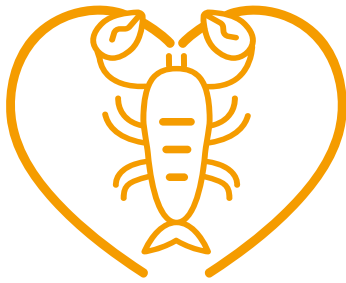


# Book of Abstracts



# CrayfIT

Regional European IAA Meeting



5 – 8  
September  
2023

Pavia, Italy

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# CrayfIT

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## Welcome message

Dear cray-friends,

On behalf of the organising team and scientific committee, it is our great pleasure to welcome you at the CrayfIT meeting, at the University of Pavia, one of the world's oldest academic institutions in a lovely town rich in history and culture.

In 1859, for the first time *Aphanomyces astaci* (the etiological agent for the crayfish plague) appeared in the Po River, not far away from here, and rapidly spread throughout Italy and Europe.

CrayfIT was supported by the Life CLAW project, co-funded by the European Union, 'Crayfish lineages conservation in north-western Apennine' (LIFE18 NAT/IT/000806). The project main objective is to conserve and enhance the stock of the endangered native crayfish *Austropotamobius pallipes* in North-Western Apennine area of the Italian regions Emilia Romagna and Liguria by a long-term conservation programme. Native populations of *A. pallipes* have undergone a remarkable contraction and decline over the last 50 years on a widespread basis in Europe, and in Italy the decline has been more than 70%. However, North-Western Italy is a hotspot for *A. pallipes* where high nucleotide diversity was detected and different lineages overlap. Three invasive alien crayfish species (*Procambarus clarkii*, *Faxonius limosus* and *Pacifastacus leniusculus*) have been also found and represent both strong competitors for the native crayfish and carriers of the crayfish plague.

It has been many years since Italy hosted an international crayfish conference, so we are delighted to be your hosts.

About ninety participants from 18 countries will guarantee an interesting programme, with their enthusiasm to share their scientific news and experience, and to have fun as well!

Daniela Ghia and Gianluca Fea

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# KEYNOTE LECTURES



## **The ecological implications of invasive signal crayfish for macroinvertebrate communities and the potential role of environmental controls**

KATE MATHERS

Department of Geography, Centre for Hydrological and Ecosystem Science, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK.

The spread of non-native species represents a global threat to freshwater ecosystem health, and it is likely that the rate of biological invasions will increase in the future. Signal crayfish, represent one of the most prevalent translocated species globally with significant ecological implications for the aquatic systems they successfully establish in. In this talk I will consider the environmental and ecological implications of signal crayfish invasion for macroinvertebrate communities, considering both taxonomic composition and community functioning, at a range of spatial and temporal scales. I will also discuss how environmental controls such as flow regime modifications and habitat availability may mediate the successful establishment of crayfish populations and the subsequent magnitude of their ecological effects, and how crayfish may alter their physical environment by modifying fine sediment dynamics. Finally, I will consider the potential for invasive crayfish to reduce the effectiveness of many commonly employed biomonitoring metrics.

## **Environmental DNA and crayfish monitoring**

**PIERRE TABERLET**

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The Arctic University of Norway, Tromsø Museum, Tromsø, Norway

My talk will begin with a brief introduction to environmental DNA (eDNA), including some definitions, the historical background to its emergence and the main topics for which eDNA is used by the scientific community, including diet studies and analyses of water, soil, sediment and air. To date, the vast majority of crayfish eDNA studies are based on qPCR or ddPCR and involve the detection of native or invasive species from filtered water. In addition, it is worth mentioning a few studies also based on qPCR for the detection of crayfish plague disease. I will present two other approaches that could potentially enable in situ detection of crayfish eDNA: LAMP and RPA methods. According to articles published to date, the DNA metabarcoding approach has not been implemented for crayfish. I will explore the latter possibility, which could enable large-scale inventories of crayfish populations, and present potential metabarcodes for this purpose. Finally, I will list best practices for securing results obtained with environmental DNA.

## Viral Diseases in Crayfish

KELLY BATEMAN

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A large number of Intranuclear Bacilliform Viruses (IBV) have been reported from multiple species of crayfish, infections affecting the hepatopancreas and midgut epithelia, cells displaying enlarged nuclei with marginalised chromatin. These viruses have been found in high prevalence in some crayfish stocks, and infections have been linked with stunted growth and low-rate mortalities. The majority of crustacean viruses have been tentatively assigned to families based upon morphological and developmental characteristics and the location within the cell. However, in recent years IBV infections in other crustacean species have been formally characterised within the family Nudiviridae. Viruses within this family are known to infect a wide array of arthropods, including hosts from various insect orders (e.g., Diptera, Lepidoptera, Coleoptera, Orthoptera) and crustaceans (Decapoda and Amphipoda). Phylogenetics analysis suggests that the family Nudiviridae can be divided into four genera, *Alphanudivirus*, *Betanudivirus* and *Deltanudivirus* containing species affecting insect hosts, and *Gammanudivirus* containing species affecting crustacean hosts. It is highly likely IBVs described in crayfish species will be classified within the genus *Gammanudivirus*, following formal characterisation. Additional sequence data from aquatic nudivirus infections, especially from freshwater crustacean species, will enhance our knowledge and understanding in the diversity between members of this family. I will present an overview of the Nudiviridae family and present a case study on the identification of a virus infection from wild caught *Crangon crangon* (brown shrimp), the optimisation of viral purification techniques from these samples, and the application of next generation sequencing to characterise the viral genome.

# ORAL COMMUNICATIONS



CrayfIT

## The European stone crayfish *Austropotamobius torrentium*: Population status, threats and advice for conservation management

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The stone crayfish, *Austropotamobius torrentium* (Schrank, 1803), the smallest native crayfish in Europe, is also one of the most threatened due to multiple stressors. Threats to *A. torrentium* include habitat loss, genetic diversity reduction, decreased gene flow, increased competition with non-native crayfish species, the crayfish plague, pollution from industry and agriculture and climate change effects. It acts as a keystone species and ecosystem engineer, by providing top-down and bottom-up control through its omnivorous diet and dwelling activities. Currently, four subspecies and a high genetic diversity are known. Despite major declines, it was assessed as Data Deficient in 2010 by the IUCN due to the lack of data at that time. In this review, we provide a review of relevant characteristics and an updated assessment of the conservation and population status of *A. torrentium* in Europe and propose an updated IUCN Red List Threat Level by analysing relevant and available publications and databases from all countries where populations occur. Although populations require special conservation measures, almost all countries have a bad or poor conservation status compared to the favourable status as described in the EU Habitats Directive. Interestingly, only three out of 87 river basin Life projects listed *A. torrentium* in the agenda as a target species. Based on our findings, we propose changing the threat level to at least “Vulnerable” but rather “Endangered”. The conservation of Europe’s remaining stone crayfish populations is in dire need of attention, and updated assessments, measures and advice for its conservation are necessary.

**Keywords:** IUCN, Red List, Astacidae, multiple stressors

**Acknowledgement.** This review was supported by discussions and exchange with many colleagues.

## National action plan for crayfish conservation in Switzerland

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Switzerland is home to four native crayfish species, which are classified at national level as vulnerable (*Astacus astacus*), endangered (*Austropotamobius pallipes* and *A. torrentium*) and critically endangered (*Austropotamobius italicus*). Due to invasive crayfish species, crayfish plague and habitat destruction the populations have declined sharply in recent decades. Since 2006, there has been a national action plan that describes measures for the conservation of native species and the management of non-native species and serves as an implementation guide for the cantonal authorities. A recent deficit analysis of the action plan, which was last updated in 2011, showed that a large part of the objectives had not been achieved. A revision of the action plan is currently underway, which, together with fact sheets, will provide comprehensive assistance for crayfish protection. In addition to measures for the conservation of native species, specific measures for the management of invasive species are outlined and a standardised field surveying and monitoring method are described.

**Keywords:** management, invasive crayfish, monitoring

**Acknowledgement.** We would like to thank the local authorities for their cooperation and input into the action plan.



## The white-clawed crayfish *Austropotamobius pallipes* complex in the Foreste Casentinesi National Park: conservation status and threats

Marco Morbidelli<sup>1</sup>, Matteo Orlando<sup>1</sup>, Davide Alberti<sup>3</sup>, Paola Ciampelli<sup>4</sup>, Antonio Zoccola<sup>4</sup>, Gianna Innocenti<sup>5</sup>, Giuseppe Mazza<sup>6</sup>, Elena Tricarico<sup>1,2</sup>

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The native white-clawed crayfish *Austropotamobius pallipes* complex is a protected species and its populations are declining in the entire Europe for several threats such as illegal fishing, climate change, water pollution and the presence of invasive alien species (such as *Procambarus clarkii* and *Procyon lotor*). The purpose of the study was to update the distribution and status of *A. pallipes* complex in the Foreste Casentinesi National Park and the State Reserves within it, comparing the current with the past situation and assessing the potential effects of the threats on the species. In 2021-2022 the sampling was carried out in the central-southern part of the Park, in 41 sites partially and previously monitored between 2012 and 2016. Crayfish were caught by hand along a 100 m transept during the night. Sampled individuals were sexed and measured, and any marks of raccoon presence, such as footprints or predated crayfish remains, were also noted. The results showed a significant reduction in populations, or the local disappearance, in the Tuscan side, whereas, in the Romagna area, the species appeared to be stable or even increase. This difference could be due to a more relevant human impact on the water courses in the Tuscan side caused by illegal poaching, water uptake, discharge in streams and invasive alien species. An efficient management of these threats and an environmental education are needed to halt the decline of the native crayfish in the Park area.

**Keywords:** alien species, biological invasion, *Austropotamobius pallipes*, *Procyon lotor*, *Procambarus clarkii*

**Acknowledgement.** This study was supported by the Foreste Casentinesi National Park and Reparto Carabinieri Biodiversità of Pratovecchio (AR).

## The last 25 years monitoring white-clawed crayfish *Austropotamobius pallipes* in a stream in Leeds, UK

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This is a 25-year study of white-clawed crayfish in a non-calcareous stream in a wooded valley in the city of Leeds, Yorkshire. Surveys were conducted in c.100m of varied, stony channel by multiple sessions of night-viewing annually (1999-2010). Contrary to earlier published literature, crayfish activity was mainly in pools and glides, rather than riffles. Variation in activity during the year was related to season and breeding. Some testing of mixed trap types was undertaken in 2005-2008. In 2009, volunteers carried out night-viewing at four more sites. From 2009 to 2022, there was a trapping session in early September each year with baited funnel traps (10 Fladen traps, sardine bait, 5 sites). Total annual catches ranged from 134 to 353; an average 70% traps had crayfish present. Between-year and between-site variations indicated possible local pollution and recovery. Large woody debris dams also influenced substrate dynamics, plus silt from an upstream impoundment and construction runoff. Trapping sessions helped train volunteer surveyors and raised community awareness. Catches from 2015 and 2016 were used to re-stock a now clean stream, formerly historically polluted. Artificial refuge traps (trials in 2020-2022) had low catches, more small crayfish, but similar detection rates to baited traps. In November 2022 separate eDNA sampling detected crayfish plague, but not signal crayfish. A salvage attempt upstream of detectable infection in April 2023 by de-watering was unsuccessful, due to ground conditions. The stream stocked successfully from Meanwood Beck will now provide donor-stock for a new ark site in an isolated reservoir.

**Keywords:** white-clawed crayfish, monitoring, survey methods, conservation

**Acknowledgement.** This unfunded study was carried out by the presenting author and from 2009 with the aid of many enthusiastic local volunteers. Artificial refuge traps were made by Matthew Childs, with funding from the Friends of Meanwood Park and the Environment Agency. Permission for survey was given by Leeds City Council. The Environment Agency (Tim Selway) carried out eDNA sampling and the emergency response to crayfish plague. Yorkshire Water has offered the new ark site.

## CRAYART: Crayfish in paintings around the world

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Understanding the inclusion of wildlife in arts can inform long-term species trends, historical species uses, and perceptions of nature. Artistic representations can also enhance nature connectedness, becoming a tool to promote biodiversity conservation. We reviewed the presence of crayfish in paintings and recorded over 400 paintings, embracing a high variability in naturalistic precision. While some art works offered details for precise identification of species, others roughly allowed identifying a crayfish. The vast majority of the pre-20<sup>th</sup> century pieces found were European. Despite the rich historical inclusion of wildlife in Japanese art, we did not find crayfish records (but several lobsters and prawns). We neither found representations of Parastacidae crayfish in Madagascar or Southern America, while they were uncommon in Australia and Papua. In Europe, the main areas of production of crayfish paintings shifted from the west (Italy, the Netherlands) before the 19<sup>th</sup> century, to the east (Ukraine and Russia). Interesting European representations of crayfish included their presence in Last Dinner 15<sup>th</sup> century Italian frescoes, their frequent prominent role in Flemish still lives (with highlights in the works of Clara Peeters), and their use by Italian satirical authors (e.g. Faustino Bocchi). There is a rich recent crayfish art tradition in North America, mainly focussed on the Louisiana red swamp crayfish, *Procambarus clarkii*. Representations of this species have notably expanded geographically together with its spread in non-native areas. Paintings are a valuable tool to interpret the utilisation and perception of biodiversity across spatial and historical dimensions.

