BOOK OF ABSTRACTS

IAA GOTLAND 2019 CRAYFISH CONFERENCE

Organising committee: Japo Jussila (University of Eastern Finland), Lennart Edsman (Swedish University of Agricultural Sciences), Rolf Gydemo (RG Fisheries Consultant), Andreas Petterson (County Administrative Board of Gotland), Petra Lindberg & Gunilla Rosenqvist (Uppsala University)

FOREWORD

We should take freshwater crayfish seriously since they are partially responsible for the European aquatic ecosystems wellbeing and health. When present in healthy populations freshwater crayfish constitute an overwhelming majority of the biomass that dwells and moves around on the bottom of lakes and running waters. This native ecosystem engineer and manager is facing dire straits, mainly due to the careless introduction of alien crayfish, construction of waterways, pollution and general destruction of the aquatic environment.

One of the key players in this complex network of natural resource management are the researchers focusing on various issues of freshwater crayfish ecology and biology. The facts and the best current knowledge should be presented and shared openly at all times, including at scientific seminars like this one. Common public and decision makers should also be informed. We cannot expect that only wise decisions will be taken in the future, but decision makers must be aware of the risks they are dealing with. Current facts and best available knowledge may be ignored but the information to support good decisions must always be available. We need to act - soon and preferably yesterday. No later than today. Let us discuss debate and publish the information required to improve the chances of the wellbeing of aquatic ecosystem thus offering a brighter future for native European crayfish.

We choose Gotland as the venue for a reason. It is a kind of a heaven for the native noble crayfish with the whole island declared an ark site for noble crayfish. Gotland is free from the invasive North American species signal crayfish and the fatal disease crayfish plague, since the county administration has promptly eradicated the five illegal occurrences of signal crayfish found earlier, with insect poison. Conditions are good for noble crayfish farming with lots of sun hours, mild climate, good water quality with high pH and plenty of calcium for the crayfish shells. Noble crayfish farming is the only form of crayfish farming that is economically feasible and with the recent EU regulation on invasive alien species it is the only form of crayfish farming permitted in Sweden. Gotland has a strong tradition of successful crayfish farms. Noble crayfish pays good prices both for crayfish for consumption and with an even better price as stocking material for restoration of populations in natural water as a conservation measure.

The presentations at IAA Gotland 2019 centres on freshwater crayfish and the usual hot topics like ecology, physiology, management, conservation, invasive species, crayfish plague and the side theme aquaculture. It also stretches out to kelp and lobster farming and subjects spans from plate tectonics over eDNA to molecular genetics. Geographically, apart from Europe it also deals with crayfish in Australia and on Madagascar. The wide variety of topics just shows how essential crayfish are in freshwater ecosystems.

This conference is a joint effort of Swedish University of Agricultural Sciences, Blue Centre Gotland, Eastern Finland University, Uppsala University, Gotland County Administrative Board, International Association of Astacology and the Swedish Rural Network.

The IAA Gotland 2019 organising team

Cover: Noble crayfish sitting on drift wood from Vivesholm carved out of Aspen tree wood by artist Anna Atte Larsson. Photo Karin Edsman

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IAA GOTLAND 2019 CRAYFISH CONFERENCE
SCIENTIFIC PROGRAM

Tuesday, 27.8.	
6 – 8pm	**Registration & Welcome Party**
Wednesday, 28.8.	
9 – 10am	**Registration & Coffee**
10 – 10.20am	**Welcome & useful hints for delegates**
10.20 – 11.40am	**eDNA**
	D. Strand: Monitoring of noble crayfish, signal crayfish and crayfish plague in Norway: benefits and challenges using eDNA (keynote)
	P. Bohman: Detecting crayfish in a drop of water
	N. Sieber, A. Zenker, R. Krieg, A. Weston, A. King: First Swiss field-validation of eDNA analysis to detect crayfish plague and crayfish
11.40am – 12.40pm	***Lunch***
12.40 – 2pm	**Crayfish Aquaculture & Ecology**
	S. Eriksson: Homarid lobster aquaculture and live handling
	J. Nightingale, P. Stebbing, N. Taylor, G. McCabe, G. Jones: Optimising aquaculture techniques for white-clawed crayfish conservation
	P. Nyström, M. Stenberg, B. Andersson, L. Edsman: Noble crayfish farming – a closer look at survival, feeding and biodiversity
	M. C. Ion, A. E. Puha, T. Suciu, L. Pârvulescu: Get a grip: unusual disturbances drives crayfish to improvise
2 – 3pm	***Coffee & Posters***
3 – 4pm	**Ecology**
	K. Kaldre, M. Hurt, R. Gross: Continuing expansion of non-indigenous crayfish species in Estonia
	I. Maguire, L. Lovrenčić, A. E. Livaić: Morphological diversity of *Astacus astacus* revealed by geometric morphometrics
	L. Pârvulescu, J. L. Pérez-Moreno, C. Panaiotu, L. Drăgut, A. Schrimpf, I-D. Popovici, C. Zaharia, A. Weiperth, B. Gál, C. D. Schubart, H. Bracken-Grissom: A journey on plate tectonics sheds light on European crayfish phylogeography
4 – 4.10pm	**The International Association of Astacology (IAA) 23 meeting in Czech 2020**
4.10 – 4.20pm	**Why not joining the IAA – Only benefits – First year free for students**
4.30 – 5.30pm	**Alien Species Workshop**
7pm onwards	**Informal Evening Program**
Thursday, 29.8.	
9 – 10.20am	**Crayfish plague**
	L. Martín-Torrijos: Chasing the crayfish plague: historical review of European crayfish decline and future perspectives (keynote)
	G. Casabella-Herrero, M. Martínez-Ríos, L. Martín-Torrijos, J. Diéguez-Uribeondo: Assessing the diversity of the crayfish plague pathogen *Aphanomyces astaci*: mtDNA and whole genome markers
	Á. J. Correa-Villalona, M. Martínez-Ríos, L. Martín-Torrijos, J. Diéguez-Uribeondo: Detection of *Aphanomcyes astaci* in *Procambarus clarkii* population of an estuarine environment, La Albufera de Valencia, Spain
10.20 – 10.50am	***Coffee***
10.50am – 12.10pm	**Crayfish plague & Other disease**
	K. Theissinger: Crayfish plague – present and future research (keynote)
	R. Hernando, M. Martínez-Ríos, L. Martin-Torrijos, J. Diéguez-Uribeondo: *Fusarium* pathogens: from turtles to human and crayfish
	J. Jussila, V. Tiitinen, J. Makkonen, L. Edsman: Eroded swimmeret syndrome (ESS): An update

