Book of Abstracts

Foreword

Leopold Füreder

On behalf of the International Association of Astacology, the forum flusskrebse, the conveners, local organizing and the scientific committee, I welcome you with great pleasure at the IAA 19 Symposium here in Innsbruck, Austria!

For the second time the IAA meets in Austria, where 40 years ago the first symposium of the IAA was held 1972 in Hinterthal and also the IAA was founded. So let us take the opportunity and celebrate the 40th anniversary of the IAA! The idea for the first European symposium on crayfish was that of Sture Abrahamsson of Sweden in the early 1970s, who also suggested forming the association of astacologists and having a newsletter. The first symposium in Hinterthal, Austria, in 1972 in fact became an international one as a number of American astacologists also attended. James Avault Jr. from the USA suggested a second meeting in Louisiana, and the International Association of Astacology took off from there. Over the four decades of existence the IAA has become an active organisation and now comprises members of more than 40 countries with an interest in freshwater crayfish.

At this year's symposium, about 130 delegates have registered and the submitted abstracts already promise to provide for an interesting and lively programme. We are proud to have some of the leading scientists and crayfish researchers to present keynote lectures on relevant and current topics. During the five-day scientific program, presenters of almost 100 oral or poster presentations have placed their emphasis on discussions about conservation, biogeography, genetics and diseases, as they are hot topics in Europe at the moment. At the same time, delegates were encouraged to present papers on all relevant crayfish topics including physiology, behaviour, ecology and all other interesting.

Prior to the symposium, two workshops take place at the University of Innsbruck. The Post-conference excursion is planned for the weekend after the scientific program September 1-2, where delegates can enjoy catching the three native crayfish species, *Astacus astacus*, *Austropotamobius torrentium* and *Austropotamobius pallipes*. In addition to scientific program we will cater a lively but not too exhausting social program. As the conference will take place in the historic center of Innsbruck, there will be several opportunities for accompanying persons as well as for delegates sneaking out of the lecture rooms to experience summer in Tyrol.

We are proud to have the Federal Minister of Science and Research Univ.-Prof. Dr. Karlheinz Töchterle, the Governor of Tyrol Günther Platter, and the Mayor of Innsbruck Mag.ª Christine Oppitz-Plörer, and appreciate the financial support of the University of Innsbruck, the Tyrolean Government (Land Tirol), the Town of Innsbruck (Stadt Innsbruck), TIWAG (Tiroler Wasserkraft AG), International Association of Astacology (IAA), Forum Flusskrebse, EWR (Elekrizitätswerke Reutte), and the Tyrolean Fishery Association (Tiroler Fischereiverband).

Leopold Füreder,
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Abstracts for oral contributions

Water is overrated: the conservation value of intermittent streams for crayfishes

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In the U.S., state and federal agencies establish "best management practices" (BMPs) for various activities such as forestry and agriculture. Typically, BMPs are non-regulatory but encourage landowners to adhere to practices, such as establishment of streamside management zones, that protect water quality and stream habitat. Recommendations on the size and nature of streamside management zones usually differ considerably between perennial and intermittent streams, with several states not suggesting any special management zones along intermittent streams. However, intermittent streams are essential to numerous species. *Hobbseus yalobushensis*, a rare crayfish endemic to a small area in Mississippi, appears to be restricted almost entirely to intermittent streams. Catch-per-unit-effort (CPUE) of *H. yalobushensis* was inversely related to CPUE of a) *Cambarus striatus*, b) total fish, and c) 2 families of fishes known to eat crayfishes. We infer that *H. yalobushensis* uses intermittent streams largely to avoid predation by fishes. Additional crayfish species, as well as some other aquatic fauna in the region, are also most abundant in intermittent streams. Conversely, other crayfish species in the state, including *Procambarus lylei*, another species of conservation concern inhabiting the same drainage, have very different relationships to fish CPUE. Therefore, comprehensive conservation planning for crayfishes must consider both intermittent and perennial streams. Future work will determine flow duration of presently-intermittent study streams and examine how changes in flow duration influence crayfish and fish populations.

Keywords: stream, intermittent, crayfish, predation, fish

State of knowledge about crayfish of the genus *Parastacus* (Crustacea, Parastacidae) in Brazil: implications for conservation strategies.

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Brazil is considered as the country harbouring the greatest biodiversity on the planet and several groups are good examples of this high biodiversity. Crustaceans include about 68,200 valid species worldwide, 2,500 of them recorded in Brazil. In particular, freshwater crustaceans represent a very diverse group including cladocerans, copepods, amphipods, isopods and decapods (crabs, shrimps, crayfishes). However this high crustacean biodiversity in Brazilian freshwaters have been diminished due to a combination of factors. Despite the evident loss of freshwater crustacean habitats, this has not been reflected on the Brazilian list of threatened species. Assessments were performed according to the International Union for Conservation of Nature (IUCN) criteria in which the risk of species extinction is assessed based on fundamental data (geographical distribution and population data) and the impact of threats. The main conclusion was that fundamental data are lacking for many groups, especially crayfishes. In South America, native crayfish are represented by the family *Parastacidae* comprising three genera (*Parastacus*, *Samastacus*, *Virilastacus*). In Brazil, the genus *Parastacus* comprises six crayfish species occurring in temperate southern regions. For the past three decades, data were mainly gathered on taxonomy, morphology, geographical distribution, reproduction biology, physiology and ecology. Despite this huge effort, fundamental population, ecological and genetic data are still lacking and some taxonomic uncertainties remain. This knowledge will provide a better understanding of the *Parastacus* group in Brazil and the degree of threats. Relevant IUCN assessments are the first step before undertaking conservation measures of freshwater ecosystems.

