Crayfish News

The Official Newsletter of the International Association of Astacology

Spring Issue
March 2022
Volume 44, Issue 1
p-ISSN: 1023-8174 (print)
e-ISSN: 2150-9239 (online)

Is this a Swedish signal crayfish or an alien marbled crayfish?

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Figure 1. Marbled-coloured signal crayfish initially mistaken for Marmorkrebs. Photo by Tomas Jansson.

In Sweden, import, transport and keeping of all live alien crayfish is banned since 2003. However, illegally introduced "Marmorkrebs" (the marbled crayfish, *Procambarus virginalis*) were found in Swedish waters, starting in 2012. This highly undesirable crayfish species in Europe, can be distinguished by its specific marbled pattern that covers its body. Marble-coloured individuals of another crayfish species, the signal crayfish *Pacifastacus leniusculus*, have also been detected in Sweden since 2006. Although incidents of finding marble-coloured signal crayfish are rather rare, if found, these morphs may be mistaken for Marmorkrebs. Similarities in the marbled pattern of both crayfish species complicate their correct identification, and hence, genetic identification may be used as a better alternative. It was indeed used as proof to convict a Swedish pet store in the Environmental Court to substantial fines due to keeping live Marmorkrebs. It is, therefore, important to inform the general public and authorities about the colour pattern similarities between these two species. Quick and accurate species identification is also crucial to take correct action.

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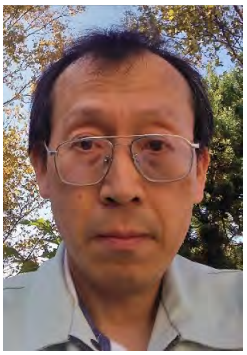
President's Corner



Tadashi Kawai, Ph.D.
IAA President (Japan)

Dear astacologists,

IAA will have two important events this early summer: the international meeting IAA23 in Czech Republic and the IAA regional meeting in Brazil. Chief Organizer of IAA23 Pavel Kozak gives an update on the meeting on page 7 of this newsletter. As Chief Organizers of the IAA Regional Meeting in Brazil at CBC-TCS 2022, Felipe and I introduce this meeting on page 8. It is not easy for astacologists in Africa and South America to attend IAA23, but this regional meeting will cover many IAA members in the southern hemisphere (see two photos).

At IAA23, we will vote for the venue of the next international meeting (IAA24). In the previous issue of *Crayfish News*, I already launched a call for suggestions for the IAA24 venue. The deadline for submitting your ideas will be extended from the end of March to the end of April 2022. At this stage, I welcome all candidates. Since this year's international meeting is being held in Europe and a regional meeting is being organized in Latin America, ideally the next meeting would be in a different region, such as North America or Oceania. This way IAA meetings can cover a broad geographical area, allowing us to attract a higher number of participants. All suggestions can be sent to me by e-mail (tadashikawai8@gmail.com).

Tadashi Kawai
Hokkaido, Japan

Figure 1. Tainã Gonçalves Loureiro (Africa)

Figure 2. Luis Amador (South America)

The International Association of Astacology (IAA), founded in Hintertal, Austria in 1972, is dedicated to the study, conservation, and wise utilization of freshwater crayfish. Any individual or institution interested in furthering the study of astacology is eligible for membership. Service to members includes a quarterly newsletter (*Crayfish News*), a membership directory, biennial international symposia and publication of the journal *Freshwater Crayfish*.

Secretariat:

The International Association of Astacology has a permanent secretariat managed by **James Stoeckel**. Address: IAA Secretariat, Room 203, Swingle Hall, Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849-5419, USA.

Tel: +1(334) 844-9249 / Fax: +1(334) 844-9208
E-mail: jimstoeckel@auburn.edu

Web page: www.astacology.org
Webmaster: **James W. Fetzner Jr.**
E-mail: FetznerJ@CarnegieMNH.org

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E-mail: lennart.edsman@slu.se

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Header photograph: Noble crayfish (*Astacus astacus)* © 2018 Karolina Śliwińska

This issue edited by **Thomas Abeel**, Managing Editor
E-mail: CrayfishNews@astacology.org

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Background

Figure 2. "Normal" coloured signal crayfish (Photo: Patrik Bohman, SLU Aqua).

