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Symposium 24

BOOK OF ABSTRACTS

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International Association of Astacology Symposium 24

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Preface

The IAA24 Symposium programme comprises a total of 106 research studies that will be displayed as oral (71) or poster (35) presentations from 125 astacologists from 26 countries, and compiles diverse topics from taxonomic and systematic studies, through pathology and genomic investigations to conservation and management of crayfish in different ecosystems of the world. These pieces of work are framed up with a Sture Abrahamsson lecture by Drs Lennart Edsman from Sweden and Japo Jussila from Finland that will review a half of a century studies on crayfish conservation and management in Fennoscandia, and key notes on crucial topics such as crayfish diseases, crayfish invasive species and crayfish conservation that will be presented by Drs Elena Tricarico from Italy, Laura Martín-Torrijos from Spain and Martina Temunović from Croatia. Cutting edge studies and implementations of new molecular tools such as Environmental-DNA, metabarcoding and genomics in astacology have substantially increased in this IAA24 programme compared to previous, and is showing how astacology investigations are incorporating multidisciplinary approaches. Thus, the IAA Symposium programme represents a fantastic update on crayfish research and an opportunity to know astacologists of reference for key topics to consult or collaborate with, and show the excellent work of the Organization Committee IAA 24 in Zagreb, Croatia.

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Map of Key Localities in Zagreb

Symposium Programme

Surnames in italic indicate student presenters

Sunday, September 15, 2024		
18:00-20:00	Registration and welcome drink	
Monday, September 16, 2024		
8:00-9:00	Registration	
9:00-9:30	IAA24 Opening ceremonies	
9:30-10:00	**Sture Abrahamsson lecture Edsman & Jussila**	All ok in Fennoscandia? Well, at least crayfisheries messed up big time
session eDNA	chair Strand	
10:00-10:15	**Baudry**	Unlocking crayfish biodiversity: eDNA metabarcoding for exhaustive monitoring?
10:15-10:30	**Burnham**	Testing substrates for an environmental DNA signal of a Critically Endangered burrowing crayfish
10:30-11:00	Coffee break - poster session	**Posters 1, 3, 5, 7, 9, 11, 13**
session eDNA	chair Grandjean	
11:00-11:15	**Bohman**	Comparison and intercalibration of eDNA protocols for crayfish and crayfish plague detection across Europe
11:15-11:30	**Strand**	Molecular detection of *Aphanomyces astaci* – experience with an improved species specific qPCR assay
11:30-11:45	**Vucić**	First application of environmental DNA metabarcoding and single-target detection of invasive and native crayfish in Croatia
11:45-12:00	**Petrusek**	Monitoring of native and invasive crayfish from environmental DNA: experience from Czech waters
12:00-12:15	***Aluma***	eDNA-based detection of invasive crayfish and crayfish plague in Estonia
12:15-12:30	**Manfrin**	Harmonizing Transboundary Monitoring of *Austropotamobius pallipes* Using Environmental DNA (eDNA): The PALLIPES Project
12:30-13:30	Lunch	
session Genetics	chair Diéguez-Uribeondo	
13:30-13:45	**Colli**	Population genomics and habitat modelling to improve the conservation status of the white-clawed crayfish *Austropotamobius pallipes* species complex in the Italian North-Western Apennines
13:45-14:00	***Pipinić***	Genetic and population characteristics of stone crayfish *Austropotamobius torrentium* (Schrank, 1803) in the cave Sušik
14:00-14:15	***Ströder***	Genetic screening of Tyrolean noble crayfish (*Astacus astacus*) - Identification of potentially endangered populations for the planning of sustainable conservation measures
14:15-14:30	***Bonassin***	Characterisation and comparative genomics of satellite DNA in freshwater crayfish
14:30-14:45	***Khan Areeba***	Characterizing the role of RNA editing in clonal and invasive marbled crayfish
14:45-15:00	**Vucić**	Coevolution of genus *Branchiobdella* with their crayfish hosts generated new species?
15:00-15:30	Coffee break – poster session	**Posters 15, 17, 19, 21, 23, 25, 27**

session Biogeography	chair Taylor	
15:30-15:45	***Huber***	Keep digging: two new burrowing crayfish species of genus *Parastacus* Huxley, 1879 (Decapoda, Parastacidae) from southern Brazil
15:45-16:00	**Westhoff**	Systematic distributional survey of endemic and invasive crayfishes in the upper Saint Francis River drainage, Missouri
16:00-16:15	***Bányai***	Has everything changed in ten years, or not so dramatically? The results of long-term crayfish monitoring programs in different Hunagrian waters
16:15-16:30	**Audo**	Reviewing fossil crayfishes
16:30-16:45	**Furse**	**STUDENT TRAVEL AWARDS**
19:00	**surprise evening (Zagreb's walks)**	

Tuesday, September 17, 2024

9:00-9:30	**Plenary lecture: Tricarico**	How behavioural studies help to control invasive alien crayfish
session Invasive species	chair Hudina	
9:30-9:45	**Riascos-Flores**	*Procambarus clarkii* in Andean waters: Ecological impacts and mitigation efforts
9:45-10:00	***Orlandi***	Spatio-temporal occurrence and abundance of *Faxonius limosus* (Decapoda: Cambaridae) in two North-western subalpine lakes (Italy)
10:00-10:15	**Hamr**	First Record of the first wild Marbled crayfish (*Procambarus virginalis*) population in North America/Canada and subsequent eradication efforts
10:15-10:30	**Mrugała-Koese**	Towards a risk-oriented national monitoring in the Netherlands
10:30-10:45	**Weiperth**	Are we really able to do anything, or are we just sitting back and watching? The history and the current status of signal crayfish (*Pacifastacus leniusculus* (Dana, 1852)) in Hungary
10:45-11:15	Coffee break - poster session	**Posters 2, 4, 6, 29, 31, 33, 35**
session Invasive species	chair Westhoff	
11:15-11:30	**Bohman**	Density estimation of signal crayfish with diving and ROV
11:30-11:45	***Fitzsimmons***	Multimethod assessment of Woodland Crayfish (*Faxonius hylas*) movement in the upper St. Francis River drainage, Missouri, USA
11:45-12:00	**Morbidelli**	Improving trapping effectiveness for controlling the red swamp crayfish *Procambarus clarkii*
12:00-12:15	**Saito**	Effects of pleopods removal on survival and sterility of signal crayfish
12:15-12:30	***Košir***	The North-American signal crayfish in the Drava river basin in Slovenia: latest spread and the first eDNA results
12:30-12:45	**Faller**	Limited efficiency of trapping on the prevention of the upstream migration of the invasive spiny-cheek crayfish
12:45-13:00	**Koese**	Crab cut, what about crayfish?
13:00-14:00	Lunch	

session Ecology & Biology	chair Kozák	
14:00-14:15	**Swedberg**	From streams, swamps, sloughs and roadside ditches: The places you will go while sampling the rare crayfishes of Texas, USA
14:15-14:30	**Magoulick**	Influence of flow regime and drought on seasonal population dynamics of co-occurring stream crayfish species
14:30-14:45	***Khan Tanya***	Seasonal microhabitat use by Brawleys Fork crayfish, *Cambarus williami*, a Species of Conservation Concern
14:45-15:00	**Klefoth**	Winter behaviour of adult noble crayfish (*Astacus astacus*) under natural conditions
15:00-15-15	**Graham**	The evolution of conspicuous coloration in crayfish
15:15-15:30	***Torrance***	Seeing the spectrum: investigating color vision in freshwater crayfish
15:30-16:00	Coffee break – poster session	**Posters 8, 10, 12, 14, 16, 18, 20**
session Ecology & Biology	chair Klobučar	
16:00-16:15	***Huber***	An overview of the knowledge about juvenile stages of the South American genus *Parastacus* Huxley, 1879 (Malacostraca, Decapoda, Parastacidae)
16:15-16:30	**Klefoth**	Winter mortality and growth of juvenile noble crayfish (*Astacus astacus*) under realistic environmental conditions – implications for conservation management
16:30-16:45	***Grubb***	No, I'm Dirty Dan! Comparative methods to explore disagreement within the *Faxonius erichsonianus* species complex
16:45-17:00	***Barnes***	Crayfish are like onions: Peeling back the layers on the burrowing behaviors of red swamp crayfish (*Procambarus clarkii*)
17:00-17:15	**Bonvillain**	Effects of environmental hypoxia on red swamp crayfish *Procambarus clarkii* population characteristics and fecundity in the Atchafalaya River Basin, Louisiana
17:15-17:30	**Breithaupt**	The role of biogenic amines in anti-predator behaviour of crayfish
>17:30	Board meeting	
20:00	**Pub quiz - Teniski tereni**	

Wednesday, September 18, 2024

9:30	Departure
12:00-15:00	Plitvice Lakes National Park
~16:00	Late lunch
~19:00	Return to Zagreb

Thursday, September 19, 2024		
9:00-9:30	**Plenary lecture: Martín-Torrijos**	Crayfish in crisis: exploring pathogens and diseases in a challenging world
session Diseases & Pathogens	chair Bielen	
9:30-9:45	**Ortega López**	Exploring the Evolution of *Aphanomyces*: Were ancestral Oomycetes such effective parasites?
9:45-10:00	***Jemmi***	Filling the gap: Starting to decrypt the genetic diversity of *Aphanomyces astaci* in Switzerland
10:00-10:15	***Pisano***	Eco - Epidemiology of *Aphanomyces astaci* in Switzerland
10:15-10:30	***Mojžišová***	*Aphanomyces astaci* in Cambarid crayfish from the native range of *Faxonius limosus*: in search of the elusive genotype group E
10:30-11:00	Coffee break - poster session	**Posters 22, 24, 26, 28, 30, 32, 34**
session Diseases & Pathogens	chair Theissinger	
11:00-11:15	**Martínez-Ríos**	Protocols for studying *Aphanomyces astaci* and its host interaction
11:15-11:30	***Tripp***	Effects of season and stress on prevalence of White Spot Syndrome Virus in *Procambarus clarkii*
11:30-11:45	**Hudina**	Crayfish pet trade as a gateway for the introduction of known and novel viruses into Europe
11:45-12:00	***Boštjančić***	Immune stimulation of the noble crayfish as a tool to study *Aphanomyces astaci* recognition
12:00-12:15	**Bielen**	Deciphering the mechanisms underlying the inhibition of *Aphanomyces astaci* by pseudomonads
12:15-12:30	**Zimmerman**	P2X as a solution to crayfish plague
12:30-13:30	Lunch	
session Ecology & Biology	chair Petrusek	
13:30-13:45	***Danilović***	Trophic insights of the invasive signal crayfish in Croatian freshwaters employing stable isotope analysis
13:45-14:00	**Ghia**	Better alone than in bad company: trophic ecology of co-occurring invasive and native crayfish
14:00-14:15	***Iqbal***	Can artificial light pollution affect the metabolic rate of *Procambarus clarkii* and *Pacifastacus leniusculus*?
14:15-14:30	***Kaur***	Efficacy of administration routes in crayfish: Comparative analysis of subcutaneous and intrapericardial techniques using fluorescein dye
14:30-14:45	***Rodgers***	Energetic cost of burrowing in red swamp crayfish
14:45-15:00	**Fogelman**	Filling in physiology knowledge gaps: Use of enzyme activity to quantify thermal ecology and evaluate differences in primary and tertiary burrowing crayfish
15:00-15:30	Coffee break	
session Conservation & Management	chair Colli	
15:30-15:45	***Jia***	Impact of land use on the presence/absence of an endangered freshwater crayfish species at local population scale on Hokkaido Island
15:45-16:00	**Ion**	Reassessing crayfish conservation: collaborative updates of *Austropotamobius* species IUCN status
16:00-16:15	**Diéguez-Uribeondo**	The strategy for the conservation of the native crayfish of Spain

16:15-16:30	***Acs***	Conservation and management strategies of the endemic idle crayfish (*Austropotamobius bihariensis* Pârvulescu, 2019): Lessons from long-term monitoring
16:30-16:45	**Forni**	The conservation status of the native crayfish *Austropotamobius palipes* complex in the Foreste Casentinesi National Park (Central Italy)
16:45-17:00	**Edsman**	Why are the crayfish too small for the crayfish party?
19:00	**Official dinner**	

Friday, September 20, 2024

9:00-9:30	**Plenary lecture: Temunović**	Application of species distribution modelling in freshwater crayfish conservation
session Conservation and Management	chair Burnham	
9:30-9:45	**Quebedeaux**	Evaluating response efforts to red swamp crayfish in Michigan, USA
9:45-10:00	**Vermeylen**	Craywatch, a citizen science project towards a better understanding of the distribution of invasive alien crayfish in Flanders: set up and initial results
10:00-10:15	**Krieg**	Misconceptions in the conservation of crayfish – what could we do better?
10:15-10:30	**Matijević**	Significance of the "Žumberak – Samoborsko gorje" Nature Park area for the conservation of the stone crayfish populations
10:30-10:45	**Furse**	Challenges in Australian Astacology: Common name confusion and much, much more. A cautionary tale
10:45-11:00	**Kawai**	Taxonomic revision of extant and fossil European Astacidae: A paleontological, molecular, and morphological approach
11:00-11:15	**Faller**	Proper interpretation of negative findings is crucial for the objective long-term monitoring of the crayfish populations
11:15-11:45	Coffee break	
11:45-12:15	**IAA24 General Assembly, different announcements and Closing Ceremonies**	
	Farewell drink(s)	

Posters

Surnames in italic indicate student presenters

No.	Surname	Title
1	**Basso**	Molecular diagnostic method for detection of *Nosema austropotamobii* (Microsporidia) in white-clawed crayfish
2	**Bohman**	Tracking signal crayfish during the cold season in Lake Vättern with acoustic telemetry
3	**Bruno**	Updated distribution and characterization of crayfish plague and microsporidiosis affecting *Austropotamobius pallipes* complex in Trentino (Northeast Italy)
4	**Bruno**	There can be only one: the two-year spread of *Procambarus clarkii* in a *Faxonius limosus* infested small perialpine lake in Trentino (Northeast Italy)
5	**Đuretanović**	Freshwater crayfish in Serbia: Update on the distribution
6	**Faith-Ell**	The Swedish hydrogen gas and crayfish project
7	**Fea**	Evaluation of the oral route of transmission of porcelain disease in signal crayfish under controlled conditions
8	**Fetzner**	A preliminary analysis of genetic diversity in the *Cambarus bartonii* species complex from the Northeastern USA
9	**Freemen**	Life history of the Pontchartrain painted crayfish *Faxonius hobbsi*, a species of greatest conservation need in Louisiana
10	**Jelić**	Urban life of the stone crayfish (*Austropotamobius torrentium*) in the city of Zagreb
11	**Gottstein**	Are deep karst springs along the Vrljika River in Croatia long-term protected refuges for white-clawed crayfish?
12	**Kouba**	Bioaccumulation kinetics of antihistamine diphenhydramine in signal crayfish
13	**Maex**	Craywatch: citizen scientists to the rescue
14	**Maguire**	Is there a future for the white-clawed crayfish in Istria?
15	**Zorić**	Morphological variability among populations of *Austropotamobius torrentium* (Schrank, 1803) from central Balkan
16	**Marguč**	Control of invasive spiney - cheek crayfish (*Faxonius limosus*) population in gravel pits near Ptuj, Slovenia
17	**Mrzelj**	Narrow – clawed crayfish (*Pontastacus leptodactylus*) in Slovenia
18	**Paolini**	Persistence of *Aphanomyces astaci* in white-clawed crayfish populations in Friuli-Venezia Giulia region (Northeast Italy)
19	**Pavić**	An opportunistic pathogen *Aphanomyces laevis* (Oomycota) is a host of new bunya-like virus
20	**Petretto**	Genomic characterisation of the white-clawed crayfish (*Austropotamobius pallipes* complex) in Italy: biodiversity assessment and support for conservation strategies
21	**Riascos-Flores**	Developing multiplex assays for crayfish detection using eDNA and ddPCR
22	**Zorić**	A contribution to the knowledge of the *Branchiobdella* species of the Balkan region
23	**Nikolić**	Morphological variability in introduced spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) occupying different habitat zones in Serbia
24	**Scheers**	Predation of invasive crayfish by diving beetles, a suitable aspect in nature based solutions?
25	**Schmidt-Posthaus**	Detection of novel RNA viruses in wild noble crayfish *(Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) in Switzerland
26	**Steen**	Exploring the native benthic fish burbot (*Lota lota*) and catfish (*Silurus glanis*) as biological control agents against the invasive marbled crayfish (*Procambarus virginalis*).
27	**Špoljarić**	More than 20 years of freshwater crayfish research in the UNESCO site Plitvice Lakes National Park
28	**Weiperth**	“New kids on the block”: crab introductions in European inland waters

29	**Barlow**	North Yorkshire crayfish forum: Collaboration for conservation
30	***Konno***	Co-evolutionary history of Japanese crayfish and ectosymbiotic branchiobdellidans from a molecular phylogeny perspective
31	***Iqbal***	Traditional model animals verses crayfish: driving revolutionary research.
32	***Lorenzoni***	Reproductive biology of *Pacifastacus leniusculus* in a small tributary of Clitunno River (Tiber River basin, Central Italy)
33	***Lorenzoni***	New record of *Astacus astacus* population in Brcanj stream (Bosnia-Herzegovina): age-class distribution and length-weight relationship
34	***Pisano***	Optimisation of a non-lethal field-based sampling method for the detection of *Aphanomyces astaci*
35	***Rusnak***	Conservation assessment of *Cambarus monongalensis and Lacunicambarus thomai* in Pennsylvania

Coffee break	**16.9. 10:30-11:00**	**16.9. 15:00-15:30**	**17.9. 10:45-11:15**	**17.9. 15:30-16:00**	**19.9. 10:30-11:00**
Presenters next to posters number	1, 3, 5, 7, 9, 11, 13	15, 17, 19, 21, 23, 25, 27	2, 4, 6, 29, 31, 33, 35	8, 10, 12, 14, 16, 18, 20	22, 24, 26, 28, 30, 32, 34

Sture Abrahamsson Lecture

All ok in Fennoscandia? Well, at least crayfisheries messed up big time

Edsman L[1] and Jussila J[2]

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The relevance of the freshwater crayfish in Fennoscandia is based on their original economic value to rural communities and resulting lively and specific culinary festivities in the form of crayfish parties. The introduction of the crayfish plague disease agent to Europe eradicated part of the native crayfish and cultural habits based on its trapping and consumption. This all happened in the early part of the 20th century and after at least 50 years the horrifying decisions was taken to bring alien species from North America to substitute the native noble crayfish populations and the fishery lost. After the initial studies and scattered stockings of the alien signal crayfish in Fennoscandia, massive stocking projects were initiated. Alien signal crayfish was introduced and promoted and it rather quickly was established to most of the water courses in southern and central Sweden and Finland as a result of licensed and illegal stockings. The warnings regarding detrimental effects of this alien species and a new very virulent strain of crayfish plague were ignored. This resulted, in a few decades, to further devastation of the remaining native noble crayfish stocks and also the discovery of lowered resistance against crayfish plague among alien signal crayfish stocks. Largely due to EU nature policies, attempts to preserve remaining native noble crayfish stocks were initiated and projects were started to eliminate alien signal crayfish stocks, although maybe now too late. These, and a few other outcomes of the often horrendous crayfisheries policies and practicalities are discussed and debated. The end result shows how vitally important it is to very carefully think before one acts.

Keywords: freshwater crayfish, crayfish plague, alien species, management, conservation

Plenary Lectures

How behavioural studies help to control invasive alien crayfish

Tricarico E

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Studying behaviour is a key factor for improving knowledge on invasive alien species and develop effective management methods for controlling them. Alien crayfish have been the subject of much research to understand their behaviour and impacts, leading to innovations and successful application of new control techniques in conservation actions. Several studies conducted to assess sexual selection, mating strategies, aggression, anti-predator and predatory behaviour, and personality were crucial for improving and developing appropriate techniques for the control of their invasive populations. Moreover, an integrated approach (e.g. coupling intensive trapping and sterile males/females release techniques or intensive trapping and native predators) is usually recommended for their management. Again, behavioural studies lie behind this approach as it is known from laboratory and field observations that, for example, adult crayfish are more vagile and tend to be trapped, while juveniles are more trap-shy but they are the preferred size preyed on by fish and aquatic birds. We the need to integrate behaviour and conservation biology in order to find the best management solutions for invasive species and thereby protect native species and ecosystems. The Louisiana red swamp crayfish *Procambarus clarkii*, one of the most widespread introduced species worldwide and highly invasive in European fresh waters, will be used to provide a powerful illustration of this integration.

Keywords: **biological invasions, management, crayfish, sexual selection**

Crayfish in crisis: exploring pathogens and diseases in a challenging world

Martín-Torrijos L

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In the era of the sixth extinction, the freshwater crayfish not only faces the major causes of destruction affecting freshwater systems (flow modification, water pollution, habitat destruction, overexploitation, climate change, invasive alien species), but also has to deal with diseases. It is therefore essential to understand crayfish pathogens and their impact on biodiversity in general.

Over the years, numerous studies have documented the causes and agents responsible for crayfish diseases through the use of traditional taxonomy, microscopy and molecular techniques. However, the global translocation of organisms for aquaculture purposes and for the aquarium trade has led to disease outbreaks in new geographic and host records, revealing previously unobserved conditions. This has contributed to the spread of certain diseases, leading to the decline and mortality of affected crayfish populations.

Historically, epidemiologists, population biologists and ecologists have used a variety of approaches to identify crayfish pathogens and understand the role of disease in shaping crayfish populations. With new molecular approaches, including environmental DNA (eDNA), genomics and transcriptomics (both host and pathogen), researchers can now focus on other aspects. Early detection of pathogenic organisms and understanding crayfish immune response to the presence of these pathogens are crucial to a comprehensive understanding of the problem.

To further understand the complex disease dynamics in freshwater crayfish ecosystems, we will examine several cases of pathogens that significantly affect a variety of freshwater crayfish species, with a particular focus on *Aphanomyces astaci*. Through this analysis, we will examine the interactions between these pathogens and crayfish populations, and how integrated approaches from a variety of disciplines can contribute to more effective management and conservation strategies for crayfish.

Keywords: *Aphanomyces astaci*, conservation, management

Application of species distribution modelling in freshwater crayfish conservation

Temunović M

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Species distribution models (SDMs) have become a useful and almost an inevitable tool in guiding species conservation strategies. They can be effectively applied in various aspects such as in identifying species distribution patterns in space and time, in habitat preservation and restoration, in assessing habitat connectivity, in predicting potential range shifts in response to different climate change scenarios, as well as in identifying invasive species threats.