Keywords: Brazil, freshwater ecosystem, native crayfish, biology, conservation

How likely is the long distance transport of juvenile crayfish by birds?

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We performed a set of experiments during the last 3 years, to assess the probability of juvenile *Procambarus clarkii* passive dispersal by waterbirds. We first evaluated the desiccation survival of crayfish analyzing the effect of environmental conditions and crayfish size on its survival time. We then evaluated the survival of crayfish under conditions simulating bird flight in mesh bags on the top of a vehicle at a constant speed. We setup another experiment using pigeons carrying crayfish on a small bag and released at several distances from a dove cot. An experiment was also performed using a dead mallard carrying crayfish on its feathers, on the top of a moving vehicle. Another set of experiments evaluated the likelihood of a juvenile crayfish taking a bird transport vector. This was first quantified using tanks, freshly dead ducks or duck paws, under still or moving conditions. An experiment was attempted using live ducks passing by two ponds, one with and one without juvenile crayfish. During our experiments, juvenile *P. clarkii* survived distances up to 150 km outside a moving vehicle and up to 62 km when transported by birds. Furthermore, juvenile crayfish were capable of clinging to duck feathers and were transported when ducks were removed from the water. We obtained evidence that the probability of transport depends on water depth and on resting time. Our results indicated that ectozoochory is possible and that, distance, size and environmental conditions may affect the likelihood of survival of crayfish during bird flight.

Keywords: *Procambarus clarkii*, invasions, dispersal, ectozoochory, birds

Evaluation of the potential of escape gaps to improve the selectivity in freshwater crayfish fisheries

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Minimum size at catch is commonly used to avoid overfishing. In the marine crustacean fisheries the use of escape gaps have been successfully implemented to reduce undersized catch. In Sweden the fishing administration have been adapting regulations and introduced the trap gaps to the inland signal crayfish (*Pacifastacus leniusculus*) fisheries, with the intention to let undersized 100 mm or smaller signal crayfish escape. We evaluate the implemented usage of 28 mm trap gaps. A morphological difference between male and female signal crayfish was found to hamper the positive effect of the implemented escape gaps. The width of female tails exceeded the limitation of the trap gap diameter 28 mm, before reached a total length of 100 mm. Male crayfish on the other hand was not limited by the carapace width until a total length of 110 mm. The catch of undersized females was found to be lower in traps with escape gaps, even at a length where the females should be physically restricted from escaping by the width of their tail. The results also indicate increased escape rate over time for both treated and untreated traps. This was probably an effect of the amount of bait remaining at time of catch. These results call for an adjusted construction, suggesting more elliptical escape gaps in order to fulfil the intention of the implementation.

Keywords: Selectivity, escape gaps, crayfish

Global warming and the agonistic behaviour of invasive crayfish

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Flexible traits such as behaviour are sensitive to environmental effects, which may range from abiotic (e.g. temperature) to biotic factors (e.g. the interactions with other species). Here we investigate the effects of two values of temperature (20 and 27 °C) on a key property of successful invaders, i.e. the agonistic behaviour, in interspecific dyads of three North American crayfish: *Orconectes limosus*, *Pacifastacus leniusculus* and *Procambarus clarkii*. We showed that the higher temperature influences the agonistic behaviour of the three species but with significant interspecific differences. Specifically, *P. leniusculus* lost most fights at the warmer temperature, whereas *O. limosus* was most often inactive. In comparison with *P. leniusculus* and *O. limosus*, *P. clarkii* appeared to be the least affected species. These results highlight the importance of temperature when studying behaviour and also suggest that the projected global warming has the potential to affect the competitive ability of invasive species.

Keywords: invasive crayfish, agonistic behaviour, global warming, competition

This work was made possible by a grant to F.G. by the Regional Council of PoitouCharentes (France) within the "Programme Régional de Bourses de Chercheur Invité" at the University of Poitiers. It is a contribution to the Marie Curie project CHAOS "Climate change and species invasion in aquatic system: a comparative perspective".

Aquatic microcosm: Stone crayfish as a potential arena for a symbionts network

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Peoples interest in the use of freshwater crayfish reaches far back in history and intensive trade of these aquatic organisms began as early as in the mid-1800s. At the same time also research on potential consummation risks caused by crayfish pathogens began. Apart from human-related aspects of research the occurrence of various metazoan organisms living epibiotic on freshwater crayfish was reported in several studies. Among these in Europe, North America and Asia the family of *Branchiobdellidans* is most popular. Formerly being considered as parasites there is today a broad consensus on their role as epicommensals feeding mainly on the "Aufwuchs" of the exoskeleton. Though occasionally observed, the occurrence of the species *Hystricosoma chappuisi* (Michaelsen, 1926), a small polychaete living on freshwater crayfish, is only scarcely described. Reports originate predominantly from Eastern Europe. In the course of a study on stone crayfish (*Austropotamobius torrentium* Schrank, 1803) in the federal state of Vorarlberg (Austria) and adjacent regions we observed *H. chappuisi* in most of the running waters together with up to four branchiobdellidan species. Biotic and abiotic requirements of the species are discussed. Moreover morphological analyses showed bacteria being socialized with the polychaete. With the presented results our aim is to 1) report the occurrence of *Hystricosoma chappuisi* in the Alps for the first time 2) discuss potential interactions between the different taxa and 3) propose that stone crayfish must not exclusively be seen as a threatened flagship species inhabiting European running waters but should also be considered as a "mobile aquatic microcosm".

