University of South Bohemia
in České Budějovice
Faculty of Fisheries
and Protection
of Waters

I.A.A.

23rd Symposium of the International Association of Astacology

June 20–25, 2022

Hluboká nad Vltavou, Czech Republic

Preface

IAA members have patiently waited from 2019 to 2022, during which the COVID-19 pandemic spread worldwide. IAA23 had to be postponed from 2020 to 2022, with our biennial meeting having a longer total gap of 4 years, during which we had a huge loss of some IAA friends through COVID infection. The pandemic has lessened, but is not yet over. Nevertheless, we are pleased to have our event in the beautiful South Bohemian region, Vodňany, Czech Republic, in June 2022, and many IAA members are happily participating in this landmark crayfish event. The Organizing Committee Chair, Professor Pavel Kozák, with his Czech Republic team, have worked hard to prepare a great symposium. As President of IAA, I sincerely appreciate their huge efforts and time spent preparing for IAA23. The Sture Abrahamsson lecture, three keynote lectures, and numerous oral and poster presentations are listed, plus great excursions and a banquet. IAA23 will long be remembered in the history of our great academic society, International Association of Astacology.

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Instructions for presenters and chairpersons

Venue for oral and poster presentations: **Aleš South Bohemian Gallery**

The duration of **oral presentations**: **20 min** (preferably 15 min + 5 min for the discussion).

Chairpersons are requested to meet the presenters in their session 15 minutes before the start of each session. PowerPoint presentations should be loaded in the morning of each respective day.

Poster authors are kindly asked to mount their posters on the allocated boards on **Monday morning**. The posters should be on display until the end of the symposium. During poster sessions, the authors should be present near their posters.

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Session: Ecology and Biology — Chairman: Lucian Pârvulescu

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Session: Invasive Species — Chairman: Susan B. Adams

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How safe is an island arksite? The example of crayfish in Ireland

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Because of its quaternary history, Ireland has fewer native and invasive species than Britain or continental Europe. Most islands lack native crayfish, unless very large (such as Tasmania and Madagascar) or recently connected to a land mass (e.g. Britain, Vancouver Island). Genetic evidence suggests that white-clawed crayfish were introduced to Ireland from France in the Middle Ages; they now occupy most suitable lowland catchments. Lake populations are frequent but vulnerable to eutrophication. Irish crayfish growth rates are high, diets change with ontogeny, and breeding biology is typical. Crayfish plague arrived in the 1980s, perhaps with visiting anglers. There was a second, larger series of plague outbreaks from 2015-2019, but no carrier species have yet been found. A 2019 meeting of European experts advised on ways and means to monitor and prevent plague spreading. In the same year, an isolated breeding population of yabbies (*Cherax destructor*), believed to have originated from dumping of aquarium specimens, was discovered in a limestone quarry lake. These occurrences demonstrate that even an isolated island arksite is not safe. Methods of controlling the dangers are suggested.

On the development of a supra-regional management tool for the protection and conservation of native freshwater crayfish (*Austropotamobius torrentium, Astacus astacus*) in the federal state of Upper Austria

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Both the stone crayfish (*Austropotamobius torrentium*) and the noble crayfish (*Astacus astacus*) are native to the federal state of Upper Austria. Conserving these endangered species demand for reliable data regarding the spread and the condition of the populations. Austria, though, is lacking a nationwide standardized tool to sample and manage freshwater crayfish.
However, gaining data on crayfish is often hampered by very low funding. Therefore, we have attempted to develop a tool, one for *A. torrentium* in lotic waters and one for *A. astacus* in lentic waterbodies, which is able to assess the conservation status of a population by simply evaluating five indicators: populations density, population structure, population development, habitat quality and state of risk. This enables a quick and comparatively cheap though reliable assessment of native crayfish populations within a wider region including both the evaluation of hazards caused by invasive crayfish species (mainly *Pacifastacus leniusculus*) and the consideration of longitudinal (e.g. barriers) and lateral (e.g. agriculture, forestry) parameters. This tool bases on the results of several projects and a master thesis in which we evaluated different sampling methods (day sampling, night sampling, eDNA, trapping and diving) in various habitats. Data collected by applying this tool will be integrated into a web-based database, accessible to all experts working in the topic of freshwater crayfish within this region. This database should then serve as basis for future projects aiming to conserve and protect native crayfish species and to manage invasive species as well as hindering the spread of the crayfish plague.

Acknowledgement
Projects were/are financially supported by the Austrian Federal Forests and the Federal Government of Upper Austria – Department of Nature Conservation.

How well protected areas safeguard noble crayfish and stone crayfish in Croatia?

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In Croatian freshwater habitats four native European crayfish species are distributed; one of them is noble crayfish and another is stone crayfish, both being endangered due to the strong anthropogenic pressure on their habitats, climate change and invasive alien crayfish species. The aim of the present study was to evaluate the effectiveness of the trans-European Natura 2000 network and nationally designated protected areas in preserving and maintaining noble crayfish and stone crayfish diversity. In order to achieve our goal, we used comprehensive species occurrence data sets and applied GIS-based approach (gap analysis) that overlays species distribution data on a map of designated protected areas. Overlaying the protected areas with the distribution of the noble crayfish revealed that designated protected areas encompass 50% of the recorded noble crayfish populations, and 73.3% of the recorded stone crayfish populations. Results of this study can serve as an assessment of the effectiveness of the protected areas in conservation of these threatened key freshwater species, and direct future conservation planning efforts.

Acknowledgement
The study was financially supported by the Croatian Science Foundation (CLINEinBIOta-IP-2016-06-2563) and Leona Lovrenčić through ESF (DOK-2018-01-9589).

Spatio-temporal effects of alien crustaceans on compositional and functional ß-diversity in Europe

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One of the most studied taxa in biological invasion is invasive alien crustaceans, one of the most species-rich and successful invaders in freshwaters. Crustacean invasions are known to cause staggering ecological impacts, among them the replacement of native species - pushing several species towards extinction, the deterioration of ecosystem functioning and services affecting human well-being, as well as economic impacts. Although the impacts of invasive crustaceans on local communities and species compositions are well studied, information on how communities change over time (i.e., beta diversity) due to crustacean invasions have remained mostly anecdotal. To investigate trends of invasive crustaceans in Europe, as well as their impacts on the invaded communities' functioning (proxied by taxonomical and functional beta diversity) over space and time, we used a recently collated dataset containing 1,816 freshwater macroinvertebrate time-series (containing 13,907 species, among which 30 where invasive crustaceans) in conjunction with 12 ecological and 15 biological traits for each species. First, we identified trends for the different groups of crustacean invaders (amphipods, crayfish, crabs, isopods, and mysids). In addition, we calculated five common community (abundance, richness, Shannon diversity, Pielou's evenness, and turnover) and four functional metrics' (functional richness, diversity, dispersion, and divergence) trends over time and investigated faunal homogenization of invaded ecosystems over time using Bray-Curtis dissimilarity. While species richness increased and communities became more diverse over time, we identified a simultaneous increase in the presence of invasive crustaceans, which were mostly driven by amphipods, while invasive mysids decreased. On a functional level, richness and dispersion of traits increased, while functional divergence only increased for biological but not ecological traits. Concomitantly, we identified that community compositions and functioning became less homogenized, driven by increasing presence of invasive crustaceans. Our results demonstrate the pressures exerted by crustacean invasions that threaten biotic communities and their functioning. Observed differences in compositional and functional changes emphasize the need also to consider functional traits when assessing the effects of biological invasions. Thus, our results will contribute to a better understanding of spatio-temporal changes in community compositions and functioning of changing ecosystems due to invasions.

Crayfisheries policies and practices – the European case

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Invasion of crayfish plague disease agent, *Aphanomyces astaci*, triggered native European crayfish conservation. Crayfish plague epidemics quickly spread over Continental Europe, then to Finland, Sweden and finally *A. astaci* was discovered also in Iberian Peninsula, Norway, Ireland, and United Kingdom by 1980s. Significant proportion of native crayfish stocks had then been lost, while industrialization and waterways construction were causing further damage to remaining native crayfish stocks. First alien crayfish introductions already gave rise to first wave of crayfish plague epidemics in late 19th century, while much later in 1960s it was decided that introductions of alien *Pacifastacus leniusculus* should be initiated. Decisions were based on presumed advantages for fishery, suitable habitat requirements and supposed resistance against *A. astaci*. Furthermore, conservation of native European crayfish species was sidelined and focus shifted toward alien crayfish stocking routine and consumption. Alien crayfish species introductions resulted in repeated waves of crayfish plague epidemics among remaining native crayfish stocks. It was soon discovered that alien crayfish were, as suspected, permanent reservoirs for *A. astaci*, some of them losing their resistance against *A. astaci* and thus struggled in European aquatic ecosystems. We introduce numerous motives behind grand mistake of introducing alien crayfish species to Europe instead of focusing on conservation of native crayfish species. False economical, biological and ecologic assumptions were used to justify a hasty introduction of alien crayfish, which has further devastated native crayfish and also permanently changed European aquatic ecosystems. Science-based warnings about alien species damage to native ecosystems and native crayfish must be taken with utmost caution. Protection of native European crayfish should be core issue, not commercial activities. We summarize main threats and actions needed to protect remaining native freshwater crayfish fauna in Europe.

Acknowledgement
Big thanks to those working to protect native ecosystems.

No way to get it out: the vain fight with signal crayfish in a small stream

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Established invasive crayfish are usually very hard to eradicate or even manage at an acceptable level. And what is the acceptable level for invasive species? One way or another, when a population of native crayfish species is pushed upstream and replaced by invasive species, some management is essential. Here we present our effort at the small foothill stream (width of ca 1.5 m, depth of max. 0.4 m) with signal crayfish population and with a fragmented upstream population of noble crayfish. In 2016, the signal crayfish was found with many big, surely more than 6 years old, animals and other size categories. It pointed out to the established population. In the following years (2017-2018) we performed 8 capture events (all possible shelters inspection and signal crayfish removal) resulting in ca. 2100 captured individuals. In 2019-2020 we increased the effort to 8 capture events per year (from April to November). However, the numbers of captured crayfish were much higher than in previous years (together ca. 10 000 individuals). The first years of eradication seemed to rather promote than limit the signal crayfish population, as the capture of bigger animals is easier than for smaller cohorts. Those subsequently increased abruptly, having more space, less competitive pressure and cannibalism by bigger crayfish captured first. To conclude, even hand searching in a small brook (vast majority of shelters were inspected) is selective and similarly as trapping, the smaller individuals remaining take advantage of the lack of competition to grow rapidly. However, continual removal can at least regulate the possible spreading of the signal crayfish in the long-term perspective. So it is worth continuing the removal despite high personal costs and no immediate visible effect. Other monitoring and upgrading of capture methods are also required.

Sterilised female pheromones do not affect male reproductive behaviour in an invasive crayfish species (*Pacifastacus leniusculus*)

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The occurrence in North-Italian inland systems of one of the most invasive crayfish species, *Pacifastacus leniusculus* requires effective management by both the EU and Italy, overall because signal crayfish populations are actually low, and the distribution area is still rather restricted. Among methods for controlling invasive crayfish species, the autocidal ones, because they interfere with reproduction, offer the advantages of being species-specific and inversely density dependent, without causing environmental changes. An ideal control technique should consider the decrease of eggs and juvenile production, and therefore the progressively ageing population until the density threshold, below which the population will cease to be self-sustaining and will collapse. Therefore, the idea of removing female pleopods (treatment) in order to remove the support for the attack of newly fertilised eggs. To address this question, signal crayfish males were exposed to water conditioned by 1) treated mature females (TM), 2) untreated mature females (UM), 3) immature females (IF) and 4) Calcium-enriched freshwater (C). The behaviour of 100 males (25 for each treatment) was recorded on video for 15 minutes before and after injection of each test water. The three behavioural categories of 'antennae moving', 'moving' and 'handling of the air-stone' were recorded. Males exposed to both TM and UM water exhibited significantly increased levels of 'handling of the air-stone' activity, than males exposed to C or IF water. These results demonstrated the female sterilisation by removing pleopods does not alter the mating behaviour in male *P. leniusculus*, and therefore could be employed to control the invasive species in management projects.

Acknowledgement
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Towards real-time global mapping of crayfish species and crayfish plague occurrence

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Accurate spatial analyses require the processing of large amounts of distribution data. Range shifts of native crayfish species in the context of ongoing invasions and the mapping of the associated diseases are rapidly running out of date, making any attempts to perform spatial data interpretations less accurate. However, accumulating new records costs precious time, during which scientific understanding and effective conservation assessments remain largely limited. Here we come with a geoportal (https://world.crayfish.ro) specifically designed for tracking the worldwide distribution of crayfish species and records of different strains of the crayfish plague pathogen (*Aphanomyces astaci*). The geoportal has built-in visualisation modules and allows users to produce their customised maps. New inputs (to be included in the data after expert validation) are easily collectable, allowing permanent updates of the database and associated species distribution maps with the latest scientifically proven records. Through this approach, we thus expect to offer a pivotal tool meant to inform the stakeholders regarding the potential expansion of the invasive species and diseases in the context of accurate monitoring of the native and endangered species.

Characteristics of stone crayfish populations along a disturbance gradient – a case study of Kustošak Stream, Croatia

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The Kustošak Stream is partially located within the Medvednica Nature Park, which is known for abundant stone crayfish (*Austropotamobius torrentium*) populations. This species is sensitive to changes in habitat so any anthropogenic impact puts stone crayfish populations at risk. This study aimed to assess how modifications of the natural features of a freshwater habitat and subsequent water quality changes affect populations of the stone crayfish in the Kustošak Stream in Zagreb, Croatia. For the study we selected three spatially close locations but under differing levels of anthropogenic disturbance: (1) no disturbance, (2) recently modified part of the stream during construction and (3) long time modified part of the stream through channelization and artificial modification of streambed. During a week-long period in September 2021, at each site, we measured physicochemical parameters of the water and collected crayfish with baited handmade traps. Caught individuals were used to estimate population size by mark-recapture method, and to analyse population structure by recording sex ratio, animal size and condition (Fulton's condition factor). We then compared all the measured population and water parameters between sites. Results showed that population density of the site without disturbance (1) was significantly higher in comparison to the two sites that are anthropogenically impacted. Also, the crayfish size structure of this site differed significantly as opposed to the two hydromorphologically altered sites. Furthermore, PLS analyses has shown that crayfish abundance (expressed as CPUE) is strongly affected by physicochemical parameters of water, including among others: oxygen, calcium ions, nitrates, nitrites, COD, pH and water temperature. We discuss our findings in the context of effects of anthropogenic disturbance on viability and persistence of this EU priority species.

The current status of crayfish in Indonesia

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The Indonesian archipelago consists of more than 17,000 islands and islets, inhabited by megadiverse flora and fauna with many endemic species. There are plenty of endemic crayfish species from the genus *Cherax* inhabiting diverse waterbodies in New Guinea, the island whose western part belongs to Indonesian territory. The total number of species there was expected to be around 100 (including at least one cave species). Therefore, New Guinea is considered a prominent crayfish biodiversity hotspot. New Guinean crayfish are attractively coloured and accordingly very popular as ornamental animals. About ten species are traded more or less commonly on the market, especially in the European Union and new species are formally described year by year. The pet trade is one of the main sources of invasive species, including freshwater crayfish and pathogens, which cause socio-economic losses and negative impacts on native biota in many regions where introduced. Indonesia was identified as the leading supplier of ornamental crayfish globally. Recently, a new threat to *Cherax* crayfish was found in Java, Indonesia: the crayfish plague pathogen (*Aphanomyces astaci*). This disease is assumed to be lethal to all crayfish species of non-North American origin. The North American species, *Procambarus clarkii*, was confirmed as the carrier and vector of the pathogen in Indonesia. The introduction of this species is alarming from the native fauna perspective, not only due to crayfish plague transmission but also because *P. clarkii* is it is often considered the most invasive crayfish species. More crayfish species were recorded to be traded on the domestic market. These crayfish are transported via numerous routes across the country. There are no effective legislative measures against non-indigenous crayfish species in Indonesia and crayfish are used by also as live fish bait by local anglers. The intensive education of the general public preceding the focused restrictions is crucial to prevent the further spreading of invasive crayfish and protect the endemic ones.

Crayfish diversity within the Tennessee River drainage: A reassessment using molecular phylogenetics

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Species identification and evolutionary relationships of crayfishes has traditionally been based on morphological traits. This is problematic as crayfish, like many crustaceans, harbour morphological plasticity and cryptic forms. Recent phylogenetic studies using molecular methods conflict with morphologically based evolutionary relationship hypotheses of crayfishes. This is likely due to traditional morphological characters being a mix of convergent and conservative traits. These phylogenetic studies have indicated that several wide-ranging species represent species complexes that house cryptic crayfish diversity as a result of allopatric speciation. The Tennessee River drainage harbours approximately 12% of North America's crayfish diversity; however, this is likely an under representation since many wide-spread species within the Tennessee River drainage have not been re-examined since their initial morphology-based descriptions for cryptic diversity. We examined the phylogenetically reconstructed relationships of two wide-spread species, *Faxonius erichsonianus* and *Cambarus striatus*, within the Tennessee River system using mitochondrial DNA to highlight potential areas of hidden crayfish diversity. Preliminary analyses from our lab indicate a mix of locally distinct and drainage wide lineages for both species.