12.10 – 1.10pm	***Lunch***
1.10 – 2.30pm	**Conservation & Farming**
	F. Gröndahl: Kelp farming an upcoming bio-based industry in Sweden
	N. Whiterod, S. Zukowski, M. Asmus: One in 100: translocations to aide the recovery of a large freshwater crayfish following significant population loss in the Murray River
	S. Palm, J. Dannewitz, L. Edsman: Colonization history and translocations explain the population genetic structure of noble crayfish in Northern Europe
	N. Green, P. Stebbing, M. Bentley, D. Andreou, R. Britton: Mechanical sterilisation of male *P. leniusculus*: Hierarchial and reproductive responses
2.30 – 3pm	***Coffee & Posters***
3 – 4.40pm	**Conservation**
	T.J. Ruokonen, E. Erkamo, T. Keskinen: Connecting conservation of native crayfish and effective exploitation of invasive crayfish – mission impossible?
	N. Whiterod, S. Zukowski, M. Asmus: Can translocations play a role in the conservation of freshwater crayfish under future conditions?
	A. Zenker, R. Krieg: Function control of a barrier in a natural stream
	J. K. M. Zimmerman, E. Grönlund, P. van den Brink: Is astacology environmental science?
	R. Gydemo: The noble crayfish, *Astacus astacus* L., on Gotland: Past, present and future
5 – 6.30pm	**Guided Visby Tour**
7pm onwards	**Informal Evening Program**
Friday, 30.8.	
9am – 4pm	**Field trip including crayfish & touristic sites**
4pm – 5pm	**Guided tour around field station Ar (aquaponics & related stuff)**
5pm – 6pm	**Taste of your country session**
6pm onwards	**Conference dinner in the form of a traditional Crayfish Party at Ar**
– late	**Buss back to Visby**
Saturday, 31.8.	
	Farwell & Departures

IAA GOTLAND 2019 PARTICIPANTS

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TALKS

DETECTING CRAYFISH IN A DROP OF WATER

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keywords: eDNA, crayfish, invasive species, environmental monitoring

Today it is possible to identify crayfish simply by taking water samples. The rapid development of DNA technology, barcode libraries and computing have cemented the way for more precise, faster and cheaper species surveys. Successful eDNA (environmental DNA) studies have increased exponentially, compared to a few years back. But identifying crayfish DNA from water samples is no easy task. There are still many questions needed to be answered before eDNA methodologies can be used more extensively, e.g. within environmental monitoring. This review presents the pros and cons of eDNA and also gives an update on the latest crayfish research that uses eDNA technology.

ASSESSING THE DIVERSITY OF THE CRAYFISH PLAGUE PATHOGEN *APHANOMYCES ASTACI*: MTDNA AND WHOLE GENOME MARKERS

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keywords: crayfish plague, *Aphanomyces*, pathogen, freshwater, invasive species, molecular detection.

The Oomycete *Aphanomyces astaci* is one of the 100 world's worst invasive alien species. This pathogen causes the crayfish plague disease that represents a mayor threat to freshwater European native crayfish. The detection and characterization of *A. astaci* is a key aspect to better understand the epidemiology of this disease and to implement effective conservation strategies. In this study, we compared two recently developed approaches to detect *A. astaci* groups based on: (i) two primer pairs specific for the mitochondrial ribosomal rnnS (small) and rnnL (large) subunits and (ii) specific primers based on the *A. astaci* genome-sequence groups. For this purpose, we tested these approaches against the RJB Oomycete culture collection, by 74 isolates of the most representative genera of freshwater environments. The results indicated that both approaches allow an efficient identification of the pathogen and its groups in both pure cultures and clinical samples. Moreover, we generated a

mtDNA sequence library of Saprolegniales that will facilitate not only correct identification of *A. astaci* haplogroups but also the identification of other oomycetes species in clinical analyses.

DETECTION OF *APHANOMCYES ASTACI* IN *PROCAMBARUS CLARKII* POPULATION OF AN ESTUARINE ENVIRONMENT, LA ALBUFERA DE VALENCIA, SPAIN

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keywords: crayfish plague, *Aphanomyces*, *Procambarus*, Saline water, invasive species, molecular detection.

The crayfish plague pathogen *Aphanomyces astaci* and its chronic carrier, the red swamp crayfish *Procambarus clarkii*, are among the 100 world´s worst invasive species. The red swamp crayfish was first introduced into Europe in Doñana National Park in 1973 from where later spread through Spain. Even though the role of red swamp crayfish as carrier of *A. astaci* and three different haplotypes (d1, d2 and d3) are well known, little is known regarding the tolerance of *A. astaci* to environmental conditions such as salinity. We described the presence of *A. astaci* in a population thriving in an estuarine environment, La Albufera de Valencia. For this purpose, cuticle samples from 40 individuals of *P. clarkii* were analyzed by applying mithochondrial ribosomal specific primers (ITS), and primers allowing the detection of *A. astaci* groups: (i) mithochondrial rnnS (small) and rnnL (large) subunits and (ii) specific primers based on the *A. astaci* genome-sequence strains. We detected the presence of *A. astaci*, and found that the pathogen belongs to haplogroup D. The results indicate that this haplogroup can survive in saline environments. Further studies on *A. astaci* salinity tolerance need to be done.