Keywords: Freshwater ecology, Polychaeta, *Hystricosoma chappuisi*, *Branchiobdellida*, aquatic conservation, ultrastructure

Thermotolerance of *Euastacus sulcatus* (Decapoda: Parastacidae)

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Euastacus is the largest of the 11 Australian freshwater crayfish genera, with 50 species described, many of which inhabit cool montane headwater streams. *Euastacus* is the World's most endangered group of crayfish with ~80% of species in IUCN threat categories. Climate change has been identified as a serious threat; however the thermal repertoire of any species of *Euastacus* has not been studied.

This study aimed to determine the thermotolerance of *Euastacus sulcatus* Riek, an abundant montane species endemic to Central-Eastern Australia. Forty crayfish were collected from varying altitudes throughout the species' distribution and were exposed to steadily increasing temperature in the laboratory, and thermotolerance was assessed using righting-response.

Behavioural response to heat stress (e.g. lethargy) was evident at ~25°C and a complete loss of righting-response (i.e. maximum thermotolerance) at ~27°C, a high temperature given this species has long been regarded as dependent on "cool" habitat. The thermotolerance exceeds, by several degrees, typical peak annual water temperature in the field and suggests other factors such as inter-specific competition with larger Euastacus and a decrease in fitness due to sub-lethal thermal stress may influence the lower-altitude limits of this species. While the righting-response method is straightforward and results are unambiguous, it is unsuitable for use on rare and endangered crayfish as subsequent fatalities may occur in experimental animals. A less intrusive, non-lethal method of tissue-based physiological and biochemical responses to thermal stress is being developed. The thermotolerance of *E. sulcatus* and ongoing thermotolerance studies on other species of *Euastacus* species will be discussed.

Keywords: *Euastacus sulcatus*, thermotolerance, environmental temperature, stress, *Parastacidae*

The importance of using multiple data types in crayfish systematics

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Crayfish have been recognised as exhibiting morphological convergence as well as both morphological conservatism and plasticity, for a range of structures in various situations. This forces taxonomists to decide on the relative taxonomic merit of often-complex morphological structures when attempting to accurately delineate species. It is widely (though far from universally) recognised that DNA sequence data can be used to suggest species boundaries, which is a line of evidence that can be used to further test phylogenetic hypotheses based on morphology. The analysis of DNA sequence data from the burrowing freshwater crayfish genus *Engaewa* highlighted previously undetected divergent lineages (candidate species), prompting further morphological revisions. Congruence of morphological and molecular characters supported the recognition of these candidate species and identified potential diagnostic characters, however, the distribution of these 'species' did not conform to the expected geographic distribution. This paper aims to highlight the role genetic data can play in species identification, discuss the reliability of morphological characters in species delineation and show how biogeography may be used to resolve incongruence between different data types.

Keywords: *Engaewa*, molecular taxonomy, morphology, systematics, biogeography

Marmorkrebs gaining ground in Europe: the role of the pet trade as invasion pathway

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The pet trade is a novel and major pathway for alien crayfish introductions into Europe. The Marmorkrebs (*Procambarus fallax f. virginalis*) is an ideal model-organism to assess the dynamics of this introduction pathway because the species was never present in the European food trade or in aquaculture, which leaves little doubt that all records of free-living individuals originate from introductions from aquaria. Here, I summarize the results of two recent studies that elucidate 1) the determinants of crayfish introductions from aquaria and 2) the spatial and temporal dynamics of Marmorkrebs records in Europe. The results clearly indicate that propagule pressure from the pet trade is the major driver of the establishment success of Marmorkrebs. Marmorkrebs entered the European pet trade in the mid-1990s and became one of the most popular aquarium species in the early 2000s. The proliferation of Marmorkrebs as pets likely increased the propagule pressure and thereby the likelihood of both introduction and establishment in nature. A high propagule pressure serves to overcome negative forces that are spatially structured, such as an unsuitable habitat at the site of introduction. For instance, Marmorkrebs failed to establish in cool, rapid-flowing brooks but eventually invaded lentic habitats. Thirteen of the fifteen European Marmorkrebs records were only discovered within the past four years, suggesting a considerable lag between introduction and detection. Five of the six known populations are located in Germany, where Marmorkrebs propagule pressure has presumably been highest.

Keywords: non-indigenous species, invasion pathway, propagule pressure, aquarium trade

Using genetic data as a catalyst for taxonomic revision

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The taxonomy of Australian freshwater crayfish is continuously being reviewed and revised, with more species being described each year. While morphological examination and comparison is traditionally the catalyst for new species to be recognised, the use of genetic techniques to elucidate the presence of diversity is ever-increasingly coming to the fore. Because of the relatively poorly studied nature of the Australian freshwater crayfish, coupled with the comparative speed and simplicity of genetic analyses, it is reasonable to suggest that genetics should perhaps take a more prevalent role in the field of taxonomy. This study originally used genetic techniques to identify cryptic diversity, primarily for conservation management purposes for the swamp crayfish *Tenuibranchiurus glypticus*, however, the levels of diversity found within this species were so large that it prompted further genetic and morphological examination. As a result, where there was only one previously recognised species, there may in fact be two distinct genera, each with at least two species, supported both morphologically and genetically. If not for the genetic review of *Tenuibranchiurus*, the morphological examination, and therefore taxonomic revision, may never have been undertaken. This is just one example of how genetics can assist the traditional morphology-based taxonomic approach to recognise previously overlooked variation and potentially new species.