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Diversification and importance of repetitive elements in Decapod genomes

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The genome size of decapods species shows huge variations, from 1 Gb in shore crab to 40 Gb in *Sclerocrangon ferox*. Such remarkable variations may be explained by a variable amount of repetitive elements, the so-called 'repeatome'. Repetitive elements fall into two main categories: tandem repeats and transposable elements (TE). Tandem repeats are classified into microsatellite, minisatellite and satellite DNA. TE have the ability to copy and insert themselves to different locations within a genome and are subdivided in Class I (retrotransposon) and Class II (DNA transposons). By their insertions, TE can lead to the emergence of new genes, modify gene expression, and promote genome rearrangements. They constitute important drivers of genome plasticity and innovation with impacts on cellular functions, adaptation and diseases. To investigate and compare the so far unknown quality and quantity of repetitive elements in Decapoda, we analysed 20 publicly available Decapoda genomes (including 4 crayfish species), plus 6 crustacean genomes as outgroup, for a *de novo* and homology based identification of repetitive elements. Our analysis reveals that the number of repetitive elements is positively correlated with genome size. In comparison to other crustacean, Decapoda genomes are characterized by a massive load in repeats with a distinct pattern of family expansion between the two suborders. The repeat content proportion in Decapoda genomes is mainly above 40%, but variable between and even within phylogenetic clades. It is especially high in freshwater crayfish (55%) and hermit crabs (75%) with a large diversity of satellite families and massive expansions of some retrotransposon. Among them, penelope elements, that can be responsible for dysgenic crosses, are particularly prevalent in freshwater crayfish. In almost all genomes, we revealed endogenous retrovirus, that can have a role in immunity system by defending against exogenous viral infections. This first large-scale exploration of the decapod repeatome establishes repetitive elements as major factor contributing to the variation of genome size in Decapoda. Analyses of some major TE and satellite families will shed the light on the evolutionary histories of the repeat landscape and their role in the dynamics of Decapoda genomes.

Cytogenomic investigation of repetitive elements in the family Astacidae

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Freshwater crayfish are considered keystone species of freshwater ecosystems and have a significant impact on the biodiversity within their habitats. Despite their important role, they are poorly investigated at a genomic and cytogenomic level, and little is known about the chromosomal evolution and genome organisation of these species. The aim of our study was to increase knowledge about the cytogenetic characteristics and genome evolution in European freshwater crayfish. Using flow cytometry we estimated huge genome sizes in two species of the family Astacidae: 18,7 Gb for *Pontastacus leptodactylus* and 17 Gb for *Astacus astacus*. In contrast, genome sizes in Cambaridae and Parastacidae range only between 2 to 6 Gb. Large genome sizes can result from the accumulation of repetitive DNA sequences: tandem repeats and retroelements. To investigate this, we performed clustering analysis of a low coverage genomic dataset to identify and characterise repetitive DNA. In the freshwater crayfish *P. leptodactylus* we observed that almost 55% of the genome consists of repetitive DNAs, with satellite DNA being the most abundant type. We mapped five of the most abundant sequence families of satellite DNA in this species on chromosomes, using fluorescence *in situ* hybridisation. The localisation allowed to identify a potential (peri)centromeric satellite family named PLSAT3-411. Based on the analysis of repetitive elements across the family Astacidae we identified unique and shared repetitive elements among different species. Some repetitive elements are preserved through long evolutionary periods and are probably associated with essential chromosomal structures, such as centromeres, pericentromeres and subtelomeres. Because a large and repetitive genome makes genome sequencing and assembly highly challenging, the identification of repeat elements through cytogenomic approaches is a suitable method for genome characterisation in freshwater crayfish.

Molecular variability of white-clawed crayfish *Austropotamobius pallipes* species complex populations of the Italian North-western Apennines assessed through mitochondrial DNA sequencing and high-throughput Genotyping-By-Sequencing

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Austropotamobius pallipes is currently considered as a species complex including *Austropotamobius italicus*, an autochthonous species inhabiting the Italian peninsula. Due to the severe contraction of the number of individuals, *Austropotamobius* populations in Italy have become isolated reproductive units, often confined to separate water basins. The Life CLAW project aims at improving the taxon's conservation status in the Italian north-western Apennines through *ex situ* breeding and restocking actions aided by molecular data. To gain knowledge on the genetic variability of the species within the project area, 948 samples have been collected from 43 populations. After DNA extraction, a fragment of the mitochondrial (mtDNA) *Cytochrome Oxidase I* (*COXI*) gene was sequenced, while the nuclear genome variation was assessed by Genotyping-By-Sequencing (GBS). Both mtDNA and nuclear DNA data showed a clear differentiation between the gene pools of *A. pallipes* in the west and *A. italicus* in the central-eastern Apennines. Remarkable variation was scored at both the mitochondrial and nuclear genomic level, despite the 74% contraction that the populations of these species have undergone in Italy during the last 10 years. The phylogenetic network reconstructed from mtDNA sequences showed the occurrence of more than 40 different haplotype variants, mostly population-specific, but accompanied by some variants shared by several populations over short geographical distances. The GBS technique detected ca. 90K and 36K Single Nucleotide Polymorphisms (SNPs) in the *A. italicus* and *A. pallipes* populations, respectively. The PCA analysis of GBS data confirmed the clear differentiation between the two lineages, together with the occurrence of a geographical structuring of diversity within each lineage. This phylogeographic pattern is in line with previous evidence and points at the effects of paleoclimatic events that affected the Mediterranean area during the Plio-Pleistocene.

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Genetic data and species distribution modelling highlight the vulnerability of the stone crayfish in Croatia

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The stone crayfish (*Austropotamobius torrentium*) is a native European species endangered by anthropogenically induced alteration of its habitats, climate change and invasive alien crayfish species. Diminishing populations' trends across its distribution range highlight the importance of developing effective conservation that will ensure its long-term survival. Aiming to guide future conservation programs of the stone crayfish in Croatia, we combined population genetics and species distribution modelling (SDM) to reveal the impact of invasive signal crayfish (*Pacifastacus leniusculus*) and climate change on the stone crayfish diversity. Population genetic analyses based on microsatellites revealed moderate within-population genetic diversity and high differentiation among populations, reflecting isolated populations with limited gene flow. Alongside strong genetic structuring, we discovered high level of inbreeding coefficient indicating homozygote excess within the majority of populations. The SDM results predicted substantial reductions of suitable habitats for both species by 2080 under two representative concentration pathway (RCP) scenarios: RCP 4.5 and RCP 8.5, respectively describing low-warming and high-warming conditions. Obtained results indicated that many populations with high and/or unique genetic diversity are located in the areas predicted to become unsuitable in the future. Further, ecological niche analysis showed that stone crayfish and signal crayfish do not exploit equivalent niches in Croatia. Our study highlights the importance of conserving remnant populations and establishing new populations in areas likely to support stone crayfish in the future (climate change refugia or ark sites), by assisted migration and repopulation approaches that can help populations overcome the risks of inbreeding and maladaptation. The results of our research emphasizes once again the importance of multidisciplinary approach in the modern biodiversity conservation.

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Location-dependent DNA methylation signatures in a clonal invasive crayfish

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DNA methylation is an important epigenetic modification that has been repeatedly implied in organismal adaptation. Adaptivity is a crucial trait for invasive species, as it allows them to rapidly respond to newly colonized environments. In invasive species, rapid adaptation often cannot be explained by the traditional selection of genetic variants, which requires longer timeframes. Indeed, several studies have explored possible epigenetic mechanisms in this context. However, many previous studies that have linked DNA methylation patterns to environmental parameters have been limited by confounding factors, such as cell-type heterogeneity and genetic variation. We analyzed DNA methylation variation in marbled crayfish, a clonal and invasive freshwater crayfish that is characterized by a largely tissue-invariant methylome and negligible genetic variation. Using a capture-based subgenome bisulfite sequencing approach that covers a small, variably methylated portion of the marbled crayfish genome, we identified specific and highly localized DNA methylation signatures for specimens from geographically and ecologically distinct wild populations. These results were replicated both biologically and technically by re-sampling at different time points and by using independent methodology. Finally, we show specific methylation signatures for laboratory animals and for laboratory animals that were reared at a lower temperature. Our results thus demonstrate the existence of context-dependent DNA methylation signatures in a clonal animal.

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Origin and propagation of the marbled crayfish

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The marbled crayfish (*Procambarus virginalis*) is a triploid and parthenogenetic freshwater crayfish species that has colonized diverse habitats around the world. Previous studies suggested that the clonal marbled crayfish population descended as recently as 25 years ago from a single specimen. However, the genetic, phylogeographic, and mechanistic origins of the species have remained enigmatic. Using population-scale phylogeographic analysis of the diploid and sexually reproducing parent species, *Procambarus fallax*, we recently demonstrated that both parental haplotypes of the triploid *P. virginalis* were inherited from the Everglades subpopulation of *P. fallax*. Comprehensive whole-genome sequencing also detected triploid specimens in the same subpopulation, which either represent evolutionarily important intermediate genotypes or independent parthenogenetic lineages arising among the sexual parent population. I will present and discuss results from our ongoing analyses that aim to resolve these questions.

Distribution of Decapods in Hungary: present status, distribution and the impacts of the non-native crayfishes, crabs and shrimps to the colonised ecosystems

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We summarise the current status of decapods in Hungary and present adverse effects of non-native representatives on the species composition in different habitats. 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 a traditional food source until the mid-20th century. In contrast, the stone crayfish (*Austropotamobius torrentium*) remains the rarest one, only at upland locations (Pilis-, Börzsöny-, Visegrádi-, Kőszegi-mountains). The number of native crayfish populations remains steadily decreasing due to anthropogenic impacts such as water pollution, habitat modifications and the introduction of non-native species. Fourteen non-native crayfish, five 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 using as bait for angling lakes, as well as restaurants. Small individuals can be transported with water of fisheries tanks. Intensive sampling of potential habitats of other non-native species (thermal springs, industrial warm water outlets, urbanised streams, and side arms of River Danube) provided the occurrence of several *Cherax* species, shrimps (*Atya gabonensis, Caridina babaulti, C. gracilirostris, C. multidentata, Neocaridina denticulata*) and crabs (*Callinectes sapidus*, *Cardisoma armatum*, *Limnopilos naiyanetri*, *Parathelphusa pantherina*). Following the first record of several non-native crayfish, the negative effects were detected in different habitats of the Carpathian Basin. Our surveys showed that the non-native crayfishes are capable of affecting species composition of colonised habitats and that several species are important prey of e.g., fishes, reptiles, birds, and mammals.

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Marbled crayfish (*Procambarus virginalis*, Lyko 2017) invasion strategies in Estonian freshwater ecosystem

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Biological invasions are crucial issues worldwide and marbled crayfish (*Procambarus virginalis,* Lyko 2017) is one of the examples of freshwater invaders spreading across Europe and beyond. Its high growth rate, reproduction, and adaptability to new habitats make *P. virginalis* a successful invader. Although *P. virginalis* is a warm water species, it can adapt well also to colder temperatures. However, the role of temperature in marbled crayfish invasion in natural habitats, has been scarcely studied. The aim of this study was to assess whether the temperature is the main factor shaping marbled crayfish trophic niche and its establishment and distribution, in the artificially warm outflow channel of the Baltic Power Plant, in the Narva Reservoir, Estonia. We hypothesised that warmer water temperature and temperature gradient along the channel affected the establishment, distribution, and trophic niche of marbled crayfish. Temperature loggers were deployed for one year along the channel to record temperature variations. Stable isotope analyses of carbon and nitrogen were performed to assess the trophic niche and diet of marbled crayfish through SIBER and MixSIAR models. Although no temperature gradient was recorded, water temperature in the channel was significantly warmer than in Narva Reservoir, providing a more suitable habitat for the establishment of marbled crayfish. Models results showed spatial and seasonal trophic niche shifts, indicating different diets and trophic positions between the head and mouth of the channel, and between summer and autumn, respectively. In particular, MixSIAR model results indicated that the proportion of marbled crayfish diet at the head of the channel is mostly represented by macroinvertebrates (54%) and macrophytes (32%), as opposed by mostly periphyton at the mouth of the channel (43%). Seasonally, marbled crayfish diets shifted from primary consumers in spring to primary producers in autumn. Our findings showed that temperature had an important role in marbled crayfish establishment in the invaded channel. However, the population distribution, represented by higher abundance at the head of the channel compared to the mouth, was likely affected by other ecological aspects, such as food sources availability, rather than temperature.

Comparison of marbled crayfish with other prominent crayfish invaders under two temperatures

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Invasive crayfish species are well-known for their adverse effects on biodiversity and ecosystem functioning. As their introduced ranges expand, sympatric populations of these species become more frequent. The question of which species and under what circumstances will dominate these communities is of great interest. Some of them are already well established and widespread. However, the parthenogenetic marbled crayfish is much less studied, yet the most outstanding freshwater crayfish invaders due to its unique reproduction mode and overall competitiveness. We conducted a series of independent trials evaluating survival, growth, claw injury and reproduction of single-species (intraspecific) and mixed (interspecific) stocks of marbled crayfish vs other invasive crayfish species. For comparability, only juveniles at the onset of exogenous feeding were used. Two temperature regimes of ~22 and ~16°C were applied for 15-18 and 45 weeks, respectively. Red swamp crayfish and common yabby (a species tested at 22°C only) grew faster than marbled crayfish in both single and mixed stocks. Marbled crayfish were superior to spiny-cheek crayfish and signal crayfish in terms of growth. Still, later species also attained considerable sizes at the end of the experiment under lower temperatures. Faster-growing species usually reached a higher survival rate and tended to negatively impair smaller counterparts by greater claw injury, delayed maturation, and reduced fecundity. Only marbled crayfish laid eggs as early as 14 and 36 weeks under higher and lower temperatures; however, eggs developed only under higher temperatures. We conclude that the success of marbled crayfish among invasive crayfish is significantly driven by relatively fast growth as well as early and frequent reproduction. These results suggest that interactions between invasive populations can unfold when invaded ranges overlap, thereby contributing to the knowledge base on complex population dynamics between already settled and newly emerging invasive crayfish species.

The use of bioenergetic population models in risk assessment and management of invasive crayfish species

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Today we face an increased need to solve complex ecological challenges, such as synergistic effects of biological invasions and climate change. Thus, assessing effectiveness of invasive species management scenarios or conservation priorities for endangered native populations affected jointly by biological invasions and climate change, is crucial. While field and laboratory studies required for such assessments are costly and logistically challenging, mathematical modelling offers a good alternative. Bioenergetic mechanistic modelling, in particular, can integrate all relevant species-specific biological and ecological information, and can be applied to identify high-risk species in a changing environment and/or management scenario with the highest impact on invasive crayfish abundances. Here we demonstrate the application of Dynamic Energy Budget (DEB) models in: i) development of a conceptual population dynamics model for evaluation of effectiveness of different approaches to signal crayfish control and ii) comparison and assessment of individual growth and reproduction of two prominent crayfish invaders (signal crayfish and the marbled crayfish) and two native and endangered crayfish (noble crayfish and stone crayfish) under changing environmental conditions. Since DEB models capture dependence of metabolism, and therefore ontogeny, on environmental and population conditions, they can be used to investigate diverse biological and ecological questions. Based on the examples of the two studies presented here, we argue that mechanistic models in general, and bioenergetics models in particular, should be at the core of adaptive management and decision-making in nature conservation and ecosystem restoration.

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Investigating the physiological basis for thermal tolerance in crayfish

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Understanding the physiological basis for thermal tolerance, and the factors that might alter tolerance is of increasing importance in the face of ongoing climate change. Aerobic scope (AS) may be an important driver underlying thermal tolerance and geographic range of aquatic ectotherms. AS represents the excess capacity of an organism to deliver oxygen in support of activity, growth, and reproduction. It can be calculated as the difference between resting metabolic rate (RMR: metabolism required for basic maintenance) and maximum metabolic rate (MMR: maximum metabolic rate an organism is capable of). AS rises, then falls, with increasing temperature. In theory, the temperature at which AS approaches zero should be similar to the upper thermal limit of an organism, because it represents the temperature at which the organism can no longer meet its aerobic energetic needs. To assess the robustness of the predicted relationship between AS and upper thermal limits in crustaceans, we measured AS and critical thermal maxima (CTM) of four crayfish species and one marine shrimp species (*Litopenaeus vannamei*). We hypothesized that each species would reach CTM when its AS approached zero. We exposed crayfish and shrimp to increasing temperatures (25–40°C) at a rate of 1–2°C/h. Organismal RMR was estimated via respirometry and MMR was estimated via the electron transport system (ETS) assay. Aerobic scope was calculated as MMR-RMR. CTM was estimated as the temperature at which individuals could be flipped over and were unable to right themselves within 30 seconds. Crayfish reached CTM between 34.7°C (*Cambarus latimanus*) and 38.1°C (*Procambarus clarkii*) while shrimp reached CTM at 38.9°C. Shrimp results provided some support for our hypothesis with AS approaching zero (i.e. 1–25% of maximum AS) at CTM. However, all crayfish species reached CTM when AS was still at >60% of maximum. This was primarily due to crayfish having a much lower RMR than shrimp, resulting in a greater AS for crayfish at high temperatures. Ongoing experiments are being conducted to gain insight as to why crayfish, as opposed to shrimp, reach their upper thermal limit while still possessing an excess amount of aerobic scope.