It is well known that individuals within the same crayfish species may differ substantially in colour. Crayfish display significant colour variation within their own species and this variation does not seem to be linked to specific geographical boundaries (Hobbs 1942). For example, the signal crayfish (*Pacifastacus leniusculus*) possess an extremely variable pattern of colouration even within the same population (Sacchi et al. 2021). The signal crayfish is native to northwest North America (Abrahamsson and Goldman 1970), but due to introductions by humans, is now the most widely spread alien crayfish species in Europe (Kouba et al. 2014). Signal crayfish are often bluish-brown to reddish-brown, and occasionally light- to dark-brown. The underside of the claws is red and the commonly occurring white-turquoise oval patch on the upper side of the claws is unique (Souty-Grosset et al. 2006; figure 2). Moreover, different colour morphs, deviating from its normal appearance, are occasionally found. For example, marble-coloured morphs of signal crayfish were recorded for the first time in Sweden in 2006 (figure 3-4), and later occasionally found in the fisheries and monitoring programs.

Figure 3. Marbled-coloured signal crayfish initially mistaken for Marmorkrebs from Lake Stora Le 2006 (above left), juvenile from pond "Tveda damm" 2013 (above right), Lake Mossebosjön 2014 (below left) and Lake Stora Loken 2017 (below right). Photos: Tomas Jansson, Kräftmannen AB.

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Figure 4. Map with locations for marble-coloured signal crayfish found and the pet store in Sollefteå

Ocular species identification

By ocular identification, we distinguished between juvenile signal crayfish and Marmorkrebs from the pond "Tveda damm". These cryptic signal crayfish (which were all females) displayed several specific morphological characteristics which are important to be able to distinguish them from Marmorkrebs. Firstly, the two pairs of postorbital ridges and the wide areola clearly rule out Marmorkrebs (figure 5). Marmorkrebs have a striking dorsal bright mid-line, which passes over the whole cephalothorax (white arrows) and may also be found in males of the sexual form of *Procambarus fallax* (white arrows). In contrast, juvenile signal crayfish do not have such a bright mid-line. An important character for

Marble-coloured signal crayfish may superficially look similar to another non-native crayfish species, the Marmorkrebs (the marbled crayfish, *Procambarus virginalis*), and may therefore be incorrectly identified as that species (Bohman and Edsman 2013; figure 3). Both crayfish species are on the European Union's list of invasive alien species of Union's concern (EU regulation 1143/2014) and thus highly unwanted in Sweden. In fact, all live freshwater crayfish of all species from outside Sweden are banned according to the Swedish Species Protection Ordinance (SFS 2007:845); Edsman 2004). The first illegally introduced Marmorkrebs in natural waters in Sweden was found in 2012 (Bohman et al. 2013). During 2013, several additional marble-coloured morphs of juvenile signal crayfish were discovered in Sweden (Bohman 2013). Therefore, to properly manage invasive alien species and to take correct and swift mitigation actions, it is imperative to have tools for quick and unambiguous species identification.

What we found and what we did

Between 2006 and 2017 marbled morphs of signal crayfish have been discovered at four different locations in Sweden (figure 3-4). Ocular species identification was performed on individuals from the site "Tveda damm" (figure 5-6). Since there were ambiguities in the species identification of these juvenile individuals, tissue samples for further genetic analyses were also taken from these crayfish. Samples were similarly taken from a crayfish suspected to be Marmorkrebs in a pet store in the town of Sollefteå 2013. The genetic analyses where done at Charles University, Prague. To genetically confirm species identification a 658 bp long fragment of the mitochondrial gene for the cytochrome c oxidase subunit I (COI) was sequenced, as recommended for DNA barcoding of animals (Hebert et al. 2003). The universal primer pair LCO1490/HCO2198 (Folmer et al. 1994) was used, following the protocols described in Mrugała et al. (2015). We also searched the Swedish Crayfish Database, which includes 823 monitoring events for signal crayfish and contains 161109 registered signal crayfish individuals (Bohman 2022). The database also includes information on colour deviations.