**Keywords:** Wildlife art, Art history, Paintings, Crayfish, Nature perception

**Acknowledgement.** This study is part of project CRAYMAP-crayfish invasions across time and space, a multidisciplinary approach (PID2020-120026RB-I00), funded by the Spanish Ministry of Science and Innovation

## Application of habitat suitability modelling for conservation management of native crayfish species in Drava and Sava Rivers

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Indigenous crayfish species (ICS) have suffered a significant decline in recent decades and there is an urgent need to assess and quantify their threats on landscape level. To date, only a few studies have been published with this specific focus. We aim to highlight the dangers arising from interacting pressures such as anthropogenic habitat degradation, fragmentation and non-indigenous crayfish species (NICS) within the Sava and Drava basins in Slovenia, Croatia, and Hungary. Within the catchment area of 75,671 km<sup>2</sup>, our analyses will assess the risks to the remaining ICS populations, predict the further spread of NICS, and identify potential habitats as suitable refugia for ICS. Our methodology consists of (1) assessing fragmentation on small catchment scale, including settlements, hydropower stations, dams, and other factors; (2) calculating a threat index inclusive of spatial distances to potential plague carrying NICS; and (3) conducting habitat suitability modeling using customized Land Cover layers with a set of various environmental parameters. The target species in this area are the ICS *Astacus astacus*, *Pontastacus leptodactylus* and *Austropotamobius torrentium*, as well as the NICS *Pacifastacus leniusculus*, *Faxonius limosus*, *Cherax quadricarinatus* and *Procambarus virginalis*. To gain valuable insights into the habitat suitability and species distribution we will utilize both recent and historical crayfish distribution data. The results will contribute to the development of a comprehensive framework for safeguarding the remaining ICS populations, predicting the spread of NICS, and help to improve sustainable management practices for freshwater ecosystems in Europe.

**Keywords:** Astacidae, WFD, red list, land use, river regulation

**Acknowledgement.** This study was supported by the Tyrolean Science Fund (Tiroler Wissenschaftsförderung - TWF).

## Successful post-crayfish plague reintroduction of *Austroptamobius pallipes* in Monte Barro (NW Italy) protected area: new challenges and role of visitors' perspective

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In Northern Italy, invasive freshwater crayfish are leading numerous local extinctions of the native white-clawed crayfish. Here, we describe the steps, the new challenges and the role of park's visitors' perspective that accompanied a successful reintroduction of a population that went extinct in the protected area of the Regional Park of Mount Barro after the illegal introduction of *Faxonius limosus* in 2013. After the removal of all spiny-cheek crayfish individuals, we started periodical surveys of the site. Prior to reintroduction we distributed a questionnaire to parkgoers to identify dangerous behaviours/categories and understand the general perspective and awareness of the visitors. From 2018 to 2020, every autumn, we reintroduced white-clawed crayfish juveniles, and we monitored population status. Of 291 visitors, most (73%) had never heard of alien species. The respondents' inclination to be favourable to conservation investments, was significantly related to their knowledge of crayfish features. Visitors' behaviours resulted not dangerous for native crayfish conservation, while those of local people living in the park were. Since November 2019 we started to record successful breeding of the reintroduced crayfish. In December 2022, the estimated density of large crayfish reached 0.61 individuals/m<sup>2</sup>, which is still lower than the density observed prior to extinction. Further actions will involve amelioration of the habitat and further reintroductions in other sites of the Park after having performed focused educational activities for the local stakeholders having the greatest potential negative impact.

**Keywords:** restoration, conservation, human dimensions, impact

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## Reconquering old territories – case studies from Austria on the re-establishment of populations after a crayfish plague outbreak

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Since the introduction of the crayfish plague (*Aphanomyces astaci*) to Austria, native freshwater crayfish were facing a massive decline in abundance. While invasive crayfish species have been spreading rapidly, the noble crayfish (*Astacus astacus*), for instance, disappeared completely from riverine watercourses and occurs nowadays only in some small stagnant waters. However, despite the trail of destruction left by the crayfish plague, there are still results that allow a positive look into the future. A long-term monitoring project, for example, shows that resettlement programs with noble crayfish worked very well in four small lakes in Upper Austria after the extinction of the original stock. Results showed evidence of increasing population densities, steady reproduction and expansion of habitats leading to a good conservation status of populations, though particular waterbodies were initially only stocked once with a few hundred individuals. Further, a small stone crayfish (*Austropotamobius torrentium*) population was detected in 2022 after a verifiable crayfish plague outbreak in 2013 without any re-establishment efforts. It can be assumed that some individuals survived the outbreak and re-established a new, maybe immune population or, though very unlikely in this particular case, stone crayfish migrate from adjacent tributaries and have been reproduced successfully within the last years.

**Keywords:** crayfish plague, re-establishment, long-term monitoring, stone crayfish, noble crayfish

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## Central/Northern Sweden- the last refuge for native noble crayfish in Scandinavia?

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The noble crayfish (*Astacus astacus*) has disappeared from more than 98% of its original sites in Sweden and the populations in southeast Norway and Finland are also highly endangered. The main risk for the noble crayfish is further illegal introduction of the alien signal crayfish (*Pacifastacus leniusculus*) which carry the crayfish plague (*Aphanomyces astaci*) which totally wipes out the native noble crayfish. Signal crayfish are far more abundant in the southern regions of Sweden and the few remaining populations of Noble crayfish in southern Sweden, (and southeast Norway and parts of Finland) mainly exists in very short geographical distance to the nearest population of signal crayfish which increases the risks of crayfish plague outbreaks. In central/northern Sweden most of the remaining populations of noble crayfish lives in much longer geographical distance to the nearest population of signal crayfish. This reduces the risk of extinction compared to the more southern populations. We do a lot of monitoring of both species in the region and introduce noble crayfish into river parts and tributaries without noble crayfish in water systems which carry noble crayfish in the main channel to create refuge-populations if some parts of the main channels are to get signal crayfish or crayfish plague. To prevent further illegal introduction of signal crayfish we spend lots of effort on information in media and lots of meetings. At some locations, we do habitat restoration targeting noble crayfish.

**Keywords:** Conservation genomics, *Astacus astacus*, genomic tools, comparative genomics

## **Environmental DNA (eDNA) Surveillance of Crayfish Plague, potential vectors, and White-clawed crayfish in Ireland, a National Monitoring Program**

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Since 2015, several crayfish plague (CFP) outbreaks have decimated White-clawed crayfish (WCC) populations in several Irish river systems. In response to the spreading plague, an environmental DNA-based national surveillance programme was established in 2018 as a collaboration between the National Parks and Wildlife Service and the Marine Institute. This study aimed to understand further and monitor the CFP outbreaks in Ireland, potential vectors, and CFP impact on the native crayfish populations. eDNA surveillance has proven to be very successful. The results of both programmes show a rapid spread of CFP within and between catchments, with positive CFP detections in 15 out of 27 catchments surveyed. Genotyping of CFP-positive samples suggests multiple possible introduction pathways are possible with multiple genotypes present. However, the overall WCC population remains largely healthy in many catchments despite the continued threat of plague. Only two of the 27 surveyed failed to record a single WCC-positive site in both the 2018-2019 and 2020-2021 cycles. To date, DNA from potential vector crayfish species has not been detected in any eDNA samples tested from CFP-positive catchments.

**Keywords:** *Austropotamobius pallipes*, *Aphanomyces astaci*, crayfish plague, environmental DNA, Ireland

**Acknowledgement.** This project is a collaboration between the Marine Institute and National Parks and Wildlife Service, Ireland.



## Influence of distance from source population and seasonality in eDNA detection of white-clawed crayfish, through qPCR and ddPCR assays

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The white-clawed crayfish (*Austropotamobius pallipes*) is an emblematic taxon of European rivers, found mainly in oxygenated streams, known to be an excellent indicator of river quality. Since several decades, population of *A. pallipes* declined in relation to anthropogenic pressure, habitat loss and competition with pests (invasive crayfish, crayfish plague). This endangered species is now submitted to conservation strategies by freshwater managers in order to survey and protect the remaining populations. In France, traditional surveys in freshwater environments were performed by electric fishing, kick-net fishing or trapping, particularly disruptive for the environment and very time-consuming. However, with the rise of molecular genetic technology, new methods based on the detection of environmental DNA (eDNA) have emerged. We present here the results of an optimized study for the detection of the endangered crayfish *Austropotamobius pallipes* in France, considering certain environmental co-factors and comparing two PCR methods (qPCR and ddPCR). After improving laboratory procedures, we were able to detect the presence of the crayfish up to 2 km downstream from a known point of presence and unfortunately highlight the disappearance of a historical population, after sampling two consecutive years. Such a level of precision is interesting because it makes it possible to precise the presence of specimens in a relatively restricted area and to orient traditional prospecting, necessary for certain additional studies. During our study, we observed better probabilities of detection during the summer period, but in a growing context of climate change, we advise to adapt the sampling year by year.

**Keywords:** *Austropotamobius pallipes*, crayfish conservation, environmental DNA, PCR methods

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## How to improve detection of crayfish and crayfish plague from water samples by comparing different eDNA methodologies

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Two of the most widespread and feared alien invasive species (AIS) in Europe are the signal crayfish (*Pacifastacus leniusculus*) and the crayfish plague (*Aphanomyces astaci*). The signal crayfish is a vector of the crayfish plague and spreads the pathogen to native crayfish species, including the noble crayfish (*Astacus astacus*). To minimize damages on ecosystems and further reduction of vulnerable species, it is imperative that AIS are detected early in the invasive process. Environmental DNA (eDNA) has proven to be a precise and accurate method for early detection of rare and invasive species. Still, there are many different eDNA methodologies used to sample and analyse target DNA from water samples, and a standard protocol is lacking. Also, several studies show difficulties in detecting crayfish by using water eDNA, mostly due to the crayfish ecology and its generally low amount of DNA shedding. To address these methodological issues, a workshop was held at the Institute of Freshwater Research in Sweden in May 2023. 26 researchers from 12 European countries participated in an experiment based on a ring test setup to detect three species (signal crayfish, noble crayfish and crayfish plague) by taking water samples from pre-prepared tanks, artificial ponds and a natural lake. The participants were divided into 12 teams using their own equipment, strategies and protocols for water sampling, DNA extraction and targeted qPCR. By comparing the results from the analysed samples, we hope to formulate useable advice and guidelines for an improved data comparability and detectability of these target species.

**Keywords:** Alien invasive species, *Pacifastacus leniusculus*, *Astacus astacus*, *Aphanomyces astaci*, ring test, qPCR

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## **eDNA monitoring as a tool for evaluating the reintroduction of *Austropotamobius pallipes* after a crayfish plague outbreak**

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The crayfish plague, a severe disease caused by the oomycete *Aphanomyces astaci*, is responsible for most population declines of susceptible crayfish in Europe. This pathogen has been devastating native populations of *Austropotamobius pallipes* since the 1970s in the Iberian Peninsula. In this study, we report a massive mortality event in one of the most important Spanish populations of *A. pallipes*. We aimed to: (i) identify the cause of the mortality, and (ii) evaluate the reintroduction viability of the species. Over the course of six months, we used environmental DNA (eDNA) and traditional trap-based methods to detect the presence of *A. astaci* or of native or invasive crayfish in order to evaluate the reintroduction viability of *A. pallipes* to the affected population. We did not capture any live crayfish or detect the presence of *A. astaci* in the reservoir water during the six months following the mass mortality event. Our analyses indicated that it was feasible to initiate a reintroduction program at the site, which will continue to be monitored for three to five years and will help improve the conservation status of *A. pallipes*.

**Keywords:** Invasive alien species, *Aphanomyces astaci*, eDNA monitoring, *Austropotamobius pallipes*, native species reintroduction

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## Exploring the Genomic World: Insights from the Noble crayfish and Beyond

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Genomics has revolutionized biology by revealing the genomic basis of phenological traits across the tree of life. Genomic data have a wide range of applications, including studying species' evolutionary history, disease genetics and assisting biodiversity assessment, conservation, and restoration efforts by providing evidence for scientifically informed management decisions. In aquaculture, genomics was successfully applied to propagate breeding stocks resilient to specific environmental conditions or diseases. Unfortunately, invasive crayfish species and their pathogens have imposed an economic and environmental burden on the European freshwater crayfish biodiversity and aquaculture. In this context, noble crayfish, one of the most farmed crayfish species in Europe, is highly exposed to pressures from invasive species and climate change. Therefore, our research is focused on providing the genomic resources as a basis for conservation actions aiming to preserve this highly important species of European freshwaters. Currently, we are sequencing and assembling the noble crayfish genome to provide an exhaustive inventory of its genetic material, allowing identification of genes, regulatory elements, genetic variations, and other genomic features that can explain resistance or susceptibility. It will be the first genome reported for any member of the Astacidae family. Its assembly presents a considerable challenge due to the enormous genome size of ~ 17 Gbp. Here, we show how the noble crayfish genome can be used to understand the evolutionary mechanisms governing genome evolution of freshwater crayfish. This fundamental knowledge will support the long-term conservation of noble crayfish in European freshwaters.