Investigating the linkages of physiology, behavior, and acute thermal stress in crayfish with varied burrowing strategies

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As global temperatures increase, climate change is a mounting threat to aquatic invertebrates. Conservation of threatened taxa such as crayfish require an understanding of thermal tolerance but there are several challenges that researchers face. These include an incomplete understanding of the relationships between physiology and thermal tolerance, as well as the difficulty of obtaining sufficient numbers of study animals for taxa that are federally protected and/or are difficult to collect (i.e. primary burrowers). In this study, we measure the physiological responses of three common secondary burrowers, and one state-threatened primary burrower, to acute thermal stress. We relate physiological endpoints to thermal tolerance and test for differences in physiology between the primary burrower and the common, secondary burrowers. All four species were initially acclimated to 25℃ and then exposed to acute thermal stress as temperatures were raised at a rate of 2℃/h. We used intermittent respirometry to measure metabolic depression (MDt – the temperature above which metabolic rate stops increasing and begins to decrease). For each crayfish, we also recorded the temperature at which they could no longer right themselves after being flipped (LOE – loss of equilibrium), and the temperature at which physical death was observed (temperature at which all movement of appendages ceased). All taxa exhibited loss of equilibrium within 3℃ of exceeding MDt and physical death within 5℃ of exceeding MDt. The upper thermal limit for the primary burrower (*C. hartii*) was within 3℃ of even the most tolerant secondary burrower (*P. clarkii*). Results suggest that MDt indicates the onset of severe thermal stress for crayfish and may provide a valuable, physiological endpoint for developing acute temperature guidelines/regulations. Results also suggest that primary burrowing species may be only slightly less tolerant of acute thermal stress than secondary burrowers and may not require more restrictive thermal guidelines for protection.

Impact of short-term pH change on anti-predatory behaviour and anxiety-like behaviour of crayfish: a comparative study

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Threat of predation is a dangerous situation that is encountered very frequently in natural environment and can induce undesirable changes in the prey's behaviour and physiology. Predation ultimately leads to death and hence, anti-predatory responses are crucial for survival. In aquatic environment signals indicating predatory threats are usually chemically transmitted through the medium of water. Changes in pH of water can interfere with the transmission of chemical signals and/or their detection by the receptor sites besides also impacting nervous processes involving central nervous system. As such, low pH can impact anti-predatory behaviour of aquatic crustaceans by interfering with the detection of predatory cues and hence increasing the chances of predation Also, detection of a predatory event can induce stress which is manifested as anxiety-like behaviour. In this study we compared the anti-predatory and anxiety-like behaviour among crayfish in response to chemical (alarm odour/blood from conspecifics) and physical (manual handling to mimic transient capture by predator) stimuli upon short-term exposure to two different pH (pH6 and pH8). Manual handling stress was used as a control stressor to determine whether short-term exposure to low pH elicits a peripheral or central response in crayfish. Hemolymph glucose levels which correspond to changes in serotonin, were analyzed to indicate stress levels. We observed that crayfish did not effectively respond to alarm odour at pH6 and were less stressed than those at pH8 as observed in behaviour trials as well as glucose level measurements. However, there was no statistically significant difference in response to manual handling in the two pH groups where animals were found to be equally stressed in behaviour observations. The results of our study indicate that short-term exposure to low pH might not involve central nervous system processes but may impact peripheral processes such as those involved in detection of alarm odour in crayfish.

When reintroduction turns to invasion: a fable about turtles and crayfish

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Alien species enter new areas through a variety of pathways, but always directly or indirectly through human activity. If an alien species manages to reproduce successfully, it may become invasive, with potential far-reaching negative consequences for humans and the environment. The calico crayfish *Faxonius immunis* has an extreme invasion potential due to its capability to walk long distances overland, its high fecundity and early sexual maturity in first summer, and its habit to dig deep corridors in the sediment where it can survive drought and frost. In addition, it is a vector of a highly virulent strain of the crayfish plague disease agent *Aphanomyces astaci*. Calico crayfish has successfully established in Southwestern Germany since 1997 and bordering Northeastern France since 2013. Biological invasions contrast with species reintroductions, which are conservation strategies where native species are deliberately returned into their original habitat. Such a reintroduction project is being carried out within the trans-bordering area between Neuburg am Rhein, Germany, and Lauterbourg, France, where wetlands have been restored since 2011 in favor of the protected European pond turtle *Emys orbicularis*. Here, the long-term success of the reintroduction of this umbrella species can be impacted by the calico crayfish presence through alteration of the habitat structure and food web. This study investigates i) the macrophyte and macroinvertebrate communities since the calico crayfish was first sighted in the area, and ii) the trophic interactions between the turtle and the calico crayfish. Crayfish monitoring revealed a significant expansion of the calico crayfish throughout the study area. Hydrobiological assessments showed that all water bodies, both natural and restored, turned highly turbid due to sediment mobilization by burrowing calico crayfish. Concurrently, both aquatic flora and fauna dramatically declined in terms of species diversity and abundance. However, we showed with eDNA analysis of fecal samples and prey choice tests that turtles prey on calico crayfish in the wild and in captivity. Since the relevance of reintroductions as ex-situ management procedures are nowadays highly debated, our results suggest the potential biological control of the invasive calico crayfish by reintroduced turtles.

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A metatranscriptomic approach to studying the RNA virosphere of freshwater crayfish

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In the era of sixth mass extinction, freshwater ecosystems are experiencing a far greater biodiversity loss then terrestrial ecosystems. Freshwater crayfish, often termed “ecosystem engineers”, play a vital role in freshwater environments. With more than 692 described species they form a highly diverse and globally distributed group of Decapoda crustaceans of monophyletic origin. Pathogens can be a major driver of the biodiversity loss in freshwater ecosystems (e.g. *Aphanomyces astaci*, White spot syndrome virus). Viral pathogens parasitize on all cellular life forms and are dominant entities in the biosphere. RNA viruses account for the majority of the virome diversity infecting Eucaryotes, but our knowledge of their distribution and diversity is limited. In this context we conducted a metatranscriptomic study of 15 freshwater crayfish transcriptome assemblies from 8 species to identify and classify RNA viruses. Identification of the potential RNA viruses was conducted with multiple BlastN searches against the NCBI non-redundant protein and RefSeq viral genome database. Clusters with potentially viral origin were annotated with InterPro5 to extract the RNA dependent RNA polymerase (RdRp) domain of each contig. RNA viruses replicate their genomes using the virally encoded RdRp, an essential part of the viral replicase complex. RdRp is the only universal gene among the RNA viruses, therefore we used it as a phylogenetic marker to taxonomically classify viral contigs. In total 160 viral contigs were identified, with 53 contigs representing putative complete viral genomes. The majority of the identified contigs were classified as Picornavirales, followed by the RNA viruses classified in the undescribed group of RNA viruses, potentially representing a novel group of RNA viruses unique to the freshwater crayfish. Lastly, we conducted a comparative analysis of the performance of the metatranscriptomic (RNA seq-based) and traditional RT-PCR approach to evaluate their efficiency in the identification of the RNA viral contigs. Our initial results suggest that a metatranscriptomic approach might underestimate the RNA virosphere diversity estimates. These findings are an initial step towards an advanced understanding of the role of RNA viruses in freshwater invertebrates.

Evaluation of microsporidia spreading in Italian white-clawed crayfish populations

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The decline of autochthonous freshwater crayfish is due to multiple factors such as the expansion of human settlements, which causes habitat modification and water pollution, the invasion of alien species and the spread of infectious diseases. Among infective agents, intracellular microsporidia represent a significant threat to the conservation of the European white-clawed crayfish *Austropotamobius pallipes* complex. *Thelohania contejeani* Henneguy, 1892, most commonly known as the agent of "porcelain disease", and the recently described *Vairimorpha austropotamobii* Pretto, 2018, cause a macroscopically similar whitening of the abdominal skeletal muscle and lead to the death of the host. In order to investigate the distribution of these pathogens, we monitored 42 populations of freshwater crayfish located in the Alps and Apennines. Overall, we performed 81 surveys, inspecting one by one 3997 freshwater crayfish (1758 males and 2239 females). A total of 95 crayfish macroscopically affected by microsporidia were recorded from 11 populations with prevalence ranging from 1 to 10 %. To confirm the suspected infection and identify the parasites involved, histological and molecular analyses followed by sequencing were performed. We evaluate the distribution of the two parasites, including their overlapping zone, reporting the data on the prevalence and the probability to detect infected specimens, to analyse which variables are correlated to these, considering factors like sex and numbers of crayfish collected. It is the first time that this analysis has been performed for *T. contejeani* and *V. austropotamobii.*

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Chytridiomycosis and crayfish: more than a double threat to amphibian conservation?

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Chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (Bd), is an emerging disease threatening amphibian populations worldwide. While mainly amphibian-based, non-amphibian hosts could play a relevant role in the dynamics of this disease. Freshwater crayfish, besides of preying on amphibians in co-occurring ecosystems, can act as alternative hosts of the diseases, but their ability to transmit Bd has gone unnoticed. In this ongoing project, we aim (1) to evaluate the susceptibility of diverse freshwater crayfish species to be infected by the Bd pathogen and (2), to analyse whether they are able to transmit Bd pathogen to amphibians. Previous results have showed a certain susceptibility of various crayfish species, and particularly, the red swamp crayfish *Procambarus clarkii* seems to increase the prevalence of Bd in amphibians but Bd-transmission remains unknown. Here, we will show the experiments conducted with the Australian redclaw crayfish *Cherax quadricarinatus*, a potentially invasive but pet-traded crayfish species often sold in diverse European countries; the signal crayfish *Pacifastacus leniusculus,* and the red swamp crayfish *P. clarkii*, two of the most widespread invasive crayfish worldwide; and the white-clawed crayfish *Austropotamobius italicus*, a species involved in actions of reintroduction and re-stocking in South European countries. In this talk, we will present the preliminary results of this project which expect to shed light on the transmission of Bd via crayfish. Under this unprecedented globalization scenario, translocations of crayfish might act as key drivers of Bd-transmission in aquatic environments, crucially affecting amphibian conservation.

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Host preference in the genus *Aphanomyces*

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The genus *Aphanomyces* (Saprolegniales, Oomycota) comprises severe pathogenic species to both plants and freshwater animals. Research in this genus has focused on pathogenicity, distribution and dispersal of the most economically important species, while other key biological questions, such as the specific host range, have lagged behind, in part because of problems in delimiting the species. In this study, we aimed to understand the host breadth displayed by the species of the genus *Aphanomyces.* For this purpose, we have first assessed species boundaries and established molecular operational taxonomic units (MOTUs) in *Aphanomyces* by using several species delimitation algorithms based on single locus data. We analysed 261 sequences (51 newly generated and 210 from the GenBank database) of the nrITS region corresponding to 20 previously described *Aphanomyces* species. The results showed that the diversity in the genus has been likely underestimated since we recovered 34 MOTUs. A database based on 1243 *Aphanomyces*-hosts records obtained from the literature and the GenBank was used to build an interaction matrix between the recovered MOTUs and their hosts. The interaction network analysis showed two main groups of *Aphanomyces* species: (i) one infecting animals in freshwater environments, and (ii) another, infecting plants in wet terrestrial soils. Within these groups, most species showed wide host ranges, especially in plant pathogenic species. However, some *Aphanomyces* species appear to be specialized to limited host, *e.g.*, *A. astaci* in freshwater decapods (order Decapoda) or *A. trifolii* in Fabaceae. Future research in the genus *Aphanomyces* should encompass all species, not only economically relevant species in order to understand the evolutionary history of host specialization in the genus.

Chasing the crayfish plague to the southeastern United States

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The oomycete *Aphanomyces astaci* is responsible for one of the most severe wildlife pandemics ever reported, affecting freshwater crayfish worldwide. This emerging infectious pathogen has caused mass mortalities of freshwater crayfish species in Europe and Asia, and threatens other susceptible species in Madagascar, Oceania and South America. Presumptions that *A. astaci* originated in North America were based on disease outbreaks that followed translocations of North American crayfish and on the identification of the pathogen mainly in Europe. These studies confirmed that *A. astaci* naturally coexists with some North American crayfish species that are its chronic carriers. We studied *A. astaci* in the southeastern US, a center of freshwater crayfish diversity. In order to decipher the origin of the pathogen, we investigated (1) the distribution and haplotype diversity of *A. astaci*, and (2) whether there are crayfish species-specificities and/or geographical restrictions for *A. astaci* haplotypes. A total of 132 individuals, corresponding to 19 crayfish species and one shrimp species from 23 locations, tested positive for *A. astaci*. Mitochondrial rnnS and rnnL sequences indicated that *A. astaci* from the southeastern US exhibited the highest genetic diversity so far described for the pathogen (eight haplotypes, six of which we newly describe). Our findings that *A. astaci* is widely distributed and genetically diverse in the region supports the hypothesis that the pathogen originated in the southeastern US. In contrast to previous assumptions, however, the pathogen exhibited no clear species-specificity or geographical patterns.

Exploring *Aphanomyces astaci*'s virulence: variability and genomic determinants

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The causative agent of the crayfish plague, *Aphanomyces astaci*, is listed as one of the 100 most invasive species worldwide. Its introduction into Europe in the 19th century caused mass mortalities among European freshwater crayfish populations leading to local extinctions. Until now, different haplogroups of this pathogen have been introduced into Europe, and their presence threatens many freshwater ecosystems. However, the interaction of crayfish and *A. astaci* does not always cause death of the crayfish but may result in latent infections, depending on the involved pathogen strain. Understanding the mechanisms of crayfish resistance and pathogen virulence is thus crucial to inform conservation actions. In this study we systematically assessed and characterized the virulence and the genomic determinants of all major *A. astaci* strains occurring in Europe. In a series of standardized infection experiments with noble crayfish, we determined the virulence of 15 *A. astaci* strains. The tested strains exhibited great variance of virulence. The percentage of mortality among challenged noble crayfish ranged between 0% and 100%. Among the strains causing 100% of mortality, the average day of death ranged between day 6 and day 16. This high virulence variability is not echoing the tentative virulence classification based on the currently used genetic markers, as some supposedly lowly virulent strains caused high mortality and vice versa. However, the age of the cultures might be a factor in reducing the virulence of more aggressive strains. The results from the controlled infection experiments will be integrated with data on hyphal growth speed and spore production of the used strains, as entities to characterize virulence. Subsequently, we sequence and assemble the genomes of all *A.* astaci strains, using a combination of Illumina short-reads and Oxford Nanopore long-reads. This will allow us to generate a representative pan-genome of *A. astaci*, to be used as reference for a genome-wide association study, to identify associations between genetic regions in the *A. astaci* genome with its respective phenotype (virulence), and to infer whether or not variation in strain virulence is coded within the pathogen´s genome. Prospectively, novel genomic markers can be developed to support advanced management strategies.

Two new invaders in freshwater ecosystems in France: the rusty crayfish *Faxonius rusticus* (Girard, 1852) and its plague agent

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Invasive alien species (IAS) are one of the biggest threats to biodiversity causing population decline or even extinctions of native species. The spread of several American crayfish species in Europe, which are non-symptomatic carriers of *Aphanomyces astaci*, an oomycete responsible for numerous local extinctions of European crayfish. In 2022, we report the first record of a new American crayfish species, the rusty crayfish *Faxonius rusticus* in the wild, in France and so far for Europe. This crayfish is considered one of the most invasive crayfish species because of its aggressive behavior and ability to out-compete and displace native crayfish in US where its native range has been extended. *Faxonius rusticus* is present in a private pond in high density (847 individuals trapped in 2021) from which it has spread into two adjacent brooks (Inières & Briane). In these brooks, this species co-occurs with the signal crayfish *Pacifastacus leniusculus*, currently the most widespread invasive crayfish species in France. This first description of the rusty crayfish is therefore alarming for the preservation of the local endangered native white-clawed crayfish for two reasons. Firstly, these species may share a large similar habitat preference for the headwaters of hydrographic basins in contact to the endemic *Austropotamobius pallipes*. Secondly, all analyzed crayfish showed high levels of infection by *Aphanomyces astaci* ranging from A2 to A5. Microsatellite analysis revealed potential co-infection by 2 strains of *Aphanomyces astaci*. As *Faxonius rusticus* could be a high-risk species for French freshwater biodiversity, actions for eradication of this species have been taken.

Prevention of vertical transmission of crayfish plague: Is the production of crayfish plague-free juveniles from infected females possible?