Combined with genetic data they can for example aid in identification of areas with suitable habitat conditions for crayfish survival in the future that at the same time harbour populations with high genetic diversity, supporting long-term viability of populations. In addition, they may help in prioritization of areas and crayfish populations at risk for developing appropriate management strategies. Combined with potential distribution and niche shift of invasive crayfish species, they can effectively identify areas where native populations may be exposed to invasion. Overall, SDMs provide valuable piece of information for informed decision-making and conservation actions for target species.

Keywords: SDM, conservation, climate change

Oral presentations

Conservation and management strategies of the endemic idle crayfish (*Austropotamobius bihariensis* Pârvulescu, 2019): Lessons from long-term monitoring

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The idle crayfish, *Austropotamobius bihariensis* Pârvulescu, 2019, endemic to Romania's Apuseni Mountains, lacks a specific conservation plan. There is an urgent need for practical recommendations for the species protection due to its recent description, which limits conservation efforts. Thirteen years of field observations and threat assessments based on the IUCN standards were used to evaluate population trends and the impact of threats. Additionally, geospatial assessments and predictive models were employed to estimate the optimal habitat and current population size under three scenarios. During our 13 years monitoring program, we found that poor forest management, extreme drought, anthropogenic development and riverbed interventions have reduced crayfish abundance. The worst-case scenario involves multiple pressures acting simultaneously at the same site, with chronic crayfish plague infection being already documented as a cofactor. Poor water quality has a significantly negative impact on the modeled population size. We predicted, based on a comprehensive survey, the total population and the suitable habitat, with 37.9% of this habitat within twelve protected areas. We conclude with recommended actions tailored to each protected area and the specific threats identified, providing a foundation for effective field implementation. A list of the most suitable Arc Sites for *A. bihariensis* is also provided.

Keywords: **extinction, protected area, species conservation plan, species distribution modelling**

eDNA-based detection of invasive crayfish and crayfish plague in Estonia

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Three invasive non-indigenous crayfish species (NICS) were detected in Estonia in the last fifteen years using traps. To control and mitigate the impacts of these NICS on the noble crayfish, which is the only native freshwater crayfish species in Estonia, rapid conservation actions are required. Recently, more studies have employed the environmental DNA (eDNA) method in Europe to detect native and non-native crayfish species. Combining the eDNA results, which have shown the capacity to detect target species without directly observing them, with trapping data, prompts rapid actions that support the conservation of endangered species. Our study aimed to assess the potential of integrating the eDNA method into an ongoing annual monitoring program for NICS and *Aphanomyces astaci* in Estonia. Using singleplex qPCR assays, all of the 139 eDNA samples filtered from 16 water bodies were screened for noble crayfish, signal crayfish and crayfish plague, and a subset screened for marbled crayfish and spiny-cheek crayfish. We detected crayfish eDNA in 9 out of 14 water bodies (where presence was confirmed by trapping), yielding 64% detection efficiency. The detection rates per sampling location varied for noble crayfish (22 -100%), signal crayfish (8 - 67%) and spiny-cheek crayfish (33 -100%). *Aphanomices astaci* eDNA was detected in one water body, while marbled crayfish were not detected at sites where their occurrence was confirmed by trapping. We suggest that with further development, the eDNA method should be integrated into the routine monitoring of crayfish and crayfish plague as a supplement to trapping.

Keywords: environmental DNA, trapping, NICS, *Aphanomyces astaci*

Reviewing fossil crayfishes

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Crayfish are an old group of decapod crustaceans: molecular clocks studies have suggested crayfishes diverged from their close marine relatives, the nephropid lobsters, 380 to 300 million years ago. As such, one would expect fossil crayfishes to be quite common. Unfortunately, fossil crayfishes are extremely rare: Terrestrial and freshwater environments are less favorable to fossil preservation. Worst, decapod crustaceans in general tend to have a rather poor fossil record. Fortunately, the existence of crayfishes can also be attested by their fossilized burrows. These burrows are recognized because they are discovered in freshwater and terrestrial deposits and have a morphology very similar to modern crayfish burrows, including scratch marks from the pereiopods and scrape marks from chelipeds. The possible oldest of such burrows is dated from around 360 million years ago. Burrows are much more abundant in the fossil record that the body fossils of crayfishes. By comparison, the earliest body fossil known so far dates back from around 220 million years ago. The trace fossil record based on burrows show that crayfishes existed even before the formation of Pangea. The body fossil record shows the order of families: first Cambaridae are attested since Late Jurassic, about 150 million years ago; Astacidae and Cambaroididae are attested since Early Cretaceous, about 120 million years ago; and the Parastacidae from the late Early Cretaceous, about 110 million years ago. Generally, this review also led us to exclude species misidentified as crayfishes, and to recognize a total of 65 occurrences in the fossil record.

Keywords: fossil, burrows, Pangea, evolutionary history, palaeobiogeography

Has everything changed in ten years, or not so dramatically? The results of long-term crayfish monitoring programs in different Hungarian waters

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Intensive research programs have documented fourteen non-native crayfish, six crab and seven shrimp species in the wild in Hungary: seven crayfish and one of these shrimp species started to colonise different water bodies of the Carpathian Basin since 2013. Our research team is focusing on long-term studies rather than the usual one-off, point-sample studies as happens in most cases in the NATURA2000 and WFD monitoring program in Hungary. The results of studies carried out over several years show that many alien species are not able to persist in all water bodies over several years. Different populations of three *Procambarus* species (*P. allenii, P. clarkii, P. virginalis*) presented the best examples, that after successful colonisation many populations collapsed or integrated the species composition. Our results show that in permanently disturbed, urbanised habitats, the non-native species can survive much more successfully, while in quasi natural, waters with high predation pressure (e.g. fishes, reptiles,

birds, mammals), they cannot spread further or the population may collapse over time. In the end our presentation will show that some methods of eradication in urban habitats often result in the opposite outcomes, even unexpected pollution and mass mortality.

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Keywords: WFD, NATURA2000, pet trade, thermal waters, urban waters

Crayfish are like onions: Peeling back the layers on the burrowing behaviors of red swamp crayfish (*Procambarus clarkii)*

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Red Swamp Crayfish (*Procambarus clarkii*) are one of the most invasive crayfish species found throughout the world. Because they live part of their lives in terrestrial burrows that can cause damage to levees and reduce the effectiveness of surface-water treatment methods, it is important to determine factors that encourage or discourage burrowing behaviors. To investigate effects of environmental conditions on burrowing activity, we built artificial burrowing chambers to manipulate groundwater level and soil type. We first tested three rates of groundwater drainage: 4cm/day, 8cm/day, and 24cm/day in ambient soil. At 4cm/day, 11 of 12 crayfish burrowed and tracked the groundwater each day for a final burrow depth of 20 cm. At 8 cm/day, 7 of 12 crayfish initiated burrows on the first day but ceased burrowing at a depth of 8 cm. At 20 cm/day, no crayfish initiated burrows, with three of them dying at the surface within 5 days. We then investigated the effects of soil type, using the optimal groundwater drainage rate of 4 cm/day, but varied the ratio of ambient soil:sand from 9:1 to 1:9. We traced each burrow after 5 days of burrowing, and used ImageJ image analysis software to calculate burrow depth, volume, and perimeter. Increasing sand content did not eliminate burrowing behavior but caused a significant decrease in burrow depth (p=0.006), and burrow perimeter (p=0.020). In conclusion, burrowing activity appeared to be more strongly affected by groundwater conditions than soil type, with crayfish abandoning burrowing behavior altogether when groundwater quickly receded a short distance below the surface.

Keywords: invasive, burrowing, crayfish

Unlocking crayfish biodiversity: eDNA metabarcoding for exhaustive monitoring?

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Freshwater aquatic ecosystems are highly important, harbouring numerous species in a limited global surface. The development of environmental DNA (eDNA) has revolutionized biodiversity monitoring in aquatic ecosystems, particularly *via* metabarcoding, based on Next-Generation Sequencing (NGS), enabling near-exhaustive taxonomic listings. However, to the best of our knowledge, no studies using this approach for crayfish detection have been published, existing research being limited to single-target methodologies. The recent development of MiDeca primers by Komai et al. (2019), targeting a ≈164 bp region of the mitochondrial 16S rRNA gene, can change the trend. While BF2/BR2 primers targeted freshwater invertebrates with inconclusive results for crayfish, MiDeca primers were designed and tested both *in-silico* and *in-vitro* on over 250 species from Dendrobranchiata and Pleocyemata sub-orders, covering most aquatic (marine and freshwater) decapods. We highlight here promising results of their application. First, the primers were optimized with mock communities including seven different crayfish species (and other decapods species), demonstrating reliable species discrimination. This was confirmed on field, detecting the invasive crayfish, *Cherax quadricarinatus*, in Martinique, confirming previous single-target qPCR results and identifying newly invaded watersheds. Native crayfish populations (*Astacus astacus* and *Austropotamobius torrentium*) were also confirmed in Croatian samples. Additionally, MiDeca primers could allow the detection of other aquatic decapod species, from *Atyidae* family for example, which includes the karstic species, *Troglocaris anophthalmus*, in Croatia, or endemic palaemonids in Caribbean. These findings are highly promising, making it a useful tool for both comprehensive monitoring and early invasion detection in a growing context of freshwater conservation.

Keywords: crayfish, environmental DNA, metabarcoding, mock communities, monitoring

Deciphering the mechanisms underlying the inhibition of *Aphanomyces astaci* by *Pseudomonas*

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Pathogens enter complex interactions with a variety of microbes during their attempts to find and invade the host. Our aim was to investigate the effects of bacterial strains naturally associated with fish and crayfish on the oomycete pathogen *Aphanomyces astaci* (Oomycota), the causative agent of crayfish plague. We collected over 300 bacterial isolates and analysed their ability to inhibit mycelial growth of *A. astaci in vitro*. *Pseudomonas* isolates (72 in total) were among the most potent inhibitors. The results of *Pseudomonas* oomycete co-cultivation assays on standard and split Petri dishes showed that the observed inhibition was mainly due to secreted diffusible compounds rather than volatiles. Next, we sequenced the genomes of the six strongest inhibitors (four from the *P. fluorescens* group, one from the *P. putida* and one from the *P. syringae* group) together with three phylogenetically related non-inhibitor isolates. The most promising candidate cluster to produce anti-*Aphanomyces* metabolites was associated with the production of the siderophore enantio-pyochelin. This is consistent with previous reports that aquatic pseudomonads secrete siderophores that bind iron and, in this way, outcompete the oomycetes. Therefore, we will further analyse the siderophore production of inhibitory and non-inhibitory pseudomonads and the effects of iron concentration in the medium on the observed inhibition. In conclusion, our results provide the basis for the development of new biological control methods for the treatment of crayfish plague and could reduce the dependence on chemicals in crayfish aquaculture.

Keywords: biocontrol, crayfish plague, *Pseudomonas* genomes, siderophores

Density estimation of signal crayfish with diving and ROV

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The invasive signal crayfish is likely to have a major impact on European ecosystems, but it is also economically important for Swedish fishermen. It is therefore important to develop methods to efficiently monitor changes in the size of crayfish stocks. In this study, we surveyed transects in three areas of Lake Vättern with both divers and uv-ROV ("underwater Remote Operating Vehicle"). We then compared the estimated population densities with catches in the sampled fishery with baited cages. We found that ROVs detected fewer crayfish than divers, suggesting that diving is a better method than ROVs. The number of undetected crayfish is, however, unknown. The number of detected crayfish correlated between diving and catches by cages. The regression of dive-detected crayfish per m2 on the number of crayfish per cage was significantly lower than one, which indicates that the proportion of undetected crayfish co-varies with the density of the population. Due to the low detection rate, it is unlikely that transect data (by diving and ROV) will lead to accurate estimates of signal crayfish population sizes. Alternative methods such as capture-recapture studies should instead be investigated.

Keywords: *Pacifastacus leniusculus*, population density, diving, ROV, CPUE

Comparison and intercalibration of eDNA protocols for crayfish and crayfish plague detection across Europe

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Analysing eDNA has proven to be an accurate method for early detection of rare and invasive species. Still, there are many different eDNA methodologies used to sample and analyse target DNA from water samples. Several studies also show difficulties in detecting crayfish by using eDNA. To address these methodological issues, a workshop was held at the Institute of Freshwater Research (SLU) in Sweden in May 2023. 38 researchers from 13 countries participated in an experiment based on a ring test setup to detect three target species (signal crayfish, noble crayfish and crayfish plague) by taking water samples from pre-prepared tanks, artificial ponds and a natural lake. The participants were divided into 12 teams using their own equipment, strategies and protocols for water sampling, DNA extraction and targeted qPCR. All teams successfully detected crayfish eDNA from the indoor tanks, while the detection rate from the outdoor ponds dropped to 36 % (noble crayfish) and 60 % (signal crayfish). This experiment shows that all laboratories can detect the target species despite a variety of approaches and equipment. But it also shows a reduced detection rate for crayfish (especially noble crayfish) as the complexity of the environment increases, and the amount of target DNA decreases. We would suggest to move forward with similar experiments focusing on blind test analyses with known amounts of DNA from target species. Through careful analysis of the results, including sources of error, we will gradually be getting closer to a more universal and further improved detection of crayfish species in Europe.

Keywords: eDNA, *Pacifastacus leniusculus*, *Astacus astacus*, ring test, *Aphanomyces astaci*, qPCR

Characterisation and comparative genomics of satellite DNA in freshwater crayfish

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Repetitive elements (RE), including satellite DNA (satDNA), are widespread components of eukaryotic genomes with essential structural and regulatory functions. They are highly abundant in the genomes of freshwater crayfish species, contributing to genome size and complexity. Due to rapid evolution and high polymorphism, REs are a valuable tool for investigating genomic diversity and species divergence, even among closely related species. The aim of our study was to characterise the most abundant satDNA sequences in freshwater crayfish species to provide insights into their genome evolution. We identified REs, including satDNAs, in 19 species representative of Astacidae, Cambaridae, Cambaroididae and Parastacidae families based on low-coverage genome sequencing. Furthermore, we investigated the chromosomal localisation of the most abundant satDNA families on chromosomes of European species belonging to the family Astacidae, which have the largest genome sizes. The proportion of REs in the genomes ranged between 40% and 65%. Based on the comparative analysis, we showed that freshwater crayfish possess both species specific and shared families of REs. The composition of satDNA reflects the established phylogenetic relationships between species, with

differences in GC content and length of satDNA sequences between the four freshwater crayfish families. The localisation of some satDNAs is preserved through long evolutionary periods and they are probably associated with essential chromosomal structures, such as centromeres, pericentromeres and subtelomeres. These findings highlight the significance of satDNA in the evolutionary dynamics of freshwater crayfish, emphasizing its potential role in chromosomal function, as well as its contribution to the overall architecture of crayfish genomes.

Keywords: satellite DNA, Astacidea, comparative analysis, cytogenomics

Effects of environmental hypoxia on red swamp crayfish *Procambarus clarkii* population characteristics and fecundity in the Atchafalaya River Basin, Louisiana

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The Atchafalaya River Basin (ARB) is the largest bottomland hardwood river-floodplain system in North America and produces approximately 90% of the wild crayfish harvest in Louisiana. However, anthropogenic modifications to the natural hydrology in the ARB have altered historic river-floodplain connectivity and reduced water circulation and flow patterns that facilitate extensive areas of hypoxia for several months throughout the annual flood pulse. Although Red Swamp Crayfish *Procambarus clarkii* can tolerate relatively low dissolved oxygen concentrations, chronic environmental hypoxia can negatively affect population characteristics. Here we compare *P. clarkii* population characteristics and fecundity between chronically hypoxic and normoxic areas in the ARB. From 2008-2009 and 2016-2022, crayfish were sampled semimonthly at multiple sample sites in the ARB during the crayfish season. Water quality and catch per unit effort (CPUE) were recorded at all sites on every sample date and sex and carapace length were recorded for all captured crayfish. Additionally, *P. clarkii* hemolymph samples were collected at all sample locations on each sample date to determine hemolymph protein concentration and *P. clarkii* oocyte number and maturation stage were compared between hypoxic and normoxic sites during 2020 and 2021. Across sample years, mean *P. clarkii* CPUE, carapace length, and hemolymph protein concentration were consistently lower at hypoxic locations. Furthermore, female *P. clarkii* from hypoxic locations produced fewer total oocytes and had a lower annual maturation index in 2020 and 2021. The results of this study demonstrate that chronic environmental hypoxia in the ARB can adversely influence *P. clarkii* population and fecundity characteristics.

Keywords: Atchafalaya River Basin, hypoxia, oocyte, red swamp crayfish

Immune stimulation of the noble crayfish as a tool to study *Aphanomyces astaci* recognition

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The seriously threatened and protected noble crayfish, *Astacus astacus*, is highly susceptible to the infection of the invasive oomycete pathogen, *Aphanomyces astaci*. Despite the varying mortality associated with different isolates of the pathogen, treatment for the infected crayfish does not exist. To investigate the impact of the pathogen infection on the noble crayfish immune system, we conducted a series of controlled immune stimulation challenges with the stimuli mimicking *Ap. astaci* infection. We utilised laminarin (β-1-3-glucan found within *Ap. astaci* cell wall), inactivated *Ap. astaci* spores and *Ap. astaci* hyphal homogenate. We analysed total and differential haemocyte counts (THC and DHC) as well as differentially expressed genes (DEGs)

in the immune stimulated noble crayfish based on RNA sequencing and transcriptome analysis. All challenges led to an immediate (at 1 and 4h) increase in the THC, followed by the decline to normal values within 8h. Granular haemocytes seem to be highly involved in response to stimuli with inactivated *Ap. astaci* spores, while the number of semigranular and hyaline haemocytes was increased in response to laminarin and *Ap. astaci* hyphal homogenate. Analysis of the DEGs in the haemolymph samples collected 4h post-immune stimulation showed 1671 DEGs in the laminarin group, 3723 in the spore group and 971 DEGs in the hyphae group. Numerous DEGs overlapped between the groups. Hierarchical clustering analysis revealed up- and down-regulated modules of DEGs. We show that immune stimulation approach can aid in deciphering the immune mechanisms associated with the pathogen recognition in the noble crayfish.

Keywords: *Astacus astacus* crayfish plague, innate immune system, gene expression, total haemocyte count

The role of biogenic amines in anti-predator behaviour of crayfish

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In crayfish, the chemical detection of predatory events triggers anti-predator responses such as slowing down and hiding in darker areas. Such stress responses have previously been characterized as anxiety-like behaviours (ALBs) if they occur in the absence of a stressor and involve an increase in the biogenic amine serotonin. Here, we study whether (i) crayfish exposure to fluoxetine, a serotonin reuptake inhibitor and common pollutant of freshwater systems, and (ii) crayfish injection with serotonin (5-HT) or dopamine (DA) lead to ALBs. We analysed crayfish behaviour in a plus-maze arena having light and dark zones. Crayfish (*Pontastacus leptodactylus*) after one week exposure to fluoxetine polluted water (1.12 µg/L) displayed ALB by spending more time in dark zones and moving less than control animals. Similarly, serotonin injection into crayfish from unpolluted water triggered ALB at both concentrations tested (1µM and 10µM) while dopamine injection elicited ALB only at the higher concentration (10µM). The anxiogenic effect of 5-HT and DA disappeared when administered together with either 5-HT1 receptor antagonists (50µM maleate salt) or the non-specific DA blocker (10µM methergine) suggesting that a parallel pathway could be involved in 5-HT and DA activation. Surprisingly, co-injection of 1µM DA with DA blockers led to ALB rather than to the bold behaviour displayed in all other blocker administrations. Overall, our results suggest a complex functional interplay among biogenic amines in anxiety-like behaviour in crayfish. They also demonstrate how pharmaceutically active substances that pollute freshwater habitats interfere with natural anti-predator behaviours in freshwater crustaceans.

Keywords: serotonin, dopamine, fluoxetine, anxiety-like behaviour, stress

Testing substrates for an environmental DNA signal of a Critically Endangered burrowing crayfish

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Effective conservation of endangered species depends on knowledge of their distributions, but species detection can often be challenging. An example of this is provided by the Critically Endangered Margaret River burrowing crayfish (*Engaewa pseudoreducta*), which is highly cryptic. Due to the burrowing habit of this crayfish, its detection currently requires a great deal of effort, the results are often non-conclusive, and, as it involves manual excavation of their burrows, the habitat of this and other species is destroyed in the detection process. In response to these challenges, this study developed and optimized a species-specific probe-based qPCR assay targeting the 16S gene region to detect the target species from environmental samples. Three test substrates were tested to see if eDNA could be detected: chimney pellets (soil expelled by a crayfish as it digs its burrow), burrow scrapes (soil lining the inside of a burrow), and burrow water (water that is filling the burrow space). Our results suggested a non-invasive eDNA-based technique could potentially replace the traditional survey method; however, there were significant differences in eDNA amplification between substrates that will be discussed in the presentation.

Keywords: burrowing, conservation, eDNA, endangered, qPCR

Population genomics and habitat modelling to improve the conservation status of the white-clawed crayfish *Austropotamobius Pallipes* species complex in the Italian North-Western Apennines

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Over the last century, the Italian populations of the White-clawed crayfish *Austropotamobius pallipes* species complex have suffered from the detrimental effects of anthropogenic pressure and the introduction of pathogen-carrying allochthonous crayfish species. This caused a severe contraction in the census size and distribution area and currently the species consists of separate reproductive units isolated in different water basins. To aid *ex situ* breeding and reintroduction activities of the EU-funded Life CLAW project, molecular characterisation and Ecological Niche Modelling (ENM) were carried out for the species in the Italian North-western Apennines. Extant genetic variation was assessed by analysing 1312 samples from 64 populations using mitochondrial COXI gene sequencing and genotyping nuclear genome SNPs by ddRAD (double-digest Random Amplified DNA). To identify areas suitable for reintroduction of the species ENM, was carried out using raster environmental layer information (Land cover/use, Elevation, Sentinel multi-spectral data, bioclimatic variables). Genomic analyses revealed i) clearly distinct gene pools for *A. pallipes* (Western Apennines) and *Austropotamobius italicus/fulcisianus* (Central-eastern Apennines), ii) a geographically structured distribution of diversity over the territory, and iii) the presence of several private mitochondrial haplotypes. Overall, these results point to the effects of Plio-Pleistocenic paleoclimatic events that occurred in the Mediterranean region combined with recent demographic changes caused by habitat fragmentation and reduction of gene flow. Based on ENM results, the sites most suitable for *A. pallipes* are small streams at intermediate altitudes. Factors such as presence of water reservoirs have positive effects, while human settlements are detrimental for the species.