HOMARID LOBSTER AQUACULTURE AND LIVE HANDLING

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keywords: *Homarus*, hybridization, deformities, early development, survival, seeding

Homarid lobsters are highly valued seafood products on the Swedish market. As the wild stock of the native European lobster (*Homarus gammarus*) has declined, the interest for aquaculture has increased and several European countries are currently conducting aquaculture efforts for conservation purposes of wild stock as well as for aquaculture food production. The aim is to reach a lobster production that is both economically as well as ecologically sustainable. In addition, the trans-Atlantic trade of live American lobster (*Homarus americanus*) is extensive, but it is also a pathway for introductions of the non-indigenous species to European waters. The two *Homarus* species can cross breed and American females with hybrid eggs have in recent years been captured in Scandinavian wild fisheries. The potential consequences of such genetic pollution to the native lobster stock, as well as state-of-the-art techniques for lobster culturing are presented and discussed.

MECHANICAL STERILISATION OF MALE *P. LENIUSCULUS*: HIERARCHIAL AND REPRODUCTIVE RESPONSES

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keywords: sterilisation, dominance, female choice, brood size

Many methods of controlling invasive crayfish are unsuccessful because they fail to target all life stages of the population. The River Barle Signal Crayfish Project uses artificial refuge traps, baited funnel traps and sterilisation of large males via gonopod removal to control *P. leniusculus* on an upland river in South-west England. The success of the sterilisation technique is reliant on the sterilised males exhibiting similar behaviours to non-sterilised males and remaining attractive to females during mate choice, and may additionally be influenced by the post-copulatory guarding behaviour of males and promiscuity of females. We designed a four stage experiment to quantify how male sterilisation affects female mate choice and promiscuity, male hierarchical status and post-copulation guarding. We also compared the brood sizes of males mated with non-sterilised and sterilised males.

KELP FARMING AN UPCOMING BIO-BASED INDUSTRY IN SWEDEN

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keywords: renewable, bioindustry, kelp farming

Kelp farming circumvents several disadvantages related to land-based biomass production, e.g. the need for fertilizers and irrigation, and does not compete for valuable arable land. In addition, seaweeds grow fast and their farming counteracts coastal eutrophication and may stimulate biodiversity. The overreaching goal of the SEAFARM project is to develop a sustainable system for the use of seaweeds/kelp as a renewable resource in a future bio-based industry for the Swedish society. The trans disciplinary research approach includes techniques for cultivating seaweeds to be used as raw material in a bio refinery for the production of food, feed, bio-based materials and bioenergy. A holistic approach is used where utilization of the resource is maximized in each step of the process cycle. Seaweeds are cultivated at the Swedish west coast and methods suitable for preservation and storage are evaluated. The obtained biomass will subsequently be fractionated in an integrated bio-refinery. The different fractions are thoroughly characterized and recovered for production of

biochemical, polymers, and food/feed additives. The residues from the bio refinery are utilized for production of biogas and biofertilizers. In parallel, a general multi-process sustainable assessment method was developed to analyse the overall sustainability of the system. The multi-disciplinary research team collaborates closely with a set of state agencies, commercial enterprises and other stakeholders in the different tasks of the project. The study presented here will summarise the results from the successful five-year project and how the SEAFARM project have boosted the development of a growing new bio based industry in Sweden.

THE NOBLE CRAYFISH, *ASTACUS ASTACUS* L., ON GOTLAND: PAST, PRESENT AND FUTURE

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keywords: noble crayfish, island Gotland, history, development

The noble crayfish *Astacus astacus*, most likely introduced to Gotland in the 19th century, became a refuge population with the introduction of the American signal crayfish to Sweden in the 1960´ies and hence further spreading of the crayfish plague. Promoting exclusivity, the commercial interest in farming as well public awareness of the unique European conditions for the survival of the species, including eradication of illegally introduced signal crayfish, seems to ensure Gotland as a stronghold for the noble crayfish. From production of live crayfish for cooking, more interest is arising in value increase by product development.

***FUSARIUM* PATHOGENS: FROM TURTLES TO HUMAN AND CRAYFISH**

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keywords: *Fusarium*, turtles, crayfish, pathogen, molecular detection, molecular taxonomy.

Fungal pathogens of the genus *Fusarium* can infect a large number of hosts causing severe diseases. The *Fusarium solani* species complex (FSSC) contains at least 60 cryptic species. Short et al (2013) identified cryptic species within the FSSC that cause human keratitis and fusariosis of sea turtle eggs, STEF, using a multi-local sequence types (MLSTs) approach based on the combined analysis of four loci, which includes nuclear DNA regions from a portion of the translation elongation factor 1-α (TEF nDNA), the transcribed internal spacing region of ribosomal DNA (ITS nrDNA), the large ribosomal subunit (LSU nrDNA), and the second largest RNA polymerase II subunit (RPB2 nDNA). Jussila et al (2013) also found a *Fusarium* species in crayfish and its complete molecular characterization can now be addressed from isolates obtained from human fusariosis and STEF cases. We describe several cases of FETC and compare with *Fusarium* infections found in crayfish.

GET A GRIP: UNUSUAL DISTURBANCES DRIVES CRAYFISH TO IMPROVISE

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keywords: *Pontastacus leptodactylus*; memory; drifting; learned behaviour.

Animals facing unexpected situations react instinctively to efficiently adapt to the changes in the environment. Persistence leads to training and learning the developed solution, which can be applied to similar challenges in the future. We experimented on adult individuals of narrow-clawed crayfish chronically exposed to the risk of drifting caused by water currents (~1 m/s). First, we compared specimens extracted from both lentic and lotic habitats, and experimental data proved that the latter is more prone to grab objects during acute exposure to water currents. Chronic exposure of specimens from lentic habitat to water currents leads to intense clumping activity compared to control group. Acute exposure in individual experiments suggests that the specimens previously kept in directional flow conditions learned to grip quickly and firmly to adherent objects regardless of the natural or artificial origin of said objects. This response was found to be significantly lower in the non-exposed group. Moreover, the gripping activity significantly reduced after the specimens returned to an environment lacking directional water currents for a period of four weeks. The results of this study suggest that crayfish can apply and learn a solution in disturbing situations. Once the disturbance disappeared, the newly developed behaviour was no longer needed and forgotten, confirming that this observed behaviour was a temporarily learned solution.