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The crayfish plague pathogen (*Aphanomyces astaci*) is recognised as one of the best-studied invertebrate diseases. However, little is known about its vertical transmission from mothers to their offspring and whether this can be prevented. We hypothesised that if *A. astaci* hyphae do not penetrate inside the eggs (i.e., the pathogen remains on its surface only, the transfer of crayfish plague can be prevented combining artificial incubation and anti-fungal treatments. Egg-carrying females of the narrow-clawed crayfish (*Pontastacus leptodactylus*) carrying eggs were caught from Lake Eğirdir, Turkey, in spring 2017. Crayfish from this population are known to be frequently chronically infected by *A. astaci*, and often develop disease symptoms and die when held in artificial culture conditions incl. high mortality of their offsprings. The batch of stripped eggs was randomly assigned into experimental groups for artificial incubation in a flow-through system. The experimental groups were as follows: 1) formaldehyde treatment 3000 ppm; 2) peracetic acid treatment 20 mg L^{-1} PAA; 3) control group I (no treatment). The antifungal treatment was applied 3 times a week. Out of the treatment groups, for some of the females, the eggs were left for maternal incubation. The females and incubated eggs and juveniles were tested using a sensitive quantitative PCR assay for *A. astaci* presence. The results confirmed that nearly all the females were infected, and *A. astaci* infection was confirmed also in the maternally incubated juveniles with their high mortality. In contrast, we observed high survival rate and low frequency of *A. astaci* infection in all artificial incubation treatments, including the Control groups, with the best result for the formaldehyde treatment. Apparently, the artificial incubation itself limits pathogen's vertical transmission. While it cannot be guaranteed that the artificially incubated juveniles are completely free of *A. astaci*, the approach seems suitable for routine aquaculture applications.

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Advances in crayfish plague research: what did we learn from application of molecular tools?

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Crayfish plague, caused by the oomycete *Aphanomyces astaci*, is the best-known disease of freshwater crayfish. It has been decimating populations of indigenous European crayfish since the 19th century and remains a key threat for their conservation. Our knowledge on the distribution, diversity, host range, reservoirs and dispersal of *A. astaci* has improved dramatically in the last two decades thanks to development and application of various molecular tools. DNA-based methods allow for example sensitive detection of the pathogen in host tissues as well as environmental DNA, or *A. astaci* genotyping in clinical samples. Most information on the crayfish plague pathogen has been nevertheless acquired in the Western Palaearctic (mostly Europe and Turkey) where its conservation relevance is undeniable. Diversity and ecological interactions of *A. astaci* in North America, from which it originates, is heavily understudied. Although considered a potential threat for crayfish species from other biogeographic areas, the risk of spreading the crayfish plague pathogen to new regions seems still underestimated by stakeholders. Only recently, crayfish plague outbreaks were unambiguously confirmed from *Cambaroides* crayfish in Japan, and presence of *A. astaci* in the red swamp crayfish *Procambarus clarkii*, widely spread for aquaculture, fisheries and ornamental purposes, has been confirmed in Indonesia (with a particular risk of spreading the disease to New Guinea) or South and Central America. In the presentation, I will highlight some of the key advances in our understanding of *A. astaci* biology obtained from DNA screening and genotyping methods, as well as their promises and limitations.

Strategies to monitor *Aphanomyces astaci* in North-western Apennine in *Austropotamobius pallipes* complex populations

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The endangered *Austrapotamobius pallipes* complex is among the autochthonous European crayfish species threatened by *Aphanomyces astaci*. Even though coexistence without evidence of mortality outbreaks has been reported in recent years by different authors, monitoring the presence of *A. astaci* within the sanitary survey of autochthonous populations is still paramount for the conservation of the species, primary objective of the LIFE+ CLAW project. A monitoring to detect *A. astaci* presence was conducted in the summers of 2020 and 2021 on thriving populations of *A. pallipes*, detected in creeks and small streams in the Italian North-western Apennine (Liguria and Emilia Romagna Regions), following two non-invasive sampling methods. The first one involved the use a thin cotton swab to collect superficial epibionts and putative *A. astaci* zoosporangia, rubbing it all over the external cuticle, focusing on the joints of the pereiopods and the melanized areas. From each sampling site, up to 30 specimens were singularly collected. A second method based on eDNA analysis was performed by filtering 4 water samples (5L/each or until clogging) through glass microfiber filters (porosity 2.7 µm) downstream of the swabbed population. Swabs and filters were stored in absolute ethanol at 4°C until processing. DNA extraction was performed via commercial kit for swabs and via CTAB protocol following Strand *et al*. (2019) for eDNA filters. Quantitative PCR targeting *A. astaci* was performed following Rush *et al*. (2020). Overall, 38 populations were swabbed, and for 17 of them eDNA samples were collected. *Aphanomyces astaci* was detected in 15 populations and, when available, the results were supported by eDNA. Moreover, *A. astaci* presence was confirmed in 14 populations via a pre-amplification of the ITS region followed by PCR and sequencing proposed by Oidtmann *et al.* (2006).

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Crayfish plague pathogen *Aphanomyces astaci*: from non-destructive monitoring to ecology and sustainable control methods

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Crayfish plague is responsible for decimating populations of native crayfish species in Europe and elsewhere, making *Aphanomyces astaci* one of the 100 worst invasive species in the world. I will present the results of our research on *A. astaci* in recent years, including advances in monitoring approaches, new insights into the biology/ecology of the pathogen, and the development of environmentally friendly control strategies. We have shown that *A. astaci* can be detected in the microbial biofilm collected from the cuticle of individual live crayfish. Thus, we contribute to the growing trend of non-destructive methods for monitoring pathogens in freshwaters. The method has yet to be compared to the direct pathogen detection in water, but it provides results consistent with conventional detection of *A. astaci* in cuticle samples. For the past five years we have been using the novel method to monitor *A. astaci* in natural crayfish populations in Croatia. We also investigated the relationship between native crayfish hosts (*Austropotamobius torrentium* and *Pontastacus leptodactylus*) and *A. astaci* group B at different water temperatures. Preliminary data from the infection trials showed lower *A. astaci*-induced mortality of native crayfish at elevated water temperature (22 vs. 18°C), suggesting that the temperature increase had a greater negative impact on *A. astaci* than on the crayfish. These results may help predict the spread of crayfish plague in future climate change scenarios. Finally, we are actively developing environmentally friendly methods to control *A. astaci* as an alternative to the toxic chemicals currently used in astaciculture. We have shown that the application of bioactive plant products such as propolis and essential oils from Mediterranean wild plants can inhibit mycelium and zoospores of *A. astaci*, while commensal bacterial isolates from the cuticle of crayfish, mainly pseudomonads, can inhibit mycelial growth.

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Resistance to crayfish plague: assessing the response of native Iberian populations of the white-clawed freshwater crayfish

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Crayfish plague, caused by the oomycete pathogen *Aphanomyces astaci*, is one of the most devastating of the emerging infectious diseases. This disease is responsible for the decline of native European and Asian freshwater crayfish populations. Over the last few decades, some European crayfish populations were reported to display partial to total resistance to the disease. The immune response in these cases was similar to that exhibited by the natural carriers of the pathogen, North American freshwater crayfish, e.g., weak to strong melanization of colonizing hyphae. We tested the degree of resistance displayed by 29 native Iberian populations of *Austropotamobius pallipes* that were challenged to zoospores of the pathogen. We measured the following parameters: (i) mean survival time, (ii) cumulative mortality, and (iii) immune response, and found that the total cumulative mortality of all the challenged populations was 100%. The integration of the results from these parameters did not allow us to find differences in resistance towards *A. astaci* among the northern and central populations of the Iberian Peninsula. However, in the southern populations, we could identify 4 distinct population responses based on an evaluation of a GLM analysis. In the first case, the similar response could be explained by the effect of a pathogen strain with a lower than expected virulence, and/or an actual increase of resistance. In the Southern populations, these differences were the consequence of either whole population or individual resistance. Individuals that survived for a longer period than the others showed a stronger immune response, i.e., presence of partially or fully melanized hyphae, which is similar to that of North American crayfish species. This might be the consequence of different mechanisms of resistance or/and tolerance towards *A. astaci*.

Genetic diversity and phylogenetic relationships of *Pontastacus leptodactylus* across its distributional range

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The narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) is the large native European crayfish species with a relatively wide distributional range. Its original distribution territory is situated in the drainage area of the Azov, Black and Caspian Seas, but it was also introduced to most western European countries as a commercially valuable species. The narrow-clawed crayfish is morphologically variable, and according to updated classification of freshwater crayfishes it presents eight species and two subspecies. The studies of *P. leptodactylus* genetic diversity are very scares and mostly from limited geographical areas. Therefore, our aim was to analyse genetic diversity and phylogenetic relationship of crayfish originating from 65 populations from 13 countries, covering well its distribution range. Our results confirmed existence of three divergent mitochondrial phylogroups corresponding to geographical areas of Central & Western Europe, Eastern Europe & Asia and Turkey. However, the nuclear clustering was not congruent with mitochondrial phylogroups. Analysis of ancestral ranges revealed Black Sea basin (Turkey, Thrace, Dnieper-South Bug, and Don Rivers) as the area of species origin. Moreover, Turkey harbours haplotypes from all three mtDNA phylogroups, while traces of introduction from Turkish populations were found in shared haplotypes in populations from Hungary and Denmark. Although species delimitation methods based on mitochondrial markers indicated up to nine OTUs (operational taxonomic unit), incongruent pattern was found using nuclear markers suggesting rather young species from evolutionary point of view.

Evaluation of pet trade regulations for management of invasive crayfish in Midwestern States

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The increasing world-wide popularity of the aquatic pet trade is causing widespread transport and introduction of non-native species, including crayfishes, into natural waters. Over 30 crayfish species have invaded North America, changing ecosystems and impacting native species. Increasing recent crayfish introductions are linked to the pet trade; this pathway requires proper attention from natural resource managers. The Missouri Department of Conservation (MDC) responded in 2014, implementing regulations to restrict sales of all crayfish species except the Virile Crayfish (*Faxonius virilis*) by Missouri bait and pet stores. However, MDC has never monitored regulation compliance by pet stores. We conducted a survey of all 125 identified Missouri pet stores in 2019 to estimate compliance. MDC Agents visited all but 2 stores and gathered evidence of crayfish sales by inspecting displayed livestock and interviewing store owners and employees. We also conducted a comparative survey of 49 pet stores in Ohio, where minimal regulations exist limiting crayfish sales. Crayfish were sold (illegally) at 17 (15.5%) of 110 Missouri stores and included 3 non-native species. Thirty-one of 49 (63%) Ohio stores sold crayfish, including 3 non-native species. Results suggest that the Missouri regulation has been effective at managing and possibly reducing pet store sales of potentially invasive crayfish. However, we also developed a brochure to educate pet store owners about the problem of invasive crayfishes, and we mailed it to all Missouri pet stores following our survey.

A multi-method approach for conserving a rare, burrowing North American crayfish (*Cambarus causeyi*)

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The Boston Mountain Crayfish, *Cambarus causeyi*, is classified as a Species of Greatest Conservation Need in Arkansas, United States of America, and is endemic to the Ozark Mountains of North America. The relatively small range and rarity of this species makes it vulnerable to extinction, and the species has knowledge gaps that need to be addressed to facilitate conservation. We utilized species distribution modeling (SDM) using the program MaxEnt and fine scale habitat modeling to analyze the distribution and habitat preferences of *C. causeyi*. Our SDM found average annual precipitation was the most important predictor of *C. causeyi* presence. We collected habitat data from across *C. causeyi*'s known range, and we used fine scale-data to ground-truth our SDM. We detected *C. causeyi* at only nine of 51 sites, potentially due to sampling outside of the peak of the reproductive season. We ran our fine-scale analysis by modeling zero-inflated Poisson generalized linear models and selecting most-supported models with AICc. Our best model included proportion of sand in the soil and the presence of a competing burrower as explanatory variables. The MaxEnt output was found to be a poor predictor of finding *C. causeyi* in our fine-scale analysis, potentially because the SDM did not account for biotic interactions and lacked accurate soil data. We also developed an environmental DNA (eDNA) assay for the detection of *C. causeyi* and tested it across a subset of our field sampling sites. *Cambarus causeyi* eDNA was found at 24% of sites, a higher percentage of sites than we were able to detect *C. causeyi* from using conventional field sampling. Our findings are promising for future applications of eDNA to *C. causeyi* population monitoring.

Linking laboratory and pond studies to evaluate control techniques for red swamp crayfish in surface waters and burrows

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Red swamp crayfish (RSC; *Procambarus clarkii*) are highly invasive in parts of North America and the world. There is a need to develop and evaluate practical control techniques for infested waterways and riparian zones. We synthesized a series of laboratory and pond studies conducted to develop and evaluate approaches for control of RSC. In chemical trials, we evaluated the efficacy of treating retention ponds and burrows with pyrethrin. In raceways and small ponds, we evaluated the ability of carbon dioxide (CO_2) to "push" crayfish and water flow to "pull" crayfish towards specific areas for improved collection. Finally, we assessed the effectiveness of physical blockers (Benseal® bentonite clay and Great Stuff® sealing foam) to trap and kill RSC in underground burrows. Pyrethrin treatments in ponds removed a significant number of RSC; however, RSC were not eradicated after a single treatment. Pyrethrin treatments in burrows resulted in >75% mortality. Carbon dioxide caused RSC to move towards pond edges and surfaces but not towards low CO_2 refuge areas. Water flow stimulated positive rheotaxis with crayfish migrating towards flow at small scales but not across larger scales. Benseal and Great Stuff induced >60% mortality in occupied burrows. Evaluating strengths and weaknesses of each technique will assist the development of integrated pest management (IPM) strategies. Chemical treatments should target all life history stages of invader populations in ponds and burrows. Carbon dioxide may increase trapping effectiveness around pond perimeters but may require development of novel emergence traps. Studies are needed to investigate flow thresholds needed to effectively attract crayfish across large distances to enhance trapping. Physical blockers require better application techniques to consistently get blockers down to burrow groundwater but may help repair riparian zones. Resource managers require practical control tools for invasive RSC. Coordinated laboratory, pond, and field studies can greatly aid in the development, evaluation, and identification of control tools that will be most effective in comprehensive IPM plans.

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Eradication attempts of invasive spiny cheek crayfish *Faxonius limosus* (Rafinesque, 1817) in gravel pits near Ptuj, Slovenia

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The spiny cheek crayfish (*Faxonius limosus*), a species native to North America, was first observed in Slovenia in gravel pits near Drava River in 2015. A substantial population with specimens of various size classes was discovered. So far, this is the only known location of the species in Slovenia and its introduction route is still unknown. The gravel pits are located in the floodplain area of the Drava riverbed, south of Lake Ptuj, and are continuously connected with the river. The area involves 10 separate gravel pits and two channels with a total water surface of 6 ha. In 2017 the Fisheries Research Institute of Slovenia started implementing measures to control and prevent the spread of this invasive crayfish. The eradication process is still ongoing as part of the LIFE-IP NATURA.SI project (LIFE 17 IPE/SI/000011). Control measures consist of electro-fishing, crayfish trapping, hand - capture, setting artificial hiding places and water drainage of one gravel pit. Fieldwork is performed during daytime and nighttime. Every collected specimen is sexed, weighed and measured (carapace length with rostrum - CLR). Females and juvenile males are eliminated on site. Since 2019, males over 23 mm CLR are marked and returned to the original gravel pit. Since the start of the eradication, 8532 individuals of spiny cheek crayfish were caught between years 2017 and 2022. The crayfish specimens were tested positive for crayfish plague (*Aphanomyces astaci*). Significant effort was used to optimize the eradication methods. By now noticeable decrease of crayfish population was observed. Also, the sex ratio was altered from 1:1 in 2017 (n=274) and 1:1,2 in 2018 (n=1633) to 1:0,67 in year 2021 (n=1578) between males and females respectively. According our data the species is contained to this area.

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Ultrastructural and biometrical features of the antenna in six crayfish species

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Antennae in crayfish are essential for gaining tactile information about the local topography, localizing food, or conspecifics. The antenna of the other Arthropoda such as insects have been the subject of many morphological studies, but there are still gaps in the research of this remarkable appendage morphology in crayfish. Therefore, we applied morphological and biometrical methods using Cryo-scanning electron and light microscopies to study antennas of six different crayfish species (*Procambarus virginalis, Cambarellus patzcuarensis, Procambarus clarkii, Pacifastacus leniusculus, Cherax destructor, Faxonius limosus*) from three families to find the potential morphological differences. The carapace length, antenna length, length and width of each segment of the crayfish antenna was determined using Image J Software. The ultrastructure results showed morphological differences in the surface of antenna and the morphology of sensory hairs among studied species. Generally, two types of sensory hairs were observed on the single antenna (with and without branches) in crayfish. Similarly, the results of biometrical measurements showed significant differences in the antenna/carapace length ratios, the number of segments, and the length and width of each segment among the studied species. The different morphology and biometry of antenna seems to be related to different habitats of studied species. It is not known to which extent are the differences species specific or condition specific. Hence, there are lot of questions related to differences among wild populations, cultured populations, and their different living conditions.

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What is this? A strangely coloured Swedish Signal crayfish or an alien Marbled crayfish?

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In Sweden, import, transport and keeping of all live alien crayfish is banned since 2003. Illegally introduced “Marmorkrebs” (the marbled crayfish, *Procambarus virginalis*) were, however, found in Swedish natural waters, starting in 2012. Being a potentially strong competitor and a possible carrier of parasites and diseases, this caused great alarm and panic. This highly undesirable crayfish species in Europen waters, can be distinguished by its specific marbled pattern that covers its body. Marble-coloured individuals of another crayfish species, the signal crayfish *Pacifastacus leniusculus*, have also been detected in Sweden since 2006. Although incidents of finding marble-coloured signal crayfish are rather rare, if found, these morphs may and have been mistaken for Marmorkrebs. Similarities in the marbled pattern of both crayfish species complicate their correct identification, and hence, genetic identification may be used as a better alternative. It was indeed used as proof to convict a Swedish pet shop in the Environmental Court to substantial fines due to keeping live Marmorkrebs. It is important to inform the general public and authorities about the colour pattern similarities that can occur between these two species. Quick and accurate species identification is crucial to take swift and correct mitigating actions.