Keywords: *Austropotamobius pallipes* complex, White-clawed crayfish, Italian North-Western Apennines, population genomics, habitat modelling

Trophic insights of the invasive signal crayfish in Croatian freshwaters employing stable isotope analysis

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The invasive signal crayfish (*Pacifastacus leniusculus*), a species of high concern in numerous European freshwaters, poses a substantial threat to indigenous crayfish species and freshwater ecosystems in general. Its widest distribution in Croatia is observed in the lowland Drava River and the karstic river Korana, where signal crayfish have been present for over a decade. Populations of the native noble crayfish and the narrow-clawed crayfish have been completely displaced in the invasion core and active invasion fronts of the rivers, with only a few or no native crayfish individuals left. As the signal crayfish is gradually invading new freshwater sections, we aim to evaluate its diet and trophic niche to estimate potential trophic dynamics over various invasion stages. We hypothesize that the trophic niche of signal crayfish in the active invasion front will differ from the invasion cores with long-time established populations. In this study, we performed stable isotope (carbon $\delta^{13}C$ and nitrogen $\delta^{15}N$) analyses on sampled signal crayfish and its major potential food source such as macrophytes, detritus, periphyton and macroinvertebrates. Preliminary analyses indicate different carbon and nitrogen isotopes variation between the signal crayfish populations, which will be further examined in more detail. Our findings will help better understanding of the trophic change of the signal crayfish during invasion and its possible impact on native freshwater communities.

Keywords: SIA, invasive species, Astacidae, trophic niche, Balkan

The Strategy for the Conservation of the Native crayfish of Spain

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The native crayfish of the Iberian Peninsula (*Austropotamobius fulsicianus syn Austropotamobius pallipes*) is listed as "vulnerable" in the Spanish Catalog of Endangered Species. The Spanish Law 42/2007 on Natural Heritage and Biodiversity entails the adoption of a Conservation Plan within a maximum period of five years. The same Law 42/2007 establishes the State Council for Natural Heritage and Biodiversity should approve the strategies of conservation of a threatened species. These strategies must include at least a diagnosis of the situation, the main threats to the species and the actions to be undertaken for its recovery. The case of *A. pallipes* fully applies to this legal mandate. In this work, it is reviewed the technical bases for the Conservation Strategy of *A. pallipes*, and the basic lines of action and the measures that must be applied for the conservation of this species in Spain. This document also aims to serve as a guiding framework to prepare or review the Plans of the Autonomous Communities of Spain, both for the management of the native species and its habitat in the Iberian Peninsula, and for actions in relation to exotic invasive crayfish species that directly condition its conservation and recovery. This strategy has taken into account all the experience from all the proposals, presentations and approaches derived from the experience formulated in the successive meetings, congresses and conferences developed and applied for this species over the last twenty years by crayfish experts and managers.

Keywords: conservation, management, *Austropotamobius pallipes*, native species, invasive species

Why are the crayfish too small for the crayfish party?

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Noble crayfish has been known to occasionally form so called ”dwarf populations”, that is very many but very small crayfish. This phenomenon has now started to appear in populations of the introduced signal crayfish. Two hypotheses are preferred to explain why this occurs.

1. Density dependent factors. Strong recruitment due to a rich year-class (or low predation rate) results in strong competition for resources, resulting in slow growth for all but a few of the crayfish.
2. Abiotic factors, such as general shortage of food or poor water quality, including toxins, do not allow fast growth in any of the individual crayfish.

To distinguish between these explanations, age was estimated by quantifying the concentration of the age pigment lipofuscin in the brain of signal crayfish from two different lakes. One lake had a population with a normal size distribution and the other lake had a population of ”dwarfs”. Surprisingly there was no difference in the growth rate of the crayfish from the two lakes. The crayfish from the ”dwarf population”, however had a shorter life span and this was the reason for their generally smaller size. Thus, none of the originally suggested causes is a likely explanation. Why crayfish in the ”dwarf population” die young remains to be explained. The usual management advice to overcome the problem with “stunted” populations, is to direct the fishery towards the smaller crayfish in order to relax competition. If the results from this study are generally applicable, such advice will not solve the problem.

Keywords: signal crayfish, stunted growth, fisheries problem, age determination, management advice

Limited efficiency of trapping on the prevention of the upstream migration of the invasive spiny-cheek crayfish

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Efficiency of a novel approach, a sterile male release technique, was tested to reduce negative impact and upstream migration of the invasive spiny-cheek crayfish. The survey was undertaken on a 500 m stretch of the Vuka River, Croatia, using 50 traps over 16 days. The same approach will be repeated twice a year for the next three years, but preliminary results are as follows. In total, 1260 males were caught, sterilised and released, while 1307 female crayfish were removed from the river. The number of crayfish caught each day changed as follows during the survey: newly caught males declined (from 90 to 68); sterilised males increased (from 0 to 79); females declined (from 108 to 66). Stated results represent a significant reduction in the reproductive capacity of the spiny-cheek crayfish population, however, the surveyed section of the Vuka River represents only a short part of its total length (less than 0.5%) and therefore reduction in the overall negative impact on the river ecosystem is negligible. Despite intensive trapping efforts on a short section of the river, only a limited reduction in the population of adult spiny-cheek crayfish was observed, so trapping is unlikely to be able to prevent upstream migration of the spiny-cheek crayfish. These preliminary results suggest that other methods to prevent the spread of the invasive crayfish should be prioritised, particularly public education to prevent accidental release and the implementation of multi-methods to slow the upstream migration of the invasive crayfish.

Keywords: invasive crayfish management, spiny-cheek crayfish, sterile male release technique, upstream migration, public education

Proper interpretation of negative records is crucial for the objective long-term monitoring of the white-clawed crayfish populations

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Reporting under Article 17 of the Habitats Directive requires member states to provide information on species population sizes (number of occupied 1x1 km grids), a short-term trend (12 years) and a long-term trend (24 years). No reliable models of white-clawed crayfish distribution in Croatia currently exist. In 2023, a project was conducted to gather field data, analyse all information and revise the existing monitoring programme for white-clawed crayfish in Croatia. The following number of white-clawed crayfish records were collected: before the year 2000 (119 positive and 0 negative), 2001-2012 (73 positive and 204 negative) and 2013-2023 (64 positive and 206 negative), but only positive findings are required in reporting. In total, these records fit into 120 1x1 km grids, but only the following number was covered during each time period: before 2000 (77), 2001-2012 (58) and 2013-2023 (40). However, using data in this form would not distinguish between real population change and the change caused by differences in applied monitoring efforts. Therefore, we applied an approach that focused on the proper interpretation of positive and negative results. Firstly, since crayfish migration is limited, all historical results were treated as positive for the period in which they were collected, but also for all the periods before the collection year. If negative records were confirmed at any point in time, in a specific grid, the respective grid would be marked as negative for the subsequent survey period. This approach resulted in the following number of 1x1 km grids with positive findings of the species: before 2000 (120), 2001-2012 (113) and 2013-2023 (66). White-clawed crayfish is disappearing throughout its range in Croatia, however, there are differences depending on the approach to data interpretation, emphasizing the importance of proper collection and interpretation of negative results during long-term monitoring programmes.

Keywords: negative records, Article 17 reporting, population decline, monitoring

Multimethod assessment of woodland crayfish (*Faxonius hylas*) movement in the upper St. Francis River drainage, Missouri, USA

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The invasion and spread of the Woodland Crayfish (*Faxonius hylas*) in the Upper St. Francis River drainage in Missouri threatens the endemic St Francis River Crayfish (*Faxonius quadruncus*) and Big Creek Crayfish (*Faxonius peruncus*) and resulted in their listing of threatened under the U.S. Endangered Species Act. Understanding the dispersal capabilities of *F. hylas* is essential to design effective management strategies and predict the timing of further spread. We are using three tracking methods to document the movement patterns of *F. hylas* for adults and juveniles, males and females, and leading edge and fully invaded sites in six streams over one year. At two leading edge sites, we tagged 20 *F. hylas* with radio tags in June 2024 and will relocate them every other week until tag batteries expire, likely in September 2024. At each of the four remaining sites, we have tagged adults with passive integrated transponder (PIT) tags and with p-chips during June and July 2024. We also tagged 80 juveniles with p-chips and additional adults and juveniles with visible implant elastomer (VIE) tags. Individuals tagged with PIT tags will be relocated monthly and p-chips every quarter until May 2025. Location data will be used to create dispersal kernels and compare between each group throughout the year to determine total displacement, upstream v downstream movements, and demographic effects on movement. These data will improve our understanding of *F. hylas* dispersal, the permeability between occupied and unoccupied habitats, and improve predictions of future dispersal.

Keywords: movement, demographics, invasive

Filling in physiology knowledge gaps: Use of enzyme activity to quantify thermal ecology and evaluate differences in primary and tertiary burrowing crayfish

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The southeast U.S. is the epicenter of global crayfish biodiversity, but these species are among some of the most threatened in the world. Life history, biological trait, and environmental tolerance data deficiencies, particularly of burrowing crayfish species, hampers efforts for accurately assessing imperilment rates and informing conservation efforts. In this study we evaluate a sublethal approach assessing potential metabolic activity of crayfish legs via an assay of enzymes of the electron transport system (ETS) to estimate organismal thermal tolerances, which will allow us to sublethally predict population risks associated with warming temperatures. Using the ETS assay we also evaluate differences in ETS activity of tail tissue for three primary burrowing (terrestrial burrows not connected to surface waters) crayfish species and three tertiary (stream-dwelling) crayfish species. We hypothesize that tertiary burrowing crayfish will have higher ETS enzyme activity (ETSmax) as they use their tail more in activities associated with their stream habitat compared to primary burrowers who use their chelae more for burrowing activities. Additionally, we hypothesize that tertiary burrowers will have a higher optimum temperature (Topt) and a wider range of optimal temperatures (ETSbreadth; 80% upper and lower boundaries of ETSmax) due to their exposure to fluctuating stream temperatures. The knowledge gained through this work will further our understanding of crayfish physiology and environmental tolerances to inform species specific adaptive capacity and climate vulnerability. This work aims to supplement the efforts of natural resource managers and conservation professionals in conserving rare, endemic crayfish biodiversity in the U.S. and beyond.

Keywords: burrower, thermal tolerance, physiology, conservation

The conservation status of the native crayfish *Austropotamobius palipes* complex in the Foreste Casentinesi National Park (Central Italy)

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The native crayfish *Austropotamobius palipes* complex is classified as a threatened species in the IUCN Red List, and is protected by various European, national and regional laws. Throughout Europe, its populations are declining due to threats like habitat loss, fragmentation, illegal fishing, climate change, water pollution, and invasive alien species (such as *Procambarus clarkii* and *Procyon lotor*). The aim of this study was to update the distribution and conservation status of *A. palipes* complex inside the Foreste Casentinesi National Park and State Reserves (Central Italy), by comparing the current situation with the past and assessing the presence and effect of potential threats to this specie. During 2021-2023, 66 samplings were conducted in the Tuscan and Romagna side of the Park, including sites that had been monitored between 2012 and 2016. The crayfish were caught by hand along a 100 m transect at night. The sampled individuals were sexed, measured, and examined for: presence of parasites, diseases, and any traces of raccoons, such as footprints or remains of preyed crayfish. The results showed a significant populations' reduction, or local disappearance, on both sides of the park, particularly in Tuscany. The different trend in the crayfish populations between two sides could depend on a more pronounced anthropic impact in the watercourses on the Tuscan side, due to poaching, water catchment, illegal discharges and invasive alien species. To address this decline,

appropriate management and conservation measures, along with environmental education, are crucial for the preservation of native crayfish populations in the park.

Keywords: *Austropotamobius palipes*, conservation measures, habitat loss, invasive alien species, anthropic impact

Challenges in Australian Astacology: Common name confusion and much, much more. A cautionary tale

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In 2020, while preparing species' nominations for conservation listing under Australian State and Federal Legislation, a series of invalid and unfamiliar common names, mainly for species of *Euastacus*, came to my attention. These common names are invalid as they are neither: 1) listed in the original taxonomic descriptions, 2) accepted by the scientific community, nor 3) names used in the scholarly literature over many years, by the experts. The invalid names have now polluted State and Federal databases, are mistaken as valid by workers unfamiliar with the Australian crayfish fauna, and of course the names are not recognised by the scholars of Australian crayfish research; creating confusion. The invalid names could cause considerable problems, could lead to negative conservation outcomes for endangered Australian crayfish, and continue to waste substantial amounts of scarce research resourcing for Australia's freshwater crayfish. In this presentation, as part of the process of setting the record straight, I will outline the valid common names and discuss the apparent origin of this avoidable problem.

But wait, there is more...

The problem is now more extensive and serious than just 'simple' invalid common names. State and Federal Government documents, legislation and the scholarly literature are also now polluted with unreliable biological and ecological information. This information is mistakenly being applied as if reliable. I will outline the known extent of the problem, provide a series of examples, discuss how the problem may be addressed, and how additional 'pseudoscience pollution events' may be avoided.

Keywords: Parastacidae, *Euastacus*, citizen "science", pseudoscience

Better alone than in bad company: trophic ecology of co-occurring invasive and native crayfish

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The North American signal crayfish has become one of the most successful invasive crayfish species in Europe. Its broad trophic niche and ability to exploit various food sources across different trophic levels, coupled with the spread of lethal crayfish disease, pose significant threats to native crayfish populations. Despite this, instances of co-occurring between invasive signal crayfish and native crayfish in invaded freshwater ecosystems remain rare, and research on their coexistence remains limited. In an Italian stream, signal crayfish was found to coexist with the native white-clawed crayfish. This study compared the trophic niches and diets of signal crayfish and white-clawed crayfish in sites where the two species co-occurred and in sites where they occurred alone. The research employed stable isotope analyses of carbon and nitrogen, utilizing SIBER and MixSIAR mixing models, along with stomach content analyses. Results revealed that when the two species were alone, they exhibited trophic niche partitioning. However, when signal crayfish co-occurred with white-clawed crayfish, their trophic niches significantly overlapped. Specifically, signal crayfish shifted its trophic niche to that of white-clawed crayfish, changing from a predatory-omnivorous diet to that of a primary consumer. Furthermore, a higher occurrence of crayfish was found in the stomachs of signal crayfish compared to white-clawed crayfish, indicating higher cannibalistic behaviour, while both species consumed substantial proportions of macroinvertebrates, detritus and periphyton when co-occuring. This study sheds light on the complex dynamics of invasive and native crayfish interactions, emphasizing the greater trophic plasticity exhibited by invasive signal crayfish during co-occurrence.

Keywords: stable isotopes, stomach content, sympatric species, trophic niche, diet

The evolution of conspicuous coloration in crayfish

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Crayfishes exhibit diverse colors, ranging from relatively cryptic tans and browns to conspicuous patterns of reds and blues. The function of these colors, especially the conspicuous colors, are unknown. Here, I present results of both phylogenetic comparative methods which explore color evolution across the crayfish phylogeny, as well as reflectance data within a blue crayfish, *Cambarus monongalensis*. Regarding the phylogenetic analysis, I found that conspicuous colors have evolved independently over 50 times; and these conspicuous colors are more common in semi-terrestrial crayfishes that construct extensive burrows. The intuitive, but not evolutionary-justified assumption when presented these results is to assume that these colors are adaptive. But contrary to this intuition, we discuss the hypothesis that coloration in crayfish is neutral. Supporting these ideas, reflectance data from *C. monongalensis* suggests that color is not correlated with size or sex. Overall, our work brings into question the traditional view of animal coloration as a perfectly adapted phenotype.

Keywords: behavior, adaptation, camouflage, crypsis, sexual selection

No, I'm Dirty Dan! Comparative methods to explore disagreement within the *Faxonius erichsonianus* species complex

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Species complexes abound in aquatic environments as newer genetics methods disagree with long-held morphological hypotheses of species relationships. North American crayfishes have seen recent taxonomic reclassification to better align morphology and genetic hypotheses, but many species complexes remain unresolved. The *Faxonius erichsonianus* complex consists of four putative species found within the Tennessee, Alabama, Tombigbee, and Coosa River systems in the southeastern United States. Previous mitochondrial analyses have recovered unresolved relationships between the four species. Given such uncertainty, we explored a combination of traditional morphological and geometric morphometric approaches to delineate members of the *F. erichsonianus complex.* We analyzed 26 traditional measurements and explored shape variation across two regions (carapace, and chelae) using 69 Form I males representing the four species. Several statistically significant traditional ratios and meristic traits were useful in delineating one to two species from the complex, but they failed to provide clear separation of all four putative species. Geometric morphometric approaches identified chelae shape as an important feature for separating species, while carapace shape evaluations were less informative. These early results highlight the use of multiple approaches to assess species separation based on morphology of closely related species.

Keywords: morphology, systematics, taxonomy

First record of the first wild marbled crayfish (*Procambarus virginalis*) population in North America/Canada and subsequent eradication efforts

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The Marbled crayfish (*Procambarus virginalis*) is an invasive parthenogenic species that has been introduced and has expanded rapidly in Europe, Asia and Madagascar. The first documented 'in the wild' detection of marbled crayfish in North America occurred near a stormwater management pond in Burlington, Ontario, Canada. Initially, a photograph of an unidentified crayfish species walking on land was submitted to the citizen science platform iNaturalist in October 2021. The identity of the crayfish was subsequently confirmed by the senior author, and a monitoring and eradication plan was developed through a partnership of governmental and non-governmental organizations. Visual searches and trapping efforts failed to detect any marbled crayfish initially in 2022, but environmental DNA (eDNA) analysis indicated the presence of marbled crayfish in the pond. Containment measures were subsequently implemented to prevent further spread to connected waters. During the winters of 2022/23 and 23/24 the pond was dewatered to attempt to eradicate the crayfish This was followed by sampling of the ponds and nearby sites in the summers of 2023 and 2024, through netting, additional trapping, and expanded eDNA monitoring in the stormwater ponds, as well as downstream and adjacent waters and wetlands. The sampling captured multiple marbled crayfish in the ponds, including young of the year confirming their continued presence and reproduction. No crayfish were captured outside the ponds to date which suggests the populations remains contained. Extensive eDNA surveys throughout Ontario are planed for the summer of 2024 and efforts to eradicate/contain the Burlington Population will continue.

Keywords: Marbled crayfish, introduction, eradication, Canada

Keep digging: two new burrowing crayfish species of genus *Parastacus* Huxley, 1879 (Decapoda, Parastacidae) from southern Brazil

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The native Brazilian crayfish fauna comprises 21 species of the genus *Parastacus* distributed in the southern region. In the last decade, 14 new species of the genus have been discovered and described. These freshwater crayfish can develop strong burrowing habits, depending on the characteristics of their habitats, and are classified under various threat categories. However, the genus is still understudied and under increasing threat of extinction mainly due to its species restricted ranges of occurrence, associated with its burrowing habits, and the fragility of freshwater ecosystems. In this contribution, we describe two new species of burrowing crayfish from the countryside of the municipality of Pelotas, Brazil. We evaluated their morphology and conservation status according to the IUCN Red List Criteria using B1 subcriterion, which takes into account the species' Extent of Occurrence (EOO), calculated based on the hydrographic basins according to the Otto Bacias shape method (level 5). *Parastacus* sp. nov. 1 differs from other burrowing species in the region by having a septagonal anteromedian lobe of the epistome, large eyes and cervical groove U-shaped; its estimated EOO is 382 km². *Parastacus* sp. nov. 2 differs by having a wide pleon and a subtriangular telson with small blunt spines on

the lateral margins; its estimated EOO is 963 km². The main threats identified include continued decline in habitat quality resulting from deforestation, habitat fragmentation, agriculture activities. Nevertheless, as we only know of one occurrence point for each new species, we suggest that both species should be categorized as Data Deficient.

Keywords: conservation, morphology, South America, taxonomy

An overview of the knowledge about juvenile stages of the South American genus *Parastacus* Huxley, 1879 (Malacostraca, Decapoda, Parastacidae)

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Freshwater crayfish exhibit parental care behavior that extends beyond the hatching of juveniles, as is the case with the South American endemic genus *Parastacus* Huxley, 1879. *Parastacus* species show complexity in parental care, which includes three post-embryonic stages. In the last decade, the genus diversity increased from eight to 23 species. Part of this hidden diversity is related to species complexes. Therefore, using additional information, such as juvenile stage morphology, might be a helpful tool to address these challenges. This work aims to provide an overview of the current knowledge about the juvenile stages of *Parastacus*. Information was obtained from literature, laboratory experiments, and observations. Our findings indicate that only five species have juvenile stage descriptions, with only one fully documented across all three stages. Descriptions are mostly short, incomplete, or cover only one stage. However, two species have detailed insights into parental care pre- and post-eclosion, and interactions between juveniles and females across stages. In our laboratory analysis, with a new species that is in the process of being described, we observed the repetition of maternal behaviors already recorded for female crayfish, and registered morphological structures of the distinct juvenile stages, such as the telson filament (stage 1) and the hooks on the dactylus of

pereopods 4° and 5° (stages 1 and 2). In the future, detailed morphological and behavioral descriptions of all stages might be an important tool to identify informative structures for differentiating *Parastacus* species, and enhance understanding of crayfish development and behavior.

Keywords: behavior, crayfish, development, freshwater, morphology

Reassessing crayfish conservation: collaborative updates of *Austropotamobius* species IUCN status

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The IUCN status is crucial for informing conservation actions on species; however, developing this status is a costly endeavor. These assessments require thorough and accurate data about distribution, range, trends, and the pressures that affect the species population. Moreover, the availability of such data is often limited or patchy, posing significant challenges. In our case study, we conducted an IUCN assessment for the Idle Crayfish (*Austropotamobius bihariensis*). During this process, the need to re-evaluate the Stone Crayfish (*A. torrentium*) emerged. In order to make sure that the IUCN status of the Stone Crayfish is both accurate and up-to-date, this presentation calls on experts in the field to join forces. The White Clawed Crayfish expert group may also find this initiative appealing since it presents an opportunity to collaborate and provide an updated taxonomic perspective.

Keywords: conservation actions, native crayfish, Red List

Can artificial light pollution affect the metabolic rate of *Procambarus clarkii* and *Pacifastacus leniusculus*?

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Artificial light pollution is an escalating concern with massive impacts on biodiversity and ecosystem functioning, particularly in aquatic environments. This study investigates the effects of artificial light pollution on the metabolic rate of two North American crayfish species, *Procambarus clarkii* and *Pacifastacus leniusculus*. Both species, known for their ecological importance and nocturnal behavior, were exposed to controlled light conditions mimicking natural daylight (1000 ± 50 lx) and artificially lit nights (20 ± 2 lx). The metabolic rate was measured using intermittent flow respirometry during both day and night cycles. Our results indicated no significant difference in metabolic rate between the control and treatment groups over a continuous 24-hour period. However, significant variations were observed during different times of the day and night, with higher metabolic rates noted under artificial night light conditions. These findings suggest that artificial light pollution disrupts the metabolic processes of these aquatic organisms, potentially due to heightened energetic demands and altered behavior caused by light pollution. This study provides critical insights into the impact of artificial light at night on aquatic species, emphasizing the need for further research into the broader ecological implications and the development of strategies to mitigate these effects on aquatic ecosystems.

