In addition to groundwater, they may emerge in surface waters, such as springs, during episodes of heightened groundwater levels following heavy rainfall events.

Our study focused on spring habitats within the Western Carpathians of Poland and Slovakia during the years 2022–2023. We sampled 118 individuals across 23 sites spanning four Carpathian Mountain ranges: 1. the Orava Beskid and the Żywiec Beskid; 2. Bukovec Mountains and Bieszczady; 3. Veporskie Mountains; 4. The Island Beskids. These sites encompassed springs in various environments, including fields, meadows, forests, and roadsides, representing both natural and human-transformed landscapes. The focus of this investigation was the genus *Niphargus* (Malacostraca, Amphipoda), stygobiontic crustacean characterised by lack of pigmentation and absence of eyes. This taxon comprises over 400 species, with *Niphargus tatrensis* being one of the most typical representatives of subterranean amphipods in the Western Carpathians. Described solely based on morphology, its range has been estimated from southern Poland to Austria and Slovenia. Initial morphological observation suggested that all specimens collected in the field belong to *N. tatrensis*. Considering the potentially limited dispersal capabilities, our objective was to explore cryptic and pseudocryptic diversity among the collected specimens.

Genetic analyses were conducted using the mitochondrial COI gene. A total of 118 barcodes were obtained, revealing 25 haplotypes. These sequences were deposited in the BOLD database (Barcode of Life Data System), where they were assigned to eight BINs (Barcode Index Numbers), roughly equivalent to species. Additionally, application of the ASAP (Automated Sequence Analysis Pipeline) method identified five MOTUs (Molecular Operational Taxonomic Units). Finally, we confronted the observed molecular diversity patterns and morphological traits. Among species identified through genetic analysis based on BINs, we measured several morphological traits to identify characteristics of a particular BIN.

Unravelling the impact of drying events and land use on the distribution of Balkan Goldenring in a Pannonian river network

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Balkan Goldenring (*Cordulegaster heros*) is listed in Annexes II and IV of the European Habitats Directive. This dragonfly species has a 3-4-year-long larval development, making the conservation of its larval habitats and surrounding areas crucial for its survival. It is well-known that *C. heros* prefers headwater habitats in shady valleys, typically with a high percentage of forest cover. In vitro laboratory experiments have also shown that the larvae of the species can survive short-term dry periods. Our aim was to investigate the importance of dry periods and land cover on the presence of the species in vivo, using a multivariate machine learning model. We focused on Bükkösi river network (SW Hungary), where both intermittent and perennial water bodies with the occurrence of *C. heros* can be found. Species presence and absence data were collected through a two-year-long seasonal macroinvertebrate sampling. Hydrological logger data were gathered from 42 streams daily, and land use data were obtained from a high-resolution national dataset (NÖSZTÉP). The percentages of different land use types were calculated for the catchment area above the sampling points. As a first step, we performed a binomial GLM to select the most important hydrological and land use variables. In the second step, we used a cross-validated XGBoost model for the selected five variables, with an 80/20% train-test split, and defined the importance of these variables. From our results, we were able to predict the occurrence of the species *C. heros* with high accuracy (0.9 AUC). Our results demonstrate, through a two-year in vivo experiment, that areas suitable for *C. heros* can be characterised by a high proportion of forest cover and a low proportion of agricultural areas in the catchment area. The species can survive and potentially recolonise areas that have been periodically dry. It can be concluded that the protection of forests in the catchment area is highly important for the species' occurrence, and while the species can survive increasing frequency of drying events up to a level, long-term drought periods and water abstraction can lead to its extinction.

Molecular diversity and diet analysis of *Gammarus roeselii* in the Lake Ohrid basin

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Lake Ohrid is recognised as the oldest preserved lake in Europe and is characterised by high biodiversity – both in the lake and surrounding springs. Among the fauna found in the lake, Amphipoda (Crustacea) play a key role in its ecosystem as detritus-eaters and food for predators. One of the amphipod species, not only found in the lake, but also widely distributed in Europe, is *Gammarus roeselii* Gervais, 1835. In Central Europe the species inhabits rivers and is characterised by low molecular diversity. In contrast, in the Balkans, the species can be found in lakes or springs and its molecular variability is high, suggesting the presence of cryptic species.

The main objective of the study is to analyse the diet of *G. roeselii* from Lake Ohrid and adjacent springs in the context of the molecular diversity of populations found in the two habitats. In the first stage of the project, we employed DNA barcoding, based on the sequence of the mitochondrial gene encoding the COI. This will enable the determination of the *G. roeselii* molecular variability in the study area. The next stage of the project will be the analysis of diet, based on DNA metabarcoding, using second-generation sequencing. Various markers will be employed (COI, 18S rRNA, ITS2, 16S rRNA) to recognise possibly wide range taxonomic spectrum found in the food content. As a result of the project, we can expect differences in diet as well as variation in patterns of molecular variability of populations occurring in contrasting environments.

Road crossings change functional diversity and trait composition of stream-dwelling macroinvertebrate assemblages

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Functional diversity is regarded as a key component in understanding the link between ecosystem function and biodiversity and is therefore widely investigated in relation to human-induced impacts. However, information on how the intersection of roads and streams (hereafter road crossings, representing a widespread habitat transformation in relation to human development), influences the functional diversity of stream-dwelling macroinvertebrates is still missing. The general aim of our study was to provide a comprehensible picture on the impacts of road crossing structures on multiple facets of the functional diversity of stream-dwelling macroinvertebrates. In addition, we also investigated changes in trait structure. Our research showed that road crossing structures had negative impacts on functional richness and dispersion, i.e., functional diversification. However, we found no significant impact on functional divergence and evenness components. We found a decrease in functional redundancy at road crossing structures. This indicates a reduced ability of the community to recover from disturbances. Finally, we found that road crossings drive stream habitat and hydrological changes in parallel with modification of the trait composition of stream-dwelling macroinvertebrate assemblages. All these results suggest that road crossings cause notable changes in the functional diversity of stream-dwelling macroinvertebrate assemblages.