**Keywords:** Conservation genomics, *Astacus astacus*, genomic tools, comparative genomics

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## On the conservation of white-clawed crayfish in the Iberian Peninsula: an analysis of the genetic diversity and structure, and its origin

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Most of the native European freshwater crayfish species are included in the IUCN Red List as threatened. The endangered species native to the Iberian Peninsula, the white clawed-crayfish (WCC) (*Austropotamobius pallipes*) requires especially attention. Currently, conservation management strategies require a better understanding of its genetic diversity, structure patterns and evolutionary history. For this purpose, we generated the two largest datasets from the whole geographical range of WCC in Europe of two informative ribosomal mitochondrial regions, *i.e.*, COI and 16S. These datasets allowed us to analyze in detail (i) the genetic diversity and structure of WCC populations, and (ii) divergence times of Iberian populations by testing three evolutionary scenarios. The results indicate high levels of haplotype diversity and a complex geographical structure for the Iberian WCC. The diversity found includes new unique haplotypes and reveals that most of the WCC genetic variability is concentrated in the northern and central-eastern regions. Although molecular dating analyses provided divergence times that were not statistically supported, the proposed scenarios were congruent with previous studies, which related the origin of these populations with paleogeographic events during the Pleistocene, suggesting an Iberian origin for these WCC. All the results generated in this study, indicate that the alternative hypothesis of an introduced origin of the Iberian WCC is highly improbable. The result of this study has allowed us to better understand the genetic diversity, structure patterns, and evolutionary history of WCC in the Iberian Peninsula, which is crucial for the management and conservation needs of this endangered species.

**Keywords:** *Austropotamobius pallipes*, genetic diversity, divergence time, conservation

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## Conservation genomics of endemic crayfish species *Austropotamobius bihariensis* populations using reduced representation sequencing

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Increased anthropogenic footprint and climate change are the leading cause of biodiversity loss in freshwater habitats. Freshwater crayfish, considered keystone species of freshwaters, are particularly at risk. The idle crayfish (*Austropotamobius bihariensis*) is an endemic species distributed over a small geographic area in the Apuseni Mountains (Romania). Being a recently described species, its conservation status is not yet determined. For an informed assessment of the species status and analysis of genetic adaptive potential, population size and gene flow among subpopulations, we used a reduced-representation sequencing approach, namely ddRADseq (double digest restriction-site associated DNA sequencing). Our samples included leg tissue of 235 individuals from 13 populations belonging to five river basins across the whole species distribution range. The results of ddRADseq suggest a moderate genetic diversity, based on the analysis of 5000 genomic single nucleotide polymorphisms. Most of the populations have low heterozygosity ( $H_0 = 0,1 - 0,3$ ), pointing to a low adaptability to environmental changes. Individual based clustering and principal coordinate analysis showed grouping of populations according to the river basins and admixture between idle crayfish populations within river basins, reflecting their close contact in the past or ongoing gene flow. Our results provide insights into intraspecific genomic diversity, population structure and phylogeographic patterns and can be used as a baseline to establish species management programs.

**Keywords:** ddRADseq, *Austropotamobius bihariensis*, conservation, population genetic structure, SNP

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## Can transcriptomic tools revolutionise our understanding of the noble crayfish immune response to crayfish plague pathogen? Facts, hopes and dreams

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Preventing the transmission of the crayfish plague pathogen is the biggest challenge for the preservation of the European freshwater crayfish fauna. Noble crayfish populations have been particularly affected by the introduction of this highly invasive pathogen. Local extinctions and collapses of natural and farmed noble crayfish stocks left an unerasable mark on its distribution in Europe. Therefore, it is crucial for the conservation of noble crayfish to understand the molecular mechanisms of species immune response towards the crayfish plague pathogen. Transcriptomic methods allowed us to identify a variety of novel genes involved in the immune response within a single experiment. At least 412 genes are differentially expressed in the noble crayfish immune response to the crayfish plague pathogen. Previously, only a subset of genes involved in the canonical prophenoloxidase pathway was known. Among them, transcriptional factors C/EBP and KLF1 seem to be strongly involved in the orchestration of the immune response. Tracing their expression profiles allowed us to understand the temporal dynamics of the noble crayfish immune response towards the crayfish plague pathogen. Furthermore, we identified over 4700 genes involved in the crayfish plague-specific and non-specific pathways of the noble crayfish immune response. The ability to mount a specific immune response to a pathogen challenge is one of the key aspects of immune memory. Induced immune tolerance of the noble crayfish towards the crayfish plague pathogen will find its application in aquaculture by aiding the breeding of resistant noble crayfish stocks and further the conservation of this emblematic species.

**Keywords:** *Aphanomyces astaci*, *Astacus astacus*, panzootic, transcriptomics, invertebrate immunology

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## Evolution of freshwater crayfish (Decapoda: Astacidea)

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Astacidea contains four superfamilies, Enoplometopoidea and Nephropoidea in the marine, Astacoidea and Parastacidea in the freshwater. Astacoidea has three families, Astacidae, Cambaroididae, and Cambaridae in northern hemisphere, Parastacidae contain only member of family Parastacidae in southern hemisphere. The two superfamilies are called as freshwater crayfish and they have contained approximately six hundred species, distribute in North, Middle, and South America, Australia with New Zealand and New Guinea, Malagasy Africa, far-east Asia, Europe. It is estimated based on fossil evidence, molecular, and morphology of post-embryonic stage, ancestor of the freshwater crayfish has been evolved from ancient of the marine Astacidea members in Mesozoic era, the single ancestor of freshwater crayfish invades into freshwater area in Super-continent Pangea from marine. This mono-invasion of the freshwater crayfish creates their several adaptations for the freshwater environment. Present study shows functional morphology of respiration and feeding and sensor, calcium metabolism of their gastrolith in stomach, their burrows in natural habitat, mating behavior and direct development of reproductive biology. Those are compared with that of Enoplometopoidea in coral reef and Nephropoidea in deep sea to clarify adaptation for the freshwater environment.

**Keywords:** ecology, functional morphology, ontogeny, paleontology, zoogeography



## Contributing factors of crayfish dispersion

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Invasive species are amongst the most relevant threats to biodiversity worldwide, particularly in aquatic ecosystems, causing loss of native species and having major socio-economic consequences. *Procambarus clarkii* is an omnivore species native to northeastern Mexico and the southern USA and it is a prominent example of a successful invasive species due to its rapid life cycle, dispersal capacities, burrowing activities, high population densities and significant individual variations in dispersal distances. These crayfish ability to disperse is known to be a key factor in determining the successes of invasion and impacts. However, there is still limited information on which factors may contribute more significantly to crayfish dispersal, namely the influence of temperature, light or presence/absence of food. Here we used a total of 212 individuals (males and females) encompassing various life stages to test for dispersal capability of the crayfish using 3 variables, temperature (16 °C and 22 °C), absence/presence of food and light/darkness, using a two-meter-long track filled with 7 to 8 cm of water and sand substrate. Early analysis of the results suggests that higher temperature and light may have a significant influence on dispersion capability while food seems less important than other factors. Combination of these factors may lead to an increase of the speed and distance travelled although further analysis is needed. Faced with current global changes and habitat alterations the study of which factors contribute to a higher rate of dispersion can prove instrumental in formulating plans to limit the impact of *P. clarkii*.

**Keywords:** *Procambarus Clarkii*, temperature, light, food, dispersion

## Signal and noble crayfish, disentangling the reasons for their rare coexistence

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The invasive signal crayfish (*Pacifastacus leniusculus*) and native noble crayfish (*Astacus astacus*) rarely coexist in the same freshwater ecosystems due to the crayfish plague carried by signal crayfish. Recently the two species were found together from a Finnish lake giving the opportunity to study their ecology in sympatry. Previous studies in Finnish lakes showed that the trophic niche of signal crayfish overlapped with the niche of noble crayfish, but was wider. Moreover, although similar significant effects were detected on littoral benthic macroinvertebrates, the effects on profundal macroinvertebrates were different between the species. However, these studies were conducted with the crayfish species in allopatry. In the present study, we aim to compare trophic niches of sympatric invasive signal crayfish and native noble crayfish and assess their effects on abundance and species richness on benthic and profundal macroinvertebrate communities, in a Finnish lake. Trophic niches and diets of the two sympatric crayfish species are assessed using carbon and nitrogen stable isotopes.

**Keywords:** Invasive crayfish, native crayfish, sympatry, trophic niches, macroinvertebrate community

## Trophic overlap between invasive red swamp crayfish and Atlantic blue crab along a steep environmental gradient

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Understanding inter- and intra-specific interactions involving invasive species is a main topic of invasion science, since invaders may become the main driver of trophic dynamics within ecosystems. This is especially applicable to decapods, which severely alter aquatic food webs due to their trophic flexibility and the high abundances they attain. We studied trophic patterns in co-occurring invasive decapods, red swamp crayfish (*Procambarus clarkii*) and Atlantic blue crab (*Callinectes sapidus*), along a 3-km steep environmental gradient in a small estuary, varying from a crab-dominated saltwater area to a crayfish-dominated freshwater one. We analysed the isotopic signatures of 67 crayfish and 66 crabs for a wide range of sizes (9-102mm length, 8-170mm width; respectively). The  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  bi-plot was strongly determined by location along the estuary. Crayfish caught upstream and crabs caught downstream were separated from those caught in the overlapping stretch, which were clustered together. Within this cluster, the isotopic niche of both species was clearly partitioned, mainly because crayfish had lower  $\Delta^{15}\text{N}$  values (i.e. lower trophic positions) than crabs, but also a narrower variability of  $\Delta^{13}\text{C}$ . Crayfish  $\Delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$  values were independent of body size along the study area, reflecting a lack of ontogenetic trophic shifts. Patterns arising from the  $\Delta^{34}\text{S}$  and  $\Delta^{15}\text{N}$  bi-plot suggested crayfish predation on animals migrating from sea or downstream reaches. This study is a pioneering exploration of trophic interactions occurring between these invasive decapods, an increasingly frequent reality in Northern African and European aquatic systems.

**Keywords:** isotopic niche, interactions, invasion, decapods, inter-specific

**Acknowledgement.** This study is part of the CRAYMAP Project (Crayfish invasions across time and space, a multidisciplinary approach, PID2020-120026RB-I00), funded by the Spanish Ministerio de Ciencia e Innovación.

## The red swamp crayfish in the Carpathian Basin: present status, distribution and the impacts of the colonised ecosystems

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Fourteen non-native crayfish, six crab and five shrimp species have been reported in the wild habitats in Hungary and seven crayfish and one shrimp of these species start to colonise the different water bodies of the Carpathian Basin. Among the new reported species in the recent years, the red swamp crayfish (*Procambarus clarkii*) is stand out. Following the first record (2015 December) of red swamp crayfish in Hungary, the first established populations were found in a tributary stream and in the estuary area of this stream in Danube River only a half year later in June 2016. Since the first observation of the red swamp crayfish an intensive survey period was started in the potential habitats in Hungary and a monitoring program was begun in the colonised habitats at the same time. During the country-wide survey program, the species was detected in 8 additional area, so nowadays four streams, one thermal water polluted channel, three side arms and circa 120 km long section of the main arm of the River Danube occur the red swamp crayfish. The results of the seven-years monitoring program showed that the red swamp crayfish has capable to completely alter aquatic macrophytes, macroinvertebrates, fish, amphibians and aquatic reptiles. The overall transformation of species composition and the environmental parameters has a significant impact on the ecological status/potential of water bodies. Our results are in line with previous findings on *P. clarkii* in Western Europe and elsewhere.

**Keywords:** invasion, urbanization, WFD, long-term effect

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## Modelling the expansion of *Procambarus clarkii* in Lombardy during the past 25 years

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Non-indigenous crayfish species are a critical threat to indigenous crayfish species across Europe and especially in Northern Italy, where mostly *Procambarus clarkii* is driving to extinction the only native crayfish species *Austropotamobius pallipes* complex by carrying the crayfish plague. This work aims to model the expansion of *P. clarkii* in the Lombardy region, from its first observation till today (1997-2022), to investigate the factors influencing its establishment and spread. Reliable observations were merged into a single dataset from various sources, including the so-called grey literature. First, we tested the relationship between the year of sighting and distance from the first point of introduction using a linear regression model. Then, we modelled its ecological diffusion in the area of first introduction using the Bayesian framework by Hefley et al. (2017) and considered the following covariates: percentage cover of water bodies, forests and urban areas along with the terrain slope. The best regression model estimated an expansion rate of 3.8 km/year (adjusted  $R^2=0.45$ ), while the Bayesian model highlighted that urban coverage is negatively correlated with the growth rate but positively with the diffusion. Therefore, the human presence makes for unsuitable habitats but favours *P. clarkii* expansion, possibly in the form of translocations. In conclusion, the colonization of the remaining Po Plain areas and the great lakes' shores seems possible in the next few years. Containment and dissemination activities are thus fundamental to avoid the further spreading of *P. clarkii* (and hence of the crayfish plague) to uplands where native crayfish populations still survive.