Keywords: artificial light pollution, red swamp crayfish, signal crayfish, intermittent flow respirometry, diurnal cycles

Filling the gap: Starting to decrypt the genetic diversity of *Aphanomyces astaci* in Switzerland

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The genetic diversity of *Aphanomyces astaci* influences host-pathogen interactions. Genetic diversity of *A. astaci* in Europe is broad and five major genotype groups (A-E) have been identified. These groups have been linked to different low-susceptibility North American crayfish species, acting as carriers, and to varying disease virulence in susceptible European crayfish species. To detect these genotype groups in Switzerland, we adapted the qPCR protocol by Di Domenico et al. (2021) into a pentaplex qPCR. We analyzed DNA from fresh tissue samples (2020-2023) and archived Bouin's or formalin-fixed, paraffin-embedded tissue samples (1991-2020). The adapted pentaplex qPCR reliably detected genotype groups A, B, D, and E in 28 out of 46 populations. Most samples with detectable genotype groups were linked to crayfish plague outbreaks in European crayfish. Only two North American crayfish populations had detectable genotype group, both B. No sample reacted positively with the assay for genotype group C. The highest genotype variability was observed in the Rhine basin, peaking between 2016 and 2020. In southern Switzerland's Ticino basin, genotype groups E and B were found, while only B was found in the Rhone basin. Genotype group A was only detected once in 2017 in the Rhine basin. Genotype group B was first detected in 1996 in both the Rhine and Rhone subbasins. Genotype D was first detected in the Rhine basin in 1991 and reappeared in later years. Genotype group E was first detected in 1994 in the Aare subbasin and later in the Rhine basin. The use of archived samples allowed the detection of genotype groups back to the 1990s, revealing high genotype diversity in Switzerland and aiding understanding of crayfish plague spread in Europe.

Keywords: crayfish plague, genotypes, multiplex qPCR, invasive, native

Impact of land use on the presence/absence of an endangered freshwater crayfish species at local population scale on Hokkaido Island

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Land-use change can be seen as a primary threat to global biodiversity. As human activities continue to shape landscapes, understanding the response of native species to changing land use becomes increasingly vital. Freshwater ecosystems are vulnerable to the effects of land use because of the interconnected nature of terrestrial and aquatic environments. Comparing to terrestrial fauna, the effect of land use on freshwater species is still lacking. This study aims to fill this gap by examining the Japanese crayfish (*Cambaroides japonicus*) population response to varying land uses. 383 presence sites and 166 absence sites of the Japanese crayfish were obtained from previous research. We created buffer zones at distances of 10m, 50m, 100m, 150m, 200m, and 300m from both presence and absence sites utilising GIS. We computed the land-use composition within each buffer zone, including forest, agricultural, and urban areas. For each distance buffer zone, we conducted the analysis using logistic regression models, to investigate the influence of land-use types on the presence/absence of Japanese crayfish. We found that no matter in which distance buffer zone, the decreasing of forest and increasing of agriculture and urban areas around crayfish-inhabiting sites have a significant effect on local populations of Japanese crayfish existence respectively. Additionally, we found that in the 50m buffer zone, the interaction effect of agriculture, forest, and urban area proportion is the best model which suggests that Japanese crayfish exhibit microscale habitat preferences, and the importance to conserve the immediate surroundings near water bodies for supporting healthy crayfish populations.

Keywords: *Cambaroides japonicas*, land-use, conservation

Efficacy of administration routes in crayfish: Comparative analysis of subcutaneous and intrapericardial techniques using fluorescein dye

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Crayfish are emerging as model organisms for various disciplines. Moreover, decapod crustaceans also exhibit pain-like reactions and heightened anxiety when exposed to harmful stimuli, leading to short-term or persistent behavioural shifts. Awareness of decapod crustacean sentience and thus, suffering calls for refinement of current laboratory protocols. This study aims to enhance the standard methodology for injecting substances into crayfish by minimizing stress-inducing manipulation. We examined the impacts of various administration routes on the persistence of injected chemicals in marbled crayfish, its excretion, and animal survival. Fluorescein dye was used as a visual marker. It was administered via three alternative injection routes – subcutaneous (SC), intrapericardial through areola (IP-A), and intrapericardial through arthrodial membrane (IP-AM). Continuous video observations were made for a four-hour period under UV light, followed by intermittent observations at 12-hour intervals over 48 hours. The highest mortality (20%) was observed in IP-A administration. The IP-A method also provided the fastest systemic distribution of the dye in the body. Results indicated visibly higher urination frequency in IP-AM compared to IP-A. SC mirrored IP-AM outcomes without any observed mortality. We conclude that SC administration proved superior to intrapericardial methods, offering the least harmful but effective approach for crayfish injections.

Keywords: administration route, injection, crayfish, fluorescein, urine

Taxonomic revision of extant and fossil European Astacidae: A paleontological, molecular, and morphological approach

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In this presentation authors will introduce recently updated taxonomic classification of the European Astacidae. The systematics were revised using the examination of fossils, molecular phylogenetic data, internal morphology (including the gastric mill, gill structure and formulae, and mandible shape), and the genital apparatuses of museum specimens. All taxonomically valid species of the three extant genera—*Astacus, Austropotamobius*, and *Pontastacus*—along with the fossil genus *Emplastron*, are listed with their morphological features. The presentation focused on *Austropotamobius fulcisianus* (formerly *Austropotamobius pallipes*, which included *A. pallipes italicus*), and the authors seek to address the taxonomy of these problematic taxa.

Keywords: fossil, molecular, internal morphology, genital apparatus, systematics

Characterizing the role of RNA editing in clonal and invasive marbled crayfish

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The marbled crayfish (*Procambarus virginalis*) is known for reproducing through parthenogenesis and for thriving in diverse environments despite genetic uniformity. A-to-I RNA editing, driven by the ADAR enzyme family, targets dsRNA and can change codons, thus diversifying the transcriptome and potentially adapting protein function to different environmental conditions. We have identified a conserved homologue of ADAR2 in the marbled crayfish genome, which is dynamically expressed in many tissues and embryonic stages. Transcriptomic analysis revealed over 600,000 unique A-to-G hyper-editing sites with an editing frequency of approximately 15 sites per 10 kilo base-pairs. Additionally, marbled crayfish have hundreds of genes recoded by ADAR2, with many recoding events occurring in embryonic stages, particularly before zygote genome activation. This may represent an important mechanism for transcriptome diversification. *In vivo* ADAR2 knockdown via dsRNA interference (RNAi) resulted in a significant reduction of the total hemocyte count, suggesting that ADAR2 knockdown impairs hemocyte differentiation and potentially affects its pro-inflammatory function. qPCR analysis of ADAR2 knockdown showed the lowest expression levels at 2 weeks post-knockdown. Preliminary plus-maze behavioural trials at 6 weeks post-injection indicate that ADAR2 knockdown animals spend significantly more time in the dark zone, an indicator of anxiety-like behaviour, suggesting a potential behavioural consequence of ADAR2-mediated RNA editing. Taken together, our findings raise the possibility that RNA editing provides a mechanism for the adaptability and invasion success of marbled crayfish.

Keywords: RNA editing, marbled crayfish, epigenetics

Seasonal microhabitat use by Brawleys Fork crayfish, *Cambarus williami*, a species of conservation concern

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Cambarus williami (Brawleys Fork Crayfish) is endemic to the Stones River and Collins river watersheds in central Tennessee. This species is listed as a state endangered (S1) species and is proposed for federal listing under the Endangered Species Act. Our aim was to evaluate predictors of seasonal habitat selection and crayfish species assemblages associated with *C. williami*. Nine 200-m reaches in three streams (East Fork Stones River, Hollis Creek, and Mountain Creek) were sampled in Spring (March 2021) and Fall (September-October 2021) seasons. Microhabitat conditions were measured and crayfishes collected from six randomized 0.5-m^2 quadrat samples per 200-m reach in the Spring and Fall seasons. Crayfish associates of *C. williami* were: *C. graysoni*, *C. rusticiformis*, *C. tenebrosus*, *Faxonius compressus*, and *F. placidus*. *Cambarus williami* was most abundant in riffle mesohabitats, moderately abundant in run mesohabitats, and least abundant in pool mesohabitats, and this pattern remained consistent across two seasons of the year. In both Spring and Fall seasons, there were no detectable differences in microhabitat use between adults and juveniles, or between adult males and adult females, or between Form I and Form II males. These results suggest that *C. williami* prefers riffle mesohabitat, although the species also occupies run and pool mesohabitats. Given the level of endemism the species displays, maintenance of quality riffle habitat within its range is likely important to its persistence.

Keywords: ecology, community, habitat

Winter behaviour of adult noble crayfish (*Astacus astacus*) under natural conditions

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Only little is known about the winter ecology of noble crayfish (*Astacus astacus*) under natural conditions. We investigated the winter behaviour of N = 80 adult noble crayfish (n = 40 female and n = 40 male crayfish, respectively), equipped with implanted PIT tags, in a small pond (525 m²) from December to March using stationary PIT antennae. Generalized linear mixed models were used to analyse crayfish activity as a function of daytime, temperature, water depth, and sex. The choice of bottom substrate was analysed using selection ratios. Tagging mortality rate after PIT injection was 14.5%, and the relative probability of winter survival was 76%. The mean minimum distance moved per day (± SD) over the course of the study was 0.4 ± 0.5 m/d. We found significant effects of daytime, temperature, and water depth on crayfish activity, with distances moved increasing at night and with elevated temperatures, and decreasing in deeper waters. Male crayfish moved about twice as often as females. However, during active periods, female crayfish moved significantly longer distances (7.5 ± 6.7 m/d) compared to males (5.6 ± 3.1 m/d). Selection ratios indicated general preferences for hiding places, coarse particulate organic matter (CPOM), and fine particulate organic matter (FPOM), whereas coarse gravel substrate was significantly avoided. Our study suggests that noble crayfish regularly show high activity rates during winter and that increasing temperatures as a consequence of climate warming might impact the autecology of *Astacus astacus* with potential ecological and fitness consequences.

Keywords: autecology, passive telemetry, crayfish conservation, winter ecology

Winter mortality and growth of juvenile noble crayfish (*Astacus astacus*) under realistic environmental conditions – implications for conservation management

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Noble crayfish (*Astacus astacus*) are endangered in many parts of Europe. Conservation efforts, including stocking measures, are widely conducted to safeguard the species and associated ecosystem functions. Due to limited availabilities of adults, juvenile noble crayfish are frequently released into lakes and rivers during autumn, usually without information on their fate and growth during the following winter. To investigate size- and density dependence of winter mortality and growth of newly stocked juvenile noble crayfish we conducted a highly replicated mesocosm experiment (N = 108 mesocosms stocked with a total of N = 360 crayfish in three density treatments) in a small artificial lake in northern Germany. The experiment was carried out at water temperatures between 0.7 °C – 9.1 °C from mid-December until mid-March and stocking densities of 20, 60, and 120 crayfish per square meter with unlimited food availability. Winter mortality rates were analysed using multiple Cox-Regression and found to be generally low and strongly size dependent. Specifically, a winter mortality rate of 9.2% was observed and crayfish with a total length (TL) above 34 mm all survived. The relative risk of mortality in dependence of TL increased by 41% - 94% (95% CI) with every decreasing centimetre of TL at the onset of winter. Increasing density had no effect on winter survival, but tended to reduce growth. Our work indicates that stocking measures of noble crayfish for conservation reasons can decrease costs and increase prospects of success if juveniles larger than 30 mm TL are used as stocking material.

Keywords: stocking, size-dependency, fisheries management, invasive species, mesocosm experiment

Coevolution of genus *Branchiobdella* with their crayfish hosts generated new species?

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Annelid commensals of the genus *Branchiobdella* Odier, 1823 live on surface or in gill cavities of freshwater crayfish. The genus includes 9 species: *Branchiobdella astaci* Odier, 1823, *B. parasita* (Braun, 1805), *B. pentadonta* Whitman, 1882, *B. hexadonta* Grüber, 1883, *B. italica* Canegallo, 1928, *B. balcanica* Moszynsky, 1938, *B. kozarovi* Subchev, 1978, and recently described *B. bulgariensis* Subchev, 2021, and *B. turkestanensis* Gelder, 2024. The objective of the present research was to get a better understanding of the phylogenetic relationships of branchiobdellidan species collected from European crayfish belonging to genus *Astacus* Fabricius, 1775, and *Austropotamobius* Skorikow, 1907, using mitochondrial cytochrome c oxidase subunit I sequences (*COI*) as a molecular marker. Previous studies observed preference of certain branchiobdellidan species for certain crayfish species. In addition, our previous work indicated congruent phylogenetic patterns within *Austropotamobius* and specific branchiobdellidan species showing similar evolutionary histories that implied their coevolution. In this study 55 unique sequences were found that were compared to 113 unique sequences for this genus present in the NCBI GenBank database. Bayesian-based analyses revealed distinct *COI* groups that could not be joined to previously known species and, combined with the results of mPTP and bPTP species delimitation methods, suggest the existence of new species. Potential new species were observed within recognized *Branchiobdella* species (apart from *B. astaci* and *B. balcanica*) present on different crayfish species indicating significant long-term coevolution.

Keywords: branchiobdellidans, *COI*, species delimitation, native European crayfish

Crab cut, what about crayfish?

Koese B

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There is no doubt that crayfish can damage vegetation. It is often said that they cut plants, which is factually correct, but different from the way most people think that crayfish cut. Often, only crayfish researches are aware that the chelae are of minor importance in the process of destroying aquatic vegetation. In this presentation, I'll discuss and demonstrate the role and power of the mandibles and give some examples of affected vegetation in the Netherlands.

Keywords: ***Procambarus clarkii, Stratiotes aloides***

The North-American signal crayfish in the Drava River basin in Slovenia: latest spread and the first eDNA results

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The North-American signal crayfish (*Pacifastacus leniusculus*), alien and invasive to Europe, is a very problematic species particularly because it transmits a fungal-like parasite *Aphanomyces astaci* – the causative agent of the crayfish plague, disease which is lethal to native crayfish species. Until our study, *P. leniusculus* distribution in the Drava River in Slovenia was known only from Dravograd at the Austrian border downstream to the artificial accumulation lake at Ptuj. Here, we present new distribution data for *P. leniusculus* Slovenia, acquired through systematic sampling campaign with crayfish traps combined with visual inspection and/or e-DNA targeting of *P. leniusculus* and crayfish plague. The survey area expanded over 142 km of the Drava River in Slovenia from the Austrian to the Croatian border. Twenty-nine locations in the Drava River and 19 tributaries were checked in July and August 2022 and 2023.

Our results show, that in the Drava River live specimens of *P. leniusculus* were recorded in 21 locations, among these four locations were new, and we detected a further spread downstream between 2022 and 2023. No specimens of *P. leniusculus* were caught or observed in the tributaries in both years, however, three e-DNA samples were positive for *P. leniusculus* presence and two for *A. astaci* in 2023. Our results show persistent and vital population of *P. leniusculus* in the Slovenian part of the river Drava, likely invading some tributaries and carrying the crayfish plague pathogen, which increases the concerns of biosafety.

Keywords: freshwater, crayfish, crayfish plague, IAS spread, new finds

Crayfish pet trade as a gateway for the introduction of known and novel viruses into Europe

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The pet trade is among the major introduction pathways of invasive non-native species and also an important pathway for the introduction of novel diseases. Freshwater crayfish are frequently translocated for aquaculture and pet trade purposes, with species from families Cambaridae (of North American origin) and Parastacidae (of Australian and New Guinean origin) commonly available on the EU and global markets. Besides the most frequently studied crayfish pathogens, such as *Aphanomyces astaci* and white spot syndrome virus (WSSV), ornamental crayfish may carry novel pathogens, which may potentially lead to the emergence of novel diseases in both native and already established non-native crayfish. This is especially problematic in the case of viruses, which represent an important group of crayfish microbial pathogens but are significantly understudied. Here we analyzed the viromes in the hepatopancreas of four crayfish species acquired in the international pet trade in Europe (*Procambarus clarkii, Procambarus alleni, Cherax holthuisi, Cherax quadricarinatus*) using an HTS-based metagenomic approach. We identified several known and novel virus sequences and further analyzed 27 longer virus-like sequences, out of which 63% (17 / 27) were similar to

6 known viruses previously associated with crayfish or crustacean hosts, including the detected WSSV in *P. clarkii*. Additional 10 virus-like sequences represented novel and divergent RNA viral sequences most similar to *Picornavirales*, *Elliovirales*, *Hepelivirales* and *Ghabivirales*. We discuss our findings in the context of species identity, their phylogenetic relationship as well as geographical origin and report results of phylogenetic analyses of selected virus-like sequences and their possible importance in terms of crayfish disease ecology.

Keywords: virome, ornamental crayfish, hepatopancreas, RNA and DNA viruses

Misconceptions in the conservation of crayfish – what could we do better?

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European crayfish populations continue to decline in numbers throughout Europe. In Switzerland in the centre of Europe four different species are native to our streams and lakes, but they are threatened by habitat destruction, water pollution, invasive crayfish species and with them the crayfish plague. Alien invasive species are one of the main drivers of biodiversity loss around the world. How can we stop them? The authors report first-hand experiences. The catch and the culinary utilisation of invasive crayfish in large rivers and lakes has not led to a decline or even eradication. Utilisation is rather an incentive and by that this has resulted in the proliferation of invasive crayfish. Containment seems more promising. By building crayfish barriers invasive species have been stopped or at least slowed down in their further spread. Drastic measures as filling up infested ponds or the use of predatory fish such as eels were particularly successful to reduce or eradicate invasive crayfish species. Helping does not always help – it sometimes makes the situation worse. Revitalizations by restoring habitats have led in some cases to the disappearance of populations. Reintroduction campaigns of native crayfish should use a critical mass of individuals and the expected water quality should be good or even better. We protect what we know. Knowledge about the genetic diversity and population structure of our native populations helps to preserve them and, if necessary, to support them so that our crayfish remain a key species as part of our ecosystem in the future.

Keywords: crayfish barriers, containment, habitat restoration, eradication

Influence of flow regime and drought on seasonal population dynamics of co-occurring stream crayfish species

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Extreme weather events like droughts can amplify threats posed by other biotic and abiotic stressors on crayfish populations. Climate change is expected to increase drought frequency and severity in many regions, but knowledge is limited on how these events will affect crayfish persistence. Here we sought to understand how seasonal drought and stream permanence influence the density, occupancy, detection, colonization, and extinction dynamics of four co-occurring crayfish species in the Spring River Drainage of Arkansas and Missouri, USA. We quantitatively sampled surface and hyporheic zones to estimate occurrence of crayfish at 23-25 intermittent and permanent stream sites in the summer and autumn of 2022 and 2023. We used multi-season dynamic occupancy models to examine crayfish population dynamics under different flow regimes in periods of flow and seasonal drought. Seasonal drying and stream permanence influenced crayfish populations, but the effects were species- and habitat-dependent. Because of its higher colonization rates and lower extinction rates, *Faxonius marchandi* populations may experience population growth or stability through seasons despite the seasonal drought conditions that occur in autumn. *Cambarus hubbsi, Faxonius punctimanus,* and *Faxonius ozarkae* appear to be more sensitive to fluctuations in water availability than *Faxonius marchandi*. Crayfish used both intermittent and groundwater streams as well as both surface and hyporheic zones across seasons, so future sampling designs and conservation decisions should consider these differences in habitat for crayfish species. Improved understanding of crayfish population dynamics can better inform population viability and identify threats to population persistence in the context of future environmental changes.

Keywords: colonization-extinction dynamics, drought, stream permanence, detection, occupancy

Harmonizing transboundary monitoring of *Austropotamobius pallipes* using environmental DNA (eDNA): The PALLIPES Project

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Austropotamobius pallipes, a decapod crustacean inhabiting ecologically valuable watercourses, is considered a sentinel species for ecosystem health. Its native range extends from the eastern Balkan Peninsula to Spain, reaching its northernmost distribution in the UK. Since 2010, it has been classified as endangered by the IUCN Red List under the A2ce criteria. Significant efforts are being made to expand knowledge of its distribution and the factors influencing its population viability. The current PALLIPES project employs environmental DNA (eDNA) techniques alongside traditional methods to monitor the distribution of *A. pallipes* in the transboundary region of Italy and Slovenia. This initiative aims to harmonize monitoring protocols and gather comprehensive data for conservation across different countries. Here, we present data on *A. pallipes* populations collected during a five-year brown (*Salmo trutta*) trout eradication campaign in Italy. Our findings demonstrate that the ongoing removal of this introduced predator, first introduced in the 1970s, supports the recovery and growth of *A. pallipes* populations. Additionally, the preliminary results from Slovenia provide a first framework for understanding the species' distribution in this area through eDNA revealing a decline in the abundance of the *A. pallipes* in comparison to past trapping data. The ongoing project confirm the decline of the A. pallipes population in Slovenia and the increase of the population size in Italy following the reduction of predator pressure. Furthermore, they underline the feasibility and suitability of eDNA to monitor threatened species, the establishment of common surveillance protocols to monitor *A. pallipes*, its main pathogen and alien competitor.

Keywords: white clawed crayfish, eDNA, distribution, management, conservation

Protocols for studying *Aphanomyces astaci* and its host interaction

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The crayfish plague pathogen, *Aphanomyces astaci,* is responsible for the decimation of the European and Asian populations of freshwater crayfish and is threatening other crayfish species in the world, such as the Australian, Madagascar, and South American species. Currently, there is a need for a better comprehension of the *A. astaci* biology and crayfish interactions, specifically the resistance and tolerance immune mechanism. This contrasts with the high number of molecular applications developed for the identification of *A. astaci* during the last decades. Investigations on *A. astaci* biology and host-pathogen interactions require a robust basic knowledge on the developmental biology of the pathogen in order to reproduce life stages and to perform infection experiments. A great piece of work in this area was carried out during the 1960's to 80's in University of Uppsala, Sweden. However, contributions in this area has been scarce since then. Thus, the purpose of this work was to update previous protocols, to generate new guidelines to reproduce key developmental biology stages of *A. astaci* and to identify crayfish populations with higher resistance and tolerance to this pathogen. This study also reviews methodologies and guidelines for the isolation and the *in vitro* production of all developmental stages as well as for the diagnosis of crayfish plague.