ERODED SWIMMERET SYNDROME (ESS): AN UPDATE

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keywords: eroded swimmeret syndrome, signal crayfish, disease, reproduction

During early 2010's it was observed during wild signal crayfish (*Pacifastacus leniusculus*) stock surveys in Sweden and Finland, that several females had traumatised swimmerets and some had lost a few or all of them. A joint venture to investigate the phenomen revealed that female signal crayfish were suffering from *Fusarium* SC infection in addition to being in most cases infected with *Aphanomyces*

astaci. The condition was named eroded swimmeret syndrome (ESS), with the causative conditions described a multiple infection by *Fusarium* SC and *A. astaci*. The ESS causes loss of eggs during incubation period and delays in hatching, both resulting in decreased reproductive success. Alien species wild stocks, both signal crayfish and red swamp crayfish (*Procambarus clarkii*), have been reported to suffer from the ESS at least in Sweden, Finland, Switzerland and Germany. The symptoms include melanisation of the swimmerets in the initial stages of the ESS, then partial erosion of one or more swimmerets and finally loss of whole swimmeret. Due to regeneration process, swimmerets can grow back and regain their function but it takes several molts. Since 2015, also male signal crayfish have shown ESS-like symptoms, with even eroded mating legs. The potential impacts of the ESS on signal crayfish ecology are discussed.

CONTINUING EXPANSION OF NON-INDIGENOUS CRAYFISH SPECIES IN ESTONIA

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keywords: signal crayfish, *Pacifastacus leniusculus,* spiny-cheek crayfish*, Faxonius limosus,* marbled crayfish*, Procambarus virginalis*

We report the continuing expansion of the non-indigenous crayfish species (NICS) in Estonia. Just a decade ago Estonia was one of the last countries in Europe where NICS were not recorded. For today, three alien crayfish species have been detected in Estonia. The first signal crayfish *Pacifastacus leniusculus* population was detected in 2008 and by now it is found in five different water bodies. In 2017, the first spiny-cheek crayfish *Faxonius limosus* was registered in the pre-estuary of River Pärnu which flows into the Baltic Sea. In 2018, the marbled crayfish *Procambarus virginalis* population was detected in the outflow channel of the cooling system of the Balti Power Plant, entering into the water reservoir of the River Narva. The dispersed pattern of distribution of NICS indicates that these populations are the result of illegal human-assisted introductions.

MORPHOLOGICAL DIVERSITY OF *ASTACUS ASTACUS* REVEALED BY GEOMETRIC MORPHOMETRICS

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keywords: noble crayfish, Croatia, lentic habitat, lotic habitat

Astacus astacus is one of four native crayfish species inhabiting Croatian freshwaters. It is known that they play a key role in the ecosystems functioning, that their populations are declining and that they are vulnerable. In order to develop adequate conservation programs for their preservation, sound knowledge of their biology and ecology is necessity. It is known that organisms adapt to specific environmental conditions in different ways and that some of these adaptations can also affect their

morphology. The aim of this research was to study morphological characteristics of *A. astacus*, and to compare populations from different regions in Croatia. Study included 230 individuals belonging to different water basins/ water body types/mtDNA phylogroups. Analyses were conducted using geometric morphometrics approach, comprising 34 landmarks on the dorsal side of crayfish cephalon. Results revealed that morphometry differs among phylogroups, among river basins and also between water body types.

KEYNOTE

CHASING THE CRAYFISH PLAGUE: HISTORICAL REVIEW OF EUROPEAN CRAYFISH DECLINE AND FUTURE PERSPECTIVES

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keywords: *Aphanomyces astaci*, native crayfish, alien crayfish, diagnostics, population crashes

The actual biodiversity crisis and the increasing trend of the species extinction have focused scientific interests on invasive alien species (IAS) and emerging infectious diseases (EIDs). In particular, the worldwide translocations of the North American crayfish, *Procambarus clarkii* and *Pacifastacus leniusculus*, acting as vectors of EIDs such as *Aphanomyces astaci*, have made authorities catalogued them among the 100 World´s Worst IAS and EIDs. This review explores the history of the introduction of *Aphanomyces astaci* in Europe, the current state of knowledge about the pathogen and European crayfish populations, the last molecular diagnostic tests and the future perspectives for European crayfish conservation.

OPTIMISING AQUACULTURE TECHNIQUES FOR WHITE-CLAWED CRAYFISH CONSERVATION

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keywords: *Austropotamobius pallipes*; conservation; aquaculture; nutrition; captive-breeding; stage-2 hatchlings; *Artemia* nauplii; cysts; gel diet, practical diet.

The white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet 1858) is endangered throughout its range in Europe. In response to this decline, captive-breeding for reintroduction is becoming a recognised conservation measure. Aquaculture has several key elements in order to maximise productivity, including stocking density, grading, refuge selection and dietary regimes. Bristol Zoological Society established an ex-situ, closed-circuit crayfish hatchery for white-clawed crayfish in 2009 and has since been researching optimal aquaculture techniques for hatching and rearing *A.*

pallipes for wild release. This presentation will summarise the results of four hatchery experiments assessing survival and growth of captive-bred, *A. pallipes,* fed different treatment diets.