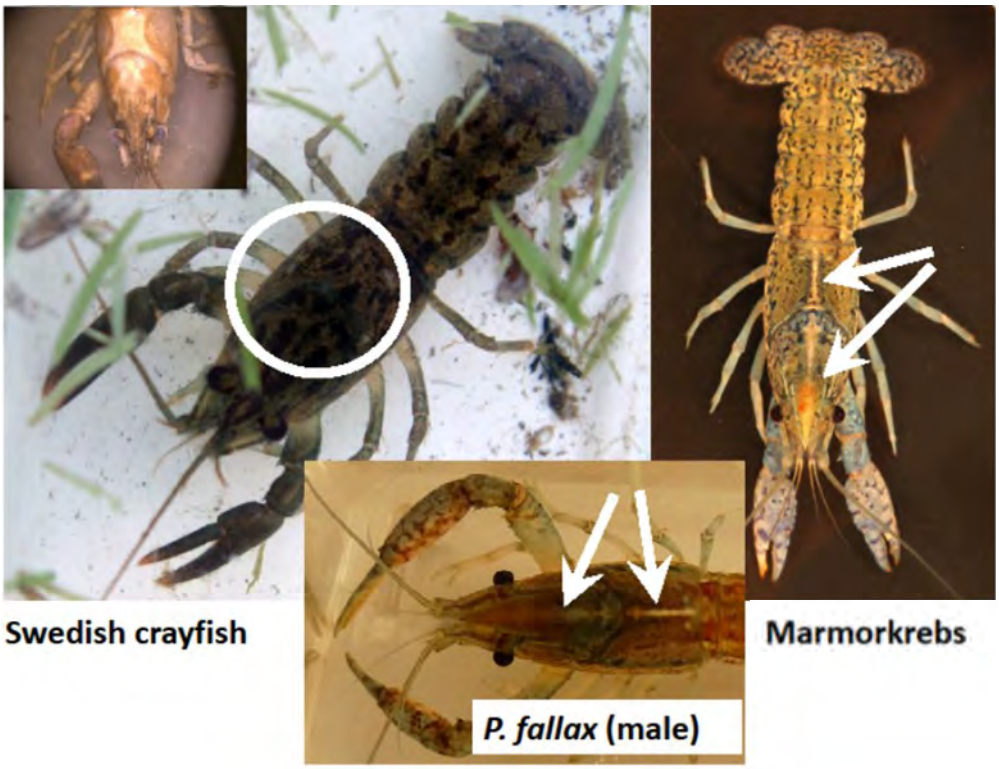

Figure 5. The photographs show the mid-line and areola on the back of a young female signal crayfish (above left), Marmorkrebs (above middle), male *Procambarus fallax* (below middle), and female signal crayfish (far right). Illustrations: Peer Martin, Humboldt University in Berlin and Patrik Bohman, SLU Aqua.

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identifying crayfish is also the width of the areola (the hourglass-shaped lines on dorsal surface of the thorax; figure 5). There are also differences in shape of the chelae and in spination of carpus and merus of the cheliped (figure 6). As observed on figure 6, both chelae of the signal crayfish are quite small, likely due to recent claw regeneration. Since signal crayfish normally have comparatively large chelae, especially compared with Marmorkrebs, their atypical small size was one of the reasons for the identification difficulties.

Genetic species identification

The COI sequence obtained from crayfish from "Tveda damm" completely matched the sequence isolated from *Pacifastacus leniusculus* (accession number: JF437997) described by Filipová et al. (2011), and hence unambiguously identify this crayfish as *P. leniusculus*. The COI sequence obtained from the crayfish individual from the pet store in Sollefteå was, however, unambiguously identified as Marmorkrebs.

Searching the Swedish Crayfish Database

None of the previously caught and measured signal crayfish in the Swedish Crayfish Database (www.kraftdatabasen.se) showed any marbled-colour morphs. Only 22 of the individuals had notes on deviating colours and then mostly notes of uniform blue or red colouration. This indicates that marbled-coloured signal crayfish are extremely rare in Swedish fisheries.