Keywords: crayfish plague, tolerance, resistance, zoospores, diagnosis

Significance of the "Žumberak – Samoborsko gorje" Nature Park area for the conservation of the stone crayfish populations

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"Žumberak – Samoborsko gorje" Nature Park in North-Western Croatia is a 334 km^2 area of mainly Dinaric karst. Protected by the national law in 1999 as the V IUCN category, it is characterized by spectacular mosaic landscapes of vast forests and grasslands and great diversity of endangered species and habitats. This secluded area hides more than 840 water springs and 260 surface and underground water flows. The stone crayfish *Austropotamobius torrentium*, the smallest autochthonous European crayfish species, inhabits central and south-eastern regions of Europe and is widespread in the Nature Park area which represents significant climate refugium characterized by high biodiversity. Twenty years of research confirm that this relatively small area is inhabited by four genetically, morphometrically and meristically distinct stone crayfish groups which represent geographically isolated and deeply divergent evolutionary significant units (ESUs). These were determined based on molecular analysis of mitochondrial and nuclear genes and morphological data analyses. The Balkan is one of the most important Pleistocene glacial crayfish refugium in Europe, and the Dinaric Karst is recognized as the hot spot of European crayfish biodiversity. However, threats like progressive habitat deterioration, decline in water quality, climate change, and the presence of alien invasive crayfish are affecting the crayfish populations. This calls for immediate prioritization in shaping and implementing consistent conservation measures in the Nature Park area, especially considering negative population trends among crayfish. In this presentation, threats and priorities in the protection of stone crayfish populations within the Nature Park will be highlighted.

Keywords: Dinaric karst, *Austropotamobius torrentium*, endangered species, climate refugium, ESU

Aphanomyces astaci in cambarid crayfish from the native range of *Faxonius limosus*: in search of the elusive genotype group E

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The crayfish plague agent, *Aphanomyces astaci* is spreading together with its original hosts, North American crayfish. The pathogen in its US native range has higher genetic variation than in the invaded ranges, mostly without clear host-specific associations. However, across most of Europe, its common genotypes are associated with specific American crayfish. Genotype group E seems to spread only by *Faxonius limosus*, presumably due to a founder effect after its single introduction to Europe. In our study, we investigated *A. astaci* genetic variation, distribution, and prevalence in the native range of *F. limosus* in eastern USA, focusing on Pennsylvania (from where it was supposedly introduced). We screened eight crayfish species from 49 sites for this pathogen: *Cambarus bartonii*, *C. robustus*, *Faxonius immunis*, *F. limosus*, *F. obscurus*, *F. propinquus*, *F. rusticus*, and *F. virilis*. *Aphanomyces astaci* was confirmed by quantitative PCR in 76% of sites in all but one of the examined host taxa. The pathogen′s mitochondrial haplogroup A was confirmed at 11 sites in *F. limosus*, *F. rusticus*, *F. virilis* and *C. bartonii*, and haplogroup B at one site in *F. propinquus*. Two additional genotyping methods based on nuclear markers confirmed the presence of at least four multilocus genotypes, none of which is known from Europe. Our failure to detect genotype group E suggests it is not dominant in the native range of *F. limosus* and may have limited spatial distribution.

Keywords: crayfish plague, eastern USA, genotyping, host specificity, prevalence

Improving trapping effectiveness for controlling the red swamp crayfish *Procambarus clarkii*

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In Tuscany (central Italy), a population of the alien red swamp crayfish *Procambarus clarkii* is present in Lake Romena threatening the native white-clawed crayfish *Austropotamobius pallipes* complex present in a national park close by. The aims of the study were to reduce the *P. clarkii* population abundance through trapping activities and to improve effectiveness by using three different trap types: two types of wire mesh traps (cylindrical and rectangular) and Artificial Refuge Traps (ART). We also aimed to assess the composition of the lake community, particularly the presence of crayfish predators (using eDNA), and the potential spread of *P. clarkii* outside the lake. The control activities conducted from 2022 to 2024 led to a decrease of at least 50% in the abundance index (Catch Per Unit Effort, CPUE) of crayfish population. Considering data of 2022 and 2023 (2024 activities are still ongoing) cylindrical traps, with larger mesh and narrower entrance, trapped the highest number of individuals (CPUE: 1.92), especially larger ones and males, while ARTs were the less efficient (CPUE: 0.25) but they captured relatively more females and smaller individuals. eDNA sampling highlighted the presence of a community mainly composed of alien species and some crayfish predators (e.g. fish). The surveys conducted in the surrounding areas revealed the presence of *P. clarkii* downstream of the lake. Control activities using different types of traps should be maintained to further decrease the population abundance, while additional monitoring activities should be carried out to better assess the spread of the species outside the lake and to promote containment activities.

Keywords: alien species, Artificial Refugee Trap, wire mesh trap, Central Italy, bycatch, crustacean

Towards a risk-oriented national monitoring in the Netherlands

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A red swamp crayfish waving on a cycle path is not a rare sight in the Netherlands. Over land or in the water, seven non-native crayfish species are currently spreading in the country. If predictions hold, almost all Dutch waters will be colonized by crayfish within 15 years. Their spread does not come without a cost. Water and conservation managers are increasingly confronted with the damage they cause, such as the decline of water quality, disappearance of aquatic vegetation and erosion of banks. In response, a preparation of the Dutch national plan to control populations of non-native crayfish has recently been initiated. In order to identify suitable control actions, however, information is needed on crayfish distribution, their densities and the damage they cause. Such a comprehensive overview is lacking in the Netherlands. Moreover, the few monitoring programs currently taking place differ in their objectives, applied methodology and scale. We explored the possibilities of establishing a national monitoring program of non-native crayfish that also takes risks into account; in other words, the probability that a crayfish species at a certain density will cause damage. Firstly, we conducted an inventory of current monitoring programs in the Netherlands. Secondly, we interviewed water managers and other stakeholders to get a realistic idea of the practical application of these monitoring programs. The outcome is a set of preconditions that should be considered if a national monitoring is to be established. We will present these preconditions and discuss their relevance to risk-oriented monitoring.

Keywords: monitoring, risk, non-native species, probability, damage

Spatio-temporal occurrence and abundance of *Faxonius limosus* (Decapoda: Cambaridae) in two North-western subalpine lakes (Italy)

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Biological invasions are among the most influential human-driven processes impacting Earth's ecosystems, with freshwaters being particularly vulnerable as transition ecosystems. Invasive crayfish impact substantially these environments and their ecosystem services. Lakes Orta and Mergozzo are two subalpine lakes (NW Italy) where the invasive American crayfish *Faxonius limosus* is well established. The current study examined the population structure, and its temporal and spatial distribution over a one-year period (2021-2022). Monthly littoral trap samples (taken in the same week in both lakes) enabled us to measure, weigh, and sex-determine all gathered individuals and asses their relative abundance measured as Catch Per Unit Effort (CPUE; number of crayfish per trap per trapping night). The population structure was then examined to determine: 1) the invasive species' abundance, 2) size variation between the two lakes, and 3) the invasive species' potential use as waste reduction strategy by producing biofilm for sustainable food packaging. The total number of individuals were higher in Lake Orta (86) than in Lake Mergozzo (57) and dominated by males (sex ratio M/F of 1.39 and 3.07, respectively). Average abundance was comparable between the two lakes, but species size distribution was greater in Lake Mergozzo than in Lake Orta. Crayfish-derived film shows higher melting temperatures, mechanical resistance and slower degradation than the commercial plastic one. Therefore, producing crayfish-based biofilm from shell-derived chitosan from *F. limosus* may be a practical strategy for waste reduction as its crayfish-derived film is qualified to be introduced into the commercial market, at least in countries where their use is legal since

they are native. Our research also contributes significantly to the understanding of the ecology of *F. limosus* in deep lakes.

Keywords: annual trend, biometry, biofilm production, freshwaters, invasive species

Exploring the Evolution of *Aphanomyces*: Were ancestral Oomycetes such effective parasites?

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Saprolegniales (Oomycetes) are a group of oomycetes that comprises plant and animal parasites and also saprotrophs. While extensive research has explored the devastating impact of plant parasitic oomycetes, particularly within Saprolegniales, limited knowledge exists regarding the evolution of lifestyles within this group. This study focuses on understanding the patterns and rates of evolution and diversification of saprotrophic and parasitic lifestyles, with a specific emphasis on the genus *Aphanomyces*, which includes the crayfish plague disease agent *A. astaci.* In order to study patterns and rates of the evolution and diversification of the saprotrophic and parasitic lifestyles, we have performed comparative phylogenetic analyses (Bayesian inference and Maximum likelihood) on 2950 bp nuclear internal transcribed spacer (ITS) ribosomal DNA sequence database, using all information available in the GenBank plus the one generated by our group. The results indicated that in the Saprolegniales there is a strong phylogenetic signal for the trait lifestyle (saprotrophic, animal and plant parasitic), meaning that the distribution of this trait is closely associated with the evolutionary history and phylogenetic relationships of the species. Our results show that ancestral oomycetes were parasites, most likely infecting plants, while the saprotrophic lifestyle seem to be a derived character that evolved later. In addition, the acquisition of the saprotrophic lifestyle appears to be first adopted by ancestral Saprolegniales species. However, plant parasitism in the Saprolegniales was lost until recent innovations acquired during events of diversification of the genus *Aphanomyces* (≈50 Mys).

Keywords: *Aphanomyces*, evolutionary history, ancestral state reconstruction, pathogens, parasites

Monitoring of native and invasive crayfish from environmental DNA: experience from Czech waters

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Monitoring crayfish using analysis of environmental DNA (eDNA) has become a common approach in recent years that can complement or replace traditional capture methods. The popularity of eDNA screening for crayfish is growing despite methodological challenges in comparison with other commonly studied aquatic animals that continuously release large amounts of eDNA into the surrounding water, such as fish and amphibians. In our research, we use low-cost do-it-yourself sampling equipment and species-specific quantitative PCR assays to detect several crayfish known from Central European waters, both native and invasive, together with screening for the crayfish plague pathogen *Aphanomyces astaci*. I will present the results of several, mostly small-scale, case studies focusing on all three North American invaders known from Czech waters (marbled crayfish, spiny-cheek crayfish and signal crayfish) as well as on the two native species (noble crayfish and stone crayfish), and highlight our experiences with the advantages and limitations of this approach. Although eDNA screening has not yet been implemented in routine biological monitoring in Czechia, we hope that recent technological advances in this field will stimulate both the demand for such analyses and the offer by applied laboratories. This may be facilitated by the availability of sampling "kits" allowing efficient eDNA collection by stakeholders.

Keywords: eDNA sampling, species-specific qPCR, do-it-yourself, *Aphanomyces astaci*

Genetic and population characteristics of stone crayfish *Austropotamobius torrentium* (Schrank, 1803) in the cave Sušik

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The stone crayfish, *Austropotamobius torrentium* (Schrank, 1803), is the smallest native European freshwater crayfish and a priority species listed on the EU's Habitats Directive. It is distributed in continental and alpine biogeographical regions in Croatia, with Northern central Dinarides presenting its biodiversity hotspot. This study aimed to determine the genetic and population characteristics of a rare subterranean stone crayfish population in the cave Sušik. A study was conducted between July and October of 2023, and a total of 181 individuals were caught at the mouth of the cave Sušik. They were sexed, and their length and weight were measured. Also, tissues (pereopods) were sampled for further genetic analysis. The results showed that the population is stable, with 1.01 individuals per m^2 and an even sex and size-class distribution. A deviation from the usual year cycle was recorded (e.g., the earlier presence of attached spermatophores in late August and a shift in the moulting period), possibly indicating an adaptation to the subterranean environment. Phylogenetic analysis of the mitochondrial *COI* gene placed all individuals into the previously established phylogroup ŽPB, within two already known haplotypes. Microsatellite analysis established poor gene flow between the Sušik population and geographically close populations belonging to the same phylogroup, with a high number of private alleles, pinpointing the isolation of the studied population. This study therefore provides a baseline for further comparisons of stone crayfish populations above and below ground, considering it is only the second subterranean population described for this species.

Keywords: native crayfish, conservation genetics, morphometry, karst cave, Croatia

Eco - epidemiology of *Aphanomyces astaci* in Switzerland

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Aphanomyces astaci is the causative agent of crayfish plague, causing high mortality in native European crayfish. On the other hand, invasive North-American crayfish are less susceptible and serve as carriers. The epidemiology of crayfish plague in Switzerland and pathogen dynamics in natural water bodies are largely unknown. Our aims were to (1) monitor 40 years of *A. astaci* occurrence in Switzerland, and (2) characterise pathogen dynamics in natural waters following an outbreak. Crayfish plague occurrence was studied both retrospectively and prospectively. Necropsy reports of native crayfish from Switzerland analysed for crayfish plague through histology, mycology and conventional PCR were evaluated (1968-2020). Archived formalin-fixed paraffin-embedded material (1991-2020) was re-evaluated by qPCR. Invasive crayfish species were captured (2020-2023) and analysed for pathogen detection by qPCR. After a crayfish plague outbreak, eDNA was investigated over 6-18 months by filtering water (1800-2400ml) along the course of water body with 0.45µm Sterivex Filter® followed by qPCR. Crayfish plague was diagnosed in 54/192 (28%) necropsy reports evaluated, with a first case from 1980. The number of positive cases increased by 15% after re-evaluation of archived samples by qPCR. *A. astaci* was detected in 4/4 populations of invasive crayfish populations already analysed by qPCR. Preliminary eDNA results from 633 water samples collected in three rivers and one pond indicate that this method can be used for pathogen detection after an outbreak, that the pathogen concentration is usually very low and that there are influences of season and water body characteristics on eDNA detection probability.

Keywords: crayfish plague, *A. astaci*, eDNA, spatio-temporal spread, oomycete

Evaluating response efforts to Red Swamp Crayfish invasion in Michigan, USA

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Invasive crayfish harm ecosystems around the world by preying on native plants and animals, destroying habitat, and outcompeting native crayfish. The most widespread invasive crayfish species is the Red Swamp Crayfish (RSC; *Procambarus clarkii*), which is native to the Mississippi and Gulf Drainages of North America but is invasive in portions of North America, including Michigan, USA. In the summer of 2017, live RSC were first detected in the state of Michigan's waters and have since been found in 9 distinct population complexes, primarily consisting of man-made ponds. Following the initial detection of RSC in Michigan, a multi-agency response plan focused on detection, control, and containment was implemented. Given the relatively isolated occurrences of RSC, intensive response efforts have been attempted to eradicate or control populations in the state. Initially, Michigan Department of Natural Resources and Michigan State University, in collaboration with other partners, focused their efforts on trapping and removed close to 200,000 RSC. Starting in 2021, innovative pyrethrin-based pesticide treatments became a primary focus of the RSC response. As part of an adaptive management strategy, catch per unit effort data have been collected through time to evaluate the effectiveness of trapping plus pesticide treatments compared to only trapping in the same ponds. We will present temporal trends in trapping data to explore what control strategy has been most effective at reducing populations of RSC. Results from this evaluation will help inform future control efforts in Michigan and other management agencies tasked with implementing control strategies for invasive crayfish.

Keywords: Red Swamp crayfish, invasive species, pesticide, trapping, *Procambarus clarkia*

Procambarus clarkii in Andean Waters: Ecological impacts and mitigation efforts

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In 2013, the invasive crayfish species *Procambarus clarkii* was introduced into the high Andean lake of Yahuarcocha in Ecuador. By 2018, the population had increased significantly, coinciding with the last recorded presence of submerged macrophytes. By 2020, the abundance of *P. clarkii* had declined, with only a few individuals detected in traps or after several days of monitoring in areas adjacent to the lake. *P. clarkii* is considered a major factor driving the lake into a hypereutrophic state, exacerbated by anthropogenic influences such as sewage and influent pollution. This crayfish species is also associated with changes in the composition of the

local macroinvertebrate and fish communities. Since 2019, various efforts have been made to improve the water quality of the lake. These efforts include the control and monitoring of influents and effluents, the use of ultrasound technology, eDNA monitoring and the reintroduction of aquatic plants. Initially, these measures resulted in a resurgence of submerged macrophytes in 2021. However, in 2023 the regrowth of the *P. clarkii* population reduced the submerged macrophytes again. Currently the species is present in twelve water bodies in the Ecuadorian highlands.

Keywords: *Procambarus clarkia*, Andean lakes, macrophytes, monitoring

Energetic Cost of Burrowing in Red Swamp Crayfish

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Red Swamp Crayfish (*Procambarus clarkii*) are one of the most invasive crayfish species worldwide. Because they live part of their lives in terrestrial burrows that can cause damage to levees and reduce the effectiveness of surface-water treatment methods, it is important to determine factors that encourage or discourage burrowing behaviors. We hypothesized that burrowing activity is energetically expensive and caloric density of crayfish would decline the deeper they burrowed. To test this hypothesis we placed 20 crayfish in artificial burrowing chambers and lowered the groundwater level at a rate of 4 cm/day for 8 days. Crayfish that refused to burrow by day 2 had a lower median caloric density than those that had burrowed 8 cm within the same time period, but this difference was only marginally significant ($p = 0.11$). Caloric density of non-burrowers significantly declined from initial values ($p = 0.022$). However, neither crayfish that burrowed 8 cm by day 2 nor crayfish that burrowed 32 cm by day 8 had a significantly lower caloric density than initial values ($p = 0.0.924$ and 0.715 respectively). Taken together, these results do not support our hypothesis. Energetic costs of burrowing were barely detectable and less than the cost of remaining at the surface. Potential explanations are that non-burrowers expend energy via frequent wandering activity and/or there is an energetic cost associated with desiccation. Additional experiments are being planned to increase our number of replicates and to video crayfish behavior and compare activity levels between burrowers and non-burrowers.

Keywords: *Procambarus clarkii*, energy, caloric density, burrowing

Effects of pleopods removal on survival and sterility of signal crayfish

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The study explores sterilization methods for signal crayfish, an invasive species posing ecological threats in non-native habitats. Signal crayfish, introduced for consumption, have rapidly spread throughout Japan, causing ecological damage and resource competition. Current population control efforts face challenges like biased catches and legal restrictions, prompting the investigation of alternative methods. One promising approach involves sterilizing crayfish by disrupting their brood care behavior, crucial for reproduction. Female crayfish carry eggs in their pleopods, and removing these could hinder their ability to care for offspring. In this study, we conducted a controlled feeding experiment using signal crayfish caught in Hokkaido from July to November 2023, and verified whether the removal of pleopods could be a cause of mortality, to what extent the pleopods would be restored by molting in the same year, whether it would inhibit brood care, and the degree of its effect. In total, 108 individuals were used. As a result, it was found that removal of the pleopods did not significantly increase mortality rates (3.8%), but did reduce brood care ability by 97.0%. Immediate molting post-pleopodal removal does not restore reproductive capacity. This research highlights the potential of pleopod removal as a cost-effective sterilization technique for signal crayfish. While further validation is needed, this method offers a promising avenue for managing invasive populations, complementing trap-based extermination efforts.

Keywords: signal crayfish, invasive species, sterilization

Molecular detection of *Aphanomyces astaci* – experience with an improved species specific qPCR assay

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Crayfish plague is caused by the parasitic oomycete *Aphanomyces astaci,* a lethal disease for European freshwater crayfish and is mainly spread through human mediated translocation of North American crayfish that act a vector for the parasite. Crayfish plague diagnostic is commonly based on *A. astaci* specific qPCR, followed by sequencing for confirmation. However, *Aphanomyces fennicus*, a newly discovered and described oomycete, is amplified with the well established *A. astaci* qPCR assay that was previously recommended by the World Organisation for Animal Health (WOAH). Consequently, this may lead to false-positive results. We have developed and published an *A. astaci* assay with improved specificity, ensuring no cross-reaction towards *A. fennicus*. The improved *A. astaci* qPCR assay has been verified across three different national reference laboratories for fish and crustacean diseases in Norway, Sweden and Finland, using the respective workflow in each laboratory. The improved assay shows similar sensitivity with the established assay and was highly specific for *A. astaci*, not amplifying *A. fennicus* or other closely related *Aphanomyces* spp., and is now recommended by WOAH. Additionally, sequences from environmental DNA (eDNA) samples collected in a Norwegian river in April 2022, suggest the presence of *A. fennicus* in Norwegian waters. eDNA samples collected in Norway during 2022 and 2023 in late May to September have been screened with both the established and improved assay. Our eDNA results suggest that *A. fennicus* is detected at lower water temperatures, and not during the eDNA sampling from late May to September for crayfish plague monitoring.

Keywords: crayfish plague diagnostics, environmental DNA monitoring, molecular diagnostics, *Aphanomyces astaci*, *Aphanomyces fennicus*

Genetic screening of Tyrolean noble crayfish (*Astacus astacus*) - Identification of potentially endangered populations for the planning of sustainable conservation measures

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Once widespread across Europe, the noble crayfish (*Astacus astacus*) in Tyrol, Austria, is now endangered and declining according to the IUCN. As keystone species and 'ecosystem engineers', crayfish play a crucial role in ecosystems, holding significant ecological, cultural, and economic value. Their primary threat is the crayfish plague, transmitted by species originating from North America. This study aims to analyze the occurrence and genetic structure of noble crayfish populations in North and East Tyrol and investigate correlations with field parameters and environmental factors. We genetically analyzed 16 individuals from each of the nine crayfish populations using mitochondrial (COI) and nuclear (10 tetranucleotide microsatellites) markers. Numerous abiotic parameters, including water chemistry, were recorded to correlate with population size and genetic diversity indices. The results revealed a separation between East and North Tyrolean populations, with a unique haplotype in East Tyrol. Populations in Krebsbach and Tramser Weiher exhibited high genetic diversity, suggesting their use as source populations for future reintroductions in Tyrol. Significant positive correlations were found between magnesium concentration and population size, and between body size and observed heterozygosity. Diversity parameters correlated with SO4 concentration and pH value, indicating a sensitivity of noble crayfish to water acidification. This research enhances understanding of environmental impacts on noble crayfish genetic diversity and highlights the need for sustainable ecosystem management. Future studies should further explore these relationships to improve conservation strategies.

Keywords: Tyrol, noble crayfish, microsatellites, COI, conservation

From streams, swamps, sloughs and roadside ditches: The places you will go while sampling the rare crayfishes of Texas, USA

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Crayfishes are a diverse and ecologically important component in Texas's aquatic ecosystems. Timely information on distribution, abundance, habitat needs, and threats is needed to effectively manage this resource. Texas currently has ten crayfish species of greatest conservation need, five of which were the focus of our sampling: *Procambarus brazoriensis, Procambarus nigrocinctus, Procambarus nechesae, Procambarus nueces,* and *Procambarus texanus*. From 2022 to 2024, we sampled almost 100 sites targeting these species and have been able to collect each of the five species. Our collections and the associated habitat data have documented possible range reduction in *Procambarus nueces* and *Procambarus texanus*. While also documenting *Procambarus nigrocinctus* and *Procambarus nechesae* might be more common than initially perceived. *Procambarus brazoriensis* is documented as a highly seasonal crayfish with collections that only occur in early spring. With our collection data, we are able to draw conclusions on range and habitat data to better estimate the conservation status for these five crayfishes and other sympatric species that were collected through our efforts.