Keywords: *Tenuibranchiurus*, taxonomy, genetics, morphology

KEYNOTE LECTURE

The legend of the crayfish plague

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Fungal and fungal-like pathogens are responsible for some of the most severe mortalities occurring in wildlife. During the last decades, natural populations of endangered animal species have experienced an increasing number of fungal infections. Some of the responsible pathogens are associated with the first ever-documented species extinction events caused by infection and represent increasing rates of biodiversity loss (Fisher et al., 2012). Probably, one of the most eloquent cases is the so-called crayfish plague caused by the fungal-like organism *Aphanomyces astaci* (Oomycetes). This pathogen is currently considered among the one hundred world's worst invasive species (http://www.issg.org), and has destroyed the majority of the indigenous populations of freshwater crayfish in Europe (Unestam, 1972; Edgerton et al., 2004, Diéguez-Uribeondo et al., 2006; Phillips et al., 2008).

The rapid and devastating effects of crayfish plague and the massive destruction of dense indigenous populations of crayfish have resulted in a number of general beliefs and myths of this disease throughout Europe. All together constitute what we have called "The Legend of the Crayfish Plague". On the other hand, slowly but efficiently, crayfish plague has become one of the best-known invertebrate diseases. Recent advances in developmental biology, cell biology, molecular taxonomy and phylogeny, genomics and development of molecular tools for identification of *A. astaci* is allowing us a better understanding of the crayfish plague behavior.

Past and current knowledge still remain overlooked or ignored by general public and also people involved in fisheries and management of freshwater ecosystems. This is causing great problems when trying to implement conservation actions. Thus, the aim of this key-note lecture is to contrast actual scientific knowledge on this disease to the content of the Legend. Through this comparison, we intend to present an update of the actual knowledge, recent developments, and innovative new technologies on crayfish plague and recommend how this information can be applied to conservation and management programs.

Distribution and biometry of native and alien crayfish in Trentino (Italian Alps)

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The distribution of native and alien crayfish populations in Trentino (Italian Alps) is poorly known and for this reason a first survey was carried out in 2010-2011. Based on historical information and recent grey literature, 46 freshwater sites were selected and surveyed. For each surveyed site and sampling date we recorded environmental parameters such as presence of refugia, type and extension of riparian vegetation, aquatic vegetation, substrate composition, and water physico-chemical parameters. Crayfish were collected with different methods according to the habitat (lakes, streams) and all individuals were measured, sexed and weighted. Variation of density and structure of crayfish populations were recorded in spring, summer and early autumn, to assess the distribution, density and seasonal dynamics of the two species. Sixteen of the investigated sites hosted crayfish populations, 12 with *Austropotamobius italicus* and 4 with *Orconectes limosus*. *A. italicus* was found in small, isolated streams and in one small isolated pond. *O. limosus* was found only in four lakes, one of which invaded only in 2011, where it replaced a previously existing population of *A. italicus*. Infestation by the parasite *Thelohania* was registered in some populations of *A. italicus*. The extinction of eleven native populations of *A. italicus* was attributed to the spread of the alien species and to the related transmission of their specific parasite *Aphanomyces astaci*, and to habitat modifications. The environmental survey allowed identifying potential refuge sites for the conservation of the native species, and the most likely future diffusion pathways of the alien species.

Keywords: *Austropotamobius italicus*, *Orconectes limosus*, crayfish distribution, invasive pathways, biometry

Assessing density, growth and substrate preference in juvenile signal crayfish using enclosure traps

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Recruitment plays an important role in development of crayfish populations, but sampling juvenile crayfish can be difficult since they do not enter baited traps. In an attempt to create a simple passive sampling tool for juvenile crayfish, the enclosure trap has been constructed earlier,. The aim of this study was to (a) collect data on growth, density and substrate preference of juvenile crayfish in the studied population, (b) assess the usefulness of the enclosure trap as a sampling tool, and (c) to provide users with guidelines on its use. Traps were set and emptied on several occasions during the summers of 2010 and 2011 in Lake Erken, in central Sweden. Both years showed an expected decrease in abundance of juveniles from first to last catch, but many more juveniles were caught in 2010. At any given date juveniles were larger in 2011, probably due to earlier hatching resulting from higher temperatures. The density of crayfish juveniles was low on silt and sand. Instead they preferred gravel and/or rocks as found in earlier studies. We summarize notes on the use of the trap, including number of traps, timing and number of samplings necessary for different sampling purposes. We believe that with the guidelines provided the enclosure trap is a useful tool in sampling juvenile crayfish.

Keywords: signal crayfish, juvenile, sampling, abundance, growth, substrate

Do the native noble crayfish and introduced signal crayfish occupy similar trophic niches in small boreal lakes?

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The North-American signal crayfish is an introduced species that has become widespread throughout Europe, including Finland, where it has often replaced the native noble crayfish (*Astacus astacus*), threatening the stability of Finnish lakes.

This study aimed to compare the trophic niches of the introduced signal crayfish and the native noble crayfish in small to medium sized boreal lakes using stable isotope data of carbon and nitrogen. We studied 8 lakes with signal crayfish and 8 lakes with noble crayfish. For each lake, stable isotope analyses were made from samples of crayfish as well as from their potential food sources.

We used mixing models to analyse the stable isotope data to quantify the food sources used by the two species of crayfish and to describe their respective feeding niches. We also compared niche widths between the two species considering the same basal resource isotope signature found in every lake.

Our data suggest that signal crayfish and noble crayfish populations do not show differences in within-lake niche widths, although there were indications of signal crayfish having a somewhat wider overall niche at the species level. Nevertheless, isotope niches of the two species strongly overlapped suggesting that the introduced species essentially uses the same resources and hence occupies a trophic niche very similar to that of the native species. This inference is also supported by the fact that the estimated proportions of basal resources (profundal, littoral and terrestrial) used by crayfish varied considerably among individual populations but did not differ consistently between species.