The court case

Armed with the genetic proofs for Marmorkrebs, the prosecutor opened up a case against the pet store owner in the Environmental court, for violation against the Species Protection Ordinance. The owner was convicted and sentenced to pay considerable fines. This was the first time that somebody was found guilty according to the targeted specific crayfish paragraphs in this legislation. The prosecutor later informed us that the genetic evidence made his court case much stronger.

Final words

The authorities and the general public should be aware that alien crayfish species may appear in Swedish waters, even if rare. Marble-coloured signal crayfish can be distinguished from Marmorkrebs, but due to the difficulties in ocular identification, genetic identification is the preferred approach, especially for juveniles. Genetic identification is also what is required as proof to open up a case in the Environmental Court and get a conviction.

Thanks goes to Chris Chucholl at Fisheries Research Station (LAZBW) for his generous help in species identification. Thanks also to Peer Martin and Gerhard Scholtz at Humboldt University in Berlin for helping out with the ocular identification and for illustrations, to Tomas Jansson for photos of Swedish marbled-coloured signal crayfish and to Charles University in Prague for the genetic analyses.

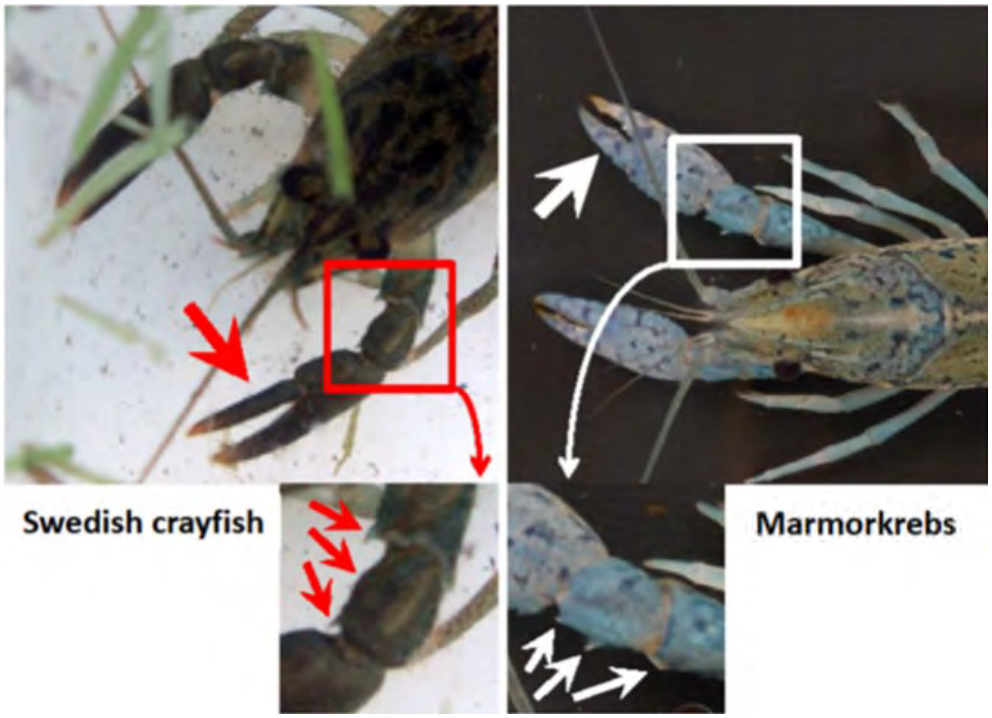

Figure 6. Differences in shape of the chelae and in spination of carpus and merus of the cheliped in young female signal crayfish (left) and Marmorkrebs (right). Illustrations: Peer Martin, Humboldt University in Berlin.

Patrik Bohman and **Lennart Edsman**
Swedish University of
Agricultural Sciences
SLU Aqua
Drottningholm, Sweden

Agata Mrugała
Independent Researcher
The Netherlands

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IAA23 23RD INTERNATIONAL SYMPOSIUM ON FRESHWATER CRAYFISH

Dear colleagues,

Dear astacologists,

We would like to cordially invite you and update some information to the postponed IAA 23 Symposium which will take place in in the charming Hotel Štekl, located near the enchanting Hluboká nad Vltavou Château in the beautiful South Bohemia Region of the Czech Republic from June 20 to 25, 2022.