NOBLE CRAYFISH FARMING – A CLOSER LOOK AT SURVIVAL, FEEDING AND BIODIVERSITY

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keywords: noble crayfish, culture, survival, feeding, stable isotopes, biodiversity

The aquaculture project "Noble crayfish farming – a handbook based on experiences with a future perspective" focuses on increasing the interest in noble crayfish farming. By marking crayfish before the growth season and emptying a culture pond at the end of the season we estimated natural morality of adult crayfish to be as high as 70%. Stable isotope analysis showed that crayfish were positioned at the top of the food web together with nymphs of dragonfly larvae. The invasive plant *Elodea canadensis* totally dominated the macrophyte assemblage but it was never found in guts of crayfish. The species richness of macrophytes, wetland birds, benthic invertebrates and amphibians in culture ponds showed that in comparison with other constructed wetlands in southern Sweden, biodiversity in noble crayfish ponds can be similar. Subsequently, noble crayfish farms can potentially play an important role in promoting biodiversity and the same time have large socioeconomic values.

COLONIZATION HISTORY AND TRANSLOCATIONS EXPLAIN THE POPULATION GENETIC STRUCTURE OF NOBLE CRAYFISH IN NORTHERN EUROPE

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keywords: genetic diversity, microsatellites, conservation, management

The noble crayfish *(Astacus astacus*) is an endangered freshwater species in Europe. Illegal introductions of American signal crayfish, a carrier of the lethal crayfish plague, is considered as the main threat. Here we report results from a large scale genetic study (15 microsatellites) of noble crayfish, covering 70 locations in the Fennoscandian Peninsula. Main objectives were to describe post-glacial colonization history and evaluate how anthropogenic effects such as stocking have affected the species' genetic structure. Results revealed three main genetic clusters corresponding to populations in northern, middle and southern Fennoscandia, with measures of genetic diversity being markedly higher within populations in the southern cluster. The observed genetic structure likely mirrors different immigration routes after the last glaciation period. Several deviations from this pattern

existed, however, which likely reflect past human translocations of noble crayfish. Our results are discussed in relation to conservation and management needs of this critically endangered species.

A JOURNEY ON PLATE TECTONICS SHEDS LIGHT ON EUROPEAN CRAYFISH PHYLOGEOGRAPHY

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keywords: Apuseni Mountains; Biogeographical pattern; Divergence time estimates; Endemic lineages; Freshwater species distribution; Molecular clock; Tisza-Dacia mega-unit.

Traditional molecular clock calibrations based on substitution rates can be imprecise, especially in comparison to divergence time estimates that incorporate accurately dated geological event from the relevant time period. We have carried out a comprehensive investigation into the phylogeography of *Austropotamobius torrentium*, including information from previously unstudied sites. Two mitochondrial (16S and COI) markers were sequenced from samples covering unstudied area in Eastern Europe (Hungary and Romania). Available sequences from GenBank were used to include known haplogroups and outgroups. Phylogenetic relationships and divergence time were estimated by substitution rate and geological calibration methods. Reconstructions of the ancestral distribution were addressed. A new haplogroup (APU) was discovered in Romania's Apuseni Mountains. This haplogroup is endemic and closely related to other haplogroups that are endemic in the Dinarides (NCD), despite their vast geographical separation (~600 km). The separation of this haplogroup is a result of tectonic displacement of the Tisza-Dacia microplate, which started in the Miocene (~15 Ma) and carried part of the *A. torrentium* population to the current location of the Apuseni Mountains, which was then cut off from the Dinarides for a period of ca. 11 m.y. by marine and lacustrine phases of the Pannonian Basin. The inclusion of the Apuseni Mts. displacement geological calibration point in divergence time analyses challenges the currently accepted crayfish evolutionary time frame for the region constraining the crayfish evolution in the area to a much earlier event (15 Ma) than the divergence time estimate based on the arthropod COI substitution rate (2.2 Ma). The previous molecular clock calibrations for the European crayfish species divergence should therefore be reconsidered.

CONNECTING CONSERVATION OF NATIVE CRAYFISH AND EFFECTIVE EXPLOITATION OF INVASIVE CRAYFISH – MISSION IMPOSSIBLE?

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keywords: *Aphanomyces astacii*, *Astacus astacus*, management zones, *Pacifastacus leniusculus*, spatial prioritization

The noble crayfish suffers the pressure of invasive crayfish and crayfish plague, and is listed as endangered in the Red List of Finnish Species. EU directive on invasive species banned signal crayfish introductions and translocations but trapping and selling continues in Finland. Co-existence of noble and signal crayfish is problematic leading often to collapse of noble crayfish population. To reach objectives set in the Finnish crayfish strategy, a pilot management plan was made in collaboration with researchers, fisheries authorities and stakeholders for Pohjois-Päijänne Fisheries District. Main goal of planning was to devote zones for conservation and re-introduction of native noble crayfish, zones where spread of signal crayfish and crayfish plague is restricted, and zones for effective commercial and recreational trapping of existing signal crayfish populations. Informative maps with clear spatial management plans help stakeholders and water owners to conserve noble crayfish populations far in to the future.

FIRST SWISS FIELD-VALIDATION OF eDNA ANALYSIS TO DETECT CRAYFISH PLAGUE AND CRAYFISH

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keywords: eDNA, crayfish plague, validation, detection, survey

Environmental DNA is seen as a helpful tool to detect either crayfish plague or native and alien invasive crayfish in natural waters. Compared to traditional surveying methods like trapping eDNA analysis has some big advantages. There is a higher chance to detect the species while disturbing less the ecosystem by sampling only water. To establish this method in Switzerland a sampling campaign was performed throughout the country to account for different stream characteristics. The results were used to prepare a risk map for crayfish plague and to update the spread of invasive crayfish populations. We will share our experiences with the sampling method, some drawbacks in laboratory work and the interpretation of the results.

KEYNOTE

MONITORING OF NOBLE CRAYFISH, SIGNAL CRAYFISH AND CRAYFISH PLAGUE IN NORWAY: BENEFITS AND CHALLENGES USING EDNA

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keywords: Crayfish plague, environmental DNA, disease surveillance, freshwater crayfish,

During the last decade, environmental DNA (eDNA) methodology has become an important non-invasive tool to monitor freshwater micro- and macroorganisms. One of the main benefits of eDNA monitoring is the possibility for temporal and spatial monitoring of several organisms from the same water samples. The simultaneous eDNA-monitoring of *Aphanomyces astaci* and relevant native and invasive freshwater crayfish species is well-suited for early-warning of invasion or infection, risk assessments, habitat evaluation and surveillance regarding pathogen and invasive/native crayfish status. While eDNA is an effective monitoring tool, there are also some challenges to consider when using eDNA for surveillance. We have developed and are now regularly using eDNA monitoring tools in the surveillance of *Aphanomyces astaci*, the causative agent of crayfish plague, and freshwater crayfish in Norway. We will present eDNA results and experience from the TARGET project, the national surveillance of *Aphanomyces astaci* and the national surveillance program of noble crayfish.