The long-lasting effect of dredging on aquatic macroinvertebrate communities in streams

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Dredging is a commonly used practice in water management to remove the accumulated sediment and vegetation from the waterbed. It has direct and indirect effects on aquatic macroinvertebrate communities, additionally it creates a modified habitat in the streambed that can be newly recolonised by macroinvertebrates. Previous studies suggest that communities can recover one year after dredging, but less is known about the potential long-lasting effects. The Space-Time Substitution Method (STSM) was used to reveal the potential long-term effects of dredging on macroinvertebrate community structure (abundance, richness and Shannon diversity and Hungarian Macroinvertebrate Multimetric Index – EQR). In 2021, nine streams were sampled during both spring and autumn following the Hungarian Water Framework Directive (WFD) protocol. Three categories were established based on the time since dredging (1, 2, and 3 years), with three streams sampled from each category, including both control and dredged sections. We found no significant differences in the richness, abundance, Shannon diversity and EQR between the control and the dredged sections. However, there were slight differences in taxonomic composition between control and dredged sites. The annual fluctuation in the proportional species richness (relative to the total number of captured species) of Odonata, Crustaceans, Gastropods, and Dipterans after dredging was observed. For Odonata, the proportion of species richness was significantly higher in all dredging categories compared to the control with no detectable trends in relation to the elapsed time. This suggests that dragonflies may be more successful in recolonising habitats following dredging. Crustaceans, Gastropods and Dipterans did not show significant differences across all categories. Plecopterans were the most sensitive to dredging, as they were absent from the community in all categories following dredging. Further studies are needed to elucidate the functional aspects of the effects of dredging on the aquatic macroinvertebrate community.

Genetic diversity of Mysida (Crustacea, Malacostraca) in brackish and freshwaters of peri-Adriatic region

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The order Mysida comprises malacostracan crustaceans that inhabit benthic and pelagic zones in various environments, including marine, brackish, freshwater, and underground waters. These small creatures are most found swimming near the bottom, sometimes forming large shoals. They can also burrow into sediments. We studied mysid populations inhabiting flowing and standing inland and estuarine waters of the peri-Adriatic region, which is a recognised biodiversity hotspot in the Mediterranean. During an extensive study across the Apennine and Balkan Peninsulas, we collected over 350 specimens from 56 sampling sites located in Italy, Croatia, Montenegro, and Albania. Based on morphological examination, these specimens were assigned to seven morphotypes within the genera *Diamysis*, *Limnomysis*, *Paramysis*, and *Siriella* of the family Mysidae. However, a genetic study of 152 individuals using the COI mitochondrial gene revealed high diversity, with the identification of over 100 haplotypes. The sequences were deposited in BOLD and assigned to 11 Barcode Index Numbers, nine of which were unique. Analyses incorporating additional mitochondrial and nuclear markers (16S, 18S, 28S, and Histone H3) were employed to elucidate species relationships within the investigated group of crustaceans. Furthermore, the newly generated sequences significantly contribute to the DNA barcode reference library for European Mysida.

Spatial distribution of two highly divergent *Wolbachia* strains associated with freshwater bug *Aphelocheirus aestivalis* (Insecta: Heteroptera)

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Wolbachia infection prevalence among aquatic insect species is approximately 52%, albeit within infected species, the presence of endosymbiotic bacteria is detected in fewer than 50% of individuals tested. Molecular analyses *utilising* single or multiple genetic markers have delineated numerous distinct lineages of *Wolbachia*, commonly denoted as 'strains'. These strains are distributed in several large clades, termed 'supergroups', and have likely diverged for over a hundred million years. Currently, *Wolbachia* strains have been classified into supergroups designated from A to U. Supergroups A and B, along with E and F to a lesser extent, are widely distributed in arthropods. Conversely, *Wolbachia* strains from supergroups C and D are prevalent in filarial nematodes. The remaining supergroups are distributed among other arthropod clades.

The present study selected *Aphelocheirus aestivalis* (Insecta: Heteroptera) as a model species. This riverine water bug is the only reported species of the monogeneric family Aphelocheiridae occurring in most parts of Europe. Preliminary studies have previously revealed the presence of *Wolbachia* in *A. aestivalis* populations.

Here, we used the *wsp* molecular marker to screen 30 samples of *A. aestivalis* collected from various European locations. *Wolbachia* was detected in all but five samples. Notably, the prevalence of selected endosymbiont varied widely, ranging from 3% to 100% among the tested samples. Subsequent analyses based on nucleotide sequences of the *wsp* molecular marker identified two highly divergent *Wolbachia* strains infecting *A. aestivalis*. These strains were classified within supergroup A and were

not previously reported in other host species. Additional analyses uncovered intricate spatial distribution patterns for these strains. In general, strain Aaesti1 predominantly occurred in *A. aestivalis* samples collected in Central Europe whereas strain Aaesti2 was more prevalent in Western European samples. Interestingly, the co-occurrence of both *Wolbachia* strains was also observed in samples collected from Central Europe. The study was supported by the National Science Centre, Poland, under research project no. UMO-2019/35/D/NZ8/00251.

***Rhyacophila fasciata delici* – a new subspecies for the Trichoptera fauna of Bosnia and Herzegovina**

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With its rich hydrological network, Bosnia and Herzegovina is widely known for its high freshwater aquatic biodiversity and endemism, with aquatic insects being a prominent biotic component. The latest national assessment lists 220 species of Trichoptera, 74 Plecoptera, and 58 species of Ephemeroptera, with 25, 11, and 5 endemic species, respectively. Due to their sensitivity to pollution, the presence and relative abundance of caddisflies (Trichoptera) are often used for biological assessment and monitoring of water quality. Notwithstanding a long history of their research since the late 19th century, modern scientific techniques still allow for the discovery and description of new species and subspecies nowadays. Regardless of its dependence on the available reference genetic sequence libraries, DNA barcoding is an essential auxiliary tool in biodiversity research. During 2019 and 2020, a study on the diversity of aquatic insects in five protected areas in the urban area of Canton Sarajevo was conducted. Collected samples were DNA barcoded using degenerated primers LCO1490-JJ and HCO2198-JJ. Although only larval samples were available, based on the generated DNA barcodes, a new subspecies of caddisfly, *Rhyacophila fasciata delici*, was discovered in the investigated areas of Dariva (river Miljacka) and Vrelo Bosne (river Bosna). Furthermore, haplotype diversity was observed. Out of nine sequenced samples, five different haplotypes were discovered: three for the specimens from the Dariva location and two for the Vrelo Bosne locality. This finding is a strong indication that the diversity of Trichopteran fauna in Bosnia and Herzegovina is far from being fully explored. It is also an important argument that additional efforts should be employed to register, describe, and protect diversity within explored areas and design additional measures of conservation and management given the high anthropogenic pressures present. We emphasise that DNA barcoding, together with morphological species identification, should be part of the biomonitoring methodology in the protected areas in Canton Sarajevo. This study once again showcases the importance of continuous research and the existence and supplementation of publicly accessible genetic databases to improve species identification and bioassessment reliability.