Keywords: habitat, conservation, threatened species, Texas crayfish

Seeing the spectrum: investigating color vision in freshwater crayfish

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The visual abilities of crayfishes are understudied. In the context of color vision, physiological evidence suggest that crayfishes are either monochromatic or dichromatic. However, there are no behavioral studies that support or refute these findings. Using classical conditioning, we test the hypothesis that crayfish are dichromatic and can differentiate between colored shelters from non-colored shelters. Shelters were provided in seven different shades of gray and four shades of blue for each crayfish in each tank to represent achromatic and chromatic stimuli, respectively. Blues were selected to have brightness values near their gray counterparts to prevent the crayfish from using brightness/achromatic cues to differentiate them. Thus far, thirteen *Cambarus monongalensis* were provided a randomized order of shelters. Shelters were randomized again every other day for sixteen days. At the same time each day, one commercial crayfish pellet was provided in a randomly chosen blue shelter. On the seventeenth day, the crayfish were no longer provided food, and their location and behavior in the tank was monitored for the next four days. Preliminary results show that crayfish resided in gray shelters more frequently than blue shelters overall.

Keywords: Cambaridae, classical conditioning, shelter color, behavior

Effects of season and stress on prevalence of White Spot Syndrome Virus in *Procambarus clarkii*

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White spot syndrome virus (WSSV) is a pathogen of concern for aquatic crustaceans. In the United States, it has been reported from Louisiana crayfish farms since 2007. In spring 2022, *Procambarus clarkii* in laboratory raceways at the E.W. Shell Fisheries Center in Auburn, AL exhibited patterns of lethargy and mortality indicative of WSSV. Subsequent qPCR assays confirmed WSSV, making this the first report of the disease in Alabama. Crayfish surveys across two watersheds from 2022-2023 showed that WSSV was widespread and seasonal in *P. clarkii* and other crayfish species across both watersheds. Most detections were recorded when water temperatures fell between 18 and 30 ℃. Because pathogens can remain relatively harmless to healthy organisms until triggered by stressful conditions, we tested whether stress associated with laboratory studies could trigger virulence in *P. clarkii.* Laboratory crayfish from a group that did not test positive but were suspected carriers of WSSV were subjected to a stressful procedure involving thoracic insertion of PIT tags. Control and tagged crayfish exhibited 100% survival over 12 weeks and the control group had only two individuals that tested borderline positive for WSSV at the end of the study. In conclusion, WSSV now appears to be endemic in parts of Alabama, infecting multiple crayfish species during the warmer months. However, neither warm temperatures nor stress automatically result in detectable levels or virulence. Additional research is needed to determine the conditions that cause WSSV to transition from a potential pathogen to a virulent pathogen that causes heavy mortality.

Keywords: white spot virus, invasives, pathogen

Craywatch, a citizen science project towards a better understanding of the distribution of invasive alien crayfish in Flanders: set up and initial results

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Research has been done sporadically on alien, invasive crayfish in Flanders for several years. As of today, there are six known non-native species in Belgium and they have a major impact on aquatic biota and ecosystems. The likelihood of new introductions of other invasive crayfish, from neighboring countries or the aquarium trade, in the future is high. In order to more efficiently manage these species, mitigate their impacts and prevent future introductions, accurate knowledge of their current distribution in Flanders is needed. This is especially important for new and more recent introductions, for example the clonally reproducing, highly invasive marbled crayfish. For this reason, the Research Institute for Nature and Forest (INBO), in collaboration with the Agency for Nature and Forests (ANB), have organized the citizen science project Craywatch. From mid-June to mid-October 2024, volunteers can sign up and set out traps at predetermined locations across Flanders to monitor the presence or absence of these crayfish. Traps are placed and checked daily for four days at each location and volunteers report their findings, along with photographic evidence, through our online platform. The observations sent in are validated by the Craywatch team. So far, we have about 350 registrations and 250 locations that will be sampled. The generated data will help gain a better understanding of the distribution of these invasive crayfish and will be used as a proof of concept for future IAS surveillance. The set up of this project, initial results and discussion will be covered in this presentation.

Keywords: invasive crayfish, citizen science, distribution, management, trapping

First application of environmental DNA metabarcoding and single-target detection of invasive and native crayfish in Croatia

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Many native freshwater species are threatened by, amongst others, the introduction of invasive non-native species (INNS). Effective management of biological invasions depends on the detection of INNS in the early stages of invasion. However, traditional methods for detection of freshwater INNS, such as trapping and hand catching, have limited success when INNS are present at low abundances. Environmental DNA has proven to be an efficient method to detect aquatic species more reliable than traditional methods. Studies on native and invasive freshwater crayfish species have shown that eDNA-based monitoring is well-suited for identifying newly formed populations in recently invaded areas. During 2023 and 2024, we performed an eDNA-based survey for detection of the invasive crayfish *Pacifastacus leniusculus* in the rivers Mrežnica, Korana, Dobra and their tributaries in Croatia. Water samples from 15 localities were filtered and single-target qPCR analysis was done on all samples, while metabarcoding was performed on samples from 5 localities. Our results showed the presence of signal crayfish upstream from the localities where it was previously recorded with traditional sampling, highlighting the upstream invasion, while metabarcoding recorded native crayfish presence in several localities close to signal crayfish. These results provide new insights on the status of native species and upstream invasion of signal crayfish. This is the first study implementing eDNA, qPCR and metabarcoding for crayfish species assessment in Croatia. The data obtained is used to inform ongoing activities of signal crayfish control in the area, which are also essential for the preservation of the native crayfish species in these rivers.

Keywords: *Pacifastacus leniusculus*, eDNA, native crayfish, management, metabarcoding

Are we really able to do anything, or are we just sitting back and watching? The history and the current status of signal crayfish (*Pacifastacus leniusculus* (Dana, 1852)) in Hungary

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The signal crayfish (*Pacifastacus leniusculus*) is among the most widespread invasive crayfish species in Europe. The signal crayfish invaded in Western Hungary as early as the mid-1990s and here we investigated the recent expansion of this species and its impact on other aquatic ecosystems. The colonization of watercourses throughout Europe by the signal crayfish resulted in negative impacts on the present aquatic communities. Our investigation in 37 water body revealed that the distribution range of signal crayfish is still in expansion in the western and north and south-west part of Hungary and in all likelihood impacting the aquatic communities in these aquatic habitats. Our results well demonstrated that signal crayfish densities were highest in different rip-rap zones and gravel banks of rivers. An unexpected result was that the different types of rip-raps under the bridges and urbanised sections of streams have a function as hots-pots for all watercourses but it was detected that dams of beavares could function as spreading barrier. Our investigation proved that the presence of signal crayfish had negative effects on a number of protected species (e.g. ICS, fishes, amphibians) and sediment transport, howewer the tested eradication methods could be effective in some cases.

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Keywords: historical datasets, ecological engineer species, road ecology, urban waters, WFD

Systematic distributional survey of endemic and invasive crayfishes in the upper Saint Francis River drainage, Missouri

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Crayfish invasions are among the top global threats to native crayfish populations. The Saint Francis River crayfish (*Faxonius quadruncus*) and Big Creek crayfish (*F. peruncus*), endemic to the upper Saint Francis River (USFR) drainage in Missouri, are currently under threat of extinction due to invasion of the Woodland Crayfish (*F. hylas*) from nearby drainages. Previous research documented reduced abundances and range reductions for both native species in reaches invaded by *F. hylas*. However, the entire ranges of the native species have not been systematically sampled, and the most recent data documenting *F. hylas* invasions are over 13 years old. Management agencies need updated distributional data to create an informed recovery plan for the two threatened species. We used stratified random sampling to select 96 sites across the USFR basin for a distributional survey conducted in 2022-2023. Our analyses incorporate presence-absence data from this survey with coarse-scale GIS data into random forest and boosted regression models and create probability of presence maps for the invasive and two endemic crayfish species. Field sampling also resulted in the discovery of *F. hylas* invasions in two major tributaries to the USFR drainage. Additionally, we used targeted sampling to locate the leading edges of invasion in six streams and documented a range expansion of *F. hylas* 1.4 km upstream from the 2009 leading edge in Orr Hollow Creek. Results from this study will be used to identify refugia for native species and locate barriers to further invasion.

Keywords: invasive, distribution, survey

P2X as a solution to crayfish plague

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River Ljungan in Sweden has suffered from crayfish plague (*Aphanomyces astacii*), resulting in the extinction of noble crayfish (*Astacus astacus*). At the same time, P2X industries such as hydrogen gas production is developing in the region. The Swedish Hydrogen and Crayfish research project explores how the residue products from the production of hydrogen gas; oxygen, and heat, can be utilized to disinfect crayfish plague and facilitate the growth and survival of crayfish. The temperature of process water varies between 48 and 73°C and could disinfect water from crayfish plague as spores of crayfish plague die from a short exposure to 60°C. Ozone disinfection and UV light are common techniques to neutralize microorganisms in drinking water. It is unclear how these techniques affect the survival of crayfish plague spores. This presentation outlines a study, in which solutions for direct or indirect water supply from hydrogen production to crayfish farms will be proposed. The goal is to ensure that crayfish farming can be freed from crayfish plague and has a temperature that favors the survival and growth of the crayfish. Laboratory experiments will test different methods to disinfect crayfish plague. The presence of crayfish plague and the survival of crayfish in the treated water will then be investigated. Crayfish plague occurrence will be measured with a real-time PCR Thermocycler before and after treatment of water. The genotype of the crayfish plague will be analyzed with TaqMan MGB real-time PCR. We will present the experimental design and would appreciate your input.

Keywords: P2X, crayfish plague, disinfection, ozone, UV light

Poster Presentations

North Yorkshire Crayfish Forum: Collaboration for Conservation

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White-clawed crayfish (*Austropotamobius pallipes*) is the only native species of freshwater crayfish in the UK and has suffered a large population decline in recent years. In order to preserve its populations the North Yorkshire Crayfish Forum (NYCF) was founded in 2019. NYCF draws together organisations with an interest and passion in saving our only native crayfish. The aim of this poster is to show the importance of working at river catchment scale through partnership working, utilising the diversity in experience and specialisms of NYCF members, and the impact that it can have on practical conservation to increase effectiveness of project work, including the activities described below. In the past five years North Yorkshire Crayfish Forum has; recruited a full-time staff member, gained members from over twenty different organisations, developed a five year delivery plan, established a group of fifteen skilled volunteers, improved base-line crayfish data, set up a captive rearing facility, and created three new crayfish ark sites. The key to this has been collaboration, and resource and knowledge sharing between partners. North Yorkshire is a stronghold for white-clawed crayfish, but populations are still facing extreme pressures and uncertainty. The NYCF's work aims to ensure the long-term survival of this endangered species. This poster will demonstrate the key to its success is continual learning and collaboration to effectively undertaking conservation across connected river catchments and across large areas like North Yorkshire.

Keywords: Conservation strategy, partnership, white-clawed crayfish

New molecular diagnostic method for detection of *Nosema austropotamobii* (Microsporidia) in white-clawed crayfish

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Microsporidia are obligate intracellular pathogens that infect a wide range of invertebrate and vertebrate hosts, often resulting in chronic disease. In the white-clawed crayfish (*Austropotamobius pallipes* complex), the most studied microsporidia are *Astathelohania contejeani* and *Nosema austropotamobii*, which cause the "porcelain disease", characterized by a typical whitish appearance of the abdominal tissues. Recent publications have highlighted that *A. contejeani* and *N. austropotamobii* can circulate within the same population and sometimes co-infect the same crayfish. Currently, specific molecular identification methods are available only for *A. contejeani*, while the detection of *N. austropotamobii* relies on generic molecular protocols, mainly based on the SSU rRNA. Although this gene commonly offers sensitivity advantages, its high conservation and repeat motifs complicate species-specific assays. Therefore, it is crucial to design molecular methods to detect *N. austropotamobii* based on other genetic regions to ensure specificity and avoid cross-reactions with other microsporidia or similar microorganisms. For this purpose, we selected the RNA polymerase II largest subunit (RPB1) gene because it exhibits enough variability among these organisms. The newly developed end-point PCR was verified and validated using more than 40 *A. pallipes* specimens previously evaluated with the published molecular protocols for microsporidia. The new method produces a single amplicon of about 400 bp in *N. austropotamobii*-affected specimens without amplification from *A. contejeani*-infected or microsporidia-negative samples. All amplicons were sequenced and compared with sequences available in the NCBI database, showing >99% similarity to *N. austropotamobii*-RPB1 gene, corroborating the specificity and reliability of this new protocol.

Keywords: RPB1, species-specific assay, microsporidiosis, validation

Tracking signal crayfish during the cold season in Lake Vättern with acoustic telemetry

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This ongoing study gives indications that crayfish movements could be successfully tracked during the cold season using acoustic telemetry. Our aim was to investigate how the accuracy of triangulation changed with the distance to the receiver and when the signal was obscured by artificial burrows. The invasive signal crayfish is abundant in Lake Vättern and is active during the cold season. It could therefore affect sensitive species and environments all year round, e.g. aggregations at spawning sites of arctic char in October and migrations to deeper, possibly more sensitive areas during autumn and winter. We investigated how to use externally attached transmitters on crayfish carapace both in the laboratory and in the field. Our results did not indicate higher mortality as a consequence of the external tags. In the field we tested different sized transmitters with various power output both on crayfish in constructed burrows and out in the open. We tested tags with both fast (20 sec) and slower (180 sec) signals. Despite signal differences we were able to acceptably triangulate the crayfish. As the experiment is ongoing, we will be able to test a more thorough and more accurate triangulation in the near future. In our follow-up experiment we will test tags with longer delays and also fewer of the fixed 20 second tags (fewer tags pinging simultaneously) which we believe will cause less signal collision.

Keywords: signal crayfish, *Pacifastacus leniusculus*, triangulation, transmitter, acoustic telemetry

There can be only one: the two-year spread of *Procambarus clarkii* in a *Faxonius limosus* infested small perialpine lake in Trentino (Northeast Italy)

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A containment campaign to prevent the spread of *Faxonius limosus* was conducted in June, July and September 2023 and repeated in 2024, in Costa Lake, a Special Area of Conservation of about 1 hectare surface area, where this IAS was first recorded in spring 2021. Unexpectedly, in June 2023 we trapped 38 adult *Procambarus clarkii* in the NW part of the lake, suggesting a recent point of introduction. *Procambarus clarkii* showed a high invasive potential, spreading quickly in 2023 to the whole lake perimeter and its emissary canal; its body size was much larger than that of *F. limosus*, with two main size classes, one (50-60 mm cephalothorax length) corresponding to the individuals introduced most likely in the fall 2022 (2-years old), and a second class (40-50 mm) representing the new generation born in the lake. In July 2024, we recorded three main size classes: about half of the *P. clarkii* population belonged to the 50-60 mm size class (2-years old), while the remaining was evenly distributed between the size classes of 40-50 mm (new generation) and 60-70 mm, i.e., older individuals (4-years old). *F. limosus* did not show significant shifts in size: in both years the majority of the individuals belonged to the 30-40 mm size class. The much larger size of *P. clarkii*, longer time span, fast dispersion ability, suggest a future dominance of *P. clarkii* and possible disappearance of *F. limosus*, with relevant risk of spread of this new, more invasive species to the upstream, hydrologically connected, water bodies.

Keywords: management of autochthonous species, invasive alien species, spiny-cheek crayfish, Louisiana crayfish, faunistic monitoring

Updated distribution and characterization of crayfish plague and microsporidiosis affecting *Austropotamobius pallipes* complex in Trentino (Northeast Italy)

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One of the causes of the decline in distribution and abundance of the endangered white-clawed crayfish *Austropotamobius pallipes* complex throughout Europe is the widespread invasion of alien crayfish and the associated spread of infectious diseases, primarily crayfish plague caused by *Aphanomyces astaci.* Although this disease usually causes mass mortality in *A. pallipes*, some wild populations appear tolerant towards *A. astaci.* Another relevant disease is microsporidiosis (porcelain disease), caused by the parasites *Astathelohania contejeani* and/or *Nosema austropotamobii.* In 2021-2024, we conducted a monitoring survey, aimed at mapping the distribution of *A. astaci*, *A. contejeani* and *N. austropotamobii* in wild populations of *A. pallipes* in Trentino (Northeast Italy). We applied non-invasive sampling methods to collect cuticular swabs from 31 of the 46 known populations, investigate the presence of *A. astaci* and if possible, identify its genotype through molecular analyses. *Aphanomyces astaci* was detected in 8 populations, and the presence of a low pathogenic genotype (genotype A) was confirmed in one of them. Thirty-three specimens from 10 populations showed macroscopic signs of porcelain disease, abdominal muscle tissues were collected and subjected to molecular evaluation. The presence of *A. contejeani* was identified in 23 individuals from 9 populations and *N. austropotamobii* was detected in 3 individuals, from 3 populations. Eight specimens collected from 6 populations were co-infected by the two microsporidians.

This study was partly supported by the EU LIFE Programme: LIFE-CLAW, Crayfish Lineages Conservation in North-western Apennine (LIFE18 NAT/IT/000806), and by the SAGA and SAGA2 collaborative projects between FEM and IZSVe.

Keywords: *Aphanomyces astaci*, cuticular swab, microsporidia, co-infection

Freshwater crayfish in Serbia: Update on the distribution

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Our study aims to build upon a prior studies of crayfish distribution by consolidating findings from our fifteen years field research and summarizing existing published data. In Serbian freshwater ecosystems, we have identified the stone crayfish *Austropotamobius torrentium*, the noble crayfish *Astacus astacus*, and the narrow-clawed crayfish *Pontastacus leptodactylus* as native species. Unfortunately, our ecosystems have been penetrated by two invasive species: the spiny-cheek crayfish *Faxonius limosus*, first discovered in 2004, and the signal crayfish *Pacifastacus leniusculus*, more recently found in 2020. Fifteen years since the last research, we have observed that the most commonly found native crayfish is the stone crayfish, documented at 206 sites in 131 freshwater ecosystems, followed by the noble crayfish (46 sites in 31 freshwater ecosystems) and the narrow-clawed crayfish (22 sites in 11 freshwater ecosystems). Field observations have shown that changes in habitat and the increasing impact of climate change (significant droughts and floods over the past decade), primarily affect native crayfish populations. Understanding species distribution is fundamental to a wide range of biological research. Gathering additional data on species distribution is essential for enhancing our understanding of biodiversity, the functioning of aquatic ecosystems, conservation planning, climate change adaptation, and the management of invasive species. Moreover, distribution data is crucial for monitoring endangered species, such as the stone and noble crayfish, which are strictly protected under the Rulebook on the Proclamation and Protection of Strictly Protected and Protected Wild Species of Plants, Animals, and Mushrooms ("Official Gazette of RS" no. 5/2010, 47/2011, 32/2016 and 98/2016).

Keywords: crayfish diversity, Serbia, indigenous species, non- indigenous species

The Swedish hydrogen gas and crayfish project

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The aim of this poster is to present a new research project called Hydrogen Gas and Crayfish (VOK). The research project was initiated by the local fisheries management NGO in Ånge municipality in Sweden. Their challenge for future generations is to be able to fish for Noble crayfish (*Astacus Astacus*) in river Ljungan since the area has been hit by crayfish plague. At the same time, Ånge municipality is situated within the Swedish green industry belt that is currently under development. This means that P2X industries such as hydrogen gas production is developing in the region. Residue products from hydrogen gas production is oxygen and heat. To ensure the long-term survival of the crayfish and sustainable fishing of crayfish in river Ljungan for future generations, the use of water needs to be governed. Also, public, non-profit associations, municipalities and companies need to be involved in the work to promote the crayfish and prevent the spread of crayfish plague. The aim of the research project is to study how water management in hydrogen gas production can be used to ensure the survival of the crayfish. In doing so, the project can contribute to a better understanding of how new P2X industries can contribute to the sustainable use of water resources, thus improving biodiversity and creating sustainable rural development. We are doing this through a blue innovation that involves creating an ark site for Noble crayfish in connection with a hydrogen gas factory.

Keywords: Noble crayfish, P2X, crayfish plague, governance, ark site

Evaluation of the oral route of transmission of porcelain disease in signal crayfish under controlled conditions

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Microsporidia are obligate intracellular eukaryotic parasites that are widespread worldwide and specialized in infecting almost all animals and plants. In populations of the endangered European crayfish, *Austropotamobius pallipes* complex, and the vulnerable noble crayfish *Astacus astacus*, the microsporidian parasite *Astathelohania contejeani* can cause a chronic infection, commonly known as porcelain disease, which ultimately leads to the death of the crayfish. The present study aims to evaluate the horizontal transmission of microsporidia under controlled conditions from native to invasive species, focusing on *Pacifastacus leniusculus*, to assess the rate and timing of infection. Sixty signal crayfish were collected and separated into two groups: treatment (20 females and 20 males) and control (10 females and 10 males). At three different time points, crayfish in the treatment group were fed with abdominal tissues from infected white-clawed crayfish. During the experiment, the specimens were housed separately in different tanks and periodically monitored for infection levels. Each crayfish was tested for the presence of *A. contejeani* using non-invasive sampling techniques and molecular biology analysis. Two infected crayfish were detected positive three months after the experiment started, and another one tested positive five months later. In all the other specimens, *A. contejeani* was never detected during whole nine-month trial. The observed infection rate was lower than that reported by Imhoff et al. (2012). However, the latter was likely overestimated since they did not test the specimens before the treatment and found infected crayfish in the control group at the end of the study.

Keywords: *Pacifastacus leniusculus*, experiment of infection, microsporidian

A preliminary analysis of genetic diversity in the *Cambarus bartonii* species complex from the Northeastern USA

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An analysis of genetic diversity in the *Cambarus bartonii* species complex from the Northeastern USA was conducted using the mitochondrial COI barcode region. This species has a widespread distribution in the streams of eastern Canada and the USA, ranging from southern Ontario, Quebec, and New Brunswick southward to northern Alabama. It is thought to represent a species complex and its relationship to *C. carinirostris* and *C. cavatus*, previously considered subspecies of *C. bartonii*, are also examined. Results suggest that sampled populations of *C. bartonii* from across eastern Pennsylvania contain the same COI haplotype, and is indicative of the type population. These populations are distinct from populations of *C. carinirostris* sampled from western and southwestern Pennsylvania, and from populations of *C. cavatus* from North Carolina and Virginia, indicating that their current elevated status as species is justified. For *C. carinirostris*, there appeared to be two distinct haplogroups in southwestern PA, possibly suggesting a previously unknown and undescribed species co-occurring in the region. Additional sampling will be needed to help delimit the geographic distributions of these species.