**Keywords:** invasive crayfish, red-swamp crayfish, Bayesian model, ecological diffusion, prediction

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## The role of temperature in marbled crayfish (*Procambarus virginalis*, Lyko 2017) invasion in Estonian freshwater ecosystem

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Marbled crayfish (*Procambarus virginalis*) is an example of a freshwater invader spreading across Europe, and understanding its establishment and distribution is crucial. This study aimed to assess the role of temperature in the invasion of marbled crayfish in the artificially warm outflow channel of a power plant in Estonia. It was hypothesized that warmer water temperature and the temperature gradient along the channel would impact crayfish establishment, distribution, and trophic niche. Temperature loggers recorded a gradient, revealing that the channel was significantly warmer than the reservoir, providing a suitable habitat for crayfish. Stable isotope analyses showed spatial and seasonal trophic niche shifts. The crayfish diet at the channel head mainly consisted of macroinvertebrates and macrophytes, while the mouth had a diet dominated by periphyton. Additionally, the crayfish shifted from consuming primary consumers in spring to primary producers in autumn. Temperature played a crucial role in crayfish establishment, but other factors like food source availability likely influenced population distribution. Overall, this research sheds light on the importance of temperature and ecological aspects in the invasion dynamics of marbled crayfish.

**Keywords** stable isotopes, trophic niche, invasive species, population dynamic, temperature effect

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## A rare observation of conjoined twins in marbled crayfish *Procambarus virginalis*

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Conjoined twins in crustaceans, including crayfish, have been predominantly observed during early developmental stages. However, cases of conjoined twins surviving into juvenile stages are infrequent and often associated with mortality resulting from complications during moulting due to morphological abnormalities. In this study, we present a unique case of marbled crayfish conjoined twins that remained viable for 36 days post-hatch, progressing into the 3<sup>rd</sup> juvenile stage with the ability to moult. The fused individuals exhibited separate complete anteroposterior body axes, conjoined along the dorsal side of the cephalothorax. Both twins demonstrated successful predation of living nauplii of *Artemia salina*, their provided food source. Nonetheless, reduced mobility led to the growth of epibionts on their body surface, ultimately hindering the moulting process. This exceptional case sheds light on the prolonged survival and functional capabilities of conjoined twins in crayfish, expanding our understanding of their developmental biology.

**Keywords:** decapods, developmental aberration, parthenogenesis, genetic anomalies

## **Controlling Marbled Crayfish in an urban pond: a dual approach involving intensive trapping and predator introduction**

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The marbled crayfish is a newly introduced invasive species in the Flanders region of Belgium, first sighted in 2017. The species has primarily been sighted in artificial ponds in urbanised areas, suggesting its introduction through the release of aquarium pets. Marbled Crayfish have not yet spread to open water systems in Belgium, presenting an opportunity to experiment with control measures within a closed system. To manage this species within the urban park pond in the municipality of Westende, a pilot study was developed. The municipality did not approve of draining the pond, and the use of chemical agents is prohibited in aquatic environments in Flanders. To comply with these restrictions, we developed an alternative management measure to decrease crayfish densities and minimise the risk of spread. A dual approach will be implemented, involving an intensive catch event followed by the introduction of two fish species, *Cyprinus carpio* and *Lota lota* to further suppress the population. Electrofishing and baited crayfish traps will be used to achieve a proportional reduction in densities at the start of the reproductive season. The introduction of fish aims at preventing juvenile crayfish from reaching the reproductive phase, by creating a year-round predation pressure with common carp preying from spring to autumn, and burbot throughout the winter. This dual approach will be tested to determine its effectiveness in controlling marbled crayfish densities. Preliminary results obtained after the first two phases of the study, namely population reduction and the introduction of the common carp, will be presented.

**Keywords:** Marbled Crayfish, invasive species management



## Once, twice, three times, or whenever possible? Reproductive biology and population dynamics studies of three invasive crayfish and one shrimp species in Hungary

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Nowadays 25 non-native decapode species have observed in the wild habitats in Hungary. Total seven crayfish and one shrimp of these species have self-sustaining population and start to colonise the different water bodies in the whole territory of Hungary. During our surveys were collected the individuals of the four most widespread non-native crayfish (*Faxonius limosus*, *Pacifastacus leniusculus*, *Procambarus clarkii*, *Procambarus virginalis*) and the red cherry shrimp (*Neocaridina denticulata*) in different water bodies (natural and urbanised running/standing waters, natural water with seasonal dynamics and thermal/industrial heat polluted waters) in order to get a comprehensive picture of reproductive biology strategy and population dynamics structure of these species. Our research aimed to answer the question of why these species can spread so successfully and rapidly in our country's very different water bodies. The results of field surveys and reproductive biology studies represent that the *Procambarus clarkii* and *Neocaridina denticulata* are active and breed in natural, urbanised and heat polluted waters throughout the year. This is proven by the fact that both juvenile, adult and female individuals with different stages eggs were collected in the colonised habitats throughout the one year long survey program. In the case of all species, it has been proven that if their habitat has heat stress, they reproduce and the different age individuals are active during the whole year. Our results can represent that several non-native crayfish could adapted well the natural and urbanised habitats and use the heat polluted habitats as invasion hotspots and steppingstones.

**Keywords:** breeding strategy, GSI, urbanization

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## The biology of the crayfish plague, *Aphanomyces astaci* (Oomycota)

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Fungal and fungal-like pathogens are responsible for diseases associated with the first documented extinction events caused by infection. One of the most notorious cases is the ‘crayfish plague’ caused by the fungal-like organism *Aphanomyces astaci* Schikora (Oomycota). This pathogen is considered among the one hundred world’s worst invasive species (<http://www.issg.org>), and has destroyed the majority of the native populations of freshwater crayfish in Europe and Asia. The rapid and devastating effects of crayfish plague have resulted in a number of generalizations and myths of this disease. Ironically, over time crayfish plague has become one of the best-known invertebrate diseases due to recent advances in developmental biology, cell biology, genomics, molecular taxonomy and phylogeny. In addition, the development of molecular tools for identification of *A. astaci* has provided a better understanding of the biology and molecular genetics of this pathogen. However, there is still a substantial lack of understanding on its biology by the general public, and also by people involved in the management of freshwater ecosystems. This can lead to difficulties when trying to implement conservation action plans. Thus, the aim of this presentation is to provide information on the main general questions regarding the biology of the crayfish plague pathogen by reviewing the current state of the art of this organism.

**Keywords:** crayfish plague, invasive species, crayfish

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## Whole-genome single nucleotide polymorphism analysis of an *Aphanomyces astaci* strain collection: searching for the genomic determinants of virulence

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*Aphanomyces astaci*, crayfish plague agent, is one of the worst 100 invasive species worldwide, and the introduction of its strains into Europe has led to impoverishment of crayfish stocks all over the continent. Several genetic markers have been developed to understand the diversity of the pathogen's strains, and the groups defined through them have often been used to predict *A. astaci*'s virulence. However, new studies on the pathogen's genetic diversity and its coevolution with its European hosts highlighted that the current markers cannot fully capture *A. astaci*'s genetic variability and are unreliable sources to infer the strains' virulence. With our study we aim to develop new markers based on whole-genome single nucleotide polymorphisms (SNPs) that will allow us to reliably predict the virulence of *A. astaci*'s strains. Using a combination of Illumina short-reads and Nanopore long-reads, we sequenced and assembled the genome of 29 *A. astaci* strains. For all strains we measured *in vitro* growth and sporulation rate. Additionally, for a subset of 14 strains we obtained virulence data through standardised infection experiments. These data, combined with ecological information and principal component analysis on SNPs, will allow us to select the strains representative of the entire collection. Genome-wide association analysis and/or association tests will then be used to individuate SNPs associated with virulence, which will be used to develop new genomic markers for *A. astaci*. The results of these ongoing analyses will provide fundamental insight on the genetic determinants of virulence and will translate into powerful tools for conservation measures.

**Keywords:** crayfish plague, intra-specific variability, virulence, GWAS, SNPs analysis

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## ***Austropotamobius pallipes* can be infected by two haplotypes of *Aphanomyces astaci*: a key example from an outbreak at an ex-situ conservation facility**

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The crayfish plague, caused by the pathogen *Aphanomyces astaci*, is a pandemic disease endemic to North America that has been devastating susceptible crayfish populations in Europe since the 19th century. In Spain, this disease has decimated populations of the native crayfish species *Austropotamobius pallipes* due to introductions of North American crayfish, which act as vectors of the pathogen. To combat against these losses, several regional governments have established ex-situ breeding programs to restock wild populations of the species. In this study, we report on an outbreak of *A. astaci* that occurred in one of the most important *A. pallipes* aquaculture centers in Spain. Using a variety of detection methods, we analyzed affected crayfish and environmental samples from the facilities over a period of six months and determined that the outbreak was caused by two haplotypes of *A. astaci*, d1 and d2, which are both associated with the North American crayfish species *Procambarus clarkii*. To our knowledge, this is the first report of a two-haplotype coinfection of *A. astaci* outside the pathogen's native range.

**Keywords:** invasive alien species, white clawed crayfish, crayfish plague, aquaculture, crustacean diseases

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## Review and systematization of microbial pathogens of freshwater crayfish

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Despite important ecological role and growing commercial value of freshwater crayfish, their diseases remain understudied. Many studies examining potential crayfish pathogens do not thoroughly address their epizootiology, pathology or biology. Here, we reviewed over 100 publications on potentially pathogenic viruses, bacteria, fungi and fungal-like microorganisms reported in crayfish and systematized them according to observations of pathogenicity, which were based on successful execution of infection trials. For 40.6% of examined studies, microbes were successfully systematized, while for more than a half (59.4%) no conclusion on pathogenicity could be made. Fungi and fungal-like microorganisms were the most studied group of microbes with the highest number of analysed hosts, followed by bacteria and viruses. Our analysis demonstrated the need for: (a) inclusion of higher number of potential host species in the case of viruses, (b) research of bacterial effects in tissues other than haemolymph, and (c) more research into potential fungal and fungal-like pathogens other than *Aphanomyces astaci*. We highlight the encountered methodological challenges and biases and call for a broad but standardized framework for execution of infectivity trials that would enable systematic data acquisition on interactions between microbes and the host.

**Keywords:** bacteria, fungi and fungal-like microorganisms, viruses, infectivity trials, potential crayfish pathogens

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## How much do crayfish plague and microsporidiosis affect the conservation status of the white-clawed crayfish *Austropotamobius pallipes* complex in Trentino (NE Italy)?

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The white-clawed crayfish *Austropotamobius pallipes* complex is an endangered species, with decreasing trend in distribution and abundance throughout Europe. One of the causes of decline is the widespread invasion of alien crayfish and the associated spread of infectious diseases, primarily of the crayfish plague caused by *Aphanomyces astaci*. Although this disease usually causes mass mortality in *A. pallipes*, some wild populations appear to be resistant or/and tolerant towards *A. astaci*. A further disease, poorly investigated and understood, is microsporidiosis, i.e., the porcelain disease, caused by the microsporidian parasites *Astathelohania contejeani* and *Nosema austropotamobii*. We present the results of a 2021-2022 monitoring survey, aimed at mapping the distribution of *A. astaci*, *A. contejeani* and *N. austropotamobii* in the wild populations of *A. pallipes* in Trentino. We applied non-invasive sampling methods to collect cuticular swabs from 17 of 44 known populations, concurrently collecting eDNA at one of the sampling sites, to investigate the presence of *A. astaci* and if possible, identify its genotype through molecular analyses. Sixteen specimens from 5 populations showed abdominal muscles with macroscopic signs of porcelain disease, tissue from these specimens were collected and subjected to molecular evaluation to confirm the presence of microsporidia and identify the species. *Aphanomyces astaci* was detected in 5 populations and the presence of a low pathogenic genotype (genotype A) was confirmed in one of them. The presence of *A. contejeani* was identified in 5 populations. In 2 of them, *N. austropotamobii* was detected in co-infection with *A. contejeani* in the same individual.

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

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## Immune response in crayfish is species-specific: differences between native and invasive alien crayfish species in the Korana River, Croatia

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The immune response plays a critical role in maintaining an individual's health by providing protection against pathogens, making it one of the key drivers of species' invasion success. In this study, our objective was to explore the immune responses of two species found in mixed populations at three sites in the Korana River: the relatively resistant native species, narrow-clawed crayfish (*Pontastacus leptodactylus*) and the successful invasive alien species, the signal crayfish (*Pacifastacus leniusculus*). We compared immune response parameters which are commonly reported in crayfish: total hemocyte count, the strength of encapsulation response and enzyme activity of phenoloxidase (PO) and total prophenoloxidase (proPO). Additionally, as the immune response is considered an important fitness component, we also compared it to body condition indices of each species: hepatosomatic index and Fulton's condition factor. Results showed that invasive signal crayfish had an overall higher immune response in comparison to native narrow-clawed crayfish. Specifically, invasive signal crayfish had significantly higher values of two immune response parameters (encapsulation response and total prophenoloxidase). We discuss our results considering the possible impact of the observed differences in immune response on the displacement of the narrow-clawed crayfish by the signal crayfish in mixed populations along the Korana River.

**Keywords:** immune system, signal crayfish, narrow-clawed crayfish, encapsulation response, prophenoloxidase

**Acknowledgement.** This work was conducted within the project "Changes in the pathogen composition and immune response during range expansion of successful crayfish invaders" funded by Croatian Science Foundation (HRZZ UIP-2017-05-1720).