Almost all Greek endemic caddisfly and stonefly species are threatened with extinction

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With today's update, the number of species at risk of extinction on the IUCN Red List has exceeded 44,000 and that is still 28% of all assessed species. The IUCN Red List now includes 157,190 species of which 44,000 are threatened with extinction. The Mediterranean region is recognised as the second largest global Biodiversity Hotspot where at least 20% of all species are threatened with extinction. In the Red Book of the Threatened Animals of Greece (2009) aquatic macroinvertebrates, including caddisflies and stoneflies, have been vastly neglected due to lack of comprehensive occurrence data. However, for the first time, Greek caddisfly and stonefly species were included in the IUCN Red List assessed for both regional and global level. Forty-eight endemic Trichoptera and fifteen Plecoptera species were assessed under the IUCN Red List criteria. Most of the endemics are located mainly in the Aegean islands, where aquatic habitats are decreasing at alarming rates due to climate change, water deficiency and increased tourism development. Nine Trichoptera species are critically endangered (CR), twenty-two are endangered (EN) and the remaining 17 are vulnerable (VU) or near threatened (NT). Regarding Plecoptera, five species are CR and ten are EN. This is the first major step towards the protection and conservation, or at least the recognition, of threatened caddisfly and stonefly species in Greece. Robust conservation measures must be implemented as soon as possible, otherwise, a significant number of species will be lost within the next years.

Impact of riverbed substrate changes on benthic invertebrate biodiversity: a case study of the Drawa River (NW Poland)

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Sediment is a crucial component of river ecosystems, and the balance of sediment transport and deposition significantly impacts aquatic biota. However, anthropogenic alterations of watercourses, such as dam construction or riverbed dredging, profoundly affect the grain size distribution of the bed sediment, and thereby indirectly impact benthic fauna. This study focuses on the influence of changes in bed sediment grain size due to the construction of riffle-pool sequences on the diversity of benthic invertebrates. The research was conducted on the Drawa River at four sites: Prosino, Kuźnica Drawska, Rzepowo, and Drawsko Pomorskie (NW Poland). Samples were collected before substrate deposition and 3 years after the interventions. Samples were taken at two points: one was subjected to restoration treatments, while the other served as a control. Throughout the study period, a total of 61 benthic taxa were recorded at all sites. Morphological changes in the river channel affected not only invertebrates collected from the directly impacted area but likely also individuals from the control sections. Restoration activities may have indirectly contributed to improving the benthic invertebrate biodiversity only at heavily urbanised sites (presumably through improvements in physicochemical water parameters), while no observable trend or even adverse effects were noted at other sites. This is attributed to sediment homogenisation and the loss of microhabitats at the intervention sites.

Financed by the Minister of Science under the “Regional Excellence Initiative” Program. Agreement No. RID/SP/0045/2024/01.

Insights into water beetle communities: a study of protected peatlands and peat mining areas

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Water beetles play crucial roles in wetland ecosystems, including peatlands. This study investigated water beetle communities in a protected peatland area and adjacent peat mining sites. The protected area encompasses the Slowinski National Park. Sample collection was conducted using water traps and a hydrological net during the spring, summer, and autumn of 2023. The study aimed to assess water beetle diversity, identify potential bioindicators, and examine seasonal migration patterns between protected and mining areas. Results showed significant differences in beetle richness, abundance, and community structure, underscoring the importance of conservation efforts in preserving these ecosystems. Furthermore, our observations revealed that peat extraction sites harbour a higher prevalence of ubiquitous species, suggesting notable environmental alterations. Surprisingly, even with the cessation of peat extraction and improved hydrological stability during summer droughts, peat ponds within the mining area failed to act as refuges for aquatic beetles typically found in the national park. The study highlights the urgent need for proactive measures to mitigate habitat degradation and preserve biodiversity hotspots, underscoring the importance of considering invertebrate fauna, particularly bioindicator species like water beetles, in peatland conservation alongside the traditional focus on flora.

Dragonflies of Slovakia – national DNA barcodes reference library (almost) complete

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Dragonflies and damselflies (Odonata) have been widely used as bioindicators of the environment in various ecological and monitoring studies. But sometimes it could be a challenge to correctly identify dragonfly nymphs, which are commonly found in freshwater samples, using only morphological characters (especially in early instars). Therefore, the use of molecular data for their accurate and rapid determination is very topical and DNA barcoding has become an essential tool in current diversity studies also of this group of insects.

Globally, there are more than 45,000 records of Odonata in BOLD database (www.boldsystems.org) available, covering around 3,500 species. Currently, 143 species are known from Europe, of which 65 are confirmed from Slovakia (45.5% of the European fauna). Despite the high number of records in BOLD database, data from Slovakia have been missing so far. The aim of this study was to process the material collected on Slovak territory, and to build the national reference DNA barcode library, considering also local genetic diversity.

Samples of odonate specimens were collected in 2021 and 2023 from 140 locations evenly distributed across Slovakia, in co-operation with the State Nature Conservancy of Slovak Republic within the project of Monitoring of animal species and habitats of community interest. Altogether 392 individuals of 62 species were collected, covering almost all our species. Processing the collected material led to a final dataset of COI sequences comprising 244 specimens (168 adults, 74 nymphs and 2 exuviae) of 62 morphologically identified species. Although the barcoding of exuviae was not successful through conventional PCR and subsequent Sanger sequencing, by using an Oxford Nanopore MinION technology we were able to obtain two barcodes out of ten collected exuviae.

The obtained dataset represents the reference database of DNA barcodes for almost complete dragonfly fauna of Slovakia. The *a priori* morphological determination of adults and nymphs was confirmed by comparing with barcodes of European

Odonata published on BOLD. The DNA determination was highly congruent with morphology except few nymphs and very closely related species. By comparing the datasets, we also tried to reveal relationships between Slovak and European specimens concerning intraspecific variability. While some Slovak samples have pooled with other European samples of the same species, others revealed distinct haplotypes suggesting specific position of our region regarding genetic diversity within species. This study was supported by the project VEGA 2/0084/21.