KEYNOTE

CRAYFISH PLAGUE – PRESENT AND FUTURE RESEARCH

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keywords: *Aphanomyces astaci*, diagnostics, immune system, susceptibility, resistance

European decapods are keystone species in freshwater ecosystems, with population trends are currently in decline. The main threat are invasive North American crayfish, which are vectors of the crayfish plague disease agant *Aphanomyces astaci*. These North American vector species are usually resistant towards the disease. In European crayfish however, the *A. astaci* infection leads to crayfish death usually within a few days to weeks, depending on the pathogen strain virulence and environmental conditions. However, recent reports from laboratory and wild indicate that *A. astaci* exposed noble crayfish can in some cases withstand an acute crayfish plague infection. In this talk I

first summarize the various molecular diagnostic methods to detect the crayfish plague disease agent and to distinguish among the different *A. astaci* genetic groups. Then I introduce our novel attempt to use gene expression data to identify target genes and molecular pathways, which underlie the defense mechanisms of the crayfish immune system under an *A. astaci* challenge and might be responsible for an increased resistance towards a crayfish plague infection. By comparing gene expression profiles of *A. astaci*-susceptible and -resistant crayfish individuals to uninfected control groups this research project will lead to important perceptions regarding the crayfish plague molecular defense response. This knowledge will help to understand the co-evolutionary dynamics behind this host-parasite system. Potentially, the finding of resistance-responsible target genes might be usable for selective breeding of resistant noble crayfish for restocking purposes in future.

ONE IN 100: TRANSLOCATIONS TO AIDE THE RECOVERY OF A LARGE FRESHWATER CRAYFISH FOLLOWING SIGNIFICANT POPULATION LOSS IN THE MURRAY RIVER

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keywords: conservation, translocation, population loss

Globally, many species have experienced population declines and are at risk of extinction. In addition to contributing to the loss of biodiversity, increased rates of extinction may disrupt ecological processes and functions (particularly when a keystone species – which has a disproportionally large effect relative to its abundance – is lost). Furthermore, with the extinction of a species, often people's (including managers and the community) connection with the species is also lost. Translocations are increasingly employed in an attempt to aid species persistence and recovery, but also to engage renewed awareness and support for species and ecosystems. Here we focus on application of translocations strategy for the large freshwater crayfish, Murray Crayfish, in the Murray River. The species is the second largest freshwater crayfish in the world, and naturally occurred widely across waterways of the southern Murray-Darling Basin. Yet, Murray Crayfish has experienced substantial decline in distribution and abundance over the past 50 years attributed to river regulation, pesticides and pollutants, habitat degradation and harvest pressure and blackwater events. Mostly recently in the Murray River, 81% population loss was experienced in areas exposed to extreme blackwater disturbance over 2010−11, and minimal recovery eventuated. Translocations are common in other freshwater crayfish around the world and, whilst illegal releases are known, targeted and strategic translocations have not been initiated for the species in almost one hundred years. This is despite translocations being proposed as a feasbile option to re-establish locally extinct populations of species some 25 years ago. This presentation the details the steps involved, lessons learnt and opportunities following the first such translocation of Murray Crayfish into areas of the Murray River that have demonstrated no recovery following the blackwater disturbance.

CAN TRANSLOCATIONS PLAY A ROLE IN THE CONSERVATION OF FRESHWATER CRAYFISH UNDER FUTURE CLIMATES?

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keywords: Reintroduction, reinforcement, assisted colonisation, climate change, viability, extinction

Globally, many species have experienced population declines and are at risk of extinction. This is particularly true of freshwater crayfish with approximately one-third of the more than 590 species described worldwide considered under threat. Most freshwater crayfish are predicted to be highly senstive to the impacts imposed by climate change, so extinction risk will increase in the future. Conservation translocations are increasingly employed in an attempt to aide species persistence and recovery; by augmenting existing populations, reintroducing into former habitats or introducing into areas outside of its natural range (assisted colonisation). Although underrepresented compared to other taxonomic groups, translocations have been directed toward some freshwater crayfish around the world. Drawing on these, and contemporary examples, we explore the feasibility of conservation translocations to lessen extinction risk for freshwater crayfish around the world as climates change in the future.

FUNCTION CONTROL OF A CRAYFISH BARRIER IN A NATURAL STREAM

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keywords: barrier, function control, *Astacus astacus*, trout, migration

A crayfish barrier in a middle sized stream called “Etzgerbach” in Canton Argovia, Switzerland, was built-in to stop future immigration of signal crayfish from the river Rhine. In order to check if the construction could hinder upstream movement of crayfish and allow fish to pass the barrier we marked local noble crayfish, trouts and bullheads with passive integrated transponder (PIT) tags. Tagged animals were recorded by two installed antennas up and downstream the barrier for one year. With this setup a passing of crayfish was not recorded. Trouts of different size were able to pass the barrier. One bullhead was recorded upstream the barrier. It can be assumed, that the weak swimmer overcame the barrier in the stomach of a predator. The tested barrier is an effective way to stop the non-anthropogenic spread of invasive crayfish. In contrast the migration of strong swimming fish species is not impeded.

IS ASTACOLOGY ENVIRONMENTAL SCIENCE?