The evolution of life history traits and burrowing in crayfishes

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Understanding what drives the diversity in reproductive strategies is among the most researched topics in evolutionary biology. Generally, species with relatively low fecundity tend to exhibit increased investment into individual offspring. The increase in investment into each offspring can manifest itself in several ways, including increases in the overall size of each egg or by parents caring for offspring before, during, or after reproduction. Because of their diversity in ecology, freshwater crayfish present an ideal group to study the evolution of life history traits. Some crayfish rely on surface water and invest minimally into their offspring. By contrast, burrowing crayfish species rely on their burrow for their entire lives; many burrowing species show an increase offspring investment by cohabiting burrows with multiple generations of young. Here, we used phylogenetic comparative methods to investigate the relationship among body size, burrowing, clutch size, and egg size in freshwater crayfishes. Interestingly, we did not find any correlation between the prevalence of burrowing our three life history traits (body size, clutch size, and egg size). These results demonstrate phylogenetic conservatism in life history traits throughout crayfishes in diverse ecological roles.

Fecundity of stone crayfish in Czechia: egg number, size and mortality

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Within the period 2016–2021, a large study concerning stone crayfish fecundity was carried out. In total, 165 females with eggs in 19 streams (3–20 females per stream, median 8) were analysed: eggs were counted, and 2–3 eggs from each female were collected and then measured (using a binocular microscope, camera MOTIC 5, Motic Images Plus 3.0 software). The clutch size (counting only complete egg set) varied between 21 and 119 (median 57), egg size between 2.1 and 3.7 mm (median 2.8). Females typically implemented two reproductive strategies: some of them had many small eggs, whereas other females had fewer bigger eggs, but not all of them. The oral presentation also evaluates if there are population differences or differences among individuals. On four streams, four females with eggs (i.e. 16 females in total) were isolated in small cages made from anodised steel and kept from October to next June. Females were periodically monitored, and changes in the number of eggs within this period were calculated. The loss of eggs during the winter was 2–26 eggs, which represented app. 6–34% of the original clutch size. Small or incomplete clutches and smaller females lost fewer eggs during winter than in case of bigger clutches or bigger females.

Can we count on birds? A study on the diet of the great crested grebe (*Podiceps cristatus*) at an invasive crayfish 'hotspot'

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The diet composition of the great crested grebe was studied over the course of three years in a fen-area in the western part of the Netherlands, inhabited by dense populations of three invasive crayfish: Procambarus clarkii, Faxonius limosus and F. virilis. In total, 579 prey captures of 3 species of crayfish, 14 fish, 4 insects, and one mollusc were observed. Out of 69 captured crayfish specimens, 66 were actually eaten (96%); composing 11% of the total diet in terms of prey items. Crayfish appear to be a 'seasonal diet', mostly preferred in May and June. Factors that may affect the seasonal preference can be the high moulting activity i.e., high availability of soft crayfish in May and June or the high demand for food for juvenile grebe, or a combination. The average time for a grebe to process a crayfish is 54 seconds, much longer than 6 seconds for an 'average fish', which may give the impression that grebes eat more crayfish than they actually do. First estimates from this area indicate that a grebe consumes up to 650 crayfish per year.

Response of burrowing crayfish species to land management practices in Missouri, USA

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Six species of burrowing crayfish have established ranges in Missouri with five of these being designated Species of Conservation Concern. Although wetlands and prairie ecosystems that support crayfish populations undergo many forms of land management, the impacts of these practices on burrowing species have rarely been evaluated. These studies evaluated the responses of burrowing crayfish populations to some of the most common practices including mowing and disking in moist-soil wetlands and burning in prairies, at publicly managed properties in Missouri. Our results demonstrated that crayfish were tolerant of mechanical vegetation management, responding more to vegetation composition and broader landscape effects. Crayfish response to prescribed burning will also be discussed in this presentation. With three more burrowing species recently recognized as occurring in Missouri and undergoing conservation assessment, it is of critical importance that we understand how to properly manage and conserve these populations. Here we will discuss how we can integrate conservation support for burrowing crayfish with current management strategies for other taxa and public lands.

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Resource use by the slenderwrist crayfish, a narrowly endemic primary burrowing crayfish in Arkansas, USA

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Narrowly endemic species are usually of high conservation concern. Understanding those species' habitat and resource needs is critical for effective conservation. The Slenderwrist Crayfish, *Fallicambarus petilicarpus*, is a primary burrowing crayfish species historically known from an approximately 200 sq. km region of south-central Arkansas, USA. To determine its diet and habitat use, we conducted stable isotope analysis on abdominal tissue from the species and measured habitat variables at field sites visited during 2021 and 2022. Our results represent some of the first available data on long-term feeding in primary burrowing crayfish and support a more herbivorous diet strategy in this species. Our field sampling also suggests the importance of low canopy cover and low vegetation height in habitat selection. During the course of our field work, we identified new populations of Slenderwrist Crayfish, however, those populations do not dramatically expand the known range of this narrowly endemic species. With little evidence for specialized dietary or habitat needs, other explanations must be explored to determine factors limiting burrowing species such as the Slenderwrist Crayfish to extremely narrow ranges.

Seasonal microhabitat use by brawleys fork crayfish, *Cambarus williami*, a Tennessee State protected species

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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 under petition for federal protection under the Endangered Species Act. Determination of federal status will be facilitated by knowledge of ecological associations of *C. williami* across its distributional range. 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-m2 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.

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The life history of the Rusty Crayfish (*Faxonius rusticus*) in a small southern Ontario stream

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A thirteen-month study of the life history was conducted in Fleetwood creek, a small stream in Southern Ontario, Canada. The reproductive biology as well as molting cycle were investigated, and the larval development was documented in detail for this important invasive species. Despite recent climate change effects such as higher maximum temperatures as well as significant short term temperature fluctuations, the overall life cycle does not appear significantly different from previous studies conducted in the 1980s and 1990s. Maturity is attained within the first summer at 19-20 mm CPL in at least 30% of the young of the year and most individuals of both sexes are mature at 25 mm CPL. Mating and spawning occurred in the first week of April. The onset of mating occurred at temperatures of 9-10°C which was somewhat higher than the 4-5°C previously reported for the species in this region of Ontario.The distribution/spread of the rusty crayfish in Ontario and adjoining provinces is also updated and discussed. The legislation, and management strategies are summarized.

Sea level rise, storm surges, and the fate of the Banded Mudbug (*Lacunicambarus freudensteini*), a primary burrowing crayfish near the Gulf of Mexico, USA

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Lacunicambarus freudensteini (Banded Mudbug), a recently described primary burrowing crayfish, inhabits a narrow band in two coastal counties in the US states of Alabama and Mississippi. Its distribution appears to be restricted by a congener (*L. mobilensis*, Lonesome Gravedigger) to the north and by geographic features in the other directions. The species is known from eight sites, some of which yielded a single specimen, and it was not found at seven other sites within its range, although other burrowers were plentiful. Some of the species' localities are at low elevations within several km of the Gulf of Mexico. A sea level rise of 2.7 m—predicted by some climate change models—would inundate two of the known localities and render others coastal. However, given the species' deep burrows, sea level rise and soil salinization may affect the species' distribution before any of its localities are actually inundated. Moreover, storm surges may present an even greater risk than sea level rise. Storm surge from a Category 3 hurricane could inundate more localities than would a 2.7 m rise in sea level. We will present spatial modeling results of the risks to the species from various degrees of sea level rise and storm surges, discuss a few of the many uncertainties, and offer suggested approaches to addressing ecological uncertainties.

Scents of sex and death. An exploration of the chemosensory world of crayfish

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Crayfish live in an aquatic world full of chemicals. They have excellent chemical senses allowing them to extract information from the environment crucial for their survival, reproduction, and resource acquisition. In this talk, I will take you on a journey into the chemosensory world of crayfish. Using examples from my own research I will discuss how crayfish find food, how they communicate with conspecifics to find mating opportunities and to repel competitors, and how they avoid lethal confrontations with predators, all based on perception of chemical cues and signals. After many years of research using odour visualisation, experimental modification, and quantitative behavioural analysis, a picture is emerging pointing to the strong reliance of crayfish on environmental odours. In riverine environments, crayfish employ a simple orientation strategy (odour-gated rheotaxis) to find food. In lacustrine environments, water currents created by crayfish's own fan organs facilitate olfactory search. Crayfish use urinary chemical signals concurrent with physical displays to settle social contests. Urinary cues are also used by males to recognize receptive females during the breeding season. Perception of conspecific blood is an indicator of an imminent predation risk. Crayfish reduce their activity and seek darker areas to avoid visual detection by the predators. The dependency on chemicals makes crayfish vulnerable to chemical pollution but also provides opportunities for management of natural, invasive, or captive crayfish populations.

From a renaissance royal whim to our beloved "native crayfish": How the Italian crayfish became a conservation priority in Spain

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In the 16th century fish and crayfish breeding in garden ponds was fashionable among European elites. King Philip II of Spain was fond of these costumes, which he had known during a trip as heir prince. The King's first attempts to import fish and crayfish for garden ponds date from 1563. Different species were then requested from either the Netherlands or France, leading to the introduction of carp *(Cyprinus carpio*) and pike (*Esox lucius*) into Spain, but not of any crayfish species. The royal interest on crayfish reactivated during the 1580s, then focussing on the Medici Tuscan court. After at least five years of diplomatic efforts, a large crayfish shipment was sent to Spain in February 1588. This documented historical introduction fits with the taxonomic identity of crayfish found in Spain, the *Austropotamobius italicus italicus* subspecies of the Italian crayfish, the one found in Tuscany. The Italian crayfish transit from an elite item to a popular one in Spain remains largely unknown. By the early 19th century, the Italian crayfish was already exploited in different areas in northern and central Spain. Thereafter the species spread across all of the country's suitable habitats through multiple introductions, often leading to high-density populations. Between 1950 and 1970 the Italian crayfish was promoted by the Spanish dictatorship in the framework of a sport-fishing program that also endorsed several introduced fish species. This led to the overexploitation-driven decline of several crayfish populations, which was used to justify the introduction of American crayfish species. The associated introduction of the crayfish plague caused a rapid collapse of the Italian crayfish populations in Spain. The Italian crayfish, now widely known as "the native crayfish", is today the main Spanish target for the conservation of freshwater biodiversity. These efforts are surprising in the context of high imperilment of the native freshwater biota. The management of the Italian crayfish in Spain may be reconsidered in the light of its non-native status, and framed into the global conservation needs of native European crayfish.

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Sampling in hard to sample habitats, a case study for assessing the status of a rare, swamp inhabiting crayfish in Illinois, USA

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Illinois is home to 24 crayfish species; however, much of our distribution and habitat needs knowledge is over 35 years old, including for the Shrimp Crayfish, *Faxonius lancifer*. *F. lancifer* occurs in the central Gulf Coastal Plain of the United States from extreme southern Illinois to east Texas and Louisianna. The species occurs in swamp-like habitats and other permanently flooded areas near creeks and lakes. In Illinois, *F. lancifer* has only been collected from Horseshoe Lake, a cypress-tupelo oxbow lake in the deep southern portion of the state. To determine *F. lancifer*'s status in Illinois, we implemented four strategies: seining, d-frame dip netting, month-long microhabitat trapping, and overnight minnow trapping. Additionally, we used species distribution models with coarse-scale habitat data to predict catchments with high habitat suitability. In 2021 we collected only three juvenile and three adult *F. lancifer* using seining and dip nets. These were the first records of *F. lancifer* in Illinois in over 20 years. Both trapping methods did not collect *F. lancifer*. Our models identified wetland areas as critical habitats for the species that will need to be visited in the future to try and identify new populations of *F. lancifer*.

The 2019–2020 Australian bushfires and genus *Euastacus*: what do we know 2 years later?

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More than 770,000 km^2 of the Australian landscape was burnt across Eastern and Southern Australia during the 2019-2020 Australian bushfires. The impacts of the fires on the landscape, its flora and fauna, and Australian society were substantial, and the recovery continues. Among the fauna potentially impacted by the widespread fires were the various species of endemic *Euastacus* whose habitats were within the extent of the fires. Subsequently, 22 species of *Euastacus* were classified as "priority species" as part of the Australian Federal Government response to the fires. In this presentation we will discuss the extent of the fires, the *Euastacus* habitats and species that were affected, and what is known about impacts to the crayfish and their habitats within those areas. We will outline responses from Australian Federal and State Governments (Queensland, New South Wales and Victoria) to aid recovery of native wildlife and their habitats from the bushfires, including *Euastacus*. Finally, the objectives of a Federally-funded project aiming to inform and support recovery of the 22 priority species of *Euastacus* impacted by the fires, plus address previously-identified knowledge gaps for the genus, will be discussed.

How alien species are threatening the native crayfish *Austropotamobius pallipes* complex in the National Park "Foreste Casentinesi" (Central Italy)

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The white-clawed crayfish *Austropotamobius pallipes* complex populations are decreasing in the Foreste Casentinesi, Monte Falterona and Campigna National Park (Central Italy), due to several factors, including illegal poaching, predatory fishes, drought, and invasive alien species. Recently, the Northern raccoon *Procyon lotor* is reported to be present in the area of the National Park and is predating on the white-clawed crayfish. Moreover, since 2015, the presence of the invasive red swamp crayfish *Procambarus clarkii* is reported at the border of the National Park. The aim of the study was to update the distribution and population status of *A. pallipes* complex in the National Park, and to assess the potential effects of those two alien species on the native crayfish. Between 2020 and 2021, during the summer, crayfish were sampled by hand or by traps in 27 sites; sampled individuals were sexed and measured. Signs of raccoon presence (e.g., footprints and predated crayfish) were also recorded. Our study confirms the impact of raccoon on native crayfish: indeed, where the invasive mammal is present, crayfish disappeared, or their populations are dramatically reduced in number, with a size distribution skewed towards juveniles. Poaching, illegal water uptake and sewage could be responsible of the decline/disappearance in a few sites, while episodes of crayfish plague outbreak cannot be completely discarded. Urgent actions should be taken into account to guarantee the survival of the protected native crayfish.

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Force and boldness a side-by-side comparison of native narrow-clawed crayfish (*Pontastacus leptodactylus*) and invasive spiny-cheek crayfish (*Faxonius limosus*)

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Native and invasive species may have reduced ability to understand the other species' signals regarding fighting abilities. If invasive species' weapons are more potent than those of the species they encounter, they may start to behave fiercer in the habitats they invaded. Here, we investigated the hypothesis that effective anatomical features of an intruder (*Faxonius limosus*) come with increased boldness behaviour, contributing to their invasion success in competition against the native species (*Pontastacus leptodactylus*). We tested the boldness of specimens representing the two species by video-based assessment of crayfish individuals' attempts to leave their settlement microenvironment. Next, we studied a series of measurements concerning chelae biometry, force and muscle energetics. The native species was less expressive than the invasive regarding boldness, even if it had larger chelae and better muscular tissue performance. In contrast, *Faxonius limosus* had better biomechanical construction of the chelae than *Pontastacus leptodactylus*. The invasive species was capable of twice superior force achievements, which might also explain its bolder behaviour. These findings suggest that factors like boldness could also play an essential role in predicting dominance in interspecific agonistic interaction. This personality trait adds to already studied aspects like intimidation display tactics (avoiding costly aggressions) and sheer claw force.

Evaluation of five trap designs for removal of invasive red swamp crayfish (*Procambarus clarkii* Girard, 1852) in Southern Michigan: catch per unit effort, body size, and sex biases

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Trapping and removing invasive crayfish is one of the most common forms of control, however gear-specific biases can limit effectiveness of such methods. We evaluated five trapping gears (Gee's minnow traps [GMT], pyramid traps [PYR], artificial refuge traps [ART], additional partition refuge traps [APART], and juvenile traps [JUV]) for their effectiveness in a *Procambarus clarkii* Girard, 1852 (red swamp crayfish) removal effort among four Southern Michigan ponds between May and October 2019. Our objectives were 1) determine which gear(s) produce the highest catch per unit effort (CPUE); 2) assess gears for body size and sex biases; 3) ascertain the degree of seasonality in gear-specific catches and biases. We found that baited GMT and PYR traps substantially outperformed the ART, APART, and JUV traps with respect to CPUE. However, catches of refuge-style traps trended positively over the season. Body size biases were prevalent, with GMT and PYR traps consistently recording individuals > 30 mm carapace length. The ART and APART traps caught relatively smaller individuals but trended towards capturing larger individuals later in the season. We observed no sex biases or biases between Form 1 and Form 2 males among the gears. Our findings support employing multiple gears for crayfish removals but demonstrated that baited traps can remove more crayfish than unbaited traps (on a CPUE basis) and both can capture body size trends by accounting for the timing of capture. Further, the lack of sex bias suggests any of these commonly used traps can be used to broadly sample females or male reproductive forms. Based on our results and recommendations, the Michigan adaptive response strategy has adopted a combination GMT-APART strategy to maximize CPUE while also targeting a wide range of crayfish sizes.