Keywords: noble crayfish, signal crayfish, stable isotopes, trophic niches, food sources

Distribution and conservation statues of South Carolina Crayfishes

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Spatial distributions of South Carolina crayfishes were geo-referenced for 3400+ museum and collection records. Collection site records are not evenly distributed across the state; some parts of the state are poorly represented while other parts have a disproportionate number of collection records. Currently 37 described species in five genera occur in the state of which six are endemic species and one an introduced species. *Cambarus latimanus* and *Procambarus troglodytes* were the two widest distributed species while the four *Distocambarus* species have the narrowest distributions. Most of the records of *Cambarus* were from above the fall line whereas *Procambarus* records occurred mostly below the fall line; the exceptions were for the primary burrowers *C. diogenes* and *C. reflexus* and the stream dwelling *P. spicatus*. Crayfish species were rarely restricted to single river systems or watershed units. The 22% of South Carolina crayfish species considered imperiled include all four of *Distocambarus* species, two *Cambarus* species and one *Procambarus* species. Several taxonomic problems exist for the state crayfish fauna and the conservation status of new species descriptions and state records require evaluation. Conservation status of crayfish will be discussed within watersheds and protected lands spatial scale context.

Keywords: crayfish distribution, conservation status, South Carolina

Synchronization of molting in the noble crayfish *A. astacus* by daily and lunar light cycles

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Temporal molting patterns of the noble crayfish *A. astacus* were studied in crowded groups, which were subjected in the laboratory to both a daily light-dark cycle (LD 16:8) and an artificial moonlight cycle. About 90% of molts occurred during the L-phase of the daily cycle with a maximum at noon. Furthermore, molts were not distributed equally over the artificial moonlight cycle. There was a clear maximum in molt frequency in the middle ("new moon") between successive periods of moonlit nights ("full moon"). To exclude that the observed lunar synchronization of molting is produced by any factor other than light, experiments with phase-shifted artificial moonlight cycles are in progress.

Successful synchronization of molting among crayfish would have several advantages in aquaculture: First of all, it would reduce cannibalism, which is a main source of mortality in crowded groups. Moreover, as crayfish reduce their food intake a few days before molting, the feeding regime can be optimized when animals molt synchronously. Ultimately, animals in similar phase of the molting cycle become available for experimentation.

Keywords: *A. astacus*, synchronization of molting, daily pattern, lunar pattern, artificial moonlight cycle

A method for long-term environmental temperature monitoring in studies of freshwater crayfish.

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Increasing environmental temperature was identified as a potential threat to freshwater crayfish over 20 years ago, in particular montane and/or cool-adapted species. Recent assessments for the IUCN Red List identified that direct and indirect effects of climate change pose serious threats to many, particularly restricted-range, species. Also highlighted was a lack of data, and that the effects of climate change should be a research priority. This lack of data hinders conservation initiatives, as some government departments do not recognise climate change as a threat due to a lack of information demonstrating that climate change is "currently impacting populations". There are numerous indicators of climate change (e.g., changes in rainfall, riparian vegetation assemblages) but most are impossible to monitor without substantial costs, particularly in the case of species in remote areas. In the context of freshwater crayfish, we identified, 1) the most logistically realistic and biologically useful indicators are simple water and ground (i.e., burrow) temperature (i.e., environmental temperature), and 2) a long-term monitoring method that would be feasible to achieve this. Over the last 2 years we have developed and field-tested a straightforward, inexpensive method of monitoring environmental temperature: the method uses commercially available equipment, is suitable for deployment in areas prone to episodic floods, and importantly, as data can be collecting at ~3-yearly intervals, it is suitable for long-term studies, over wide areas, and at remote sites. We outline our method, discuss the equipment used and costs involved, and present the data from our first 2 years of monitoring.

Keywords: Climate Change, environmental temperature, long-term monitoring

Crayfish in the European Alps: Distribution, threats and future challenges

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Mountainous regions, like the European Alps, are hotspots of biodiversity and due to a complex mosaic of specific habitats harbour many specialized plants and animals. Although European crayfish diversity is generally low compared to other continents, three out of five native crayfish species have a natural occurrence in the Alps. Nevertheless, crayfish distribution is limited in the Alps, due to the location of many freshwaters at high elevations, steep environmental gradients and a relatively cold climate throughout the mountain range. At the same time, crayfish are among the most widely introduced species in the Alpine countries. The aim of this study was i) to review the historical and modern crayfish distribution in the Alps, ii) to comment on actual threats and iii) to define appropriate recommendations for the protection and/or wise management of indigenous crayfish populations.

Although the distribution of crayfish in the Alps has been significantly modified by humans, populations of indigenous crayfish species have been recorded from many Alpine countries. The suitable crayfish habitats are mainly found in the valleys, where also human settlements and their urban, industrial and agricultural areas are located. In the past, human activities promoted the spread of crayfish as food in the Alpine region and historical notes showed that crayfish were also found and harvested in remote Alpine areas. Although, protected in many regions, most of them are threatened by several reasons and a steady decrease has been observed. Here we assessed how well landscape metrics can explain the distribution of indigenous crayfish species. We particularly considered potential anthropogenic impacts and other threats (non-indigenous crayfish species) being responsible for the condition of crayfish populations. Threat intensity and history analysed on a catchment and habitat scale was explaining population quantities and qualities of the individual species in selected regions. Future challenges will include catchment-scale risk assessments, in order to identify threats and to guide effective action plans.