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keywords: Astacology, Environmental Science, Sustainable development, Environmental issues, Ecosystem service

Environmental Science aims to study the relationships between human beings influence on and dependence of nature, the interaction and integration of ecological, social and technical systems as well as the strategies for sustainable use of natural resources. The linking of social, ecological and economical aspects in Environmental Science can provide useful tools for the study, management and conservation of freshwater crayfish from a broad social perspective. Therefore, it is of interest to explore the relationships between Environmental Science and Astacology. By analysing the aims of the International Association of Astacology and the content of some volumes of Freshwater Crayfish from an Environmental Science perspective we found that Astacology is operating within the field of Environmental Science. The focus is mainly on identifying underlying mechanisms of environmental issues. We will point out unexplored scientific perspectives and propose some advice of how to study freshwater crayfish from a broad systems perspective.

POSTERS

EFFECT OF PHOTOPERIOD ON JUVENILE REDCLAW (*CHERAX QUADRICARINATUS*) PERFORMANCE IN A CLOSED AQUACULTURE SYSTEM

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keywords: aquaculture, photoperiod, RAS, redclaw

An experiment was carried out to determine the optimal photoperiod for indoor farming of juvenile redclaws. Crayfish with an average body weight (ABW) of 0.96 ± 0.66 g were stocked into a recirculating aquaculture system (RAS) at a density of 50 crayfish.m^{-2}. During 15 weeks, the crayfish were exposed to four different photoperiods: hours light/dark (L:D) 24:0, 16:8, 12:12 and 0:24. In this experiment, the highest growth rates were obtained when redclaws were reared in constant light (L:D 24:0), resulting in the highest total biomass and ABW. Highest survival was obtained in the L:D 24:0 and 0:24 treatments. Redclaws farmed in continuous dark conditions, showed the poorest growth rates. These results suggest that juvenile and pre-adult redclaws should be farmed under continuous light conditions in order to improve growth performance and survival rate. The effect of photoperiod on adult redclaws will be evaluated in a future experiment.

ECOLOGICAL PLASTICITY AND COMMERCIAL IMPACTS OF INVASIVE MARBLED CRAYFISH POPULATIONS IN MADAGASCAR.

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keywords: marbled crayfish, Madagascar, habitat diversity, epigenetic, crayfish plague, farming.

The marbled crayfish (*Procambarus virginalis*) is a monoclonal, parthenogenetically reproducing freshwater crayfish that has formed multiple stable populations worldwide. Madagascar hosts a particularly large and rapidly expanding colony of marbled crayfish in a variety of ecosystems. Here we provide a detailed characterization of the habitats of five populations in Madagascar. Our data show that marbled crayfish can tolerate a wide range of ecological factors. The adaptation to these different habitats is associated with specific epigenetic variations. Further analyses also showed sympatry with endemic crayfish species, but no evidence of the crayfish plague pathogen. Finally, we document fishing, farming and market sales of marbled crayfish in Madagascar. The commercial value of the animals is likely to result in further anthropogenic distribution. Taken together, our results provide a paradigm for the complex network of factors that promotes the spread of marbled crayfish.

CAN SPECIES DELIMITATION METHODS HELP IN RESOLVING STONE CRAYFISH TAXONOMY?

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keywords: *Austropotamobius torrentium*, ITS2, biodiversity, ABGD, TCS, GMYC, bPTP, *BFD

Stone crayfish is a freshwater species that inhabits small waterbodies at higher altitudes of central and south-eastern Europe. Previous research revealed that stone crayfish presents genetically highly diverse species, and recently a new crayfish species, formerly considered stone crayfish, was described. In this comprehensive study we included previous samples and new ones collected from 69 previously unsampled locations in Croatia, Slovenia and Macedonia. In order to assess the genetic diversity of the stone crayfish we analysed mtDNA and nuDNA. Results confirmed Dinaric Karst as a hot spot of the stone crayfish diversity and revealed previously unrecognised genetically divergent populations. Considering the high diversity present within the species, a wide range of species delimitation methods were applied on the mtDNA dataset. Different methods resulted in different numbers of operational taxonomic units within the stone crayfish. The results of this study are a step forward towards the future species description within the stone crayfish species complex.

EVOLUTIONARY HISTORY OF *AUSTROPOTAMOBIUS TORRENTIUM*

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keywords: stone crayfish, molecular clock, conservation, paleohydrogeology

Austropotamobius torrentium is a freshwater crayfish species distributed across central and south-eastern Europe, with the highest genetic diversity recorded in the northern-central Dinarides. Previous studies have shown that evolutionary history of *A. torrentium* was shaped through different paleohydrologic and paleogeologic events from Miocene onwards. In order to connect current genetic structure of *A. torrentium* with paleogeographic events divergence time estimates were applied on the large-scale mtDNA dataset. Different calibration approaches were applied, including substitution rates, geological events, and for the first time hydrogeological event of the paleo-Danube and paleo-Tisza rivers connection. We presumed that this event could have had a strong influence onto *A. torrentium* expansion into nowadays areal. Results obtained by different calibrations yielded mostly congruent results and confirmed complex evolutionary history of *A. torrentium*. Application of acquired results in the future *A. torrentium* conservation and management programs is discussed.

MATING STRATEGIES OF INVASIVE VERSUS INDIGENOUS CRAYFISH: MULTIPLE PATERNITY AND PARTHENOGENESIS AS DRIVER FOR INVASION SUCCESS?

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keywords: *Faxonius limosus*, mating system analyses, microsatellites, invasive species, *Pontastacus leptodactylus*, Danube

This is the first study comparing the reproductive strategies of an invasive (*Faxonius limosus)* and a sympatric indigenous crayfish (*Pontastacus leptodactylus)*. We investigated the contribution of parthenogenesis and multiple paternity to the invasion process of the River Danube. Using microsatellites, we genotyped offspring and their mothers of 11 clutches of *F. limosus* and 18 clutches of *P. leptodactylus*. While no evidence of parthenogenesis was detected, multiple paternity was observed in both species, with a higher incidence in *F. limosus'* clutches (45%) than in *P. leptodactylus'* (11%). Additionally, for *F. limosus,* the incidence of multiple paternity in the invasion front was three times higher than in the older invaded river sectors. Our results suggest that multiple paternity is a driver of *F. limosus'* invasion. Furthermore, they highlight a modulation of *F. limosus*' mating strategies, suggesting that *F. limosus* can adapt its mating behaviour to different environmental stimuli.