Keywords: *Cambarus bartonii*, genetics, phylogeography, species complex, taxonomy

Life history of the Pontchartrain Painted Crayfish *Faxonius hobbsi*, a species of greatest conservation need in Louisiana

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Crayfish are key components in freshwater ecology and trophic webs, are fundamental in determining ecosystem structure and function, and are considered a keystone species in many aquatic systems. However, crayfish extinction rates are higher than those of other freshwater fauna, including fish and amphibians. Louisiana lacks basic ecological information on most crayfish, including species of greatest conservation need (SGCN). To improve conservation and preservation efforts of crayfish, gathering information on ecological and life history characteristics is critical to determine conservation status assignments for crayfish. Therefore, the purpose of this project is to examine the life history characteristics of the Pontchartrain Painted Crayfish *Faxonius hobbsi*, a SGCN in Louisiana. Crayfish were sampled monthly in two streams in the Tangipahoa and Tchefuncte River basins using backpack electrofishing, seines, and dipnets. All collected crayfish were identified to species, sexed and carapace length measured. Additional morphometric characteristics were documented for all *F. hobbsi* collected including sex, reproductive form, wet weight, and carapace, orbital, abdomen, palm, and propodus lengths. The results from this study will provide the first comprehensive life history information for *F. hobbsi*. This information can aid resource managers with species management and conservation efforts and be incorporated into Louisiana's future ecosystem, watershed, and stream management decisions.

Keywords: conservation, *Faxonius*, life history, stream

Are deep karst springs along the Vrljika River in Croatia long-term protected refuges for white-clawed crayfish?

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Over the last three decades, multiple anthropogenic pressures such as pollution and hydromorphological changes, the disappearance of riparian vegetation, climate change, the presence of invasive species and crayfish plague outbreaks have radically reduced the number and density of crayfish populations in karst rivers of the Adriatic catchment in Croatia. This study aimed to determine the distribution patterns of crayfish in the Vrljika River in southern Dalmatia after the major crayfish die-off in Imotsko polje in the late 1990s. The Vrljika River with four tributaries, two karst springs and protected karst lake Prološko Blato were monitored over two years (2009 and 2010) at 20 surveyed sites using hand search and baited LiNi traps. A total of 221 specimens of two crayfish species were observed during eight nights at 6 sites. No crayfish were found in the Vrljika River. Indigenous white-clawed crayfish was detected at six sites, while introduced noble crayfish was found at two sites. The mixed populations were recorded at two sites and showed equal to twenty times lower abundance of noble crayfish introduced from Bosnia and Herzegovina. The most important refuges for the indigenous crayfish population were large deep karst springs Vrila and Vir, where the highest catch per unit effort was recorded. Over the last 15 years regular monitoring by Croatian Waters and recent local studies have not confirmed presence of crayfish in the Vrljika River. Further research requires ecological data collection with a main focus on hydromorphological state of the river and the long-term conservation of native species.

Keywords: distribution, mixed populations, white-clawed crayfish, noble crayfish, Vrljika River

Traditional model animals versus crayfish: driving revolutionary research

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Highlighting the limitations of traditional mice models in accurately mirroring human health conditions, this study underscores the importance of diversifying animal models in biomedical research. Due to their complex neural circuits and advanced stem cell systems, crayfish emerge as a pioneering model. They offer critical insights into neurobiology and cellular differentiation, which are vital for advancements in neurodegenerative disease and cancer research. In contrast to traditional models, crayfish biological mechanisms are more like those in humans. This makes them essential for studying how tumours behave and testing new treatments that could be used more widely in humans. Position of crayfish alongside non-traditional models like ferrets, guinea pigs, and zebrafish, each serving niche purposes from studying respiratory diseases to genetic mutations and addressing the challenges of model selection, including the need for model-specific research methodologies to ensure validity and applicability, this review highlights the untapped potential of crayfish. Emphasizing their robust neurobiological and cellular features, we argue for including crayfish in the repertoire of biomedical models, projecting their role in pioneering future biomedical discoveries, and enhancing the translatability of preclinical research to human health improvements. Calls for crayfish to be used more in high-impact biomedical research and shows how they can act as a translational bridge, possibly speeding up the creation of new therapeutic strategies that directly affect human health. As such, crayfish emerge not merely as an alternative but as a necessity for bridging the translational divide, heralding a new era in the pursuit of precision medicine and sustainable research models in the high-stakes arena of biomedical science.

Keywords: biomedical diversification, cancer research, heat shock protein, neurobiology, regenerative medicine

Urban life of stone crayfish (*Astropotamobius torrentium*) in Zagreb City

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Zagreb City spreads from the Medvednica Mountain to the plain of the Sava River. The city's freshwater habitats are reduced to several semi-artificial lakes used for recreation and over 30 streams. Majority of upper reaches of the streams are located in the Medvednica which is protected as a nature park and is part of NATURA2000. Here, the streams have favourable conditions and host numerous stone crayfish (*Asutropotamobius torrentium*) populations. As streams enter the city, strong urban influences occur (concrete channelling, regulating, pollution). Most of the Zagreb urban streams are highly modified and are not favourable habitat for the stone crayfish, even though it also occurs in some parts of the urban streams. The main disadvantages of the habitat in such urban streams for the species are the lack of hiding places, reduced shade, low water levels during summer and pollution. Since habitat restoration in the urban area is not always possible, there are simple measures to improve habitat conditions in modified urban streams. In 2023, the Zagreb City Nature installed concrete blocks with holes (crayfish houses) in the canalized part of the Dolje Stream (NATURA2000 site) to test their use as hiding places for stone crayfish. We also reduced the mowing frequency around the stream, which lead to accumulation of sediment on the concrete bottom and initiated regular collection of waste from the stream originating from surrounding houses. We also constantly conduct education on the importance of protecting the urban habitat of the stone crayfish. Here, we present the results of our ongoing management campaign.

Keywords: stone crayfish, urban streams, urban pressure

Co-evolutionary history of Japanese crayfish and ectosymbiotic branchiobdellidans from a molecular phylogeny perspective

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The Japanese crayfish, *Cambaroides japonicus*, and its 11 species of obligate ectosymbiotic branchiobdellidans are endemic to Hokkaido and northern Honshu Islands in Japan. We conducted a comprehensive sampling of the host and its branchiobdellidans and sequenced each taxon for mitochondrial COI, 16S rRNA, nuclear 28S rDNA and ITS1. The results of COI were used to compare the phylogenies of the host and their ectosymbionts. Japanese crayfish show clear genetic differentiations among local populations, indicating an eastern and a western lineage on Hokkaido that are separated by the Hidaka mountains, while those on northern Honshu are part of the western lineage. In contrast, there is no significant difference in the species composition of most branchiobdellidans between the two major host lineages. A phylogenetic analysis revealed that the divergence age among branchiobdellidans estimated from the COI was earlier than the divergence time of the two lineages of Japanese crayfish. Considering the distribution patterns of branchiobdellidans and crayfish lineages, most species of branchiobdellidans would have diverged before the crayfish colonized the Japanese archipelago. However, the intra-specific phylogenetic relationship within the *Cirrodrilus cirratus* complex and *C. sapporensis* do show a clear divergence between eastern or western Hokkaido, and this is consistent with the distribution and divergence age of the host crayfish lineages. This study suggests that Japanese branchiobdellidans have maintained a high species diversity that, for the most part, occurred before the crayfish dispersion in Japan.

Keywords: Branchiobdellida, Japan, phylogeography, biogeography, co-evolution

Bioaccumulation kinetics of antihistamine diphenhydramine in signal crayfish

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Identification of bioaccumulative chemicals in aquatic organisms has historically relied on experimental work describing the bioaccumulation of organic compounds in fish, but it has remained largely understudied in other taxa. Considering the prominent roles of freshwater crayfish in aquatic ecosystems and further attributes they possess (e.g., relatively large size, sensitivity, and easy handling), they can serve as suitable model organisms. The present study aimed to estimate ratio- and kinetic-based bioconcentration factors and derive bioaccumulation parameters of the diphenhydramine (DPH) in signal crayfish *Pacifastacus leniusculus*. DPH is an antihistamine with multiple modes of action, ionisable as a weak base (pKa = 8.8) and with evidence of bioaccumulation observed in various matrices globally. In a 10-day bioconcentration experiment (7 days of uptake and 3 days of elimination, pH levels 6.7 and 8.7), we analysed the concentration of DPH in crayfish hemolymph and whole body using liquid chromatography with tandem mass spectrometry, followed by calculation of kinetic-based bioaccumulation parameters. Both modelled and observed DPH effects revealed that water pH directly influenced bioaccumulation kinetics. The ratio-based bioconcentration factor was 1-2 orders of magnitude lower than in previous studies on fish. The empirically derived apparent volume of distribution for DPH was markedly higher in crayfish (416–451) than in fish (0.3–7) and humans (3–4). Bioaccumulation models driven by empirical data obtained for signal crayfish advance the science of invertebrate bioconcentration of ionisable organic contaminants, providing valuable insights for future studies in the ecotoxicology area.

Keywords: *Pacifastacus leniusculus*, bioaccumulation model, ecotoxicology, ionisable pharmaceutical

Reproductive biology of *Pacifastacus leniusculus* in a small tributary of Clitunno River (Tiber River basin, Central Italy)

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Pacifastacus leniusculus (Dana, 1852) is an invasive crayfish of European Union Concern present in Italy. *P. leniusculus* has been recorded for the first time in the Clitunno river (Tiber River basin, Central Italy) in 2020 and, in accordance with the European regulation n° 1143/2014 a population eradication plan has been immediately drawn up as part of the LIFE IMAGINE 19 IPE/IT/000015 project. A total of 535 individuals were caught through electrofishing and trapping from November 2022 to June 2024 from Fosso Vecchio, a small tributary of the Clitunno river, and were analyzed to investigate reproductive biology. Individuals were euthanized, measured (total length, cephalothorax length and weight), sexed and analyzed. Pleopodal eggs from 35 females were counted, weighted, and measured while gonads were extracted, weighted and females' developmental stage was attributed. The number of pleopodal eggs per females ranged from 6 to 481, a positive significant relationship between mean diameter of eggs and cephalotorax length was found. Gonadosomathic index (GSI) was calculated for both sexes. *P. leniusculus* females showed the highest GSI mean value in September (mean 3.7 ± SD 2.1), while males GSI mean value was higher in October (mean 0.9 ± SD 0.4). Females with pleopodal eggs were found from October to January, only one female with juveniles attached was caught in February. Our results contribute to expanding the knowledge on reproductive biology of one of the southernmost populations of *P. leniusculus* introduced in Italy, and results may be useful in increasing the effectiveness of control plans for this invasive species.

Keywords: crayfish, reproduction, GSI, ovarian stages

New record of *Astacus astacus* population in the Brcanj Stream (Bosnia and Herzegovina): age-class distribution and length-weight relationship

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Astacus astacus (Linneaus, 1758) populations significantly globally declined in the last decades and the species has been classified as Vulnerable in the IUCN Red List. In 2022 a population of *Astacus astacus* was discovered in the Brcanj Stream, a chalk stream located in a karst polje in the Blidinje Nature Park (Bosnia and Herzegovina) during investigation for the NaturBosnia project. With the aim of expanding knowledge on this species a total of 226 crayfishes were sampled with electrofishing during three sampling campaigns between 2023 and 2024; individuals were sexed and cephalothorax length (CL), total length (TL) and weight (W) were measured to assess age-class distribution and determinate the length-weight relationship (LWR). Cohorts were estimated by Bhattacharya's method based on the CL frequency distribution data using the software FiSAT II. Individuals with one or both claws missing were excluded from the LWR analyses and the equation was estimated separately for both sexes. There was no significant difference in the LWR between sexes, and the relationships for the total sample showed a positive allometric growth (b>3). The population was numerous and composed predominantly of younger individuals: the scarcity of individuals composing older age classes could be due to overharvesting, observed during our fieldwork. *A. astacus* is native in Bosnia and Herzegovina, but this is the first record of its presence in this area, so the origin of this population should be clarified to broaden knowledge on the real distribution of the species.

Keywords: crayfish, Balkan Peninsula, length-weight relationship, age structure

Craywatch: citizen scientists to the rescue
Mapping the distribution of invasive alien crayfish in Flanders

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Research has been conducted sporadically on invasive alien crayfish in Flanders for several years. As of today, there are six known non-native species in Belgium and they have a major impact on aquatic biota and ecosystems. The likelihood of new introductions of other invasive crayfish, from neighboring countries or the aquarium trade, in the future is high. In order to more efficiently manage these species, mitigate their impacts and prevent future introductions, accurate knowledge of their current distribution in Flanders is needed. This is especially important for more recent introductions, for example the clonally reproducing, highly invasive marbled crayfish, and potential new introductions. For this reason, the Research Institute for Nature and Forest (INBO), in collaboration with the Agency for Nature and Forests (ANB), have organized the citizen science project called Craywatch. From mid-June to mid-October each multi-year, volunteers can sign up and set out traps at predetermined locations across Flanders to monitor the presence or absence of these crayfish. Traps are placed and checked daily for four days at each location and volunteers report their findings, along with photographic evidence, through our online platform. The observations submitted are validated by the Craywatch team. So far, we have received about 350 registrations and determined 250 locations that will be sampled. The data generated will help gain a better understanding of the distribution of these invasive crayfish and will be used as a proof of concept for future IAS surveillance.

Keywords: invasive alien crayfish, citizen science, distribution, Flanders

Is there a future for the white-clawed crayfish in Istria?

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The white-clawed crayfish (WCC) *Austropotamobius pallipes* sp. complex is one of four native European species distributed in Croatia. The WCC populations inhabit waterbodies draining Adriatic Sea, including Istria – peninsula characterised by diverse geology and hydrology. Historical data on WCC in Istria are scarce (two references; one from 17th century, and the other from the beginning of 20th century). Studies on its distribution started at the beginning of 2000's, while more intense studies on biology, ecology, phylogeny/phylogeography and diversity of WCC populations in Istria started from 2012. Results of molecular studies established existence of two divergent WCC lineages in Istria, highlighting its position as a hotspot of WCC diversity, as is also the case with numerous other aquatic species present there. Generally, number of populations and their size are shrinking, even though the results of consecutive monitoring at the same location can be contradictory (e.g., in 2022 no records of crayfish, in 2024 crayfish are recorded). Remaining populations are mainly present in isolated hardly accessible upper courses, while lower courses are frequently canalised, water is used illegally and/or legally for irrigation of agricultural land. Further, observed problems are pronounced extended droughts, the consequence of ubiquitous climate changes, as well as the potential danger of the introduction of alien invasive crayfish species and their pathogens, which would further worsen the situation. We discuss our findings in the context of future urgent steps (such as ensuring minimum water flow, creation of climate refugia, education of different stakeholders) that are needed to conserve and improve status of WCC in Istria.

Keywords: *Austropotamobius pallipes* sp. complex, monitoring, phylogeography, conservation, diversity hot spot

Control of invasive spiny - cheek crayfish (*Faxonius limosus*) population in gravel pits near Ptuj, Slovenia

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In LIFE-IP NATURA.SI project, we are implementing measures to reduce population of invasive spiny-cheek crayfish (*Faxonius limosus*) in order to prevent its spread to the nearby Dravinja River basin (Natura 2000 site) where native stone crayfish (*Austropotamobius torrentium*) resides. Invasive *F. limosus* is located approximately 500 m from Dravinja River in gravel pits adjacent to the main Drava River. So far, this is the only known location of this species in Slovenia. Monitoring showed that by reduction actions, we are successfully preventing *F. limosus* population increase and thus its spread to targeted Natura 2000 site. We expect that without our reduction actions, the growth of the population would be exponential. Implemented reduction measures consist of different methods: electro-fishing, hand capturing, trapping, water drainage, setting artificial refuge traps (ART) and mechanical macrophyte removal. Until 2024, we have removed 14.060 specimens within 1.721 catch reduction actions. In year 2023, we were especially successful in capturing brooding females (N= 428) meaning we additionally eliminated approximately 104.860 of crayfish eggs. On average, one female carried 245 eggs (N=40). Eliminating brooding females is essential for a better success in population control. For effective control of *F. limosus*, using combination of methods is crucial, since their effectiveness varies according to the situation on the field. In 2023, the most effective methods were the mechanical macrophyte removal and the capture by ART, while in 2020, the most effective method was electrofishing. High water levels and floods, pose a great threat for *F. limosus* dispersal to new areas.

Keywords: *Faxonius limosus*, population control, removal methods

Morphological variability among populations of *Austropotamobius torrentium* (Schrank, 1803) from central Balkan

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The Balkans is *Austropotamobius torrentium* (Schrank, 1803) hotspot. In its central parts (Serbia) three main stone crayfish phylogroups are present: CSE (Central and South-East European), SB (Southern Balkans) and LD (Lika and Dalmatia). In order to assess the morphological variability of the stone crayfish we analyzed ten populations from the central Balkans. A total of 145 adult crayfish (total length over 60 mm) were measured during our 2017 field study and 21 linear parameters and individual weight were taken. All measurements were standardized by postorbital length. Of analyzed crayfish 80 were males and 65 females. Performed non-parametric tests (Kruskal-Wallis) have shown that males and females differ in majority of parameters, including those related to weight. Discriminant analysis showed that abdominal and claw widths were the most important for sex separation, with the first one having larger values in females, while the second one being larger in males. Regarding populations, non-parametric tests showed that only stable parameters among male populations were a few characteristics of abdomen, claws and weight, while in female populations those were a bit more numerous. Canonical discriminate analysis was used to assess spatial morphological variability among studied populations (10 populations for males, and 8 for females). The results have shown that separation among male samples is more pronounced than in females. Alongside the first root a clear distinction of Uvac (CSE phylogroup) and Rasina (SB phylogroup) populations from the rest can be observed, while along the second root these two populations differ. Considering that analyzed populations belong to all three main phylogroups

our results suggest that morphological variability of the stone crayfish is related more to specific environmental conditions (adaptations) than to separate phylogenetic lineages.

Keywords: linear morphometry, sexual dimorphism, endangered species, Serbia stone crayfish

Narrow – clawed crayfish (*Pontastacus leptodactylus*) in Slovenia

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Pontastacus leptodactylus is European species with three main phylogroups. Slovenia (SI) mostly lays outside of its native range, but European haplotypes could naturally expand their areal in to SI. *P. leptodactylus* was firstly detected in SI in 2017 in Lava pond (Savinja River). In 2021, another population of this species was detected in River Pivka, near Postojna cave sink hole. Genetic results show that both populations originate from Asian phylogroup, which confirms non-nativeness of the species in SI. Additionally, we detected a concrete case of human introduction of the species in the wild; via social media. The origin of the species could be a grocery store that traded with live specimens. Another possible introduction route is cross-country transport; Financial Administration of SI have confiscated 68 *P. leptodactylus* specimens transported by Ukrainian driver. In order to prevent spread of *P. lepodactylus* in SI, Fiseheries Reasearch Institute (SI) is implementing measures for population control by removal of specimens. In Lava pond, we are removing females and returning sterilized males, while in the Pivka River, we are eliminating all captured specimens, including males. Dominant males predate on crayfish juveniles, including juveniles of the same species. Since in Pivka River native noble crayfish (*Astacus astacus*) resides, returning of *P. leptodactylus* males would be groundless - *A. astacus* takes over the predatory pressure. The results show implemented measures impact. In Lava pond, we detected significant decrease of *P. leptodactylus.* In Pivka River, the implemented measures resulted in increased number of *A. astacus* in the catch.

Keywords: *Pontastacus leptodactylus*, measures of control, Asian phylogroup

Persistence of *Aphanomyces astaci* in white-clawed crayfish populations in Friuli-Venezia Giulia region (Northeast Italy)

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The oomycete *Aphanomyces astaci* is the etiological agent of crayfish plague, which is responsible for mass mortality events in European freshwater crayfish species. However, recent evidence indicates that these crayfish species exhibit tolerance towards strains belonging to the genotype A of this pathogen and records of coexistence in wild populations are increasing. An example was documented in Friuli-Venezia Giulia region (Northeast Italy) during the LIFE-RARITY project (2011-2014), when healthy populations of the endangered *Austropotamobius pallipes* complex tested positive for *A. astaci* by molecular analysis, although genotyping was unsuccessful. In 2020 and 2023, another monitoring of 5 thriving populations in the same area was performed, taking advantage of the non-invasive sampling techniques developed during the LIFE-CLAW project and the improvements in the molecular diagnostic protocols for *A. astaci*. Approximately 30 crayfish from each population were caught and the external cuticle was swabbed to collect putative *A. astaci* hyphae and zoosporangia. A total of 164 swabs were processed using a commercial kit and the DNA extracts were analysed by quantitative real-time PCR (Rusch et al. 2020). *A. astaci* was detected in 4 populations, 2 of which were also analysed during the LIFE-RARITY project and tested positive. The presence of *A. astaci* was further confirmed in one population by sequencing (Oidtmann et al., 2006). Finally, the successful application of the real-time PCR assays designed by Di Domenico (et al. 2021) supported the initial hypothesis that *A. astaci* genotype A was circulating in white-clawed crayfish populations in Northeast Italy.

Keywords: coexistence, monitoring, swabs, genotyping

An opportunistic pathogen *Aphanomyces laevis* (Oomycota) is a host of new bunya-like virus

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Oomycetes are fungal-like microorganisms that cause significant disease outbreaks of freshwater animal species, including crayfish, threatening biodiversity and food safety. High-throughput sequencing (HTS) has recently facilitated the discovery of numerous oomycete viruses, whose impact may range from asymptomatic infections to reduced virulence in their hosts. However, all oomycete viruses known to date have been found in plant-associated species. In freshwater environments, *Saprolegnia* and *Aphanomyces* spp. are important oomycete pathogens of fish and crayfish belonging to the order Saprolegniales. Here, we aimed to screen for the first-time *Saprolegnia* and *Aphanomyces* isolates for viruses. We have isolated total RNA from 24 *Aphanomyces* spp. and 14 *Saprolegnia parasitica* axenic cultures, pooled the samples into two pools, and sequenced them on Illumina platform. A bioinformatics pipeline filtered low-quality reads and aligned the sequences with viral and host genomes. Next, a *de novo* assembly of viral reads was performed to reveal a RNA-dependent RNA polymerase (RdRp) with distant homology to the order *Bunyavirales* in one of the pools. Virus-specific primers were designed for reverse transcription-polymerase chain reaction (RT-PCR), confirming the virus's presence in an *Aphanomyces laevis* isolate from the signal crayfish in Finland. *Aphanomyces laevis* is usually considered a saprotrophic species associated with fish and crayfish that can in specific circumstances turn to an opportunistic pathogen. Our data are the first evidence of a viral sequence in freshwater oomycetes from the order Saprolegniales.