Keywords: *Astacus*, *Austropotamobius*, aquatic conservation, management, environmental protection, species action plans

STURE ABRAHAMSSON MEMORIAL LECTURE

Crayfish as global invaders: world distribution, impact on human wellbeing, and possible 'cures'

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There is a long history of intentional or accidental introductions of crayfish. During the last few decades, however, the human-aided movement of nonindigenous crayfish species (NICS) has increased as a result of the exponential growth in the volume of international trade and diversification of pathways of introduction. Ten and 21 crayfish species have been introduced outside their native ranges between and within continents, respectively, but most introductions are of six species now widely diffused in the world (*Astacus leptodactylus*, *Cherax destructor*, *Orconectes limosus*, *O. rusticus*, *Pacifastacus leniusculus*, and *Procambarus clarkii*); one of them, *P. clarkii*, accounts for over 40% of all introduction events recorded (56). Global introductions, particularly for aquaculture and ornamental purposes, have begun to homogenize naturally disjunct distributions of crayfish families.

The purpose of this presentation is twofold.

First, after having described the current world distribution of NICS and the motives of their introduction, the impacts on ecosystem services of the most diffused ones (i.e. *P. leniusculus* and *P. clarkii*) will be analyzed, including the loss of provisioning (e.g. reductions in edible native species), regulatory (e.g. lethal disease spread), supporting (e.g. changes in ecological communities), and cultural (e.g. loss of festivals celebrating indigenous crayfish) services. Second, I will discuss recent research advances in both predicting invasiveness of nonindigenous crayfish and improving control/eradication of the invasive ones, which, if applied, have the potential to reduce future damage to human wellbeing.

Keywords: nonindigenous crayfish, distribution, ecosystem services, predictions, control/eradication

Isolation of microsatellite markers in *Aphanomyces astaci*: new perspectives for the identification of pathogen strains in infected crayfish

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Aphanomyces astaci, the oomycete carried by North American crayfish, is responsible for outbreaks of crayfish plague in native European crayfish. Until now, the identification of strains was based on the analysis by random amplification of polymorphic DNA (RAPD) studies from pure pathogen cultures. In this study, we developed microsatellites markers from pure DNA of B strain isolated from *Pacifastacus leniusculus*, and tested their variation in representatives of other genotype groups of known for this pathogen. Fourteen loci chosen for further analyses, which are in most cases specific for *A. astaci* and do not cross-react with other oomycete species commonly found in crayfish, distinguish all 5 genotypes groups of *A. astaci* as identified by RAPD, with the presence of several diagnostic alleles. The application of these molecular markers on DNA isolated from native crayfish individuals infected during plague outbreaks (from *Astacus astacus*, *Austropotamobius torrentium* and *A. leptodactylus*) confirmed the suitability of microsatellites in assessing variation of *A. astaci* directly in host tissues. Finally, preliminary test on DNA from North American crayfish infected by the pathogen show that this methodology was also efficient for hosts with moderate to high agent levels. These molecular markers offer new perspectives in studying of diversity, sources and dispersal pathways of the crayfish plague pathogen.

Keywords: crayfish plague, *Aphanomyces astaci*, microsatellites

Genetic diversity and population structure of noble crayfish (*Astacus astacus* L.) in Northern and Central Europe inferred from microsatellite markers

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This study represents the first large scale population genetic survey of the most common and the most highly appreciated freshwater crayfish in Europe, the noble crayfish. We focused primarily on the Baltic Sea area (Estonia, Finland and Sweden) where the largest portion of the remaining populations exists and where a main responsibility for the species' conservation thus resides. To allow comparisons, samples from the Black Sea catchment (Germany and the Czech Republic, Danube drainage) were also included. A total of 633 specimens from 18 locations were analysed by using 10 newly developed microsatellite markers. Two highly differentiated population groups were identified corresponding to the Baltic Sea and the Black Sea catchments, respectively. The Baltic Sea catchment populations had significantly lower genetic variation and private allele numbers than the Black Sea catchment populations. These results are in accordance with the results of recent mtDNA study and can be explained by founder effects and population size bottlenecks associated with post-glacial re-colonization of formerly glaciated northern areas in Europe. Within the Baltic Sea area, a clear genetic structure was revealed with population samples corresponding well to their geographic origin, suggesting little impact of long-distance translocations. Only a few exceptions to this pattern (two outlier samples) were found, that either reflect undocumented translocations or presence of additional, not yet identified, within-country genetic heterogeneities. The clear genetic structure strongly suggests that the choice of stocking material for re-introductions and supplemental releases needs to be based on empirical genetic knowledge.

Keywords: *Astacus astacus*, genetic differentiation, population structure, microsatellite DNA, conservation genetics

Crayfishes of Manitoulin Island

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The distribution of crayfish species found on Manitoulin Island in Lake Huron, Ontario, Canada was documented. The major water bodies on the island were surveyed using hand and net sampling over a period of three days in 2007 and again over two days in 2012. The island is geographically unique as it is considered a refugium, similar geologically to regions located in more southern regions of Ontario. Overall, fourty eight sites were sampled on the island, as well as smaller adjacent islands and mainland. *Orconectes propinquus* and *Orconectes virlis* are the two native species found on the Island. *O. propinquus* was found alone in sixteen locations while *O. virilis* was the only species at seven localities. In addition, the two species were in sympatry in fourteen other locations. The introduced species *Orconectes rusticus* was found in eight localities of which one was shared with *O. propinquus*. All three species were also present on the adjacent mainland to the north of the island. Crayfishes of the genera *Cambarus* and *Fallicambarus*, which can be found in other parts of Southern Ontario, were not found on the island. The significance of the overall distribution is discussed in terms of past surveys, as well as the present and future impact of the spread of the introduced *O. rusticus* in term of model scenarios of possible spread in Southern Ontario and Quebec.