PRESENCE OF TWO OOMYCETE PATHOGENS IN POPULATIONS OF THE INVASIVE SIGNAL CRAYFISH ALONG ITS RANGE IN THE KORANA RIVER, CROATIA

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keywords: invasive species, signal crayfish, pathogenic oomycetes

Introduction and spread of invasive alien species may result in transmission of pathogens to susceptible native counterparts and lead to their populations decline. We analysed the presence of two aquatic oomycete pathogens, *Aphanomyces astaci* –causative agent of crayfish plague and *Saprolegina parasitica* –causative agent of saprolegniasis in fish and an oportunistic crayfish pathogen, in populations of the signal crayfish along its invasion range in the Korana River, Croatia. We collected mixed epibiotic microbial communities from carapace of 110 crayfish by swabs. Next, DNA was isolated and PCR-based pathogen detection conducted. *A. astaci* was detected by previously developed specific primers in 6.4% of crayfish along the entire invasion range (invasion core, upstream and downstream fronts). To detect *S. parasitica*, we developed specific primer pairs and demonstrated their specificity and sensitivity using genomic DNA of *S. parasitica* and related oomycetes. However, *S. parasitica* was not detected in any of the examined individuals.

THE EFFECT OF DIFFERENT FEEDS ON SURVIVAL AND GROWTH IN NOBLE CRAYFISH (*ASTACUS ASTACUS*) JUVENILES

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keywords: survival rate, growth rate, crayfish feed, artificial diet, feeding rate

Crayfish farming has a great potential to diversify aquaculture production in Estonia. At present, there are about 20 crayfish farms that are growing noble crayfish, the only native crayfish species in Estonia. In order to further develop such aquaculture activity, optimized artificial diets are needed. In this study, we tested two experimentally manufactured diets differing for the relative proportions of proteins and carbohydrates. Diet A contained 42% proteins and 19% carbohydrates and Diet B contained 34% proteins and 34% carbohydrates. Carp feed (ALLER CLASSIC VITAMAX, 3 mm), used trivially for feeding crayfish in farms in Estonia, was given to the control group. Feeding trials with juveniles (mean weight 0.3 g) were carried out for 211 days in triplicate per treatment (total of 60 crayfish per treatment). The feeding rate was 2% of the body weight. Growth rate was the highest in the Carp feed group, but survival was higher in both experimental diet groups (>63%).

FIRST INSIGHT INTO GENETIC STRUCTURE OF STONE CRAYFISH POPULATIONS IN CROATIA REVEALED BY MICROSATELLITE MARKERS

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keywords: *Austropotamobius torrentium*, conservation, genotyping, Southeast Europe, diversity

The stone crayfish *Austropotamobius torrentium* (Schrank, 1803), a freshwater crayfish species with intricate evolutionary history and high genetic diversity, is threatened due to anthropogenic pressure, climate change and spreading of invasive crayfish species and their pathogens. Maintenance of genetic diversity and the preservation of the integrity of a native species are one of pivotal conservation genetics goals. In order to develop appropriate conservation and management programs on the local and regional level, the aim of our study was to reveal the current population genetic structure of the endangered stone crayfish. We conducted a microsatellite analysis of 300 individuals from 15 sampling sites. Preliminary testing included 57 recently developed microsatellite markers. Ten out of 57 microsatellite loci were polymorphic and were used in further analyses. We also used seven additional, previously developed loci, cross-amplified on other Astacidae crayfish species. The obtained results enabled the identification of local genetic diversity hotspots, as well as definition of evolutionary significant and management units, which will be given priority in conservation programs.

MONITORING OF CRAYFISH PLAGUE IN THE PLITVICE LAKES NATIONAL PARK

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keywords*: Astacus astacus, Austropotamobius torrentium,* Oomycetes, latent infection

Waterbodies of the Plitvice Lakes National Park are inhabited by two indigenous crayfish species, noble and stone crayfish. Native European crayfish populations are in decline, amongst others due to the lethal disease crayfish plague caused by pathogen *Aphanomyces astaci*. Presence of this pathogen was previously recorded in the Plitvice Lakes, but those analyses included small sample size. Thus, our goal was to analyse the presence of *A. astaci* on both crayfish species during two years monitoring period, using larger sample size, at multiple locations within the Park. For *A. astaci* detection we used

non-invasive sampling method based on collecting swabs of mixed epibiotic microbial communities from crayfish carapace. DNA was isolated from swab samples and PCR was used to detect the presence of the pathogen. Positive samples were classified into semi-quantitative categories of pathogen load by qPCR, and microsatellite genotyping identified presence of strain A (As-genotype). Obtained results will be used in future management plans aiming to protect vulnerable native species in NP, with focus on prevention of unintentional spread of the pathogen.

NON-INVASIVE DETECTION OF *APHANOMYCES ASTACI* ON INDIVIDUAL CRAYFISH

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keywords: pathogenic oomycete, native crayfish species, biofilm

Pathogenic oomycete *Aphanomyces astaci* is transmitted mainly by invasive North American crayfish, and causes the crayfish plague, a disease mostly lethal for native European crayfish. Detecting the pathogen in endangered native crayfish populations before the disease outbreak would provide a starting point in the development of effective control measures. However, current *A. astaci*-detection protocols either rely on eDNA isolated from large volumes of water or, if focused on individual animals, include crayfish killing. Thus, we have developed a non-invasive method that detects *A. astaci* DNA in microbial biofilm associated with the cuticle of individual crayfish, bypassing the unnecessary killing step. Efficiency of the new method was confirmed by PCR and qPCR and the obtained results were congruent with the traditional invasive method. We propose that the new method should be used in future monitoring of *A. astaci* presence in endangered European native crayfish individuals.