Nematomorpha – parasites that can manipulate

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Nematomorpha are a poorly known type of invertebrates. They are called “horsehair worms” in English literature because of old beliefs that they come from horses’ hair. There are approximately 360 species within this phylum, and for a long time they were incorrectly classified as nematodes due to their great similarity to this group of animals. The life cycle of Nematomorpha can be simple or complex. The simple life cycle includes mainly insects like Orthoptera, Coleoptera or Mantodea as definitive hosts. During the complex life cycle, Nematomorpha develop a cyst stage and in this form, they can be found mainly in Trichoptera, Diptera or Ephemeroptera, which play a role of intermediate hosts. The final host, for example mantis, become infected after eating the mentioned above hosts of the parasite. Nematomorpha are located in the abdomen of the final host and can be found in all organs except the intestine. Larval form of this parasite feeds on body fat and gonads which makes the host infertile. When the parasite becomes the adult form, it makes a strong need for the host to be near water. Once the host enters the water, the Nematomorpha escape into the aquatic environment through the host’s anus or break through the flexible covering on the abdomen between the host’s posterior segments. After that, it can mate with other Nematomorpha and the life cycle ends. Most often the host dies in water reservoir but sometimes it can escape and then still live, but it cannot reproduce or live like other invertebrates which never had parasite inside them. There was some information that Nematomorpha can be accidentally found in humans, however these parasites do not have enough adaptations to live inside the human body. The biggest problem during study of the biology and ecology of Nematomorpha is difficulty of finding them in nature. Larval stages are situated inside hosts bodies and the host does not have any visible symptoms, while adult forms are very hard to find because they are very thin and hard visible in water.

Macroinvertebrate assemblages as a reflection of the impact of pesticides used in agricultural landscape

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An intensive agricultural land use has had broad impacts on the entire landscape, including small streams, with direct and indirect impacts on macroinvertebrate communities. Monitoring of the impact of pesticides on freshwater ecosystems is quite challenging due to their low concentrations and an irregular occurrence in time, which depends on their application and a consecutive rainfall. Monitoring of pesticides using passive samplers is possible, but this is relatively expensive, problematic, and ambiguous with uncertain results with respect to the challenging water concentration calculation. More effective is monitoring of the pesticides in sediments that represent a sink and hotspots of chemical contamination (especially for insecticides that are usually hydrophobic) as well as habitats for many macroinvertebrate species. As a substitute for demanding monitoring with uncertain results, the calculation of indices based on the composition of the macroinvertebrate community can be used. The Species At Risk index (SPEAR pesticides) was designed to determine pesticide effects where low values indicate pesticides-affected community. This index is determined by the log abundance proportion of macroinvertebrate taxa, which are sensitive to pesticides. The aim of our presented study was to evaluate the specificity of this index at 79 small agriculture streams (3 and 4 Strahler number with a catchment area of up to 145 km² and an elevation from 190 to 550 m a.s.l.) using data from 1997 to 2022 in the Czech Republic. The streams were selected regarding the diffused source of pollution (typical for agricultural land use with concurrent exclusion of point/municipal pollution), characterised by a high nitrate to total nitrogen ratio (greater than 0.95). The results of the study indicate that the value of the SPEAR index decreases significantly with higher proportion of arable land and increases significantly with the higher proportion of forest land. With higher proportion of arable land, the abundance of Ephemeroptera, Plecoptera and Trichoptera species (EPT), and value of index of food types (RETI index) also significantly decreases and simultaneously the abundance of Diptera species and Saprobic index significantly increases. Similarly, our results showed that streams with smaller catchment area and higher slope at higher elevation are more sensitive to the effect of pesticides compared to lowland streams with higher catchment area. Also, over time, the percentage ratio of individuals of sensitive groups of taxa in the sample (EPT) decreases, especially in catchments with a higher proportion of arable land. In the same way, the SPEAR index decreases (deteriorates) over time, most significantly in catchments with a lower representation of arable land. The study has been supported by the project of the Technology Agency of the Czech Republic SS02030027.

Preliminary studies demonstrate high endemism of Black Sea supralittoral Isopoda

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There are gaps in the study of species that live above the waterline (supralittoral) in terms of their identification, taxonomic status, and ecological and evolutionary studies. Many of these species are poorly studied and have issues with their naming. In the past, it was thought that the “Mediterranean” fauna in the Azov-Black Sea basin was made up only of Holocene immigrants and did not form an endemic component. However, modern molecular studies have shown that some species, highly adaptable to specific salinity conditions in Pontic region, represent an older complex and form genetic lineages that are isolated from the Mediterranean basin, sometimes even at the species level.

This study investigated some isopods from the supralittoral zone in the Black Sea basin. Morphological method and molecular DNA barcoding of COI gene region were used for the analysis. The obtained molecular data were registered in the public repository BOLD, where they were assigned a BIN (Barcode Index Number) based on genetic distance to other similar sequences in the database. The standard genetic distance for invertebrates is more than 2%, meaning that animals have different BINs.

The findings revealed the isopods *Armadilloniscus ellipticus* (BIN: BAAK0054), *Tylos ponticus* (novel unique for science BIN: AFC8363), and *Ligia cf. italica* (novel unique for science BIN: AFD2226). *Tylos ponticus* and *Ligia cf. italica* are unique Pontic BINs, showing the marine ecosystem biota of Ukraine are the sources of endemism of the biota of the Azov-Black Sea basin. The conservation status of *Tylos ponticus* and *Ligia cf. italica*, considering they appeared to be unique Pontic lineages, needs to be reassigned in Europe. Preliminary, areal of *T. ponticus* may be shortened now to only Pontic region, as originally it was described from there, and its local conservation status may be reassigned to the protected species. The conservation status of *Ligia cf. italica* in Europe is not expected to change despite a slight decrease in range without the Black Sea. However, the new Black Sea *Ligia cf. italica* now may be considered localised and vulnerable.

Parasites hot pot in a biodiversity hot spot: first report of microsporidians infecting *Gammarus* species from ancient Lake Ohrid

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Microsporidia are a phylogenetically diverse group of ubiquitous endoparasites related to fungi, that infect hosts from all major metazoan taxa and in all environments. They are particularly frequent in aquatic ecosystems and commonly found in freshwater gammaroid amphipods. These parasites influence the host behaviour and physiology, including predation, thermal preference, and tolerance to pollutants as well as alter biological processes, such as the distortion of the sex ratio. Moreover, concomitant dispersion with hosts, and further spill over to local species, has been reported. The aim of our study was to detect, assess the prevalence and identify putative microsporidians infecting *Gammarus* species flock from ancient Lake Ohrid (North Macedonia). The small subunit rRNA gene (i.e., 16S) of microsporidians from gammarids collected in the lake and from surrounding watercourses were analysed to detect the parasites and investigate their taxonomy and diversity. This is the first survey of microsporidians infecting the endemic species flock in Lake Ohrid. The results show that more than 50% of studied gammarids are infected with the parasites. Microsporidians were found in all studied species (both coming from the endemic flock and local *G. roeselii* and *G. balcanicus* populations) and in the full depth range of the lake (down to 280 metres) as well as in surrounding springs. The species of microsporidians were identified through blast searches as *Dictyocoela muelleri*, *D. roeselium*, *D. duebenum*, *Nosema* sp., and *Microsporidium* sp. Both private haplotypes and putative endemic lineages were recovered. Their effect on the endemic communities has yet to be assessed.