Future studies should explore the virus's effects on its oomycete host, transmission modes, and host range.

Keywords: *Bunyavirales*, oomycete viruses, high-throughput sequencing (HTS), RNA-sequencing, RNA-dependent RNA polymerase (RdRp)

Genomic characterisation of the white-clawed crayfish (*Austropotamobius pallipes* complex) in Italy: biodiversity assessment and support for conservation strategies

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Two species of White-clawed crayfish of the *Austropotamobius pallipes* complex inhabit the Italian peninsula: *A. pallipes* and *Austropotamobius italicus/fulcisianus*. Once widespread over the country, the range of *Austropotamobius* spp. has significantly decreased because of the introduction of diseases by non-native crayfish species and of anthropogenic pressure. A total of 1272 samples from 62 populations were collected in the north-western Italian Apennines to characterize genetic variability with both mitochondrial DNA sequencing and nuclear ddRAD (double-digest Random Amplified DNA) markers as part of the LIFE CLAW project. These analyses revealed the presence of gene pools specific to *A. pallipes* in the west and *A. italicus/fulcisianus* in the central-eastern Apennines. Within species, a lack of gene flow between different population groups was suggested by the clear correspondence between gene pool geographical distribution and water basins extension. Building on these findings, and thanks to our involvement in the National Biodiversity Future Centre (NBFC) funded by the Italian National Recovery and Resilience Plan (PNRR), we have extended the crayfish sampling area to the rest of Italy. So far, 1049 samples have been collected from 55 populations and various Italian regions including Lombardy, Friuli-Venezia Giulia, Tuscany, Umbria, and Basilicata. Moreover, consistent with the goals of the NBFC, namely the monitoring, conservation, restoration, and valorization of biodiversity in the Italian and Mediterranean landscapes, we are also targeting with the same genomic approach a number of freshwater fish species, including the Italian chub (*Squalius squalus*), the Italian riffle dace (*Telestes muticellus*) and the Italian barbel (*Barbus plebejus*).

Keywords: White-clawed crayfish, *Austropotamobius pallipes* complex, freshwater fish species, genetic variability, biodiversity

Optimisation of a non-lethal field-based sampling method for the detection of *Aphanomyces astaci*

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Aphanomyces astaci is the causative agent of crayfish plague, responsible for the decline of European crayfish. North-American crayfish, however, are resistant carriers. *A. astaci* DNA is detected by qPCR/PCR after extraction from the exoskeleton, sampled post-mortem (lethal destructive method - LDM). As killing of endangered species is controversial, we aimed to evaluate two non-lethal field-based sampling methods for *A. astaci* detection. Seventy crayfish from three asymptomatic carrier invasive species populations and one native crayfish species population undergoing a crayfish plague outbreak, were euthanized and sampled with three methods: (1) non-lethal, non-destructive method (NNM) using a dry interdental brush stored in a buffer solution, (2) non-lethal, destructive method (NDM) collecting separately one pleopod and the setae of two uropods, and (3) LDM collecting one eye, the setae of two uropods, and abdominal cuticula as a pool. DNA was extracted and qPCR for *A. astaci* was performed. In the symptomatic native crayfish population, *A. astaci* was detected in all animals with both LDM and NNM. The NDM and LDM similarly performed detecting *A. astaci* DNA in 2/3 of the invasive species populations. Field-based non-lethal sampling methods can be used to detect *A. astaci* at population level, but the minimal sampling size required for pathogen detection has to be determined. The NNM needs to be optimized in order to be used efficiently to detect carrier status. The NDM might represent an efficient/reliable field-based sampling method.

Keywords: crayfish plague, interdental brush, welfare, conservation, oomycete

Developing multiplex assays for crayfish detection using eDNA and ddPCR

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The use of environmental DNA (eDNA) for crayfish detection has increased worldwide. The number of projects and publications significant rise with the use of techniques such as PCR, qPCR and ddPCR, as well as the increasing use of next-generation sequencing technologies like Nanopore and Illumina. The choice of technology and tools is influenced mainly by research objectives, researcher expertise and available funding. At INBO, we aim to develop an eDNA-based monitoring system for the detection of nine crayfish species in Belgium. Our approach begins with and in-silico study using sequences and primers available in the literature. Subsequently, new sequences will be generated for the target individuals to design specific-target primers and probes. These primers will be tested individually and in combination, culminating in a multiplex assay in a ddPCR for crayfish detection from environmental samples.

Keywords: eDNA, crayfish, multiplex, ddPCR

Morphological variability and condition of introduced spiny-cheek crayfish populations occupying different habitats in Serbia

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The non-native species *Faxonius limosus* belongs to the family Cambaridae and is the first invasive crayfish species to be introduced into Europe. In recent decades, it has been observed in the entire part of the Danube River in Serbia, as well as in the Sava, Tisa, Velika Morava rivers and in the Ćelije Reservoir. The study included 111 specimens (48 from the Danube River and 63 from the Ćelije Reservoir). The analysis includes measurement of 21 morphometric parameters per crayfish, and fitness indices were also calculated. The results partially fit within the known range of variation and represent preliminary data for both habitats. Results showed significant differences between males inhabiting different habitats, while females differed mainly in measurements of cephalothorax. Males were in better body condition compared to females, while both sexes in the Danube River had higher fitness indices compared to the Ćelija Reservoir population. We can assume that the observed morphometric differences are the result of crayfish adaptation to the specific ecological conditions in the different habitats.

Keywords: *Faxonius limosus,* morphometric parameters, crayfish constant, Fulton's body condition, Danube

A contribution to the knowledge of the *Branchiobdella* species of the Balkan region

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Branchiobdellidans are clitellate annelids that occur mainly on astacoidean crayfishes as obligate epibionts. In Europe, all native species belong to the genus *Branchiobdella* Odier, 1823, which is represented by nine species. Of these, five species have been recorded in the Western Balkans *Branchiobdella astaci* Odier, 1823, *B. parasita* (Braun, 1805), *B. pentadonta* Whitman, 1882, *B. hexadonta* Grüber, 1883, *B. italica* Canegallo, 1928, and *B. balcanica* Moszynsky, 1938. The aim of the work is to improve our knowledge of the occurrence of these species in the Balkan region. Samples of *Austropotamobius torrentium* were collected from three streams: Miljevka, Govza and Bistrica, belonging to the River Drina basin in the municipality of Foča in south-eastern Bosnia and Herzegovina. Based on the morphological characteristics of the jaw - shape and dentation, the following species were identified: *Branchiobdella parasita* and species from the *pentadonta* complex. Since the two species of the *pentadonta* complex, *B. italica* and *B. pentadonta,* are morphologically very similar and both were previously found on *A. torrentium*, an exact identification of the species is possible using molecular-phylogenetic analyses that are planned in upcoming period. The obtained results are consistent with the literature data. However, if confirmed, the finding of *B. italica* on stone crayfish from the Black Sea basin would be of particular importance. Until now, the distribution of *B. italica* was restricted only to the watercourses of the Adriatic Sea basin.

Keywords: *Branchiobdella*, Bosnia and Herzegovina, *Austropotamobius torrentium*, Black Sea Basin

Conservation assessment of *Cambarus monongalensis* and *Lacunicambarus thomai* in Pennsylvania

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Previous large-scale burrowing crayfish collection efforts in western Pennsylvania have suggested that *Cambarus monongalensis* and *Lacunicambarus thomai* have limited distributions throughout the state. The limited distribution of these primary burrowing crayfish may potentially impact their conservation status, possibly making them highly threatened in the state of Pennsylvania. However, focused research targeting these specific species is necessary to confirm these suspicions and produce final conservation assessments and S-ranks for the state. This study aims to compile previously collected Pennsylvania records of *C. monongalensis* and *L. thomai* from unpublished sources, reports, publications, and museum specimens. These historical sites will then be checked in the field to ensure that the target crayfish are still present. Furthermore, a map displaying the updated known distribution can then be made to direct further collection efforts determining any range expansions in the state. Overall, the results of this study will provide the information needed to accurately assess the conservation statuses of both *C. monongalensis* and *L. thomai* in Pennsylvania.

Keywords: conservation, distribution, burrowing crayfish

Predation of invasive crayfish by diving beetles, a suitable aspect in nature based solutions?

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Predaceous diving beetles (Coleoptera: Dytiscidae) are worldwide one of the main groups of aquatic predators, and in smaller waterbodies they often act as top predators. Although even the largest species still measure less than 5 cm in total length, these insects are able to kill much larger prey items than themselves. Large diving beetles are present all over the world, with most species having rather wide distributions and a good dispersal ability by flight. Invasive crayfish often invade habitats where larger predators like fish are absent, and where macroinvertebrates dominate. The effects of crayfish invasions on aquatic insects are very poorly studied. The other way around, however, seems almost to have been ignored all together. To get an preliminary idea of the suitability of large diving beetles as nature based solution for invasive crayfish control, an exploratory experiment was set up with the large diving beetle *Cybister lateralimarginalis* and the marbled crayfish (*Procambarus virginalis*). *Cybister lateralimarginalis* is a veracious beetle, which is known to attack nearly all aquatic animals, including fish, amphibians, insects and snails. Therefore it was expected that smaller crayfish could prove suitable prey items. The first results are, however, less straightforward than expected and have raised more questions than answers.

Keywords: Invasive crayfish, insects, diving beetles, predation, natural based solutions

Detection of Novel RNA Viruses in wild Noble Crayfish (*Astacus astacus*) and Signal Crayfish (*Pacifastacus leniusculus*) in Switzerland

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European native crayfish populations are undergoing a strong decline due to environmental factors and the introduction of highly competitive non-native species. Pathogens are an additional threat to native crayfish. Non-native crayfish often act as carriers for devastating diseases. However, aside from *Aphanomyces astaci*, other infectious agents are still widely unknown. This study aimed to investigate viruses present in seven populations of wild noble crayfish (*Astacus astacus*) and 29 populations of signal crayfish (*Pacifastacus leniusculus*) in Switzerland, through high-throughput sequencing. Sequence analysis revealed the presence of 11 novel RNA viruses (one bunya-like, four hepe-like, two dicistro-like, three picorna-like, and one permutotetra-like) in the noble crayfish samples. In signal crayfish, preliminary results revealed sequences from 21 potential viruses (including novel and already described RNA viruses). The discovery of novel bunya-like viruses in noble crayfish as well as in signal crayfish without associated mortality or macroscopical alterations is of particular interest, since they are closely related to the bunya-like brown spot virus, described in 2019 from diseased native white-clawed crayfish (*Austropotamobius pallipes*) during a mass mortality event in France. It seems that these closely related viruses have very different impacts on their respective hosts, raising the need for further investigations on virulence factors and host susceptibility towards these viruses. This study provides a basis for future investigations, permitting to gradually fill the knowledge gap in crayfish viral diseases.

Keywords: native crayfish, non-native crayfish, virus, disease

Exploring the native benthic fish burbot *(Lota lota)* and catfish *(Silurus glanis)* as biological control agents against the invasive marbled crayfish *(Procambarus virginalis)*

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The marbled crayfish got a foothold in Flanders since 2017, and the number of populations is steadily increasing, often times in secluded aquatic systems in urbanized areas. Unfortunately managers are often reluctant to apply drastic measures as draining and dredging. Therefore, we conducted preliminary experiments to investigate the efficiency of benthic fish as a biological control measure. Predation experiments using catfish (*Silurus glanis*) and burbot (*Lota lota*) were performed in a temperature- and light-controlled laboratory setting to test their efficiency as predators of invasive marbled crayfish. The experimental setup consisted of a total of 24 tanks, each containing one fish and two size classes of marbled crayfish (n=6). Experiments ran separately for both fish species. The tanks were checked daily, and eaten crayfish were replenished daily for five consecutive days. Our findings indicate that both burbot and catfish show potential as control agents for managing invasive crayfish populations. Burbot, with its cold-water habitat preference and nocturnal hunting behaviour, contrasts sharply with the more opportunistic and aggressive predation strategies of the catfish. These differing predation strategies and biological traits suggest varying levels of effectiveness and applicability in diverse ecological contexts. Future research will expand on these findings through functional response experiments, where we aim to assess the predation efficiency of native benthic fish (catfish, eel, and burbot) on different invasive crayfish species. These studies will provide deeper insights into the ecological interactions between native fish predators and invasive crayfish, informing more targeted and effective management strategies for invasive crayfish control.

Keywords: marbled crayfish, predation experiment, burbot, catfish, invasive crayfish control

More than 20 years of freshwater crayfish research in the UNESCO site Plitvice Lakes National Park

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Freshwaters within the Plitvice Lakes National Park harbour two native European species; *Austropotamobius torrentium* and *Astacus astacus*. While *A. torrentium* is presumably native here, *A. astacus* was introduced in the past for commercial purposes, and established stable populations in the lakes and gradually displaced *A. torrentium* populations. Populations of *A. astacus* are chronically infected with *Aphanomyces astaci* genotype A, but are viable, while *A. torrentium* populations mainly inhabit streams feeding the lakes. Until now *A. astaci* was established in only one *A. torrentium* population, which resulted in drastic reduction of population size. *Austropotamobius torrentium* was originally recorded in five localities, while more recently, intensive research established three more populations in small streams, some of them running through basophilic pit bogs. In this presentation we will show results of more than 20 years of research that indicates the dynamics of crayfish populations conditioned by the: i) presence of *A. astaci* genotype A, ii) changes caused by hydrogeological processes that are part of the dynamics of the lake system, and iii) possibly by phenomena such as climate change.

Keywords: noble crayfish, stone crayfish, crayfish plague, Croatia

Present status and distribution of decapods in Hungary

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Ornamental aquaculture and the related pet industry are known to be important sources of non-native species even the reason of decline of several native species populations worldwide. During our work we summarised the current occurrence data, and present the actual distribution of all decapods in Hungary. Currently, three endangered native crayfish species occur in Hungary. The narrow-clawed crayfish (*Pontastacus leptodactylus*) and the noble crayfish (*Astacus astacus*) are common species and were a traditional food source until the mid-20th century. In contrast, the stone crayfish (*Austropotamobius torrentium*) remains the rarest one, only at four upland locations. The number of native crayfish populations steadily decrease due to anthropogenic impacts such as water pollution, habitat modifications and the introduction of non-native species, including ornamental species. Fourteen non-native crayfish, eigth crab and

five shrimp species have been reported from Hungary. The most common introduction pathway is the release or escape from aquaria, garden ponds and their using as bait for angling, as well as food items for restaurants. Small individuals can be transported with the fisheries' tanks water. Intensive sampling of potential habitats of other non-native species (thermal springs, industrial warm water outlets, urbanised streams, and side arms of River Danube) established the occurrence of several *Cherax* species, shrimps and marine and fresh water crabs.

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Keywords: native and ornamental species, pet trade, thermal and urban waters, occurrence maps

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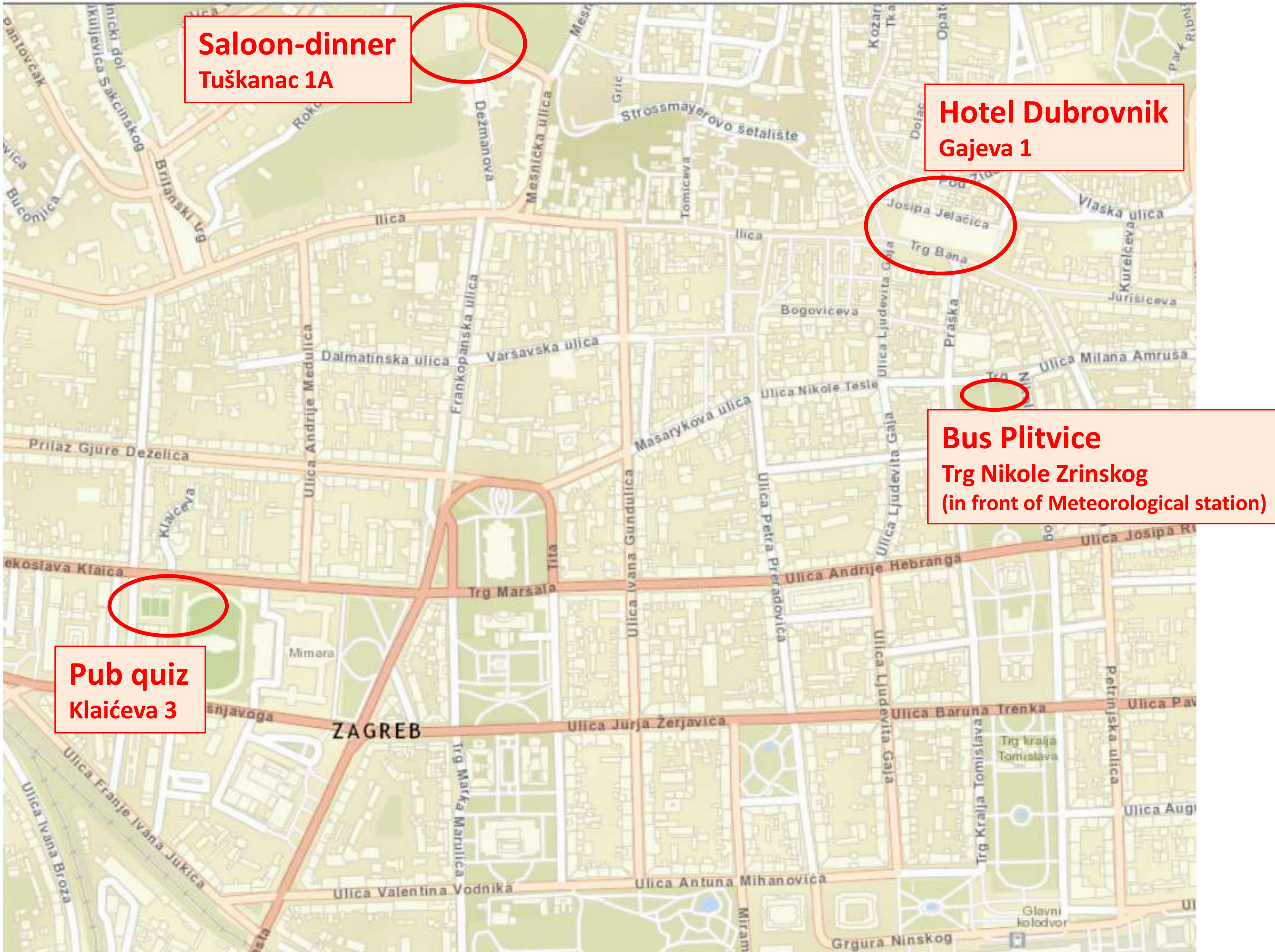
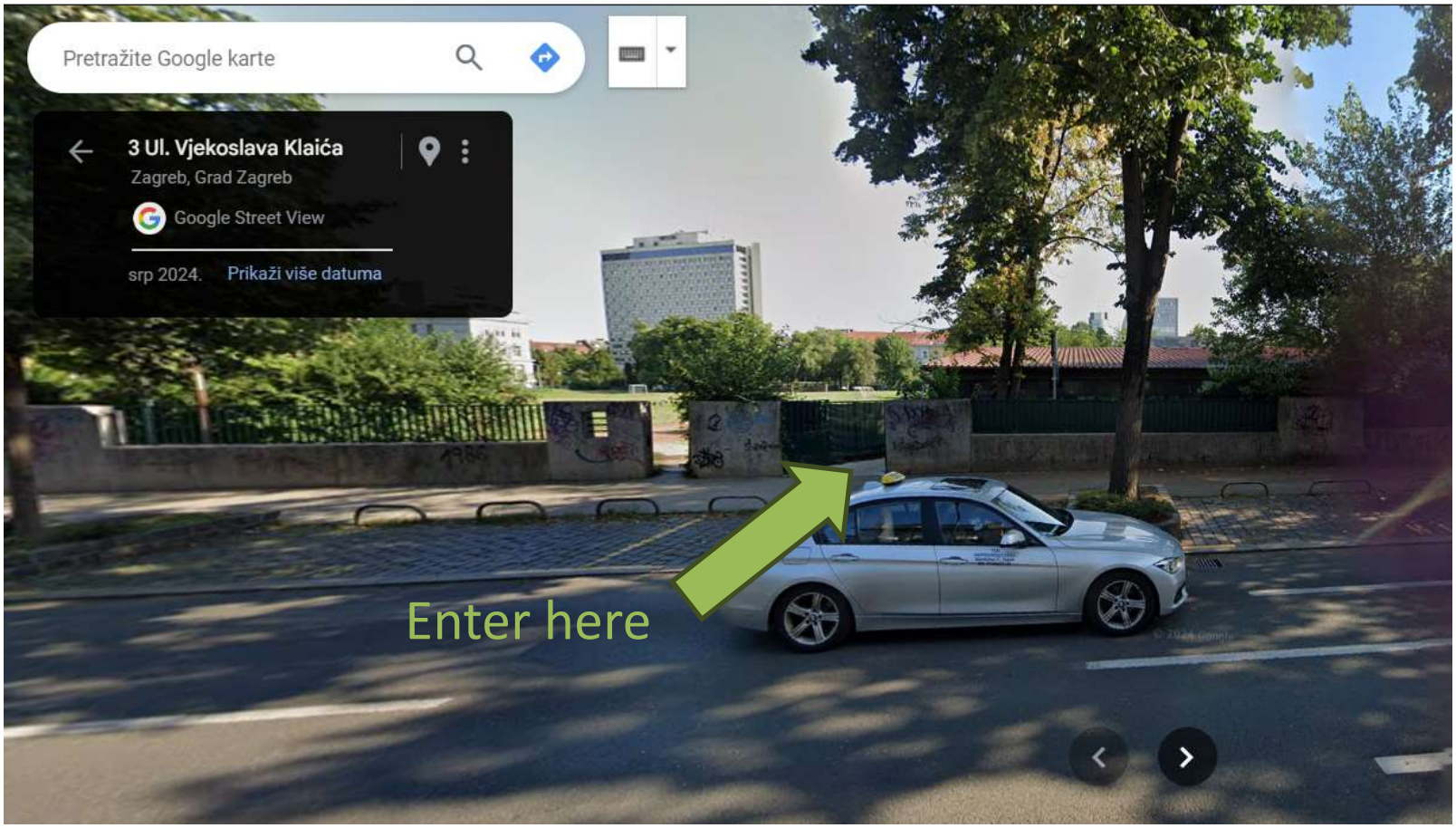

Saloon-dinner
Tuškanac 1A
Hotel Dubrovnik
Gajeva 1
Bus Plitvice
Trg Nikole Zrinskog
(in front of Meteorological station)
Pub quiz
Klaićeva 3
ZAGREB

Bus for Plitvice Lakes – Trg Nikole Zrinskog 3

Pub quiz Ul. Vjekoslava Klaića 3



Dinner - Saloon – Tuškanac 1A