Important information related to the Covid19 or other unexpected situation:

- Possibility of online participation – conference fee 220 EUR;
- Refund for registration without cancellation fee until April 30 and 50% until the end of May 2022;
- Online access will also be allowed when you register correctly and do not come for unexpected reasons. We will return the difference from the registration, including trips and official dinner (not accommodation and transport – this is under your control);
- Suppose the government bans on-site conferences (we do not expect that, given that the Covid19 situation is typically calm here in the summertime), the entire conference will be switched to online. The difference in the conference fees will be fully refunded.

IMPORTANT DATES

- Early registration: till March 31, 2022
- Abstract submission: till April 15, 2022
- Regular registration: till April 30, 2022
- Late registration: till May 31, 2022
- Online registration: till May 31, 2022

For more information, visit www.IAA23.com

We promise not only a high-quality scientific program with several keynote speakers but also a carefully prepared social program including trips to the experimental facilities of the FFPW USB, natural crayfish locality in the Šumava National Park, the Hluboká nad Vltavou Château, beer tasting in the Budweiser Budvar Brewery, the Český Krumlov UNESCO Heritage castle and wooden rafting.

We believe the program will be interesting for both delegates and accompanying persons.

On behalf of the Organizing Committee,

Pavel Kozák

IAA regional symposium in Brazil

The XI Congresso Brasileiro Sobre Crustáceos (CBC) and The Crustacean Society (TCS) are organizing a joint Summer Meeting, which will be held from 6th to 9th June 2022 in Brazil - all 100% online. The theme will be "Tradition and Innovation: Integrative Approaches to Crustacean Studies". A regional IAA symposium will be held at the joint meeting (i.e. Symposium 4: Integrative Studies of Freshwater Crayfish in South America, see below) on 8th June 2022 from 9:00 -13:00 local time in Brazil. By organizing this first event of the IAA in South America, we hope to encourage the study of freshwater crayfish in this region.

For more information and registration, please visit **cbc-tcs.com**.

Tadashi Kawai
Japan

Felipe Ribeiro
Brazil

XI CBC TCS Summer Meeting
June 06-09th, 2022
Brazil

Integrative Studies of Freshwater Crayfish in South America

Symposium 4 at XI CBC - TCS Summer meeting

Freshwater crayfish (Decapoda: Astacidea) encompass almost 700 species distributed worldwide, except in continental Africa and Antarctica. They can be found in several kinds of limnetic habitats, such as streams, lakes, wetlands, swamp forests and caves. In South America, several new endemic species have been discovered and described in the last years using traditional morphology and integrative taxonomy and also the development of behavioral and genetic studies. The advance of integrative studies of South American crayfish will contribute in the study of evolution of freshwater crayfish in the world. However, in this situation, the presence of alien crayfish in South America, especially in Brazil, brings new serious threats for conservation of the native biodiversity and ecosystems. Present symposium will bring the newest information of integrative studies of Parastacidae in South America, taking into account systematics, population genetics, biogeography, distribution modelling, behavior, habitat characterization, conservation and alien crayfish species. This symposium will reveal all research developed with crayfish in South America and encourage new students to work in the field of astacology.

This symposium is coordinated by Tadashi Kawai of Hokkaido Research Organization (Japan) and Felipe Ribeiro of Universidade Federal do Rio Grande do Sul (UFRGS) and Universidade do Estado do Mato Grosso (UNEMAT) and comprises seven lectures:

- **Tadashi Kawai** of Hokkaido Research Organization: *Evolution of freshwater crayfish (Decapoda: Astacidea)*;
- **Felipe Ribeiro** of Universidade Federal do Rio Grande do Sul (UFRGS) and Universidade do Estado do Mato Grosso (UNEMAT): *Integrative taxonomy and cryptic diversity in Parastacidae: history, development and future trends*;
- **Tainã Gonçalves Loureiro** of Cape Peninsula University of Technology / Global Ocean Accounts Partnership: *Double trouble: astaciculture and pet trade as vectors for the introduction of exotic freshwater crayfish in South America*;
- **Luis Amador** of The University of New Mexico (USA) and the Universidad Austral (Chile): *Systematics and population genomics of the burrowing crayfish Parastacus nicoleti in Southern Chile*;
- **Kelly Gomes** of Universidade Federal do Rio Grande do Sul (Brazil): *Habitat diversity and conservation of freshwater crayfish in Brazil: an approach based on current distribution and future predictions*;
- **Augusto Huber** of Universidade Federal do Rio Grande do Sul (Brazil): *Freshwater crayfish in Brazilian Protected Areas: conservation, diversity and threats*;
- **Célio Magalhães** of University of São Paulo, Ribeirão Preto (Brazil): *An overview of the decapod crustacean fauna of South America.*