What's the count? The basic descriptive characteristics of the macroinvertebrate communities in the six European case studies of the DRYvER project

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Intermittent stream sections are globally more prevalent than permanent ones, and in the future, with ongoing climate change and increasing demand for water, it is expected that dryings will get more frequent and severe. In the framework of the DRYvER project (H2020 – 869226) we processed a series of quantitative aquatic macroinvertebrate samples taken from drying river networks (DRNs). Samples were taken on six occasions in 2021–2022 from six European DRNs (Croatia, Czechia, Finland, France, Hungary, Spain). On each DRN we had between 20 to 25 sampling sites which were distributed on stream sections to represent the proportion of permanent and intermittent sites across each river network. Multihabitat sampling was conducted at each sampling site from a selected reach of 50–150 m, depending on a maximum mean wetted width of the riverbed. Quantitative samples were taken with either Surber or Hess samplers but only one type of sampling device was used in each DRN. Sampled area varied according to stream width between 0.5–1.5 m² in case of flowing conditions or between 0.1–0.5 m² if only pools were present at a site. Macroinvertebrates were processed to the genus level, except for taxonomically difficult groups which were identified at the family or higher levels. From the 639 processed samples we identified nearly 1.4 million individuals which belonged to 321 genera of 141 families of 17 higher-level taxonomic groups. We provide an overview of the basic characteristics of the macroinvertebrate fauna of each DRN and the selected methodological problems that arose during the work, such as the problem of higher-than-genus taxonomic level identification, for which our solution could serve as an example for future ecological studies.

Factors shaping caddisfly assemblages in the springs of Polish Pomerania

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Polish Pomerania hosts a great abundance of spring areas, and the diversity of underground water outflows is huge. The springs can be found in the forests on the moraine slopes, in stream and river valleys, or in the vicinity of lakes. The study was conducted in the spring and autumn of 2022 and its aim was to determine the factors shaping caddisfly assemblages in the springs of Polish Pomerania. Three samples covering 500 cm² each were collected at every studied water outflow, using a small net. The total number of water outlets was 35, located in 9 different areas. All springs were assigned to one of the following categories: helocrene, heloreocrene, and reocrene. Some of them formed singular water outlets, while others formed spring-fed mires. We measured the amount of dissolved oxygen, conductivity, hardness, pH, the concentration of nitrogen and phosphorus (total, inorganic, and organic forms), and the amount of chlorophyll-a and calcium. The studied springs varied regarding water chemistry properties.

The following questions were tackled: What is the diversity of caddisflies in the forest springs of Polish Pomerania? Which environmental factors influence the composition of trichopteran communities? Are there any factors that could endanger more sensitive caddisfly species?

Fifteen species of Trichoptera in the larval stage were identified. Most of them belonged to the genus *Sericostoma*. Nonetheless, *Chaetopteryx villosa* and *Crunoecia irrorata* – the latter protected by the Polish law and serving as indicator of undisturbed springs – were also abundant. Additionally, certain species rarely found in Pomerania, such as *Beraea pullata* and *Ernodes articularis* were noted. For each species that occurred in sufficient numbers we created distribution models in relation to different environmental variables, to better understand the underlying factors eventually shaping caddisfly communities. Noteworthy, we found that the abundance of *Crunoecia irrorata* shows an inverse relationship with nitrate concentration, supporting the indicator value of this species as an early sign of anthropogenic pressure. Research on the impact of anthropogenic pressures on Trichoptera communities is crucial for environmental protection and water quality assessment, as caddisflies play a fundamental role in the functioning of aquatic ecosystems and are sensitive to anthropogenic disturbances, such as nutrient loading which often translates into a deterioration of water quality and ecological integrity.

Our study is one of the most comprehensive research on the topic in northern Poland, where data are extremely scarce. Moreover, our study is particularly timely as spring ecosystems are currently facing severe anthropogenic pressures and require urgent conservation actions. Overall, we contribute solid fundamental knowledge on the biodiversity and ecosystem functioning of the springs in the area, with implications for their conservation.

The same species, different habits? Food consumption and preference by distinct phylogenetic lineages of a Ponto-Caspian invader *Dikerogammarus villosus*

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Dikerogammarus villosus (Sovinsky, 1894) is a Ponto-Caspian amphipod rapidly expanding in European fresh waters. Due to the high level of carnivory and voracity, this species poses a significant threat to biological diversity. In its invasive range, it originated from two genetically distinct sources: the Danube and the Dnieper deltas, spreading in Western (the Western Lineage) and Eastern Europe (the Eastern Lineage), respectively. These phylogenetic lineages exhibit phenotypic differences. We aimed to compare food consumption and preference (willow leaves, fish tissue and alive chironomid larvae, offered separately or together) between these lineages across five seasons. Each time, consumption was measured after 24 h of the experiment. In all seasons, amphipods preferred chironomid larvae. However, the amphipods from the Western Lineage consumed relatively less plant tissue than those from the Eastern Lineage. An especially high consumption rate was exhibited by large-sized amphipods from the Western Lineage. Thus, the amphipods from the Western Lineage consume more food and are more carnivorous, while their counterparts from the Eastern Lineage have a more flexible diet. As a consequence, the Western Lineage may cause a higher threat to macroinvertebrate communities, while the Eastern Lineage may be more successful in spreading in new habitats due to higher phenotypic plasticity. These differences can be explained by the different origins of both lineages as well as phenotypic disparities between them. Our study will help to predict the patterns and consequences of the further spread of invasive amphipods in Europe.

Small hydroelectric power plants and macroinvertebrate communities: are there patterns of change?