## Hemolymph microbiomes of co-occurring native and invasive crayfish in Korana River (Croatia) are species-specific

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Host-associated microbial communities are an important determinant of individual's health and fitness. In crayfish, microbes inhabit different tissues of healthy individuals, including the hemolymph. Microbial communities of the hemolymph and the host's immune system are normally in homeostasis, but under stressful conditions, opportunistic bacteria may proliferate and lead to dysbiosis or bacterial septicemia, adversely affecting crayfish health. Recently, host-associated microbiomes have been highlighted as one of the factors influencing the success of invasive crayfish species. However, existing studies have been focused only on the invasive species, while it is unknown to which extent the microbiome is shared between the native and invasive crayfish species that come in contact during biological invasions. Using 16S rRNA gene amplicon sequencing approach, we analyzed the hemolymph microbiome of two crayfish species co-occurring in the Korana River, Croatia: the invasive signal crayfish and the native narrow-clawed crayfish. Our results show significant differences in composition and abundance of hemolymph microbial communities, showing that the hemolymph microbiome is species-specific despite the prolonged contact of the two species. Proteobacteria was the most abundant phylum in the signal crayfish, while Firmicutes dominated the narrow-clawed crayfish hemolymph microbiome. The microbiome of the signal crayfish exhibited significantly lower richness and phylogenetic diversity. Additionally, a higher relative abundance of potential bacterial pathogens was recorded in the hemolymph microbiome of the signal crayfish. Our findings offer an insight into differences between hemolymph microbiomes of the invasive and native crayfish species and present a baseline for assessment of their contribution to health and condition of crayfish.

**Keywords:** *Pacifastacus leniusculus*, *Pontastacus leptodactylus*, bacteria

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## Stone crayfish reproductive success in the context of water chemistry

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This study delves into the reproductive success of stone crayfish (*Austropotamobius torrentium*) within the context of various water chemistry parameters. A unique cage experiment was set up, featuring four streams, each hosting a cage of 16 egg-bearing females, originally caught in their respective locations, resulting in a total of 64 females across all streams. The females remained in their original streams (and cages) for the entire reproductive season (from October 2022 to June 2023). To evaluate the water's properties, samples were collected three times throughout the season. Comprehensive water analysis included measurements of pH, Biochemical Oxygen Demand (BOD<sub>5</sub>), Potassium Nitrate (KNK4.5), Ammonium (NH<sub>4</sub>), Nitrate (NO<sub>3</sub>), Nitrite (NO<sub>2</sub>), Oxygen (O<sub>2</sub>), unsolved particles, Phosphorus (P), Phosphate (PO<sub>4</sub>), Magnesium (Mg), Aluminium (Al), Iron (Fe), and Calcium (Ca). This contribution aims to elucidate whether and to what extent these water chemistry parameters impact the overwinter mortality and hatchability of stone crayfish eggs. Our findings will contribute to understanding how environmental factors, especially water chemistry, play a critical role in the reproductive success of this species. The outcomes of this study may pave the way for enhanced conservation efforts and resource management strategies for these endangered crayfish.

**Keywords:** stone crayfish, overwinter mortality, hatchability, reproduction

**Acknowledgement:** We sincerely thank the Nature Conservation Agency of the Czech Republic for their financial support for this study.

## Effects of chemical cues and prior experience on predator avoidance in crayfish

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Multisensory stimuli provide organisms with information to assess the threat present in the surroundings. The impact of the chemical predatory cues combined with experiences gained between species previously unknown to each other was not fully understood. We investigated the foraging and behaviour of naïve and experienced marbled crayfish (*Procambarus virginalis*) juveniles in response to the chemical signals of pumpkinseed (*Lepomis gibbosus*) alone, and during the active foraging on the marbled crayfish juveniles. Naïve and experienced crayfish juveniles were exposed to three different conditions: the water inlet came from 1) a tank with predator actively foraging on their conspecifics, 2) the a from tank with predator only, and 3) from an empty tank. Crayfish behaviour in arenas with food and shelter was video recorded for 4 hours. The positions of crayfish in specific zones in the arena (inlet, transition, and shelter zone) were localized every five minutes. Feed consumed was measured after the trial. The results obtained indicated that crayfish exposed to the combined stimuli avoided the inlet zone and preferred the shelter zone of the arena to a greater extent than did those crayfish exposed to predator-only cues and the control. Regardless of the stimuli, experienced crayfish showed significantly reduced foraging. Crayfish juveniles were more cautious because of previous experience rather than due to scent perception. Our findings imply that crayfish respond to threat-associated odours with the greatest potency when visual or tactile cues are present, but previous experience with predator can cause high precaution.

**Keywords:** predation, crayfish, fish, kairomones, alarm cues

**Acknowledgement.** This study was supported by the Grant Agency of the South Bohemian University, project No. 072/2023/Z.

## The effect of polyphenols on protein levels of crayfish feeds and the growth and survival of noble crayfish (*Astacus astacus* L.) juveniles

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Polyphenols are good candidates for crayfish feeds due to their health-promoting effects. Under certain conditions, polyphenols can affect the digestibility of proteins. To test the hypothesis can polyphenols lower the amount of digested proteins in *Astacus astacus* juveniles and affect crayfish growth and survival, we designed the six diet experimental groups with low protein content (28-35%) and high proteins (36-42%) with and without polyphenols. Four aquarium trials and one cage trial with *A. astacus* juveniles were conducted in 2018-2022 to assess the difference between the feeds. All four aquarium trials with juveniles (mean weight 0.2-0.3 g) were carried out for 205-212 days in triplicate per treatment (a total of 60 crayfish per treatment). A cage trial with juveniles (mean weight 0.4 g) was carried out for 119 days in a pond in six replicates per treatment (a total of 60 crayfish per treatment). The feeding rate was 2% of the body weight. The growth rate was significantly greater in the high protein content (36-42%) diet groups but the survival was significantly higher in the low protein content (23-35%) diet groups. The content of polyphenols in the feed did not affect protein digestibility in *A. astacus* since there were no statistical differences between the growth rates and survival in the high proteins diet groups and in the low proteins diet groups with and without polyphenols.

**Keywords:** protein, polyphenols, survival rate, growth rate, crayfish feed.

**Acknowledgement.** The study was financially supported by the European Maritime and Fisheries Fund (EMFF) (project No. 821017780002).

## Why the offspring of marbled crayfish follow unfamiliar dominant females rather than their own mothers?

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Parental care comes with significant costs that lead to the evolution of sensory mechanisms that facilitate offspring recognition to prevent the resource misuse. In certain bird species, adoptions have been described in same-sex parents who repeatedly and successfully care for their adopted offspring. To date, such adoption-like behaviour has only been reported in vertebrates including mammals, birds, and amphibians. Here we present findings for an invertebrate, a clonal marbled crayfish (*Procambarus virginalis*) that show how the offspring of one female – the loser of a competitive interaction – are adopted by another mother, the winner of a combat between these two mothers. The dominant mother walked through the arena with her tail extended, actively accepting juveniles from both clutches while the subordinate mother crouched in one corner and was ignored by her offspring. It was surprising as we previously found that juvenile of marbled crayfish can recognise between familiar (mother and siblings) and unfamiliar relatives. However, after a fight, the clutches of the two interacting mothers chose only the dominant female. Our study thus suggests that the known impact of the maternal bond (with a brood pheromone impact) was outweighed by either the pheromones indicating dominant and/or subordinate females (social deprivation status) or by the visual cues provided by the extended tail of the dominant female. The offspring of the subordinate mother always decided to follow the dominant mother rather than their own. Future studies need to determine whether juveniles' choice is guided by chemical, visual or multimodal stimuli.

**Keywords:** hierarchy, communication, maternal care, adoption, crayfish

# LIFE PROJECTS



**CrayfIT**

## The management plans for alien crayfish of Union concern in Italy

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Six species of alien crayfish have been included in the list of Union concern according to the Regulation EU 1143/14. For these species, in addition to ban on trade, prohibition to release into the environment (etc..), there are obligations to eradicate, where possible, or contain/control the populations with the aim to minimize and mitigate their impacts on biodiversity. In Italy, the Regulation is implemented through the legislative decree n.230/17. According to art.19, which provides the definition of effective management measures for each species by the Ministry of Environment, national management plans have been drawn up for the four crayfish species already present in Italy. By now, two of these national management action plans (for *Procambarus clarkii* and *Procambarus fallax f. virginalis*) have been officially adopted with a Ministerial Decree, after consultation with the regional authorities. The plans include management targets to be achieved at national and regional level, possible containment methods (and their effectiveness) as well as monitoring techniques to apply.

**Keywords:** crayfish of union concern, national plan, management, monitoring

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## The LIFE projects on alien crayfish in Italy

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Since 1996, the LIFE program cofinanced numerous projects on invasive alien species in Italy (at least more than 50) and over 30% are related to freshwater environments, underlying the vulnerability of these ecosystems. Invasive alien crayfish, particularly the red swamp crayfish *Procambarus clarkii*, together with alien sliders and fish, are among the most frequent target taxa of the projects in fresh waters, confirming the need to manage them for protecting the native biodiversity and favouring the resilience of invaded water bodies. Here, the results of two LIFE projects on *P. clarkii* will be presented: LIFE RARITY (LIFE/10/NAT/IT/000239; 2011-2014) aimed at controlling the red swamp crayfish (also through innovative techniques such as male sterilization) and supporting the populations of the native crayfish *Austropotamobius pallipes* complex in Friuli Venezia Giulia (North Italy); LIFE SOS TUSCANY WETLANDS (LIFE+11/NAT/IT/00094; 2012-2019) aimed at stopping the loss of biodiversity of Lake Sibolla and Ramone marsh in northern Tuscany, controlling some invasive alien species (coypu, red swamp crayfish, false indigo bush, black locust) and restoring the habitats. Both projects showed how an integrated management is recommended to successfully tackle the populations of *P. clarkii*.

**Keywords:** LIFE projects, *Procambarus clarkii*, invasive alien species, management, native crayfish

## The return of the crayfish in Borrello: a story of reintroduction after the crayfish plague in the Verde River

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The freshwater crayfish *Austropotamobius italicus* (Faxon, 1914) ssp. *meridionalis* is native of peninsular Italy. They have a key ecological role in the conservation of the ecosystems. The main threats for the species are growing human exploitation of water resources, pollution, climate change, poaching, voluntary or accidental introduction of non-native crayfish outcompeting the autochthonous species and often carrying diseases, like ‘crayfish plague’ (*Aphanomyces astaci*), introduction of fish species potentially carrying parasites, especially if produced in facilities together with allochthonous crayfish. In July 2013, crayfish plague affected the Verde River, completely wiping out the native crayfish population, which the LIFE CRAINat project considered the most important in the region. Thanks to yearly releases from the breeding centre in Borrello, which was created under the LIFE CRAINat and lately supported by Rewilding Apennines, in 2021-2023 CPUE reported the comeback of the native crayfish to the Verde River. Since 2013, the reintroduction of crayfish has been carried out in several phases, under the CRAINat After LIFE Conservation Plan, with a variable number of individuals transferred to selected areas, focussing on the distribution of individuals to maximise the chances of restocking. The effectiveness of the reintroduction programme was evaluated through field observations. Preliminary results are encouraging with a gradual increase in the crayfish population and signs of natural reproduction reported. This comeback of crayfish after the plague is an example of success in wildlife conservation. Thanks to the efforts of the LIFE CRAINAT and Rewilding Apennines, the species is slowly recolonising its original habitat.

**Keywords:** *Austropotamobius italicus meridionalis*, native crayfish, breeding centre, Verde River, wildlife comeback

**Acknowledgement.** This study was supported by a financial contribution from Rewilding Europe and Fondation Ensemble.



## **LIFE GREEN4BLUE ecological connections to support Natura 2000 network and contrast invasive alien species**

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Climate change, land transformation, intensive water and land use, and their pollution are among the causes that have contributed most to the degradation of habitats, reducing their biodiversity rate and capacity to provide traditional ecosystem services. But also the clandestine importation and release or their illegal dissemination on the natural territory of alien wildlife species for both commercial and pseudo-amateur purposes have contributed and contribute to the erosion of the biodiversity of environments, endangering their ecosystem balance. This is the context for LIFE GREEN4BLUE – GREENing the BLUE canals infrastructure of Reno basin to enhance ecosystem connectivity and services (LIFE18 NAT/IT/000946), a project which aims at the environmental upgrading of reclamation canals located between the provinces of Bologna and Ferrara in order to enhance their role as green and blue infrastructures in connecting natural areas of the territory (Natura 2000 Network). The project is implemented through innovative management that integrates hydraulic safety, support for plant and animal biodiversity, control of 2 invasive alien species (*Myocastor coypus* and *Procambarus clarkii*), monitoring and study of the interactions between soil, water and sediment components, and landscape protection. In the first three years of the project, channel modification actions were initiated in specific stretches, including *Procambarus clarkii* control action.

**Keywords:** green-blue infrastructures, I.A.S. contrast, immunocontraceptive, fresh water habitats

**Acknowledgement.** This study was supported by the European Funds through Financial Instrument for the Environment, LIFE Nature and Biodiversity (LIFE18 NAT/IT/000946).