Figure 1. The seven speakers at the symposium. From left to right: Tadashi Kawai, Felipe Ribeiro, Tainã Gonçalves Loureiro, Luis Amador, Kelly Gomes, Augusto Huber and Célio Magalhães.

LIFE CLAW PROTECTING THE NATIVE *AUSTROPOTAMOBIUS PALLIPES* IN ITALY

Native populations of white-clawed crayfish *Austropotamobius pallipes* have undergone a remarkable contraction and decline over the last 50 years on a widespread basis in Europe (Souty-Grosset et al. 2006), and in Italy the decline has been about 74% in the first decade of the 2000s (Holdich et al. 2009). Residual populations of *A. pallipes* have been increasingly confined to small high gradient streams and headwater, where the crayfish IAS have not yet been expanded and the habitat has been less influenced by human activities (Ghia et al. 2013). However, North-Western Italy is a hotspot for *A. pallipes* where high nucleotide diversity was detected and different lineages overlap. Therefore, any conservation strategy for *A. pallipes* in Northern Italy must take into account the complexity of the biogeographic pattern and the progressive isolation of local demes (Bernini et al. 2016). Three invasive alien crayfish species have been also found and represent both strong competitors for the native crayfish and carriers of the crayfish plague. The most widespread Cambaridae in Italy, *Procambarus clarkii* and *Faxonius limosus*, have been spreading overall the Po river plain area and represent a potential threat overall the project area, especially in the hill zone. The newly established signal crayfish *Pacifastacus leniusculus* has been recently detected at the headwater of two river basins, which still host some residual white-clawed crayfish populations. Its bioecological attributes leave no doubt that many more Italian lakes and streams will become occupied by signal crayfish, causing strong negative impacts on freshwater biodiversity and ecosystem functioning (Ercoli et al. 2021).

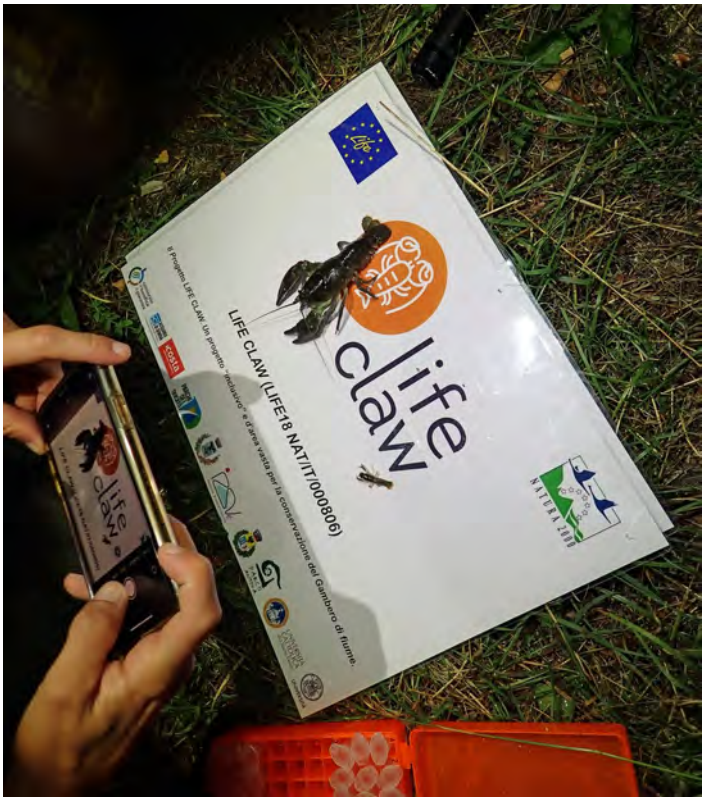

Figure 1. A volunteer taking a photo during a night-time crayfish sampling.