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The crayfish tale – an educational video

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We are presenting an educational cartoon for past, present and future children and youngsters and all those interesting in the fascinating and complicated history of the freshwater crayfish in Europe. The storyline is based on actual facts and knowledge as it is presented in the scientific papers. The cartoon is based on beautiful artwork and rhyming verses. The crayfish tale covers an enormous time span from long before the dinosaurs appeared on earth until today and how we should act in the immediate future not to destroy more of our remaining native freshwater crayfish stocks and native ecosystems, for that matter. The tale also introduces freshwater crayfish and their fundamental role as keystone species in the aquatic ecosystems, highlighting facts such as that their total weight constitutes more than 80% of what is moving about at the bottom of a water system. The crayfish tale tells about the mistreatment of native crayfish by polluting and destroying their habitats in lakes and running waters, by introducing other competing alien species and furthermost by introducing the fatal, lethal, and mortal disease crayfish plague with these alien crayfish species. The crayfish tale also advices how we humans must behave from now on, in order not to finally lose these essential species and consequently the wellbeing of our freshwater ecosystem. If we start to look after our crayfish and waters, then the future will look after us. The fundamental lesson of the crayfish tale is that we all should carefully think beforehand, while afterwards is usually too late.

Abstracts of Poster Presentations

Legions of lesions: sick crayfish in Montana, USA

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We are conducting the first statewide survey of crayfishes in Montana, USA, and have found interesting, but mysterious, patterns of disease and mortality. We sampled for six weeks during summer 2021 and also obtained voucher specimens from others sampling for different purposes in the state. We sampled 116 sites, obtained additional crayfishes from another 249 collections (unique combinations of site x date x sampling method), and found five taxa. Virile crayfish (*Faxonius virilis*) are native to parts of the state east of the Continental Divide, and signal crayfish (*Pacifastacus leniusculus*) may be native to the state west of the divide, where virile crayfish are widespread and invasive. Throughout summer 2021, which was marked by extreme drought, high temperatures, and extreme wildfires, we occasionally found dead crayfishes and received reports from fish biologists of more dead crayfishes than normal at several locations. Nonnative virile crayfish west of the divide sometimes had brown lesions that we hadn't noticed earlier when sampling in eastern Montana. Two lakes in particular had very high incidences of virile crayfish with lesions, but some nearby lakes did not. Many lesions appeared to be mild, whereas others were severe, extending completely through the cuticle or evidently causing deformities. One crayfish with severe lesions was moving very slowly, but usually we saw no obvious behavioral effects. Several crayfish had broken or eroded pleopods, but that was not the norm. Interestingly, we rarely saw lesions on signal crayfish, even when they were syntopic with virile crayfish that had lesions. We suspect that the lesions may result from multiple pathogens and stressors operating synergistically. We will include photographs and welcome discussion about what may be causing the lesions.

The potential risk of freshwater crayfish *Cherax quadricarinatus* in Java Island, Indonesia

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The potential risk is an attempt to show the likelihood and severity of the possible adverse effects of exposure to an agent of an invasive species on an ecosystem. The purpose of this study is to analyze the potential risk of invasive. This study is designed to give information on the hazards posed by invasive species and how to avoid them. This research uses a purposive sampling method for interviewees. Information about the process of entering invasive species and how to prevent it is expected to be an essential part of managing invasive species in the area. The potential risk of Freshwater Crayfish *Cherax quadricarinatus* in Java Island, Indonesia, is at a moderate risk level. The point of entry for these invasive species is inadvertently separated from cultivation activities, regardless of their community intentionally and of unknown origin. Policymaking at the central level needs to be accelerated and adjusted to regulate invasive species introduction activities at the regional level. In addition, the preparation of a population control plan for invasive species also needs to be prepared.

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Crayfish species-genetic diversity correlations in fragmented streams

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Unlike population genetic diversity, species diversity is often assessed for conservation planning. Due to parallel influences upon species and genetic diversity, focusing conservation efforts on species diversity should also protect genetic diversity, if there are strong positive species-genetic diversity correlations (SGDCs). Habitat connectivity impacts SGDCs, with connected habitats increasing species and genetic diversity by facilitating the introduction of new species and alleles. Conversely, fragmented habitats should interrupt this relationship. If true, then conservation planning based solely on species diversity may fail in regions where its success is critical (i.e., highly modified, fragmented habitats). To address this important knowledge gap, we will use mitochondrial cytochrome oxidase subunit I gene and ISSR markers to test whether there is strong positive SGDCs for crayfishes in southeastern, USA streams that have been fragmented by impoundments, versus unimpounded streams where connectivity has been maintained. We will assess the correlation between local environmental factors and strength of SGDCs to enable predictions for unstudied areas. We will gain a better understanding of how using species diversity as a proxy for population genetic diversity might impact crayfish conservation and how habitat fragmentation impacts these relationships.

Impacts of a super-invader, the invasive crayfish *Cherax quadricarinatus*, on riverine ecosystems: evidence from stable isotope analysis in Martinique

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The Martinique Island, belonging to lesser Antilles archipelago, in the Caribbean, is known as a biodiversity hotspot, hosting many endemic species. However, this biodiversity is threatened by human activities which disturb habitats and are responsible of the introduction of invasive species such as the Australian crayfish *Cherax quadricarinatus*. This species has been introduced into many tropical and subtropical areas, for aquaculture or aquarium purposes from which they have been released into the natural environment and is now well distributed in Martinique. Crayfishes can have significant impacts on their environment due to their omnivorous and opportunistic feeding diets. By using Stable Isotopes Analysis (nitrogen (δ15N) and carbon (δ13C)), we aimed to analyze (i) the trophic position of *C. quadricarinatus* in Martinique and, (ii) the effect of this species on local communities. Ten locations have been sampled including five crayfish-free and five with crayfish. In each site, we have collected samples from other representative feeding groups (shrimps, fish, mollusks, and macrophytes). Our results show that *C. quadricarinatus* seems to occupy an intermediate position in the trophic chain, which is consistent with other studies on invasive crayfish species and explained by its omnivorous and opportunistic nature. Interestingly, its presence, in invaded rivers, seems to induce a strong competitive pressure with a change in the isotopic signature of the native shrimps, probably due to a dietary specialization. The crayfish seems to exploit all the resources in the trophic chain and the shrimps, less competitive, are forced to shift their diet to exploit other available resources. This could, in the long term, lead to shrimps' population displacements.

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Distribution and habitat assessment of crayfish in the Tickfaw, Tangipahoa, and Tchefuncte River basins, Louisiana

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Although crayfish are key components in freshwater ecosystems throughout Louisiana, there is a paucity of basic ecological information on most crayfishes in the state including species of greatest conservation need (SGCN). This lack of crayfish distribution and habitat preference information makes conservation actions difficult for scientists and resource managers. Therefore, the purpose of this project was to examine the distribution and habitat requirements for crayfishes in the Tickfaw, Tangipahoa, and Tchefuncte River basins in Louisiana. Fifty-five 1st to 4th order streams were sampled from June to October in 2020 and 2021 using backpack electrofishing, kick seining, and dipnets. A total of 2,326 crayfishes representing ten species were collected. The Pinelands Creek Crayfish *Procambarus vioscai* was the most abundant and common species collected while the SGCN Pontchartrain Painted Crayfish *Faxonius hobbsi* was one of the rarest species collected. Multivariate analysis of variance indicated a significant difference in habitat characteristics between streams with and without *F. hobbsi* (Wilks' Lambda = 0.52, $P < 0.001$). Streams with *F. hobbsi* were characterized by sandy substrate, significantly higher dissolved oxygen ($F_{1,53}=9.95$, $P=0.003$), discharge ($F_{1,53}=7.38$, $P=0.009$), and in-stream wood ($F_{1,53} = 13.04$, $P = 0.001$), and significantly lower specific conductance ($F_{1,53} = 4.06$, $P=0.049$), turbidity ($F_{1,53}=10.15$, $P=0.002$), and temperature ($F_{1,53}=14.58$, $P<0.001$) compared to streams without *F. hobbsi*. Additionally, *F. hobbsi* did not co-occur in streams with Red Swamp Crayfish *Procambarus clarkii*. There was no significant difference in any habitat variables among *F. hobbsi*, Lowland Painted Crayfish *F. palmeri*, and *P. vioscai* and all three species were associated with relatively high-quality aquatic habitats. Conversely, *P. clarkii*, Southern White River/White River Crayfish *P. zonangulus/acutus*, Shrimp Crayfish *F. lancifer*, and Cajun Dwarf Crayfish *Cambarellus shufeldtii* were associated with lower quality habitat characteristics. Results from this study provide novel information on crayfishes and habitat requirements, including the SGCN *F. hobbsi.* The information collected will aid state and federal resource managers with species conservation assessments and preservation efforts, help prioritize stream and watershed locations for future sampling efforts and establish a quantitative biological and ecological data set on Louisiana crayfishes.

Strong need for including native crayfish species into SEE river restoration, management and conservation

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Although a large number of rivers stretches in Southeast Europe (SEE) have a pristine status compared to the rest of Europe, these ecosystems and their freshwater biodiversity are perceptibly threatened. Since crayfish species are known to cover a wide spectrum of ecological functions they are considered as keystone species and ecosystem engineers. Therefore, their decline may substantially impair local biodiversity and ecosystem services. In this presentation we provide a brief overview of restoration studies considering macroinvertebrates in general but also with a special focus on the status and threats for native freshwater crayfish and their habitats in SEE. Different forms of physical habitat degradation as well as invasive alien crayfish species are simultaneously threatening endangered populations all over SEE. Next to the spread of the crayfish plague, invasive species are known to outcompete native species within a short period of time. Even though all native crayfish species are listed in the IUCN Red List, till today none of the 33 LIFE rehabilitation projects performed in SEE, has crayfish as target species in their agenda. Furthermore, SEE countries rarely designated Natura 2000 sites for native crayfish, *e.g.* for the native *Austropotamobius pallipes* just 11 and 26 sites in Slovenia and Croatia, respectively. We propose future studies to assess the distribution and functional role of crayfish species in SEE, as well as to develop habitat suitability models for these species during future conservation projects.

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Circadian behaviour of parthenogenetic marbled crayfish (*Procambarus virginalis*) as influenced by its reproductive status

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Circadian rhythms are known for physiological and behavioural changes that follow the 24-hour cycle. Over the last few decades, marbled crayfish *Procambarus virginalis* Lyko, 2017 has been used as a novel laboratory model. Various studies have frequently assessed its behaviour, including circadian rhythm. However, few of them have dealt with its activity patterns as influenced by the reproductive status. It is well known that egg-carrying crayfish females reduce their activity, but the performance of females with developed glair glands has not been studied in detail. In marbled crayfish, the frequently reproducing species, females with glair glands represent a substantial part in the laboratory stocks. Hence, we decided to compare the circadian rhythm of intermoult immature, egg-carrying and glair glands-having females of marbled crayfish. Animals were acclimatised to experimental conditions for 48 hours under the Light: Dark (14L:10D) regime and then placed in the experimental arena (400 x 285 x 75 mm) and video-recorded for 24 hours. The alternative with shelter absence (only sandy bottom provided) and presence (sandy bottom and acrylic shelter provided) was evaluated. The groups of parthenogenetic marbled crayfish displayed rhythmic behaviour in terms of activity, distance moved, and velocity. Recordings were analysed using a multi-arena module by EthoVision® XT 15.0 and statistically assessed. Detailed results will be presented at the conference. This study could provide valuable information on marbled crayfish ethology for future long run experiments.

The LIFE CLAW for protecting the native *Austropotamobius pallipes* in Italy

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Native populations of white-clawed crayfish *Austropotamobius pallipes* have undergone a remarkable contraction and decline in Europe and in Italy the decline has been about 74% in the first decade of the 2000s. Residual populations of *A. pallipes* have been increasingly confined to small high gradient streams and headwater, where the crayfish IAS have not yet been expanded and the habitat has been less influenced by human activities. In late 2019, the EU financial programme LIFE funded the project 'Crayfish lineages conservation in North-Western Apennine – LIFE CLAW' (LIFE18 NAT/IT/000806). The project's main objective is to conserve and enhance the stock of the endangered crayfish *A. pallipes* in North-Western Apennine area of the Italian regions Emilia Romagna and Liguria by a long-term conservation programme. In the first two years (2020-2021) we carried out an extensive survey on 270 stream stretches within the project area (twenty-eight Natura 2000 sites), in order to update the status of conservation of native crayfish and the presence of IAS populations. We found 27 populations of *A. pallipes* and a few populations of most widespread Cambaridae in Italy, *Procambarus clarkii* and *Faxonius limosus*, and the newly established signal crayfish *Pacifastacus leniusculus*. Other 47 native crayfish populations detected outside the Natura 2000 sites will be deserved of the upmost consideration. A distribution map is needed for the effective implementation of the concrete conservation actions, which will be carried out during the following three years.

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Aquaculture of the noble crayfish *Astacus astacus* in Belarus: controlled conditions and the use of mathematical expression for stocking density as tools for optimization

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The noble crayfish *Astacus astacus* (Linnaeus, 1758) is one of the most valuable commercial crayfish species in Europe. A perceptible decline in *A. astacus* population has led to its inclusion in the list of endangered ones, thus legislatively preventing its fishing. In an attempt to offset this crisis, the present study involves artificial rearing of *A. astacus* fingerlingswith a view to optimize its productivity in Belarus. Initially, the new-born *A. astacus* larvae were reared from early July to early October (" summer period") in open-air trays at temperatures 12 – 25°C. In the successive "winter period" (early October – early April) the grown-up individuals were reared in aquaria under temperature range of 18 – 20°C. As per our new strategy, a continuous 9-month rearing of the new-born *A. astacus* under controlled conditions, resulted in attainment of marketable size in Belarus one year earlier. The results of our present study also brought out an equation between geometric mean (N, ind.·m^{-2}) of density and the average body weight (W, mg) as variables for individuals aged 3.5 - 4 months [W = 424 – 103·lgN], and those aged 9 months during winter rearing [W = 1376 – 278·lgN]. The study has also contributed valuable information on possible stocking densities to optimize aquaculture for different age groups (larvae and juveniles) under varied experimental conditions. Interestingly, the average weights [(N_g = (N_o · N_d)$^{0.5}$] of the experimental individuals showed a significant decrease in its body weight against increased planting density ($p<0.05$). Our computation using planting density and grow-out as the variables, combined with the results of our laboratory experimentation, has led us to evolve a mathematical expression which can predict the initial density (N_o) of *A.astacus* in aquaculture: $N_o = \frac{N}{\sqrt{S.}}$, where N is the density of individuals at the end of period of rearing calculated from the above equations; S - average survival of individuals in aquaculture expressed in fraction of unity.

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Captive care and rearing of the imperiled big sandy crayfish (*Cambarus callainus*)

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The Big Sandy crayfish (*Cambarus callainus*) is native to the upper Big Sandy River basin within West Virginia, Kentucky, and Virginia, USA. This species was listed as federally threatened in 2016 due to its limited range, population declines, and the increase in nearby anthropogenic stressors. To protect this species, both captive holding and rearing efforts are being conducted. Thus, in July of 2021 we begun captive care for *C. callainus* collected from a bridge construction site located in the Tug Fork River of McDowell County, West Virginia. Individuals are being held in care until the construction is complete and their habitat is no longer facing anthropogenic stressors. Three females that were captured had sperm plugs and became ovigerous in captivity. Eggs from these females hatched in September and 52 juveniles were independent at stage 4 within 30 days. Over 50% of young born in captivity have survived to 70mm carapace length. The next stages of recovery will be to implement captive breeding to reintroduce areas of historic range. A combination of all these ex-situ efforts should provide an increase in population as well as habitat restoration throughout the watershed.

Economic costs of invasive crayfish

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Invasive alien crayfish are known for their profound impacts on recipient ecosystems. Yet, the monetary dimension of their invasions has remained unknown. To fill this crucial knowledge gap, we aimed to quantify and analyse available costs associated with invasive crayfish globally across taxonomic, spatial, and temporal descriptors using the InvaCost database. Costs of aquatic crayfish invaders were limited to the period between 2000 and 2020, totalling US$120.5 million in reported costs. Their vast majority (99%) was attributed to representatives of Astacidae and Cambaridae. These crayfish-related costs were unevenly distributed across countries with a strong bias towards European economies (US$116.4 million; mainly due to the signal crayfish in Sweden), followed by costs reported from North America and ultimately Asia. The costs were also largely predicted or extrapolated, thus not based on empirical observations. Despite the obvious limitations and lack of data for invaded regions in Africa or South and Central America, the costs of invasive crayfish were found to increase considerably over the past two decades, averaging US$5.7 million per year. Despite the well-known damages caused by invasive crustaceans, data limitations regarding this groups' costs prevent a full accounting of this groups' economic damages. Further cost reports are needed to assess the true magnitude of monetary costs caused by invasive crayfish.