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The production of clean energy to reduce greenhouse gas emissions, diversify energy supplies, and reduce the pressure on fossil fuels is among the 17 goals of the Sustainable Development strategy. Hydropower, being the most traditional and cost-effective source of clean energy, supplies about 70% of the world's energy needs and is considered the market leader in clean energy. However, today, it is known that hydroelectric power plants, even the small ones (SHPs), cause river fragmentation and affect the quality of aquatic systems by altering water flow velocity and sediment transport capacity. These changes impair the state of aquatic habitats leading to negative consequences on biotic communities. Macroinvertebrate communities are pivotal in aquatic food webs, linking the trophic level of producers with higher trophic levels. By bringing together species (mainly insects) with different strategies, they also contribute to detritus breaking down and transferring matter and energy provided from several sources. The diversity of species and traits make them effective indicators of the state of aquatic systems. Since the macroinvertebrates are also easy to collect, they are commonly used in monitoring programs. While some studies focused on the response of macroinvertebrate communities' structure to SHPs construction and operation, little is known about their functional responses. Our research focused on identifying the effects of 33 intake points of 17 SHPs from the Carpathian Mountains in Romania on both the structural and functional patterns in macroinvertebrate communities. To explain the spatial and temporal differences in macroinvertebrate communities, the values of distinct indices were compared and linked to the hydromorphological changes assessed for the upstream and downstream sections of intake points. Such results are valuable for informing managers who assist the decisions in the energy sector and fostering their dialogue and collaboration with water managers and researchers.

Pressure of monkey goby (*Neogobius fluviatilis* Pallas, 1814) predation on benthic macroinvertebrate assemblages in the newly invaded Nemunas basin, Lithuania

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Almost a decade has passed since the entry of the Ponto-Caspian species monkey goby (*Neogobius fluviatilis* Pallas, 1814) into the Nemunas basin, but there is still very limited knowledge about the impact of this invasive species on the river ecosystem. Recently, there has been an increase in the abundance of this invasive fish, which may have a significant impact on invertebrate communities. The aim of our study was to determine the *N. fluviatilis* dietary spectrum and feeding preferences on benthic macroinvertebrates. This knowledge would allow us to predict which groups of benthic macroinvertebrates are most likely to be affected by this invasive predator in the newly invaded Nemunas basin.

Three sites with different biotopes were selected for the samples of gobies gut content analysis and benthic macroinvertebrate assemblages assessment – one in the Nemunas River, the other in its two major tributaries, the Neris and Nevėžis rivers. In total, 131 specimens of *N. fluviatilis* were taken for gut content analysis followed by benthic macroinvertebrate assemblages investigation at each sampling site.

The food spectrum of the monkey goby was broad. Analysis of the stomach contents showed that the diet of *N. fluviatilis* is dominated by the bivalve molluscs (Sphaeriidae), larvae of Chironomidae, mayflies (Ephemeraidae, Baetidae) and net-spinning caddisflies (Hydropsychidae). Results also indicated a significant ontogenetic diet shift. Macroinvertebrate samples collected from the study sites showed that Gastropoda, Amphipoda, Chironomidae larvae, Oligochaeta and Hirudinea were dominant in the communities. According to Ivlev's selectivity index, the monkey goby was found to have a strong preference for the Chironomidae, Sphaeriidae, Ephemeraidae and caddisflies (Trichoptera) larvae ($E > 0.4$). Meanwhile, it avoids ($E < 0.5$) Gastropoda, Amphipoda, Isopoda, Zygoptera and *Dreissena*. Also, although there were quite abundant Dytiscidae, Corixidae, Nepidae, Hirudinea, Megaloptera and Oligochaeta in the river sites, they were not found at all in the stomachs of gobies. However, there was no significant abundance decrease of Chironomidae, Sphaeriidae, Ephemeraidae and Trichoptera larvae in macroinvertebrates samples from these rivers in the last ten years, comparing pre- and post-invasion periods of *N. fluviatilis* in these rivers.

Phylogenetic position and genetic diversity of *Branchiobdella kozarovi*, an ectosymbiont of the narrow-clawed crayfish

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This study aimed to resolve the phylogenetic position of the crayfish leech *Branchiobdella kozarovi*, which has not been examined previously. We sampled *B. kozarovi* from crayfish obtained from 37 freshwater localities in Eastern Europe for this purpose. We also included data from previous research (41 further locations) to define the species' distribution range, using QGIS. The external and internal morphology was studied using a stereomicroscope. For phylogenetic analysis, genomic DNA was isolated and the COI and 16S genetic markers were used to reveal evolutionary relationships between European species of branchiobdellidans. Maximum likelihood analysis clustered *B. kozarovi* as a sister branch to the clade of *B. balcanica*. This contrasts with previous research based on morphological features that has suggested that *B. kozarovi* is relatively close to *B. pentadonta* and *B. italica*. To visualise intraspecific evolutionary relationships and haplotype diversity we used PopART v.1.7 with the median-joining (MJ) network approach. The population parameters for possible population expansion were calculated using DNAsp. We detected two divergent mitochondrial haplogroups of *B. kozarovi*. The first occurs in eastern Ukraine and neighbouring territories, and the second was found in the South Bug and Dnieper Lyman in Ukraine. Our data reflect the distribution of haplogroups of the host crayfish, *Pontastacus leptodactylus*, rather than that of geological factors, aquaculture facilities or displacement from habitats by species more resistant to crayfish plague infection. Our study provides evidence that *B. kozarovi* is a long-lived ectosymbiont of *P. leptodactylus* and is specifically endemic to that host, being rarely reported from other crayfish hosts. Populations of *P. leptodactylus* are found widely in Eastern Europe, the Middle East, and number of European countries. Our research suggests that its endemic crayfish symbiont also occurs more widely than previously reported.

Insights into the phylogeography of epigeal freshwater malacostracan fauna in the Periadriatic region: a case of the water louse (Crustacea: Isopoda)