In late 2019, the EU financial programme LIFE funded the project 'Crayfish lineages conservation in North-Western Apennine – LIFE CLAW' (LIFE18 NAT/IT/000806). The project's main objective is to conserve and enhance the stock of the endangered crayfish *A. pallipes* in North-Western Apennine area of the Italian regions Emilia Romagna and Liguria by a long-term conservation programme. Specific objectives of the project are:

1. to establish four *ex situ* breeding facilities for restoration of *A. pallipes* populations during the project and after the end of the project, in order to ensure the long-term survival of this species against major threats;
2. to protect and increase the stocks of *A. pallipes* populations most significant for the conservation of the species' genetic variability in North-Western Apennine;
3. to counteract the dispersal of crayfish IAS (and crayfish plague, consequently), by intensive and continuous removal of crayfish IAS along the invaded areas as well as by construction of physical crayfish barriers at the invasion fronts to stop the spread upstream of signal crayfish within the two involved sites;
4. to establish a crayfish zonation map in order to identify the watercourses suitable for crayfish as well as to promote the ban of the continuous release of salmonids that alters the ecosystem balance;
5. to create an open dialogue with the stakeholders, not only to increase awareness, but also to discourage inappropriate introduction of IAS and to reduce the probability of deliberate translocation into other rivers;
6. to constitute a network among local stakeholders and communities for the conservation of the species.

One more objective of the project is to transfer "best practice" techniques and develop a concrete conservation strategy plan for native crayfish, which could be exploited in other contexts in Italy and Europe.

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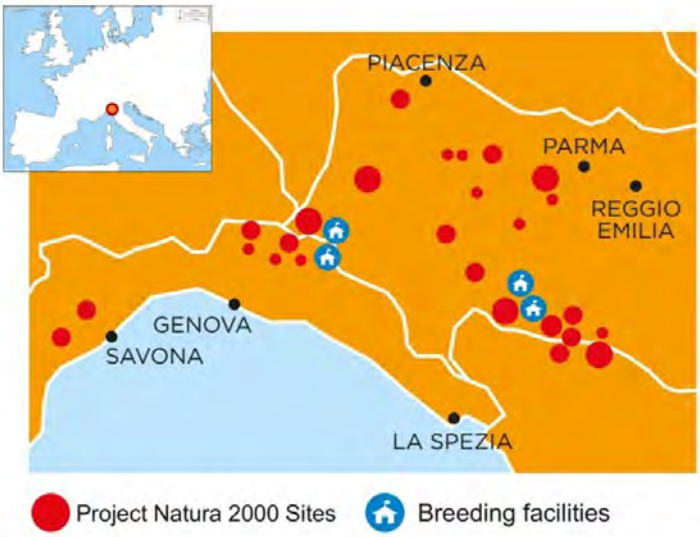

Figure 2. Map showing the project's Natura 2000 sites and breeding facilities.

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In the first two years (2020-2021) we carried out an extensive survey within the project area (twenty-eight Natura 2000 sites), in order to update the status of native and crayfish IAS populations, and to verify the occurrence of the crayfish plague agent in both native and crayfish IAS populations.

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Figure 3. High-gradient stream inhabited by white-clawed crayfish.

Figure 4. Clay-rich stream inhabited by white-clawed crayfish.

Figure 5. Crayfish voluntary team training course.

Figure 6. Tissue samples (pereopod) for genetic characterization and for veterinary analyses.

Figure 7. Reproductive white-clawed crayfish for breeding facilities.

Figure 8. Young-of-the-year white-clawed crayfish.

Figure 9. Signal crayfish trapped from Lake Brugneto.

Figure 10. Red swamp crayfish trapped from low-land watercourse.

(Continued from page 10)

The development of a biodiversity conservation awareness in the local communities is a fundamental step to achieve the objectives of the project. Therefore, a preliminary action focused on training stakeholders from fish associations and Voluntary Ecological Guards, in order to set up a stable team prepared to support project beneficiaries during the breeding activities and crayfish IAS captures, along the project lifetime as well as in the Afterlife.