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Comparison between lipid and protein oxidation progress in the tail and claw muscles of signal crayfish (*Pacifastacus leniusculus*)

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In general, shrimp as well as crayfish are extremely perishable food products owing to the microbial development and enzymatic activities of tissue throughout post-mortem condition. The deterioration of seafood quality is associated with protein degradation, lipid oxidation and autolytic activity. Consequently, freshness is the main critical attribute while the quality of seafood is evaluated. The present study compared for the first time quality of tail and claw muscle in signal crayfish (*Pacifastacus leniusculus*). Forty eight signal crayfish (*Pacifastacus leniusculus*) of approximately 30 g body weight and 90–100 mm in total length were used in the present study. Muscles from both tail and claw were taken from each crayfish. Each type of muscle was kept separately in a plastic pack under dark and cold conditions +4°C. Proteomic, microbial, and chemical analyses were performed separately in the tail and claw muscles at 0 time and after 1, 2, 4, 8 and 12 days. Two specified protein bands in the tail muscle at 140 and 36–40 kDa and one specified protein band at 107 kDa in the claw muscle were identified. Predominantly, more fainter protein bands were detected in the tail muscle compared to the claw muscle. The western blot indicated that myosin heavy chain (MHC) was heavily oxidised, particularly in the tail muscle compared to the claw muscle. Higher enzyme calpain activity and oxidation progress were observed in tail muscle compared to claw one. Both muscles were spoiled after 12 days storage with respect to total viable counts. K value as an indicator of freshness showed edibility quality condition in both muscles after 12 days storage. We would suggest that in the first days, calpain activity is the main reason for protein degradation, while protein oxidation dominates for the rest of the time. Lipid–-protein oxidation progress revealed that probably, protein oxidation started earlier than lipid oxidation in both muscles.

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Static and dynamic exposure of an antidepressant sertraline at environmental concentration alter the behavior and biochemical parameters of clonal marbled crayfish

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Pharmaceutically active compounds the emerging contaminants has direct and indirect effects on aquatic biota even at very low concentrations. The aim of this study was to assess the effect of antidepressant sertraline on clonal marbled crayfish *Procambarus virginalis* at different exposure paradigm, static (constant) and pulse. Crayfish in the 1st experimental group was continuously exposed to environmentally relevant concentration (~1 µg.l^{-1}) of pure sertraline compound for 21 days. In the 2nd experimental group the concentration of sertraline will be three times higher, but applied every third day i.e. Day 1: crayfish exposed to 3 µg.l^{-1}; Day 2 & Day 3: crayfish in aged tap water without tested compound; Day 4: crayfish exposed to 3 µg.l^{-1} and continued the process for 21 days to mimic pulse exposure of surface water. The pulse-exposed crayfish walking velocity was significantly lower than control and static exposure crayfish without available of shelter. In presence of available shelter, treated crayfish activity decreased significantly than control crayfish. While time spent outside of shelter was significantly higher in exposed crayfish than control crayfish. The concentration of sertraline in exposed crayfish were detected in hepatopancreas and nervous tissue. The bioaccumulation factors of sertraline were higher in hepatopancreas than nervous tissue. The oxidative stress response and antioxidant biomarkers of hepatopancreas and gill of exposed crayfish change significantly ($P < 0.05$) than control group. The results suggested that the low environmental concentrations of the tested compounds at variable exposure systems could alter the behavior and biochemical parameters of crayfish and also bio-accumulate in crayfish tissue during 21days of chronic exposure.

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Methamphetamine, sertraline, and mixtures of six compounds influence the marbled crayfish's biological parameters

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Pharmaceutically active compounds (PhACs) are ubiquitous in the aquatic environment worldwide and are considered as emerging contaminants for various aquatic organisms. Psychoactive compounds have become one of the most concerning types of contaminants because of their continuously increasing concentration in the aquatic environment. Evidence shows that pharmaceutics even in low concentrations found in many aquatic environments, they can have an effect on organisms that are not their target. To determine the effects of exposure to environmentally relevant levels on nontarget organisms, we analysed the cardiac activity of 2nd development stage of juveniles of marbled crayfish (*Procambarus virginalis*) and to acute stress during 96 hours for heart rate and 192 hours for locomotor at 3rd development stage response exposure to sertraline, methamphetamine and a mixture of 6 compounds (citalopram, oxazepam, sertraline, tramadol, venlafaxine, and methamphetamine) (MIX) at a dose of 1 µg L−1 of each compound. Heart rate was recorded for 5 minutes, and locomotion was recorded over a period of 15 minutes. Methamphetamine-exposed and MIX-exposed juveniles showed a higher cardiac and locomotion response to stress than control group whereas in case of sertraline the cardiac and locomotion was lower than control group. The study results also suggested that environmental concentration of individual compounds and the compounds in combination can alter the biology of non-target aquatic organisms, which may lead to disruption of ecosystem processes due to their reduced caution in stressful conditions. Further research is needed to look at different chemical mixtures, exposure systems, and habitats, as well as molecular and physiological processes that may provide useful information to elaborate the changes.

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The effect of different feeds on growth and survival of noble crayfish (*Astacus astacus* L.) juveniles

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Crayfish farming has a great opportunity to diversify organic aquaculture. There are about 20 crayfish farms that are growing the native crayfish species noble crayfish *Astacus astacus* in Estonia. At present, optimal artificial feed for noble crayfish is needed to develop such aquaculture activity and increase production in an environmentally friendly way. Four aquarium trials (1-4) were conducted in 2018-2022 with *A. astacus* juveniles to determine an optimal diet. In the feeding trials 1-3, two experimentally manufactured diets (A and B) differed in the relative proportions of proteins and lipids. In the first trial, diet A contained 42% proteins and 10% lipids, and diet B 34% proteins and 10% lipids. In trial 2, diet A contained 28% proteins and 11% lipids, and diet B 30% proteins and 11% lipids. In trial 3, diet A contained 39% proteins and 12% lipids, and diet B 39% proteins and 13% lipids. Carp feed (ALLER CLASSIC VITAMAX, 3 mm) as diet C, used trivially for feeding crayfish in farms in Estonia, was given to the control group (33% proteins and 7% lipids). In trial 4, diet A contained 40% proteins and 4% lipids, diet B was replaced by commercial sturgeon feed (Alltech Coppens INTENSIV, 3 mm) with 40% proteins and 12% lipids, and diet C (carp feed with 33% proteins and 7% lipids) experimentally manufactured polyphenols additives were added. All four feeding trials with juveniles (mean weight 0.2-0.3 g) were carried out for 205-212 days in triplicate per treatment (total of 60 crayfish per treatment). The feeding rate was 2% of the body weight. In trials 1-3, the growth was significantly greater in the carp diet group (C), but survival was higher in both experimental diet (A and B) groups. In trial 4, two months before the end, the growth and survival were greater in the experimentally manufactured diet group (A). Both survival and growth were the lowest in the sturgeon feed group (B).

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The toughest fighter is the mother – Interactions of marbled crayfish in different reproductive stages

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Competition for resources is an essential factor in the social interactions among many species. The hierarchy for utilising these resources is maintained usually by size, fitness, and aggressiveness. In crayfish, the disputes for shelter are frequent and intensive because of its high importance. It acts as a first-line defence against predators and competitors. Hence, it is the first and foremost resource to be acquired. Usually, the stronger individual occupies the better or the available shelter easier through agonistic encounters. More hidden factors affecting the readiness for fighting and fight intensity can be personality or simply higher motivation to fight successfully. Therefore, we studied the agonistic interactions for shelter and activity in female dyads at various reproductive stages: females with eggs (E), with glair glands (G), and non-reproductive ones (N). To see the differences among those three groups, we arranged six combinations (E vs. E, G vs. G, N vs. N, E vs. G, E vs. N, and G vs. N) in which dyads of similarly sized females interact in experimental arenas. The interactions and activity of opponents were recorded for 24 hours (12 h daylight and 12 h dark) using digital video recording systems. The number of contacts, fights, avoidance, tail flip, initiator and winner of the fight, and dominance establishment were analysed visually. The activity, velocity, distance moved, and shelter usage was analysed using EthoVision® XT 13.0 software. The results suggest that the females bearing eggs successfully won the maximum agonistic bouts and subsequently spent most of their time in the shelters. Females with glands show similar patterns but not to such an extent. Conversely, non-reproductive females lost most of their fights and were more active in the arena. The females with eggs can therefore be considered more aggressive and protective of their shelters, which indirectly maximises the fitness of their brood and shows maximum resource holding potential.

Practical considerations on mark-recapture studies in urban areas

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Water authorities in the Netherlands are increasingly confronted with the (public) demand to decrease invasive crayfish densities. However, trapping methods are exclusively intended for professional fishermen, which delays and limits the abilities of water authorities to respond. To address this problem, water authorities teamed up with researchers, ecologists and fishermen to design an efficient and 'exclusive' trap for crayfish with less legal restrictions. The designed models will soon be tested in the field. In order to find suitable study sites for the tests, we performed mark-recapture studies in 2021 at three locations in urban areas in the Netherlands to estimate crayfish population densities. We present the outcomes of these estimates, together with distance-recordings. The challenges of mark-recapture studies in urban areas are discussed and some practical advice are listed.

Mapping the scientific research on crayfish behaviour: A bibliometric analysis

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The study of crayfish behaviour, either in laboratory experiments or in natural settings, has been a long time interest in the field of astacology. As large freshwater macroinvertebrates, the crayfish are relatively easy to collect and rear. Besides having complex social interactions, they are often regarded as bioindicators and are sometimes migratory species with invasive potential; hence crayfish are adequate key model animals. The discipline of animal behaviour has transformed over time, becoming more integrative and interdisciplinary, with some new topics arising and developing. However, not all animal group models followed the same trends. This study aims to present an essential insight into the spatial and temporal trends toward modern studies that integrate aspects of crayfish behaviour. We used scientometric analysis on a database of over 700 articles published in the last three decades in 189 journals. We explored the main research topics identified by authors' keywords and their prominence over time, considering the institutional and authors' collaborations based on co-citation and co-occurrence networks. The rise in the production of scientific papers is not continuous, reaching a maximum in 2018. The leading journal (by the number of papers), the *Journal of Experimental Biology,* is followed closely by the *Marine and Freshwater Behaviour and Physiology*. Almost half of the papers have at least one author from the USA. Over time, the most intense collaborations were between Italy and the USA. Teams from countries like the Czech Republic, Brazil, the People's Republic of China, and Romania show new interest in the subject. There is a core of topics closely interconnected like aggression, social status, competition, movement patterns, shelter, invasion effects and communication in general. We observed a niche topic, behavioural toxicity, that rose in the 1990s and was fully established by the late 2000s. More recently, other niche topics emerged, like sexual competition and specific communication, symbionts and parasites' effects on host behaviour, drugs and addiction. Overall the information provided by this bibliometric approach revealed compelling details about the related, well-established topics and promising new ones.

Signal crayfish as a threat for European ectosymbionts – overlooked biodiversity losses

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Biological invasions are considered one of the major threats to native biodiversity, especially in freshwater ecosystems. The decline of native crayfish biodiversity has been well described in Europe, but the fate of their associated biota remains largely overlooked. Branchiobdellids (Annelida, Clitellata), crayfish ectosymbionts, are forced to switch from locally extirpated native species to new hosts. Our observations included lowered abundances or absence of branchiobdellids and their cocoons on alien signal crayfish (*Pacifastacus leniusculus*) compared to sympatrically coexisting native noble crayfish (*Astacus astacus*) in a small foothill stream. Two additionally inspected localities originally with noble crayfish but replaced by signal crayfish exhibited an absence of previously monitored branchiobdellids. Subsequently, we conducted laboratory experiments in which we individually infested individuals of signal crayfish and noble crayfish with individuals of *Branchiobdella parasita* or *Branchiobdella pentadonta* in separated aquaria. The probability of branchiobdellans survival was significantly higher on noble crayfish for both symbionts. Intensive and more effective grooming of signal crayfish could be a reason behind together with the smoother body surface in signal crayfish. Results from the field supported by laboratory experiments suggest branchiobdellans limited capacity to deal with host species alternation. Together with the progressive decline of native European crayfish, we concluded that the native branchiobdellids could be the next declining taxa due to alien crayfish species occurrence.

Responses of signal crayfish *Pacifastacus leniusculus* to pulse exposure of pesticides at environmentally relevant concentrations

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Although pesticides are often discharged into surface waters in pulses as opposed to a sustained release, the effect of episodic pollution events on aquatic invertebrates is largely unknown. We monitored change in heart rate and distance moved to assess the immediate response of signal crayfish Pacifastacus leniusculus to short-term exposure to environmentally relevant concentrations of metazachlor (MTZ), terbuthylazine (TER), and thiacloprid (TCL). A significant increase in heart rate and distance moved was detected in four of six crayfish exposed to 20 µg/L MTZ and in two of six exposed to 6 µg/L of TCL and 4 µg/L of TER. A significant correlation between heart rate and distance moved was found in all exposed groups. Increased heart rate was detected at 118±74 s, 189±179 s, and 146±22 s post-exposure to MTZ, TER, and TCL, respectively. A significant difference was only found with MTZ exposure when analyzing mean heart rate and distance moved in groups. These results suggest that exposure to high pesticide concentrations may lead crayfish to attempt to avoid contaminated areas. With lower concentrations, crayfish not exhibiting an escape response may continue to be exposed to lower, but potentially harmful, levels of pollutants. Further study is required to determine whether brief pesticide exposure leads to delayed adverse effects on freshwater crayfish.

Comprehensive measures for the management of the invasive alien signal crayfish (*Pacifastacus leniusculus*) (Dana, 1852) in Slovenia

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The signal crayfish (*Pacifastacus leniusculus*) is the most widespread invasive alien crayfish in Slovenia, which poses a serious threat to our indigenous species. In Slovenia, it was first discovered in the Mura river in 2003, followed by the discovery of the species in 2007 in the Drava river. Currently, it is present along the entire main course of the Mura river, as well as in some of its tributaries (Bolt stream near Ceršak, rivers Ščavnica, Ledava and Kučnica). In order to reach strategic goals and legislative obligations at the national and EU level, we are developing a system for the management and control of the signal crayfish. In the first phase, a survey analysis was concluded to determine if angling clubs and regional managers of protected areas recognize the signal crayfish as a threat. Based on the survey's results, national monitoring and literature, further actions are planned. Anglers often encounter signal crayfish and are aware of the threat. They are willing to participate in the implementation of measures and in population reduction activities. According to the current Slovenian legislation, capture of non-native crayfish is allowed only with established fishing techniques (e.g. fishing rod), excluding traps, which are the most effective in capturing crayfish in large rivers. On the other hand, the use of traps could lead to the abuse in fishing for endangered species and potentially risk the spread of diseases (ex. crayfish plague) and alien species. The management strategy will integrate relevant stakeholders and neighboring countries. It will propose an intervention concept and solutions to limit dispersal of the signal crayfish from the initial source of Mura and Drava river to additional tributaries and other watersheds. Furthermore, the strategy will also include monitoring of crayfish plague with measures to prevent and limit the disease.

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Effects of temperature and limb amputation level on limb regeneration parameters in marbled crayfish

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This study was undertaken to investigate the limb regeneration ability of the marbled crayfish (*Procambarus virginalis*) by inducing different levels of limb amputation and evaluating its impacts on the regeneration process at two temperatures. Altogether, sixty crayfish were used in the experiment. The six treatments consisted of two temperatures (14 and 24 °C) combined with three limb autotomy levels, including intact (control), medium (last two pairs of walking legs) and high (all limbs except last pair of walking legs) level autotomized animals. The level of amputation (medium and high) significantly affected the limb regeneration rate. However, the low temperature was a significant factor for inhibiting the regeneration process of marbled crayfish. The crayfish regeneration rate was not only affected by different temperatures but also by different levels of limb amputation.

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Diversity and distribution of *Aphanomyces astaci* in a contact zone of multiple exotic crayfish species in Hungary

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Ornamental trade has become an important introduction pathway of alien aquatic species worldwide. Correspondingly, there has been an alarming increase in the number of established crayfish of aquarium origin in Europe over the previous decade. The oomycete *Aphanomyces astaci*, the pathogen causing crayfish plague responsible for serious declines of European crayfish populations, is dispersed with introduced North American crayfish. The role of ornamental taxa in introducing and spreading different genotypes of this pathogen in open waters remains unclear. We investigated the distribution, prevalence, and diversity of *A. astaci* in Budapest, Hungary which became the hotspot of aquarium crayfish establishment thanks to local thermal waters. We screened for *A. astaci* in seven host taxa from 20 sites sampled between 2018 and 2021: five cambarids (*Procambarus virginalis, P. clarkii, P. alleni, Faxonius limosus, Cambarellus patzcuarensis*), one parastacid (*Cherax quadricarinatus*) and one native astacid (*Pontastacus leptodactylus*). The pathogen was confirmed in *P. virginalis, P. clarkii, P. alleni,* and *F. limosus* at six sites. Genotyping was successful only in individuals from two different brooks where multiple host species coexisted (Dera and Barát) but revealed unusual patterns. *A. astaci* mitochondrial haplogroup "b", previously detected only in *Pacifastacus leniusculus* or crayfish plague outbreaks, was detected in *P. virginalis* at both sites, and in both *F. limosus* and *P. virginalis* sampled from a thermal tributary of Barát in 2018. In contrast, haplogroup "a" was detected in coexisting *F. limosus*, *P. virginalis* and *P. clarkii* sampled from Barát just a few hundred metres downstream in 2020. Additional genotyping methods indicated that a previously unknown *A. astaci* strain was associated with a-haplogroup in this brook. One *F. limosus* individual was co-coinfected by both haplogroups. The results indicated multiple sources of *A. astaci* in Budapest, likely directly associated with introduction of ornamental species, interspecific transmission of this pathogen among ornamental hosts, and potential for a quick spatial or temporal turnover of dominant *A. astaci* strains at a certain locality. This highlights that in regions with high richness of potential *A. astaci* hosts, host taxon/pathogen genotype combinations become unpredictable, which would prevent reliable genotyping of pathogen sources in local crayfish mass mortalities.