## Ecostreams for Life-ecosystem based restoration and management of boreal rivers (LIFE19 NAT/SE/000333)

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Rivers and streams in the boreal Sweden are in general heavily affected by anthropogenic activities, mainly from forestry and hydropower. All rivers targeted in this project are heavily influenced by measures to facilitate large-scale timber floating. Habitat heterogeneity and biodiversity of algae, invertebrates and fish, as well as aquatic productivity have diminished. Six river systems in the three counties Jämtland, Västernorrland and Västerbotten in central/northern Sweden are included in the LIFE-project. 22 dams and 199 dysfunctional road culverts are to be removed/modified to increase the free moving of fish and other aquatic species. Actions are taken to reduce the risk of (illegal) introduction of the alien signal crayfish (*Pacifastacus leniusculus*) which by the disease crayfish plague (*Aphanomyces astaci*) they carry are the major threat for the native, critically endangered noble crayfish (*Astacus astacus*). Signal crayfish are far more abundant in the neighbouring counties south of the project area. Habitat restoration of 140 km of stream habitat and wetlands are planned as well as creating at least 470 spawning sites for salmon and brown trout. Re-establishment of freshwater pearl mussels and noble crayfish at some locations are to be executed. Adaptive plans for sustainable management of fish populations will be implemented in all six river systems.

Species targeted: Atlantic salmon (*Salmo salar*), Freshwater pearl mussel (*Margaritifera margaritifera*), Brown trout (*Salmo trutta*), Noble crayfish (*Astacus astacus*), Otter (*Lutra lutra*), Small liverwort (*Scapania carnathiaca*, *Scapania massalongi*), Sculpin (*Cottus gobio*).

**Keywords:** Mediterranean trout, biological invasions, hybridization, genetic assignment, endangered species conservation

**Acknowledgement.** This study was supported by the European Funds through the Financial Instrument for the Environment, LIFE Nature and Biodiversity (LIFE19 NAT/SE/000333).

## **LIFE STREAMS project: the key role of genetics in conservation actions for the recovery of native Mediterranean trout in Italy**

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The Mediterranean trout is critically endangered in Italy; particularly, hybridization with invasive Atlantic trout represents a main threat to the species, additionally to flow modifications, water pollution, overexploitation, habitat alterations and climate changes. The LIFE18NAT/IT/000931 STREAMS project aims to recover the native Mediterranean trout in 6 pilot protected areas in Italy. In order to restore the biodiversity of Mediterranean trout populations, we focused on the following conservation strategies: i) genetic and demographic characterization of the trout populations in the study areas; ii) eradication of alien populations and selective fishing; iii) captive breeding of wild native individuals; iv) restocking or reintroduction of eggs, alevins and fry obtained in captivity; v) publication of Guidelines for the conservation and management of native Mediterranean trout, to support conservation and managing strategies for the species at national level. In this presentation, we focus on the genetic workflow used to achieve LIFE STREAMS main goals: i) optimization of a standardized multi-marker protocol by leveraging national literature and collaborating with ichthyologists and geneticists; ii) genetic characterization of waterbody populations through the analysis of the mitochondrial control region and the nuclear LDH-C1 gene; iii) individual genetic characterization based on the analysis of 15 microsatellite loci to determine species assignment and hybridization levels. We have already analysed over 3500 fin samples from 134 sites. The initial data has allowed us to identify different levels of alien genome introgression in investigated rivers and discover some pure population of Mediterranean trout that should be recognized as genetic refuges and considered for reproduction and restocking purposes.

**Keywords:** Mediterranean trout, biological invasions, hybridization, genetic assignment, endangered species conservation

**Acknowledgement.** This study was supported by the European Funds through the Financial Instrument for the Environment, LIFE Nature and Biodiversity (LIFE18NAT/IT/000931).

## Small fish, small streams, big challenges: conservation of endangered species in tributaries of the upper Po river

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The conservation status of 6 six Annex II fish species of the Habitats Directive: *Lethenteron zanandreae*, *Protochondrostoma genei*, *Chondrostoma soetta*, *Sabanejewia larvata*, *Telestes muticellus*, *Cottus gobio* target, according to the latest Article 17 DH Assessment Report (2013-2018) is rated as unfavourable: five species in U2 and one species in U1. The project objective is to prevent the decline of six freshwater fishes, through restoration of longitudinal connectivity for 122 Km making currently fragmented by 16 barriers; Habitat improvement in 13 strategic points; Restocking of the population of five species; Alien species control and reduction in 440 Km in the 20 Natura 2000 targeted sites in Piemonte in the northern part of the Po river basin; Construction of an allochthonous biomass reuse through an agreement with a pet food company. In particular, restocking will be carried out in areas where 32 residual sub-populations are present, enabling their reinforcement, and the reintroduction of 47 new sub-populations will be carried out in areas where the species are extinct. An innovative technique using telemetry for the identification of breeding sites of *S. glanis*, will also be implemented, which will allow the application of timely action with high effectiveness to contain this invasive and interfering species. A wide approach of the stakeholders is foreseen both with active participation in the conservation actions (fishermen, volunteers, students, citizens), as well as involvement of local community to increase the value of natural resources through a Citizen Science approach.

**Keywords:** river restoration; freshwater, Invasive Alien Species, restocking

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# POSTERS



CrayfIT

## Reference genomes for non-model invertebrates: Giant genome sequencing of the endangered *Austropotamobius bihariensis*

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Reference genomes of non-model invertebrates represent the future of biodiversity conservation and are a cornerstone of conservation genomics. They are a valuable resource for the protection of highly endangered species like the idle crayfish, endemic to the Apuseni mountains (Romania). Unfortunately, freshwater crayfish are represented with only three sequenced genomes (2-6 Gbp), none of which belong to the European Astacidae, particularly understudied due to their large and highly repetitive genome composition. In fact, we estimated the genome size of the idle crayfish to 12.7 Gb. With this study, we aimed to optimise extraction, preparation and sequencing steps of the idle crayfish high molecular weight (HMW) DNA for long-read sequencing platforms. We evaluated the suitability of four DNA extraction protocols (MagAttract HMW DNA kit, DNeasy blood and tissue kit, phenol-chloroform extraction and salting out protocol) and three DNA purification methods (pre-extraction sorbitol wash, salt-chloroform wash and bead purification). Additionally, we tested two long-read library preparation approaches: with PCR step and PCR-free preparation. DNA samples with optimal quality were used for library construction and sequencing on Pacific Biosciences and Oxford Nanopore sequencing platforms. Our approach, comparing different extraction, library preparation and sequencing methods, allowed us to identify critical points in the long-read sequencing protocols. Even though challenging, the effort of sequencing non-model invertebrate genomes is extremely important towards an unbiased genome representation across the tree of life.

**Keywords:** *Austropotamobius bihariensis*, genome, long-read sequencing, conservation genomics

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## Preventing the spread of *Faxonius limosus* in Trentino: management challenges, experimental protocols and new threats

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Two Alien Invasive crayfish species are present in Trentino (NE Italy): *Procambarus clarkii* and *Faxonius limosus*. Both species were accidentally introduced in the first decade of this century with fish restocking; their introduction caused the extinction of populations of the native *Austropotamobius pallipes*. *Procambarus clarkii* was so far recorded only in one small lake at 950 m asl, whereas *Faxonius limosus* has colonized a group of 5 lakes at 450 m asl, over an area of about 80km<sup>2</sup>. The containment of *Faxonius limosus* is therefore difficult, given its presence in a high number of lakes, three of which are hydrologically connected. The first containment campaign to prevent its spread was conducted in summer 2023 in the downstream-most of the three connected lakes, Costa Lake, a small lake of about 1 hectare surface area. The efficiency of traps with different mesh size was tested in a 10-day campaign in mid-June, a more extensive one-week campaign was conducted in July. The use of small size (1 cm) mesh was the most efficient trapping system, as the small body size of the individuals probably allows them to escape from the standard, larger size (3x2 cm) mesh; crayfish were collected mainly in the canal which acts as the emissary of the lake, connecting it to the main river network. Unexpectedly and unluckily, individuals of *Procambarus clarkii* were collected in the NW part of the lake, suggesting a recent, point introduction, of unknown origin, which represent a further threat for the area.

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

**Acknowledgement.** This study was supported by funding from the “Rete di Riserve del Fiume Brenta”, and from the “Servizio Sviluppo Sostenibile e Aree Protette, Provincia Autonoma di Trento”.

## The management plan of *Austropotamobius pallipes* in Trentino

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The management plan for conservation of the crayfish *Austropotamobius pallipes* complex in Trentino, in the Eastern Italian Alps, is one of the outcomes of the European Project Life+T.E.N. The monitoring of the species and its habitats represent the first result of the management plan application. Two-hundred-twenty-four water bodies were surveyed in 2019-2021, and 79 of these resulted unsuitable for *A. pallipes* due to their environmental characteristics. In each remaining site, which were confirmed to be suitable, we georeferenced a 100 m transect, representative of the water body, where we recorded the main physical characteristics of the habitats, the physical-chemical parameters of water and, in streams, macrobenthos to calculate the Extended Biotic Index. The presence/absence of the species was evaluated along each transect and was detected/confirmed in 42 of them. Counts method was applied to 35 populations to measure the abundance calculated as CPUE. In 28 populations, all animals were collected and biometric data, sex, presence of visible infestations by parasites and pathogens were recorded, to assess the populations' structure and their conservation status. The main threats for the populations of *A. pallipes* we detected are represented by habitat alteration and the presence of the two invasive alien species *Procambarus clarkii* and *Faxonius limosus* in one and six water bodies, respectively. The populations status and the detected threats suggest a management strategy that increases the ecological conditions and connectivity of the aquatic habitats and ensures the control of the spread of alien species and their pathogens.

**Keywords:** management of autochthonous species, invasive alien species, *Aphanomyces astaci*, faunistic monitoring



## The potential spread of the Louisiana red swamp crayfish (*Procambarus clarkii*) in Casentino (Tuscany, Central Italy)

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The red swamp crayfish, *Procambarus clarkii*, is an invasive alien species, native to the lentic environments of the southern United States and northern Mexico. In 1993, the species was reported in Tuscany (Central Italy) where it became widespread, especially in the lowland areas. The Casentino is the highest part of the Arno river valley and mainly includes mountains. In 2015, *P. clarkii* was reported for the first time in the area, at lake of Romena, an artificial fishing pond close to the National Park of Foreste Casentinesi. The presence of the species represents a threat to the Park, that hosts the protected native crayfish *Austropotamobius pallipes* complex, vulnerable to the crayfish plague, caused by the oomycete *Aphanomyces astaci*, potentially carried by *P. clarkii*. In 2022 a monitoring session was carried out in order to evaluate the distribution and status of the species at the lake after one year of intensive control activities. The results confirmed the presence of the species in the lake, even if at low abundance, but unfortunately, also along the emissary of the lake (Fosso Camboffoli) that connects to the Fosso delle Pillozze, a tributary of the Arno river. The species has not yet been reported in the Arno river in that area, but this is particularly worrying given the potential impact that *P. clarkii* could cause not only to the native crayfish, but also to amphibians and other macroinvertebrates.

**Keywords:** *Procambarus clarkii*, invasive alien species, potential impact, native crayfish

## Signal crayfish (*Pacifastacus leniusculus*): a real nightmare for Italian native crayfish

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Aquatic native communities are increasingly threatened by the presence and spread of invasive alien species. Since 2009 signal crayfish has been spreading in the Valla stream, north-western Italy, threatening the white-clawed crayfish population still inhabited the headwater and fish populations as well. To control invasive species, it is indeed required to develop suitable management methods, also assessing the specificity of different environments. To address that, during summer 2019 we carried out removal actions, but differentiated for stretches in order to assess whether decrease of juvenile production, and consequently the progressively ageing of the population, could be an effective method of containment. Night-time hand catching and electrofishing were conducted to remove all crayfish (2 sites – total removal TR) or to remove only juveniles (2 sites – juvenile removal JR). Two control sites were sampled, without any removal. Comparing CPUE values, an overall decrease occurred in all the sites, particularly in JR sites. Further studies are needed, however, to better understand this process and manage the control of this invasive species. The containment of invasive species would also entail restoration of ecological conditions and a rapid recolonisation by the numerous macroinvertebrates, still present in the aquatic environments.

**Keywords:** *Pacifastacus leniusculus*, non-indigenous species, freshwater invasion, white-clawed crayfish, Italy.

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## Evaluation of the red swamp crayfish (*Procambarus clarkii*) standard metabolic rates using intermittent-flow respirometry

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The standard metabolic rate (SMR) is an essential physiological measure that establishes the minimum level of aerobic energy metabolism. It serves as a basis for estimating daily energy expenditure and is pivotal in comprehending energy balance and metabolism. In order to assess the standard metabolic rate of crayfish, the typical approach involves quantifying rates of oxygen consumption. Oxygen consumption serves as an indicator of metabolic activity since organisms utilize oxygen to generate energy. For the measurement of crayfish's standard metabolic rate (SMR), we employed an intermittent respirometry system specifically designed to study oxygen consumption. This system operates in cycles to determine respiration rates. It incorporates specialized sensors and equipment to monitor oxygen levels and calculate consumption accurately. We selected 18 randomly from 36 collected crayfish from Shell Fisheries Research Station in Auburn, Alabama, USA, to calculate the SMR. We hypothesize that experiments lasting only 12 h after crayfish have acclimated to the respirometer are enough to evaluate SMR reliably. SMR was calculated using the MLND method for 3 (24 HR), 7 (DAY), and 4 (NIGHT) individuals, for all other individuals, SMR was calculated using the  $q_{0-20}$  method. In support of our hypothesis, we found no significant difference between day and night estimates of SMR ( $t=0.47$ ,  $d.f.=17$ ,  $p=0.65$ ). Mean SMR $\pm$ standard deviation was estimated to be 63.33 $\pm$ 40.91 for the 24-hour experimental period, 65.35 $\pm$ 41.89 for the day period, and 65.35 $\pm$ 41.89 for the night period. These findings indicate that the 12 hours duration provides sufficient for accurate determination of SMR. Calculating the standard metabolic rate of crayfish over a period of 12 hours allows for sufficient time to capture a representative sample of their metabolic activity while minimizing the influence of any fluctuations and ensuring reliable data collection.