Now, we are getting ready for the effective implementation of the concrete conservation actions, which will be carried out during the following three years. In addition, we are planning two meetings addressed to the scientific (crayfish) community:

- in September 2022, there will be a 2-days international meeting focusing on the joint use of molecular genomics and GIS-based approaches to aid wildlife conservation (organised by Università Cattolica, Piacenza);
- in summer 2023, a 3-days European meeting on crayfish conservation (organised by University of Pavia).

The beneficiaries of the project are: national and regional parks (Ente Parco nazionale dell'Appennino tosco-emiliano, Ente di Gestione per i Parchi e la Biodiversità Emilia Occidentale, Parco Naturale Regionale dell'Antola), two municipalities (Comune di Ottone and Comune di Fontanigorda), a public law organisation devoted to drainage, flood control, protecting waters (Consorzio di Bonifica di Piacenza), an organisation managing the Acquario di Genova (Costa Edutainment) and three research institutes/universities (Istituto Zooprofilattico Sperimentale delle Venezie, Università Cattolica del Sacro Cuore and Università degli Studi di Pavia).

Daniela Ghia and **Gianluca Fea**
University of Pavia, Italy

VISIT WWW.LIFECLAW.EU FOR MORE INFORMATION

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John Smyth, who died this February, was for 15 years an important figure in Irish crayfish conservation. A businessman and rainbow trout farmer who exported to the London market, and latterly a processor of crabmeat for Irish restaurants, he had an intuitive understanding of the health requirements of captive fish and shellfish and developed systems for monitoring and depurating shellfish.

Perhaps as a retirement project, he turned his attention to freshwater crayfish, and everything he could find about their marketing and captive rearing. He quickly discovered that the white-clawed crayfish, *Austropotamobius pallipes*, although then widespread and often abundant in Ireland, was internationally considered threatened, and so was protected by European law. It could be reared in captivity and, looking ahead, he could see a situation where a vital conservation tool would be captive rearing, as it had proved in France and Spain, and then in Britain.

In many ways he was a non-academic - a fish farmer who had caught the 'bug' - the fascination with crayfish shared by everyone in the IAA. John built his own rearing tunnels and enlisted the expertise of international experts. Tomas Policar came from the Czech Republic to oversee and develop the impacts of raising and manipulating temperatures through the early life cycle, eventually achieving control of the breeding cycle, so that maturity could be achieved much earlier than in nature. Catherine Souty-Grosset also visited from France - examples that collaboration between academia and the private sector on crayfish is possible and a good way to develop.

John's projects were substantially funded by Interreg and ERDF. They were also successful. Advances in rearing technology (buildings, equipment, techniques) were made and he achieved a lot by collaborating with known crayfish experts. John really enjoyed this work - at a time when most people are retired, he was just starting getting involved in a whole new area.

All this was expensive, and he looked for partnerships. He presented his case at IAA meetings in 2015 in Germany (Landau) and Spain (Olot), but government conservation bodies in Ireland distanced themselves from his activities. This attitude was surprising, to say the least, given that in 2015 a major outbreak of crayfish plague occurred in the cross-border Erne system, the main river holding crayfish in Northern Ireland, and it was followed by a series of outbreaks in most of the Irish Republic's Special Areas of Conservation for crayfish.

As is well known, Ireland is the only European country still without North American alien crayfish, which carry and spread crayfish plague. This implies that a plague outbreak isn't necessarily perennial (as it is in Britain and Scandinavia) but, if carefully monitored, could be managed and even controlled. John Smyth showed that he could hold and breed captive stocks in isolation, until they could be repatriated to their source regions or to ark sites. Sadly, his expertise was largely ignored or dismissed – a startling own goal for Irish conservation.

Perhaps John Smyth's legacy is that the new round of EU Horizon funding or EMFAF funds could be used to do something like this again; academic expertise and private sector practicality, working together for the biodiversity interest of crayfish. Action now is needed in Ireland in particular, where there is still an opportunity, albeit limited, to protect Europe's last viable populations of white-clawed crayfish.

Julian Reynolds
Honorary life member IAA

with help from **Martin Flanigan**

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