Key Words: *Orconectes*, Ontario, distribution, invasive species.

Ultrastructure of sperm of *Pacifastacus leniusculus* using cryo-scanning and transmission electron microscopy

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In order to study ultrastructure of spermatozoon of signal crayfish, we obtained fresh males spermatophores by means of electrical stimulation and prepared them for observation with cryo-scanning and transmission electron microscopy. The acrosome complex was located at the anterior of the sperm cell and was divided into two main parts: (1) the main body of the acrosome, consisting of two shoulders and arms organized into three layers of differing electron densities with extended parallel filaments and (2) the sub-acrosome zone occupying the central area of the acrosome complex, with two separate electron-dense parts.

Each radial arm consisted of microtubules located with no specific symmetry and extended along the axes of the radial arms in parallel bundles. The nucleus contained fibers and dense granules. Extensions of radial arms penetrated into the nucleus and were observed as parallel networks of microtubules within granular material of the nucleus. An obvious membrane separated the nucleus from the acrosome and sub-acrosome zone. The tegument of the spermatozoon, which contained the nucleus, acrosome, and radial arms, comprised at least two membrane layers. The spermatozoon ultrastructure of signal crayfish is compared with other species.

Keywords: signal crayfish, cryo-scanning electron microscopy, spermatozoon

Occurrence, distribution and demographic composition of the noble crayfish, *Astacus astacus*, in the Upper River Kamp System (NW Lower Austria)

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The River Kamp System is the second largest left-side tributary to the Danube. The Kamp drainages the „Waldviertel" (wood quarter) in NW Lower Austria, and the bedrock is composed of crystallin. The hilly and diverse landscape is a rural, quiet area with only small villages.

The Upper stretch of the River Kamp System was mapped for the occurrence of freshwater crayfish by the first author. The stream order in the Upper Kamp ranges from 1 – 6, and the rivers of the study area are the following: 1.) Kleiner Kamp, 2.) Großer Kamp (after their confluence „Kamp"), 3.) Purzelkamp, 4.) Zwettl.

Totally, 96 sites were visited (mostly in 2011) many oft hem for several times. Noctural torching, hand catching, and trapping were combined. Several a-biotic characteristics were noted. From these, stream order, slope, substrate composition in the bed and on the banks (natural or artificial), current velocity (slow, medium, fast; homogenous or heterogenous flow pattern), and the land use (forest, meadows, farming, villages, wetlands) were the most important ones.

At 81 sites no crayfishes were recorded. Fifteen sites were found to be inhabited by crayfish: 4 with noble crayfish (*Astacus astacus*), 11 with signal crayfish (*Pacifastacus leniuscus*).

At the individual level, the situation looks a little bit better for the IC: 32 *A. astacus,* compared with 49 *P. leniusculus*. Noble crayfish were found with eggs/embryos, which confirms that they still reproduce successfully. At the best site, 27 specimens of the IC were counted. The numbers are, of course, underestimated, as very small juveniles could not be caught and conted. Average *A. astacus* males had a length of 102 cm (47 g), *P. leniusculus* males had a length of 96 cm (44 g). Values for females are as follows: 99 cm (35 g), and 95 cm (34 g), for *A. astacus* and *P. leniusculus*, respectively. Before putting the animals back, a number of body variables were estimated. Noble crayfish are larger (not significantly) in the Upper Kamp System than signal crayfish.

An interesting fact is that most of the stream sites with crayfish occurrences were found downstream of ponds, where water temperatures were usually warmer. Signal crayfish were not found to burrow, but it was astonishing enough that they also occur in artificially modified streches, which is not the case for the IC. Crayfish plague outbreaks were not detected during the study period.

Although NICS are dominating by occurrence (sites) and number, reproducing ICS still can be found. Special conservation efforts are needed not to loose our noble crayfish populations. The best site with 27 *A. astacus* specimens caught requires our special focus.

Efficient cooling decreased post-harvest boat transport mortality of the commercial crayfish catch

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The post-harvest boat transport mortality was studied and boat transport methods were developed in an applied project during summers 2010 – 11 in Lake Saimaa, Finland. The transport and ambient conditions during trapping were first monitored in 2010 to evaluate factors behind catch mortality during transports and holding in commercial depots. It was discovered that the crucial factors were air temperature during transport and water temperature during trapping, indicating that boat transport stress would be an issue. To minimise boat transport stress and mortality, several transport methods were tested the following summer 2011. The commercial signal crayfish (*Pacifastacus leniusculus*) catch was monitored during boat transport over a whole crayfishing day and a rapid cooling method was compared to existing conventional boat transport methods. It was discovered that the cooling of the catch rapidly from ambient temperature, +20 – +26˚C, to +5 – +7˚C would eliminate mortality and enable a 100% survival of the graded, prime quality, healthy catch. The improvement achieved was estimated as being up to 10% compared to conventional transport methods. The improved method includes cooler boxes equipped with -20˚C cooling units on the bottom, a mesh to prevent crayfish from freezing and a plastic bag to ensure cool and moist environment during the transport.