**Keywords:** Crayfish, standard metabolic rate, intermittent respirometry

## Towards protecting an endemic crayfish species in Romania: distribution, conservation status and threats

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The newly discovered European crayfish species, the endemic idle-crayfish, has been documented to inhabit the tributaries of the Criş rivers in the western Apuseni Mountains (Romania). Here the species encounters significant challenges, predominantly from the adverse impacts of urbanization and stream regularization. Furthermore, the invasion of North American crayfish species represents a significant hazard due to their role as carriers of the *Aphanomyces astaci* pathogen, which causes the crayfish plague. The Ministry of Research, Innovation, and Digitization (Romania) has granted funding for the project titled "Idle Crayfish: from the unknown to the next generation of species conservation assessment" through the UEFISCDI (PN-III-P4-ID-PCE-2020-1187) program. The project's objectives include assessing the conservation status of idle crayfish populations using the relevant criteria of the IUCN Red List of Threatened Species and establishing conservation measures for these populations. The field survey spanning from 2020 to 2023 confirmed known populations and revealed the presence of the species in a new river basin. Based on distributional criteria, with an extent of occurrence of 3800 sq. km, an area of occupancy of 128 sq. km, and the threat posed by invasive species and crayfish plague, this species can be considered endangered. A species distribution map will be presented, highlighting the areas covered by protected areas and potential ark sites. By incorporating the findings from this extensive field assessment, additional population genetic data, and distribution modeling techniques, we can potentially acquire the necessary tools to design and implement effective conservation measures for the idle-crayfish populations.

**Keywords:** *Austropotamobius bihariensis*, idle crayfish, conservation status

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**Observations on the morphology of museum specimens of the European Astacidae: *Austropotamobius fulcisanus* (Ninni, 1886) and *Austropotamobius torrentium* (von Paula Schrank, 1803) (Crustacea: Decapoda)**

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Museum specimens of European Astacidae, *Austropotamobius fulcisanus*, *Austropotamobius pallipes italicus* (Italian *A. fulcisanus* in the present study), and *A. torrentium* are observed, their description, diagnosis, and illustration provided. Morphometry of type series of *A. fulcisanus* in Italy are analyzed and the taxonomic status of *Austropotamobius* crayfish in northern Italy examined.

**Keywords:** geographical variation, morphology, type series

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## Contrasting endemic and cosmopolitan patterns in the evolution of *Austropotamobius* crayfish lineages

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Understanding the evolutionary history of *Austropotamobius* crayfish species has been a subject of ongoing debate. In our study, we took a comprehensive approach by considering all lineages and COI haplotypes that are publicly available. Molecular methods, including PCR, electrophoresis, and CO1 DNA sequencing, were employed to analyse the genetic variability within crayfish populations. By carefully analysing their distribution and historical context in relation to well-documented geological events and genetic data, we examined the key evolutionary events that likely led to the divergence of crayfish lineages. Our findings refine existing theories and contribute to a better understanding of *Austropotamobius* populations and why some remained endemic while others have a cosmopolitan trait. We hope that our study provides valuable insights for similar studies and will inspire further research in exploring evolutionary histories beyond crayfish.

**Keywords:** *Austropotamobius*, endemic species, spatial ecology, spatial patterns, species distribution

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## Could environmental features influence the presence of the endangered white-clawed crayfish (*Austropotamobius pallipes*)? First insights from northwest Italy

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The Avigliana Lakes Nature Park, located in the lower Val di Susa (Piedmont Region, Italy) is characterized by a remarkable variety of landscapes: three different yet interconnected biotopes consisting of two lake basins (Great Lake and Small Lake), hills, and the Mareschi wetland. The Park is a Natura 2000 Site (ZSC/ZPS IT1110007 - Laghi di Avigliana), as it hosts many habitats and species of conservation interest. These include the native crayfish *Austropotamobius pallipes*, assessed as Endangered (EN) in the IUCN Red List and included in Annexes II and V of the Habitats Directive 92/43/EEC. The presence of the crayfish is historically reported in some tributaries of Small Lake, but the population shows a decreasing trend, as in the rest of Italy. Between July 2022 and July 2023, seven sampling sites were chosen for *A. pallipes* based on reports, previous data, and targeted surveys. At each site, data on the presence and abundance of the crayfish were collected, chemical and physical water parameters were measured, the STAR\_ICMi index was calculated, and water and sediment samples were taken for ecotoxicological laboratory essays. The aim of the study was to identify a possible relationship between the presence of crayfish and the biotic and abiotic environmental characteristics, also in order to detect suitable areas for its reintroduction or translocation. We observed a correlation between the presence of *A. pallipes*, some taxa of benthic macroinvertebrates and some abiotic parameters.

**Keywords:** bioindicator, water quality, habitat, macrozoobenthos, ecotoxicological essays

## Effect of crayfish plague outbreak on genetic variation in a noble crayfish population – results of repetitive monitoring

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Introduction of diseases into natural populations can cause dramatic declines. One of such diseases is crayfish plague caused by pathogenic oomycete *Aphanomyces astaci* that has caused havoc in native freshwater crayfish populations across Europe. The noble crayfish *Astacus astacus* is a native European species who has suffered from a serious population decline, amongst others due to the lethal disease crayfish plague. This study aimed to assess the effect of crayfish plague outbreak on the genetic diversity of the noble crayfish population from the gravel pit Motičnjak (north-west Croatia). This gravel pit was dug on the oxbow of the Drava River, and the noble crayfish population in it represents remnants of the native populations historically widely spread in the region. Crayfish were sampled three times during the period of six years, encompassing a crayfish plague outbreak. This provided an opportunity to compare genetic diversity before (2016), during (2018) and after the outbreak (2022). Genetic diversity was assessed using 15 microsatellite loci, and the results revealed that there was no significant change in genetic diversity during the study period and no apparent signs of a genetic bottleneck caused by crayfish plague, though a trend of decreasing private allelic richness was observed after the crayfish plague outbreak. Results are discussed from the conservation point of view.

**Keywords:** *Aphanomyces astaci*, *Astacus astacus*, genetic diversity, Croatia



## Knocking on a natural reserve's door: impact and management of non-native crayfish in a protected area

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Invasive Alien Species cause impacts in all kinds of ecosystems and their impacts is even worst in protected areas where the local biodiversity finds shelter against anthropogenic pressures. Here we present the results of a crayfish management program in a wetland within the integral natural state reserve 'Bosco Siro Negri' (province of Pavia, Lombardy, NW Italy), one of the best-preserved relicts of oak-elm floodplain forest. A preliminary risk assessment was conducted on crayfish species already occurring or potentially arriving in the next future in the area, to inform on the possible management strategies towards eradication or prevention of these IAS. In particular, we addressed *Procambarus clarkii* and *Faxonius limosus*, already occurring, and *Pacifastacus leniusculus* and *Procambarus virginalis*, not occurring yet. The four crayfish species were evaluated using three different tools: the Generic Impact Scoring System for defining the potential and effective impacts, the Invasive Species Effects Assessment protocol for the definition of the effects on the ecosystem services and, finally, the Non-Native Risk Management tool for the evaluation of the eradication techniques feasibility. Results indicated that all four crayfish shared the same potential and actual impacts, both on environmental and economic sector and, comparing all impacts to all potential benefits on the ecosystem services, *P. clarkii* exhibits the highest impacts but also few potential benefits in provisioning services. Finally, among all the evaluated eradication techniques, trapping strategy reached the highest overall evaluation of feasibility, while X-ray sterilisation, biocontrol, electrofishing and habitat modification were considered less suitable strategies.

**Keywords:** wetlands, invasive species, horizon scanning, ecosystem services, eradication

## Biological invasion status and risk screening of the crayfish in an integral natural state reserve in northern Italy

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Risk screening of alien species already present or likely to arrive in a target area is a valuable procedure to identify the risks associated to their presence or entrance in a new environment. In this context, we inspected the wetland of the Bosco Siro Negri, a small residual floodplain forest of high conservation interest, performing surveys along one-year to verify the presence of invasive crayfish. For this purpose, we used funnel baited traps and artificial refuge traps (bricks), which provide habitat shelter for small specimens. All captured specimens were frozen, then analysed for sex and biometric variables and the body condition factor was calculated to determine the population health. The survey results indicated only the presence of *Procambarus clarkii*: in total, we collected 23 specimens, which were generally bigger and heavier in May 2022 and, for both sexes, the body condition factor indicated a decrease of the population health. Out of the 23 specimens, 7 were collected using funnel baited traps (5 males and 2 females) and 16 using bricks (9 males and 7 females): as expected, individuals collected using traps were larger-sized than those captured with bricks. Finally, we performed a risk screening analysis of the species already present, as well as of other possible “door knocker” crayfish listed in the EU list of Union Concern, by applying the AS-ISK tool. All the selected crayfish were ranked with high-level risk of invasiveness, and this information could be useful for the management of the natural state reserve.

**Keywords:** wetlands, invasive species, biometry, AS-ISK, invasiveness

## **Molecular detection of *Astathelohania contejeani* in *Austropotamobius pallipes* complex populations in Spain: a preliminary study**

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Although microsporidiosis is often an overlooked pathology compared to other diseases, such as aphanomycosis, this parasitic infection has a relevant impact on crayfish health. *Astathelohania contejeani* is the most reported microsporidian parasite of native crayfish in Europe and was detected in Spain (Álava) in 1997 through microscopic examinations. Since then, no further evidence of its presence in the Iberian Peninsula has been documented. The present study aimed to investigate the occurrence of *A. contejeani* infections in *Austropotamobius pallipes* complex in Spain by applying molecular biology techniques. The analyses were performed on DNA extracted from the abdominal muscle tissue of 83 specimens: 22 originated from Álava, while the others were collected from several locations throughout Spain (Navarra, Girona, Granada, Cuenca, Teruel, Aragón and León). In addition, samples of environmental DNA from the Navarra region (15 from the Ebro River and 8 from the Leurza reservoir) were evaluated. Molecular detection of *A. contejeani* was based on species-specific amplifications of the small subunit ribosomal RNA (SSU rRNA) gene. Firstly, we screened all samples with a recently developed protocol based on Real-Time PCR. Then we applied a nested PCR followed by Sanger sequencing to exclude false positive results. *Astathelohania contejeani* was not detected in this limited set of samples, which did not contain macroscopically affected specimens. However, the molecular approaches implemented in this preliminary work, if combined with an extensive sampling, appear suitable for further studies to evaluate the presence of this parasite in native crayfish populations in Spain.

**Keywords:** Microsporidiosis, Spain, eDNA, Real-Time PCR

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## Oomycete isolates from native and invasive crayfish species in Croatia

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Various oomycetes are known to colonise the crayfish cuticle, but for many of them the effects on the host are poorly studied. The aim of this study was to study diversity of oomycete isolates from the exoskeleton of different native and invasive crayfish species. Live crayfish were caught at seven different sites in Croatia and from five different crayfish species that were either confirmed as, or are possible carriers of *A. astaci*. Oomycetes were cultivated from pereopods and abdominal cuticle inoculated on PG1 solid growth media, with hyphal transfers to new media till pure cultures were obtained. Identification of the isolates to species level was performed by sequencing of the internal transcribed spacer (ITS) region followed by phylogenetic analysis. We detected four oomycete species originating from two crayfish species, out of which two belonged to the genus *Saprolegnia* (three *S. australis* isolates and two *S. turfosa* isolates, all originating from *P. leniusculus*) and two of which were from the genus *Aphanomyces* (two *A. astaci* isolates originating from *A. astacus* and five isolates from *P. leniusculus*, 15 *A. repetans* isolates originating from *A. astacus* and 28 isolates from *P. leniusculus*). Based on previous studies, some of the detected species are saprophytic (*S. turfosa*, *A. repetans*), while others are opportunistic (*S. australis*) or primary pathogens (*A. astaci*, the causative agent of crayfish plague). Since they were sometimes found on the same host individual (as with *A. astaci* and *A. repetans* from the same *P. leniusculus* individual) further in vivo studies are required to inspect their effects on crayfish hosts, either individually or in combination.