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Our main goal was to investigate the historical molecular biogeography of the morphospecies *Asellus aquaticus* (L.) inhabiting the Periadriatic region, i.e., the areas surrounding the Adriatic Sea (the Apennine Peninsula, southern Alps, and their foreground, western Dinarides and Hellenides). Our working hypotheses tackled the three main stages of colonisation of inland waters by crustaceans. The early stage (since ca. 40 Ma), when the emergence of landmasses (above mentioned mountain ranges as well as islands and peninsulas, e.g., Apulia, Calabria) enabled colonisation of newly established inland habitats by the organisms of Tethyan and/or Paratethyan origin. The middle stage (ca. 6–5 Ma), when the massive desiccation of main water bodies during the Messinian Salinity Crisis (Lago Mare episode) promoted habitat shifts in organisms tolerant to oligohaline conditions, divergence by the habitat loss, as well as exchange of taxa between formerly isolated river systems. The late stage (ca. 2.5–0.016 Ma), when the fluctuations of sea level and salinity during the Pleistocene glaciations caused the regression of Adriatic Sea and, therefore, connection of previously separated hydrological networks within the one huge system of River Po. The phylogenetic analyses of the mitochondrial marker (COI) suggested that the oligohaline ancestor of *A. aquaticus* originated most probably in the Paratethys Sea, as the main centre of diversity within the genus is located in Asia and within the morphospecies – in the Pannonian Basin, respectively. In the Periadriatic region, the most widespread European lineage, i.e., the nominotypical subspecies *A. aquaticus aquaticus*, is present. Nevertheless, the area is inhabited by several distinct lineages forming putative cryptic species, e.g., the Apennine, Slovenian-karstic, Montenegrin and Ohridian one. Many of them are spatially more or less restricted, except the first mentioned, which is distributed from southern Alps to northern Apennine Peninsula, but was also found in Germany, France and in a single locality in Dalmatia. The molecular clock analysis suggested the Late Miocene divergence of the Apennine lineage from the Pannonian ancestor lineage, i.e., overlapping in time with the formation of the Apennine Peninsula. The Pleistocene lowering of sea level enabled the colonisation of Dalmatia via the Po River network. On the other side, the lineages inhabited the Ohrid and Skadar Lake systems originated from the Balkan ancestors in Pliocene/Pleistocene (ca. 3–2.5 Ma), which corresponds with the date of development of the two lake basins. Similarly, the estimated time of the beginning of local karstification in Slovenian Dinarides is in line with the divergence time of lineage inhabiting this unique habitat (4–5 Ma).

Macroinvertebrates under pressure: can a Ponto-Caspian invader affect them more than a native?

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The introduction of a non-native species can cause particularly severe disruptions to community dynamics and food webs, primarily through direct predation and competition for habitat and food resources. Numerous studies have demonstrated the superiority of non-native predators over their native counterparts, potentially resulting in more significant negative impacts on local prey populations. Therefore, we aimed to estimate the predatory pressure on macrozoobenthos assemblages by investigating the diet and feeding preferences of the non-indigenous invasive round goby *Neogobius melanostomus* (Pallas 1814) and the native European bullhead *Cottus gobio* (Linnaeus, 1758) in the Ploučnice River (Elbe River tributary, North Bohemia, Czech Republic), where co-occurrence of both fish species has been observed. We investigated the diet composition and potential food niche overlap of these species to shed the light on their impact on the local macrozoobenthic communities. Moreover, by combining stomach content analysis with laboratory studies on feeding efficiency and gastric evacuation rates of *Neogobius melanostomus*, we aim to provide a more accurate estimate of the local predation pressure exerted by the invader on specific macrozoobenthic assemblages. The research presented is part of an ongoing investigation aimed at comprehensive monitoring of changes at the sites over the years, including the relationship between benthic macroinvertebrate communities and prey composition in areas where native and invasive species coexist.

Unravelling the habitat preferences of the Ponto-Caspian amphipod *Chaetogammarus warpachowskyi*

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In the 1960s, several species of peracarid crustaceans were intentionally transferred from the Dniiper basin to Lithuania to improve fish-feeding conditions. Transported together with the larger-bodied Ponto-Caspian amphipods, the tiny *Chaetogammarus warpachowskyi* became the second most widespread alien amphipod in the country. However, its small size and specific habitats make its detection and proper sampling problematic, leading to poor knowledge of its ecology. Our aim, therefore, was to investigate the habitat of this enigmatic species. We studied the amphipod assemblage composition in the main mesohabitats of a lake and a river inhabited by abundant populations of *C. warpachowskyi*, and then tested its substrate choice in the laboratory. The field sampling was performed in the summer and autumn. We defined five main mesohabitats in the lake site: emergent vegetation/roots below knee-depth, emergent vegetation/roots above knee-depth, *Dreissena* beds with some *Chara* at knee-depth, *Chara* beds at waist-depth, and pebbles at ankle-depth. Four main mesohabitats were defined at the river site: emergent vegetation/roots on the flat bank, emergent vegetation/roots along the steep bank, submerged vegetation on the sandy flat bank, and logs and stones along the steep bank. In the lake, *C. warpachowskyi* was the most abundant in the *Dreissena* beds with some *Chara* at knee-depth, and in the river, most of it occurred in submerged vegetation on the sandy flat bank. In both study sites, it was the least abundant in emergent vegetation, which was occupied by the larger Ponto-Caspian amphipod *Pontogammarus robustoides*. The substrate choice was tested in square-shaped aquaria with a petri dish in each corner, each filled with a different substrate: submerged vegetation, plant detritus, sand, or pebbles. The distribution of 50 initial specimens was evaluated after 24 hours. Most specimens chose submerged vegetation. We repeated the experiments after adding 10 specimens of *P. robustoides* and slight changes could be observed – without changing its main preference for submerged vegetation, *C. warpachowskyi* then favoured the sand a little more at the expense of plant detritus and pebbles. Our results confirmed the clinger lifestyle of *C. warpachowskyi* previously inferred from morphology. As such, this alien amphipod introduced a novel amphipod functional role, making ecological consequences more likely. We also acknowledge that the presence of a larger competitor may adjust its distribution across different habitats. The study was funded by the Research Council of Lithuania, Project No. S-SV-23-192.

Diversity patterns overview of mayflies in Serbian hilly and mountain streams

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Mayflies (Order Ephemeroptera) are amphibious insects with aquatic larvae and short-living terrestrial adults. They are characterised by higher species diversity in lotic habitats, especially the upper reaches of fast flowing streams and rivers and highland rivers. The analysed dataset in this paper included 44 sites of hilly and mountainous small to medium sized streams with predominantly hard bottom substrate (gravel, stones and rocks) classified in the three water body types (WBT) according to the Serbian national legislation: WBT3 – altitude up to 500 m (24 sites); WBT4 – altitude above 500 m (6 sites) and WBT6 – small streams outside the area of the Pannonian Plain that do not fall under WBT3 and 4 and are not covered by the regulation on the establishment of surface and groundwater bodies (14 sites). Samples were collected in spring and fall 2019 using the kick and sweep sampling method from all microhabitat types according to European standards (EN 27828:1994) with an FBA hand net (mesh size 500 µm). The biological material was pooled and transferred to sample containers and preserved with 70% ethanol. Data analyses were performed using ASTERICS 4.04 software package and FLORA software version 2013.