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A comprehensive review on the redclaw crayfish: A prominent aquaculture and pet-traded species with invasive potential in tropical and subtropical areas

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The redclaw crayfish (*Cherax quadricarinatus*), native to northern Australia and southern New Guinea, is among the largest freshwater decapods. It matures early and is considered highly prolific as females may lay over one thousand eggs in a single clutch. Despite generally preferring slow-moving streams in its native range, it has a wide environmental tolerance, making it capable of establishing populations when introduced to a wide range of other conditions and habitats. These biological and ecological features render it a highly suitable and popular species for aquaculture worldwide, being the second most important crayfish species economically (after the red swamp crayfish *Procambarus clarkii*). Adding to that, its unique coloration fuels demand and value among aquarium enthusiasts, making it attractive for the aquarium pet trade. Today, redclaw is widely translocated (67 countries/territories) and various established wild populations (22 countries) have been reported on every continent except Antarctica. Information on its potential or observed impacts, however, is sparse and often anecdotal. To address this gap, this comprehensive review compiles all available information on this species, covering its taxonomy and description, biology and ecology, native and non-native ranges accompanied with documented introduction pathways. Built upon these, we conducted biological and socio-economic classification and species distribution modelling. We reveal a lack of thorough impact assessments for this species despite sufficient indications of major observable impacts at local scales. We call attention to the importance of managing the use of this prominent introduced species in aquaculture and aquarium pet trade.

Conservation and taxonomic assessment of an undescribed *Creaserinus* species in coastal Virginia

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Creaserinus fodiens (Cottle 1863), commonly known as the digger crayfish, is a primary burrowing species that inhabits complex burrows in wetlands, seasonal pools, wooded floodplains, and roadside ditches. Historically, *C. fodiens* has been found from Ontario, Canada following the United States down to Texas and across four Atlantic slope states including Virginia, Maryland, North Carolina, and South Carolina. The Atlantic slope clades are geographically isolated from other *C. fodiens* populations by the continental shelf on the East coast and by the Blue Ridge Mountains. Despite having a wide geographical range, little research has been done on the Atlantic slope clades of *Creaserinus* spp. Recent genetic and morphological data suggests that the Virginia population of C. *fodiens* could be described as a different species. Additional morphological and genetic data from gill samples will lead to a better understanding of where the separation in classification lies among the Virginia population. A conservation and taxonomic assessment for *Creaserinus fodiens* along the Virginia Atlantic slope will be constructed from the results. These assessments will contribute to our understanding of global crayfish biodiversity and provide important biological insight to the management and conservation of these species.

Distribution of microsporidiosis in *Austropotamobius pallipes* complex populations in North-western Italy

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Microsporidiosis, also known as porcelain disease, is a chronic parasitic infection that can affect most European crayfish species. In Northern Italy, in the endangered *Austropotamobius pallipes* complex, this disease can be caused by two different microsporidia: *Thelohania contejeani* Henneguy, 1892 and *Vairimorpha austropotamobii* Pretto, 2018. The aim of the present study was to evaluate the distribution of microsporidiosis in the white-clawed crayfish populations of the North-western Apennines and to identify the microsporidian parasites causing the infection. Between 2020 and 2021, we monitored *A. pallipes* in 38 sampling sites located in Liguria and Emilia Romagna Regions; 16 crayfish showing macroscopic signs of microsporidiosis (*i.e.* the typical white appearance of the abdominal muscles) were collected to confirm the suspected infection through histological and molecular analysis. Following standard histological protocols, sections of formalin-fixed cephalothorax and abdomen were examined after staining with Haematoxylin and Eosin. Total genomic DNA was extracted from 25-30 mg of abdominal muscle tissue using a commercial kit and then amplified by end-point PCR, with primers targeting the small subunit ribosomal RNA (SSU rRNA) gene. To detect *T. contejeani* we applied a species-specific assay with primers from El-Matbouli and Soliman (2006). To detect *V. austropotamobii* we used generic microsporidian primers from Weiss *et al*. (1994). PCR products were analysed by Sanger sequencing.
Fourteen sampling sites had at least one specimen with macroscopic signs of microsporidiosis. The histological and molecular analyses confirmed the presence of microsporidian parasites and identified *T. contejeani* in all the suspected specimens. Prevalence of affected specimens ranged between 0.7-10% among the positive populations. Histology confirmed in the majority of the samples the infection of the skeletal, cardiac and intestinal musculature with inflammation and melanisation of affected fibres.
One specimen revealed a case of co-infection of the two species *T. contejeani* and *V. austropotamobii*, which showed extensive skeletal infection with little inflammation and only mild cardiac lesions, and this result was further confirmed by molecular analyses.

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Bacterial isolates associated with crayfish cuticle show inhibitory activity against *Aphanomyces astaci*, the crayfish plague pathogen

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Oomycete pathogen, *Aphanomyces astaci*, causative agent of crayfish plague, has pronounced negative impacts both on native European crayfish populations and on crayfish aquaculture. Infection is established when the hyphae of the pathogen overcome the crayfish immune response and penetrate the cuticle. During this process pathogen interacts with the resident epibiotic bacteria on the cuticle surface that may exhibit antagonism towards *A. astaci* and thus contribute to the host defence. We have collected bacterial isolates from the cuticle of four native European crayfish (*Astacus astacus, Austropotamobius torrentium, Austropotamobius pallipes* and *Pontastacus leptodactylus*) and one North American invasive crayfish species (*Pacifastacus leniusculus*). Results of the 16S rRNA gene sequencing showed that the most of the isolates belonged to the genus *Pseudomonas*, followed by *Aeromonas* and *Flavobacterium*. Next, inhibitory activity of the isolates against *A. astaci* mycelium was tested by *in vitro* plate assays and the isolates were classified as inhibitors (36%) or non-inhibitors (64%). Majority of the inhibitors belonged to the genus *Pseudomonas* (62%). Our results add to the limited knowledge of bacteria-oomycete antagonism on the surface of crayfish cuticle, important for the development of biocontrol measures in the astaciculture.

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Aspects of the population ecology of the Spinycheek Crayfish *Faxonius limosus* in a lowland river ecosystem in northern Belgium

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Invasive crayfish can have severe impacts on aquatic ecosystems, especially on water quality and macrophytes. The Spinycheek Crayfish (*Faxonius limosus*) occurs in Belgium since 1962 and is currently widely distributed and the most common crayfish species. We investigated a lowland river-system in northern Belgium on the presence of this species. We present some aspects on the population ecology of the Spinycheek Crayfish and compare catch efficiency of two different trap types. Two types of baited traps (metallic cylindrical trap and green mesh folding house trap) were used at four sites. Both were deployed pairwise, equally distributed along stretches with and without aquatic vegetation. These were, depending on the river, emptied every day for 23 to 48 consecutive days in spring 2021. Each caught individual was marked using both nail polish and a permanent marker. In total 151 individuals were caught with no apparent differences between either trap types. However the traps caught differently sized by-catch. The number of male crayfish caught was almost triple that of females, suggesting that the latter were less actively foraging in spring. Numbers of trapped individuals were much higher where aquatic vegetation was lacking. The number of trapped individuals closely followed the water temperature, indicating that this species is barely active when temperature drops below 10°C. Our results suggest that, although already long established, Spinycheek Crayfish density may still be quite low in these lowland rivers, in which case their impact is also likely to remain limited.

An account of the invasive non-native crayfish in Belgium

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The negative impact of invasive alien species (IAS) is especially severe in aquatic ecosystems. Non-native invasive crayfish are worldwide one of the most destructive invasions known to date, locally depleting resources in aquatic environments and changing the fauna and flora of ponds and rivers. Despite alarming reports from other European countries, their occurrence and impact on aquatic ecosystems and biodiversity in Belgium remains poorly understood. Recently the presence of several new non-native crayfish came to light and the number of records and distribution of known species is increasing. To monitor their presence and distribution a dedicated surveillance network needs to be set up, allowing early detection of new species or new populations in uninvaded areas and facilitating management actions by authorities. We provide an overview of the non-native crayfish and their distribution in Belgium based on available data. We present trends and status of the different species. These baseline data are essential to inform risk assessment, defining risk management options and aid in future monitoring.

Population genetics of an undescribed member of the upland burrowing crayfish complex, *Cambarus* aff. *dubius*

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The *Cambarus dubius* complex, which includes several putative species referred to as *C.* aff. *dubius,* has a range spanning West Virginia, Virginia, and Kentucky, USA. This species complex contains three montane subpopulations, each with distinct color morphs wedged within the Allegheny Plateau and Ridge and Valley physiographic regions of central Appalachia. Color morphs in this complex are atypically distributed at both the watershed and geographic level. Phylogenetic and population genetic methods will be used to explore genetic relationships within this complex to assess the evolutionary history and conservation status of this species complex. We hypothesize that landscape, specifically ridge and valley geography has affected the phenotypic attributes and genetic structure of this species complex. Thus, we can compare the population structure of this species' subpopulations to test for contrasting genetic patterns that would typically be exhibited by allopatric populations. We plan on utilizing genetic techniques to understand gene flow and effective population size in these montane crayfishes. Ultimately, we hope to determine the network structure, genetic connectivity, and genetic distinctiveness of this Appalachian endemic species.

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The crayfish tale – An educational video

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We are presenting an educational cartoon for past, present and future children and youngsters and all those interesting in the fascinating and complicated history of the freshwater crayfish in Europe. The storyline is based on actual facts and knowledge as it is presented in the scientific papers. The cartoon is based on beautiful artwork and rhyming verses. The crayfish tale covers an enormous time span from long before the dinosaurs appeared on earth until today and how we should act in the immediate future not to destroy more of our remaining native freshwater crayfish stocks and native ecosystems, for that matter. The tale also introduces freshwater crayfish and their fundamental role as keystone species in the aquatic ecosystems, highlighting facts such as that their total weight constitutes more than 80% of what is moving about at the bottom of a water system. The crayfish tale tells about the mistreatment of native crayfish by polluting and destroying their habitats in lakes and running waters, by introducing other competing alien species and furthermost by introducing the fatal, lethal, and mortal disease crayfish plague with these alien crayfish species. The crayfish tale also advices how we humans must behave from now on, in order not to finally lose these essential species and consequently the wellbeing of our freshwater ecosystem. If we start to look after our crayfish and waters, then the future will look after us. The fundamental lesson of the crayfish tale is that we all should carefully think beforehand, while afterwards is usually too late.

The LIFE RIPARIAS project: reaching integrated and prompt action in response to invasive alien species

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Tackling Invasive Alien Species (IAS) requires a coherent approach across administrative boundaries, especially so in continuous aquatic habitats. LIFE RIPARIAS targets a selection of invasive aquatic and riparian plants, and freshwater crayfish (species of *Cabomba*, *Elodea*, *Hydrocotyle*, *Lagarosiphon*, *Ludwigia*, *Myriophyllum*; *Heracleum*, *Impatiens*, *Lysichiton*; *Faxonius*, *Pacifastacus*, *Procambarus*). Its goal is to optimize the management of these IAS at river basin scale within a multiregional pilot area in Belgium (Dyle, Senne, and Marcq river basins). LIFE RIPARIAS aims to achieve 1) a data infrastructure for early warning and management reporting on emerging and widespread IAS; 2) decision support for prioritizing management actions by maximizing consistency, efficiency and resource allocation; 3) an evaluation of management actions; 4) the sharing of expertise and best management practices. A novel evidence-based workflow for decision making on IAS management at the river basin scale will be developed, translating national management objectives to concrete actions at site level and maximizing cost effectiveness. The decision support tool will be made available to IAS managers across the country as well as to other Member States. Active participation and cooperation between decision makers, field managers and the public are essential. The ten project partners include public bodies, public research institutes, academia and associations, and are all committed to work together to tackle the many challenges ahead. Additionally, species identification sheets, booklets and best practice management guides will aid identification and management in the field. Information and training sessions will spread knowledge as widely as possible. There will also be volunteering opportunities for citizens who wish to actively contribute to surveillance and management.

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Night migration pattern of stone crayfish in six small streams in Czechia

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Following the Pollhammer method (2014), we monitored movements of 60 stone crayfish in six streams (i.e. 10 marked specimens in the Chocenický, Kornatický, Skořický, Mítovský, Přešínský brook and left triburaty of the Mítovský brook). Each specimen was marked using Lightstick MINI 4. A 25-m-long section was laid out in each stream, and the section was monitored by four GoPro cameras, one frame every minute. An obtained recording was analysed using Tracker Video Analysis and Modelling Tool. It enabled us to visualise individual movement patterns for every single crayfish. Considerable differences in individual patterns were found. Whereas some individuals moved only slightly in the vicinity of their primary shelter (typically a big stone), and the total distance of their movements was close to only one meter, others spent a night continuously walking for tens of meters. Only a part of the analysed sample applied homing behaviour and returned to the same shelter. The migration pattern seems to be independent of size, shelter structure and stream morphology (including frequency and size of stones, various depths and width of the stream), and sex, but more analyses will be conducted.

The effect of non-native crayfishes for the amphibian and aquatic reptile population in different habitats in Hungary

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Global amphibian and reptilian declines have been well-documented phenomena for decades. Besides global change or anthropogenic habitat destruction and fragmentation, the negative effect of invasive non-native species is a major driver behind them. The amphibian and aquatic reptile populations have declined drastically in the last twenty years in Hungary, but the exact reasons are not always clear. Our team surveyed several habitats that non-native crayfish have successfully colonised in the research programs and where the amphibian and aquatic reptile populations severely altered or disappeared. In most cases, we did not have exact information about the real impact of these species on amphibian and aquatic reptile populations. So, we started with more detailed monitoring of the amphibian and reptile populations in different aquatic habitats colonised by non-native crayfish, comparing newly obtained findings with historical data. Based on our results, the large population density of the red swamp crayfish (*Procambarus clarkii*), spiny-cheek crayfish (*Faxonius limosus*) and marbled crayfish (*Procambarus virginalis*) have both direct and indirect adverse effects of the amphibian and aquatic reptile population. In tributary streams of River Danube, where the red swamp crayfish or several non-native crayfish co-occurred, the newt and some frog species, and the dice snake (*Natrix tessellata*) population disappeared in a short time. The poisonous toads (e.g. *Bufo bufo*, *Bombina bombina*) lost those breeding areas where the red swamp crayfish have a dense population (8-10 individuals/m^2). At the same time, the results of field and autopsy studies confirm that the marsh frog (*Pelophylax ridibundus*), dice snake and grass snake (*Natrix natrix*) feed the adult fresh moulted and juvenile individuals of *Procambarus* sp.. Based on our results, we can confirm that the effects of non-native crayfish are complex and adverse for the amphibians and aquatic reptiles.

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Conservation hot-spots or barriers: the effect of stream sections under bridges for the populations of native and non-native crayfish in Hungary

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Most biological studies on linear infrastructures focus on fragmentation, road kills, invasion by exotic species, effect of air, noise and chemical pollution on animal behaviour or the effect of preventing measures. Relatively little attention is given to the habitat function of different types of streams and channels under the road, motorway and railway bridges. In the last decades the results of monitoring indicated that not only the ecological status of water bodies are changed negatively by human activities, but also the population of several native aquatic species, for example all native crayfishes, the narrow-clawed crayfish (*Pontastacus leptodactylus*), the noble crayfish (*Astacus astacus*) and the stone crayfish (*Austropotamobius torrentium*) are substantially reduced in whole territory of Hungary. Recently the researches have few information about the role of aquatic habitats in streams beneath and around bridges. In 2013 a research program was started, which focus the crayfish assemblages were surveyed by different methods twice a year around 138 bridges of whole territory of Hungary. Each sampling area is a section consisting the neighbouring upstream and downstream from the bridge as well, and the sampling sites were characterised by 11 environmental variables. Apart from different intensive anthropogenic effects on the catchment area of lowland and hilly running waters the non-native crayfishes (*Faxonius limosus*, *Pacifastacus leniusculus*, *Procambarus clarkii*, *P. virginalis*) were high density in the ripraps of bridges and upper section. We were able to confirm, that the noble crayfish could only be caught under the bridges, where the current velocity and sediment composition were optimal for them in the polluted and well regulated running waters. The stone crayfish occured in those bridge crossed sections in higher density in Börzsöny and Visegrád-mountain, where the forest harvesting were intensive in the drainage area, because the current velocity and the sediment composition were optimal at different water levels. The results presented that aquatic habitats under bridges had key role in conservation of native crayfishes in modified and natural streams and in the successful spread of non-native crayfish species in colonised running waters.

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