**Keywords:** white-clawed crayfish, monitoring, survey methods, conservation

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## Presence, habitat use and population structure of *Austropotamobius pallipes* complex in the mountain streams of the Cellina watershed in the north-eastern Italian Prealps

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The white-clawed crayfish *Austropotamobius pallipes* complex experienced a decline in Italy in the last decades, whose reasons range from habitat fragmentation and pollution to the lethal effects of the oomycete *Aphanomyces astaci*, carried by introduced species as the red swamp crayfish *Procambarus clarkii*. A combination of these factors restricted the range of *A. pallipes* to pristine mountain streams. The hydrographic basin of the Cellina mountain stream in the Prealps of western Friuli (northern Italy) represents an interesting study site to gather information on the ecology of *A. pallipes* because downstream the water sinks underground shaping the “Magredi” dry grasslands, creating an effectively closed water system to the invasion of *P. clarkii*. This study focuses on the state of the *A. pallipes* population and its habitat preferences, to extend information on the distribution of this species in the area, try to explain why past reintroductions in some areas failed, and individuate candidate areas for conservation and for future reintroductions. The work is centred in the area around the Andreis village, which appears to have the richest population of crayfish of the Cellina basin. Samplings were performed four times, from June to September 2022, in 14 stream sites, catching a total of 3583 individuals both at night by hand and using baited traps. The presence of *A. astaci* was assessed through molecular assays, exploiting non-invasive sampling techniques (cuticular swabs and eDNA). Crayfish abundances were compared across sampling methods and investigated in relation to the morphological, hydrological and ecological features of the streams.

**Keywords:** *Austropotamobius pallipes* complex, conservation, habitat use, population structure, capture efficiency

**Acknowledgement.** This study was possible thanks to the effort of many volunteers that contributed to the sampling part: Cesare Maria Balzani, Giulio Dainelli, Giovanni Mecenero, Emma Pini, Andrea Pizzolato and Matteo Trevisan from the University of Padova and Michelangelo Dondi from the Politecnico of Milano. The sampling part was carried out in the area of the Parco Naturale Dolomiti Friulane and of the Riserva Naturale Forra del Cellina. The molecular detection of *A. astaci* was supported by LIFE-CLAW project (LIFE18 NAT/IT/000806).

## The illegal trade of marbled crayfish in Belgium: an ongoing risk for further spread

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The marbled crayfish (*Procambarus virginalis*) is a well-known invasive species. This parthenogenetic species originated from the aquarium trade and has been introduced in many European countries. Because of its highly invasive character this species was included on the list of Invasive Species of Union Concern in 2016 because of its potential as a highly invasive species. This resulted in a total ban of the trade, breeding and keeping of marbled crayfish in the European Union. To ascertain if marbled crayfish are still being traded in Belgium, the major online classified platforms were regularly monitored from 26-04-2022 to 01-08-2023 for the sale of the banned marbled crayfish. The results of this survey show that the marbled crayfish is still commonly being traded online and indicate the high risk of further spread.

**Keywords:** marbled crayfish, *Procambarus virginalis*, trade, legislation, enforcement

## Characterisation of the noble crayfish immune response to the crayfish plague disease agent and its analogues

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Recent findings point to the existence of immune memory in invertebrates. Some species show an improved immune response to pathogen challenge after previous nonlethal exposures to the pathogen or its analogues. This mechanism, although little studied in freshwater crayfish, could have significant applications in aquaculture. Here, we explored the effects of immune priming on the noble crayfish in the context of *Aphanomyces astaci* panzootics. Noble crayfish were primed with: i) crayfish saline, ii) beta-laminarin, iii) inactivated *A. astaci* hyphal suspensions and iv) inactivated *A. astaci* zoospores. We then monitored changes in the following immunological parameters: total hemocyte count (THC), differential hemocyte count (DHC), total protein content and gene expression of prophenoloxidase, immune system activator C/EBP and immune system repressor KLF1. Hemolymph sampling was conducted 1, 4, 8, 24 hours and 16 days post-priming. Our results indicate that THC was increased 4 hours post-priming in groups ii-iv and returned to normal values 8h post-priming. The number of granular hemocytes increased markedly in group i, ii and iv. We observed no changes in total protein content. Additionally, we detected down-regulation, followed by up-regulation of C/EBP, as well as up-regulation of KLF1 in later timepoints. Our results indicate that the changes in the monitored immune parameters of the noble crayfish are short-term and mostly return to normal values within 24 h. It remains to be seen if priming can induce *A. astaci* resistance as a long-term effect and can therefore be exploited as a tool for the conservation of this endangered species.

**Keywords:** *Astacus astacus*, immune priming, *Aphanomyces astaci*, gene expression, innate immunity

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# LIFE CLAW POSTERS



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## LIFE CLAW – Crayfish lineages conservation in north-western Apennine (Italy)

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Native populations of white-clawed crayfish *Austropotamobius pallipes* have undergone a remarkable contraction in Europe, and in Italy the decline has been more than 70%. In late 2019, the EU financial programme LIFE funded the project 'Crayfish lineages conservation in North-Western Apennine' (LIFE18 NAT/IT/000806). The project area includes 28 Natura 2000 sites in North-Western Apennine area of the Italian regions Emilia-Romagna and Liguria. In the first two years, we carried out an extensive survey on ICS and NICS populations and their habitat status to update the knowledge about their distribution, to molecularly characterize *A. pallipes* populations, to monitor the occurrence of the crayfish plague agent and to model *A. pallipes* habitat. Then, to improve the conservation status of *A. pallipes*, an integrated strategy of interventions has been implemented, including the containment of crayfish IAS (*Procambarus clarkii*, *Faxonius limosus* and *Pacifastacus leniusculus*), habitat restoration interventions, and ex-situ breeding and restoration of *A. pallipes* populations. The establishment of four breeding facilities will guarantee the production of about 10500 juveniles and the establishment of at least 12 new viable populations. Moreover, a broad communication campaign is creating an open dialogue with stakeholders, both to increase awareness, and to discourage the introduction of IAS. Finally, the long-term objective of the project is the establishment of a conservation programme, based on a 'crayfish zonation map' thus updating the Conservation Measures and the Management Plans of Natura 2000 project sites.

**Keywords:** native crayfish, IAS, reintroduction, *Aphanomyces astaci*, Europe

## **Assessing *Austropotamobius pallipes* species complex biodiversity through population genomics and habitat modelling in the Italian North-western Apennines**

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The EU-funded Life CLAW project (LIFE18 NAT/IT/000806) is addressing the conservation status of the crayfish *Austropotamobius pallipes* species complex in the Italian North-western Apennines. There has been a severe contraction in both the census size and distribution area of the species in Italy during the last century due to anthropogenic pressure and the introduction of allochthonous crayfish. Consequently, *A. pallipes* is now confined to individual water basins harbouring isolated reproductive units. To assess the amount and distribution of extant diversity, a DNA molecular characterization was performed on 1209 samples from 53 populations from Italy and Croatia through mitochondrial COXI gene sequencing and nuclear SNPs genotyping by Genotyping-By-Sequencing. Ecological Niche Modelling was applied to raster environmental layer data (Land cover/use, Elevation, Sentinel multi-spectral data, bioclimatic variables) to identify areas suitable for reintroduction. Molecular data show a clear differentiation between the gene pools of *A. pallipes* in the Western Apennines and of *A. italicus/fulcisianus* in the Central-eastern Apennines, together with several lineage- and population-specific mitochondrial haplotypes and the geographical structuring of diversity was identified by SNP genotype analysis. This pattern likely resulted from paleoclimatic events that affected the Mediterranean area during the Plio-Pleistocene period combined with recent demographic changes due to habitat loss and reduced gene flow. Habitat suitability modelling performed by ENM indicated that the most suitable sites for *A. pallipes* are small streams of intermediate altitude and that while water reservoirs have positive on the occurrence of the species, human settlements have negative effects.

**Keywords:** White-clawed crayfish, SNPs, Genotyping-By-Sequencing, ENM

## Implementation of outdoor ponds for *Austropotamobius pallipes* ex-situ breeding activities of the LIFE CLAW project

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Within the LIFE CLAW project (LIFE 18 NAT/IT/000806) outdoor artificial ponds for crayfish rearing have been implemented at two of the project's breeding facilities. The area around the pond, enclosed by a protective fence, has approximately a surface of 50 m<sup>2</sup> (10 m x 5), with a maximum depth ca. 80-100 cm in the center, providing a water depth of about 50 cm. The ponds are supplied by spring water, in addition to the main water supply line a backup line has also been implemented. Hollow construction bricks were positioned at the bottom of the ponds to provide shelter to the crayfish. The ponds have been built with a bowl shape allowing a complete emptying of the pond in case of sanitary interventions. A drain positioned at the bottom of the pond allows the removal of water and facilitates the mixing of the water. The ponds' banks and bottom surface have been shaped and adequately smoothed in order to accommodate a waterproof geomembrane. The ponds have also been provided with wooden and metal frames that hold a dragonfly protection net. A barrier (PVC pipe) has been positioned all around the upper edges of the pond to prevent the crayfish from escaping, while a metal fence has been placed all around the pond to ensure an adequate level of safety. Altogether, the metal fence, the barrier and the protective net worked and fulfilled their function, as in the first two years of *ex-situ* breeding no infractions or predations have been observed.

**Keywords:** *Austropotamobius pallipes*, white-clawed crayfish, ex-situ breeding, outdoor ponds, North-Western Apennines

## **Monitoring of *Aphanomyces astaci* during the LIFE CLAW project in *Austropotamobius pallipes* complex populations**

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LIFE-CLAW is an ongoing project aimed at the conservation of *Austropotamobius pallipes* complex in the Italian North-western Apennine. To achieve the restoration of the native crayfish populations, it is crucial to assess their health status. In particular, we focused on the presence of the pathogen *Aphanomyces astaci*, which can cause severe outbreaks in European native populations, although their coexistence without evidence of mortality has recently been reported. Therefore, between 2020 and 2022, a monitoring to detect *A. astaci* was carried out in the project area (Liguria and Emilia Romagna Regions), exploiting protocols based on non-invasive sampling techniques and molecular biology assays. Sampling was performed by combining two complementary methods: the concentration of *A. astaci* zoospores by filtering the water through glass microfiber filters and the collection of hyphae and zoosporangia directly from the external surface of the crayfish cuticle with thin cotton swabs. After DNA extraction from both filters (environmental DNA) and swabs, samples were analysed by quantitative PCR (TaqMan), calculating the starting concentration using a standard curve with 100-fold dilutions of synthetic DNA. Positive samples were then subjected to end-point PCR followed by Sanger sequencing to support the results. The presence of the pathogen was detected by qPCR in 20 of the 51 evaluated sampling sites, confirming the detection by sequencing in 14 of them. In conclusion, this sanitary monitoring allowed the identification of native populations from which to collect crayfish broodstocks for the LIFE-CLAW breeding facilities to increase the stocks of autochthonous populations.

**Keywords:** Conservation, Non-invasive samplings, eDNA, Cuticular swabs, Molecular detection

## Training and participation of volunteers for monitoring and containing invasive alien crayfish

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Biodiversity loss has been caused by global warming and the spread of invasive alien species. The LIFE project 'Crayfish lineages conservation in North-Western Apennine' (LIFE18 NAT/IT/000806) aims at conserving and improving the populations of the native freshwater crayfish *Austropotamobius pallipes* in the North-Western Apennine area, through a long-term conservation programme. To this end, a key role is to stop further spread of IAS. Thanks to the participation of trained volunteers in the trapping activities of invasive alien crayfish *Procambarus clarkii* and *Faxonius limosus*, a control action of these species has been implemented. The first year of trapping sessions (May to November 2022) achieved an overall capture of 47,131 individuals of *P. clarkii* in seven ponds and one open channel ecosystem. Starting from June 2023, trapping activities have been combined with an experimental female sterilisation, which should achieve the decrease of eggs and juvenile production, and consequently the progressively population ageing. In total, from mid-May to mid-August 2023, more than 8,100 individuals of allochthonous crayfish were caught. The crucial role of volunteers from fish associations and Voluntary Ecological Guards contributes to support partner beneficiaries during the crayfish IAS captures and to settle a trained stable team along the project lifetime as well as in a long-term conservation programme. Moreover, volunteers play the fundamental role of conservation message multipliers towards local communities.

**Keywords:** red swamp crayfish, spiny-cheek crayfish, massive trapping activities, pleopods, IAS control

## Strategies for the containment of *Pacifastacus leniusculus* in Liguria (North-western Italy)

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The EU-funded Life CLAW project (LIFE18 NAT/IT/000806) aims to conserve the native *Austropotamobius pallipes* species complex in the North-western Apennines. Due to anthropogenic pressure and the introduction of allochthonous crayfish, *A. pallipes* has experienced a severe contraction in both population size and distribution area in Italy during the last century. Consequently, *A. pallipes* has been now confined to small basins harbouring isolated reproductive units. In the Liguria region, *A. pallipes* is threatened by *Pacifastacus leniusculus* populations occurring in two aquatic ecosystems: Valla Stream and Brugneto Lake. Since 2019, integrated actions have been started to contain this species through trapping and hand catch activities. In particular, survey activities were first focused on tributary streams of Brugneto Lake to assess the possible occurrence of signal crayfish and its spread further than the lake. Then traps were placed in the lake using an electric boat. Valla Stream has been monitored also to assess the occurrence of a white-clawed crayfish population. In addition, in Valla Stream during the summer 2023, for the first time in Italy, three artificial barrier systems were specifically designed and built to limit the upstream spread of *P. leniusculus*, towards the stretch still inhabited by *A. pallipes*. Since 2021, more than 2,500 signal crayfish have been removed by trapping activities from water bodies: more than 1,000 from the Valla Stream and 1,500 from the Brugneto Lake. Activities will continue also to check the functionality of the barriers.

**Keywords:** White-clawed crayfish, signal crayfish, artificial barriers, control activity, Liguria

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