Insects were the most dominant group of the community, recorded at the studied sites corresponding to the communities typical for the selected watercourses. Mayflies were recorded at all sampling sites as a significant component of the community. They were represented by 8 families and 30 taxa. The family Baetidae was presented with the largest number of species (12) and the species *Baetis rhodani* (Pictet, 1843) was the representative of the Ephemeroptera group with a significant abundance in all three groups of studied sites. The species *Ecdyonurus (Helvetoeticus) subalpinus* Klapálek, 1907 was only found in WBT4, but it was the most abundant species of the Ephemeroptera group, along with *Baetis (Baetis) alpinus* (Pictet, 1843).

Alpha diversity was assessed within the Ephemeroptera community in relation to WBT using Shannon entropy, Species richness and Equitability. The highest values of Shannon entropy and Species richness showed WBT4, while communities in WBT3 and WBT6 showed lower values. Slightly higher Equitability was observed in WBT4 than in the other two groups. Total beta diversity, and its components, were analysed using the procedures described by Baselga. Beta diversity was similar in all three groups. The Ephemeroptera communities in WBT4 showed the lowest value for nestedness with the highest species turnover. Beta diversity was slightly higher in WBT3, but also the nestedness component was slightly higher. Total beta diversity was slightly lower at altitudes over 500 m. Lower values of alfa and higher values of beta diversity in WBT3 leads to the conclusion that these communities were under various pressures that cause species loss (higher nestedness) which can be explained through the position of these sites in the vicinity of human activities.

Benthic sample fixative as a source of DNA for metabarcoding: insights from the Tatra Mountain lakes

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Glacial lakes of the Tatra Mountains (W Carpathians, Slovakia) are unique but very vulnerable ecosystems with specific and relatively isolated invertebrate communities that are challenging to access for sampling. DNA metabarcoding can benefit the assessments of their biodiversity and ecological status by providing high-quality data and minimising the lengthy manual sorting and morphological identification of specimens. Processing the fixation medium (usually ethanol), instead of the valuable invertebrate samples themselves, offers enhancements through simple protocols that allow subsequent focus on specific organisms detected among the preserved specimens.

In this study, we present proof of the concept by analysing freshwater invertebrate samples collected from 15 Tatra lakes in September 2020. Subsamples of their fixation medium were passed through filters with 0.45 µm pores and further processed by salt-extraction of the captured DNA. Mitochondrial COI marker was amplified using the BF3/BR2 primers and the resulting 180 libraries were sequenced on the Illumina MiSeq platform. We obtained a total of 15,037,762 pairs of demultiplexed reads, eventually clustered into 827 invertebrate OTUs (operational taxonomic units) with 406 species identified. Promising targets for further taxonomic or population studies were detected, including unique genetic lineages without any record in the reference barcode database.

We considered fixative subsamples from three different intervals of benthos fixation, in order to analyse the impact of fixation time on the metabarcoding outcomes. Utilising generalised linear models, we identified a two-week period of samples fixation time as more appropriate than a day or month of fixation. Additionally, we confirmed the previous suggestions about the role of body size and sclerotisation for fixative DNA, finding that large freshwater species were captured more efficiently than meiofauna or terrestrial taxa. These effects emerged from the detailed presence/absence data rather than from coarse per-sample records of taxonomic richness, which highlights that metabarcoding studies and optimisation efforts can take advantage of such robust metrics to investigate even subtle trends.

Moreover, the fixative DNA metabarcoding was able to reflect the lake-specific environmental conditions. The dissimilarities in community structure clearly distinguished the forest lakes from (sub)alpine lakes, which corresponds with the accumulation of nutrients in the lower zone. This distinction remained even after combining the metabarcoding datasets from various sampling strategies (lake water, sediment, several approaches to processing the benthic samples and their fixative). The comparison revealed an even broader range of taxa, as well as high consistency of fixative with benthic samples. Supported by the project VEGA 2/0084/21.

Mayfly fauna (Insecta: Ephemeroptera) from the Karadak Mountains in Kosovo

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The faunistic and taxonomic research of mayflies in Kosovo is still in progress. The mayfly fauna of the Karadak Mountains in Kosovo was poorly investigated. To overcome this gap, we selected nine sampling sites that represent the major streams and ecological features of the Karadak Mountains running waters. Kosovo is separated into two ecoregions, Dinaric Western Balkan (ER5) and Hellenic (ER6). The majority of Karadak Mountain is in the Hellenic Ecoregion (ER6), and the freshwater habitats in this area are part of the Black Sea and Aegean Sea Basins.

These nine sites were sampled in the Karadak Mountains from June to September 2017. During our investigation, we discovered 19 species belonging to 10 genera and 6 families. The most diversified families were Heptageniidae (2 genera and 9 species) and Baetidae (1 genera and 4 species). The following families are, for the moment, only represented by a single species: Potamanthidae and Leptophlebiidae. The most widely distributed species of mayflies in Karadak mountains were *Baetis rhodani* (Pictet, 1843), *Baetis alpinus* (Pictet, 1843), *Ecdyonurus vitoshensis* (Jacob & Brasch, 1984), *Ecdyonurus submontanus*, (Landa, 1969), *Ecdyonurus starmachi* (Sowa, 1971), *Ecdyonurus macani* (Thomas & Sowa, 1970), and *Rhithrogena braaschi* Jacob, 1974) *Rhithrogena* gr. *semicolorata* (Curtis, 1834), *Ephemera* cf. *parnassiana* (Linneus, 1758), *Serratella ignita* (Poda, 1761), and *Quatica ikonovski* (Puthz, 1971).

Finally, we want to define each species' ecological preferences and level of vulnerability in the Karadak mountain region to provide an important tool for running water management and river quality assessments. This research adds to our understanding of the distribution of mayflies (Ephemeroptera) in Kosovo's Karadak mountains.

Invasive and non-native species of aquatic invertebrates in the lower reach of the Ohře river

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Invasive species of macrozoobenthos may have a significant negative impact on aquatic ecosystems as competitors of the original representatives of the fauna, that in extreme cases can completely replace them. The reason may be the absence of natural enemies to keep them under control, strong food competition or the introduction of pathogens. They can also cause ecological and economic damage. During the regular monitoring carried out for the needs of the WFD, we found such species in the main tributaries of the Elbe River in the Czech region – Ohře, Bílina, and Ploučnice, which are managed by the Povodí Ohře, s.p.

According to previous findings and information found in the literature, we provided in 2021 an oriented survey of the occurrence of invasive species of macrozoobenthos in the lower reach of Ohře River, from the mouth to Elbe River in Litoměřice to Nechanice reservoir.

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