Keywords: Post-harvest handling, signal crayfish, mortality

Specific features of cardiac responses of crayfish *Pontastacus leptodactylus* Esch. to non-toxic and toxic stimuli

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In numerous studies it was revealed that animals express significant individual variations in physiological responses to toxic exposure, which reduce its application in interpretation of ecotoxicological experiments. This fact could be explained by differences in tested organism's physiological status. The aim of the study was investigation of possibility for using cardiac activity characteristics of crayfish *Pontastacus leptodactylus* Esch. as markers of their physiological state in norm and under toxic environment. Authors suggested specific approach with the use of functional load to assess crayfish status, based on heart rate monitoring, which provided to divide test-organisms into groups in accordance with their physiological status. Some of the criteria and test-stimuli as an addition to previously suggested (Kuznetsova et al., 2010) for such selection are determined. Methodological approach based on standardized functional load proposed in the study could be applicable in selection of reference group of organisms before toxic exposure. The importance of using organisms from reference group as biosensors in bioelectronic systems of surface water quality control is discussed. An influence of oil product on the crayfish (*Pontastacus leptodactylus* Esch.) heart rate was conducted on the reference group organisms. It was shown that crayfish responded to pollution level of 33 mg/l crude oil suspension by sharp increase of used physiological state markers – heart rate and stress index.

Keywords: crayfish, physiological state, circadian rhythm in heart rate, antiortostatic suspension, oil exposure

A national inventory of freshwater crayfish in the Netherlands in 2010

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In late summer 2010, about 200 volunteers contributed to a national crayfish survey with the following goals: mapping the distribution of crayfish, defining the ecological niche of a long established crayfish species (*Orconectes limosus*) and investigating the effects on water quality and physical structure of the habitat by recently entered species, in particular *Procambarus clarkii*. In order to relate crayfish data with environmental data, already existing sampling sites of the waterboards (regional water authorities) were used, provided by the "Limnodata Neerlandica". The Limnodata Neerlandica is a national database which contains quality measurements of all waterboards of the last three decennia. Through a website, volunteers were able to apply for one of the preselected sampling sites from this database.

A total of 294 sampling sites, distributed throughout the Netherlands, were sampled. Crayfish were caught at 30% of the sites (n=89), divided among four species: *O. limosus*, *O. virilis*, *P. clarkii* and *Astacus leptodactylus*. Although many new sites with crayfish were found, the overall distribution as known prior to the inventroy didn't changed for any of the four species. However, the study provided the first overview of sites of where can be assumed that crayfish do not occur with a high level of certainty.

The spiny cheek crayfish was not found in waters with a pH<6.4 and hardly any crayfish were found in waters with a chloride content higher than 300 mg/l. No significant relations were found between recently established crayfish species and changes in water quality parameters.

Keywords: Netherlands, Limnodata Neerlandica, crayfish, *Orconectes limosus*

How does choice of analysed material affect molecular detection of the crayfish plague pathogen in North American crayfish?

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North American crayfish potentially carry the crayfish plague pathogen A*phanomyces astaci*. Knowledge if their populations are infected is important for conservation of native European crayfish, and for studies of biology of this important oomycete invader. Molecular methods, which are in continuous development, seem the only efficient approach for routine testing of its presence in American crayfish. A key part of any such protocol is the choice of most suitable material for pathogen detection. We used parasite-specific real-time PCR targeting the ITS region in *A. astaci* genome to assess three factors: tissue selection, analysis of various melanised spots, and testing small individuals. We analysed various subsamples from adult *Orconectes limosus* and *Pacifastacus leniusculus*, and mixed-tissue samples from small-sized *O. limosus*. From 22 adult *O. limosus* found infected, 20 contained parasite DNA in limb joints, 19 in soft abdominal cuticle, 18 in tail fans, 16 in pleopods, but only seven in eye stalks and five in gills, suggesting that the last two tissues are inappropriate for pathogen detection. Large (> 2 mm) black heavy melanised spots observed in *P. leniusculus* from Finland and Hungary tested for *A. astaci* mostly positive (80 % from 29 spots), unlike small (< 2 mm) reddish to blackish spots usual in *O. limosus* positive only in 36 % from 45 samples. Thus, manifestation of infection seems species-specific and black spots should be included among tested tissues. Parasite detection in 14 small (21 to 49 mm) *O. limosus* showed that even these individuals may carry *A. astaci*.

Keywords: invasive crayfish species, *Aphanomyces astaci*, melanin depositions, body parts, crayfish size, real-time PCR

Aquaculture of *Astacus astacus* in a recirculating aquaculture system and the effects of lighting on early juveniles

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Several studies have investigated physiological mechanisms in light perception and the entrainment of circadian rhythms in freshwater crayfish. Much less information is available on the possible benefits of lighting for crayfish culture, although its direct and indirect effects have received considerable attention in aquaculture of fish. Present studies on different light regimes in crayfish culture largely neglect their possible benefits on growth of periphyton, despite this food source – and associated invertebrates – constitute an important element in the diet of adult omnivorous crayfish and may even be of greater importance for early juveniles.

We studied the effect of different lighting methods (natural sunlight, fluorescent light tubes, LED lighting, no lighting) in the intense rearing of juvenile noble crayfish *Astacus astacus*. The study was performed in a recirculating aquaculture system consisting of 12 circular tanks (3.125 m^2×0.8 m). 6000 artificially incubated stage 2 crayfish were stocked into the RAS at densities of 160 Ind/m^2. Animals were fed a formulated diet for crayfish two or three times a week, depending on water temperature and moulting stage. Periphyton growth in each tank was assessed after four and eight weeks, when the experiment was terminated. Mortality, growth rate and stable isotope ratios of crayfish were affected by lighting regime and differences can partly be explained by periphyton characteristics. Our results illustrate the potential of periphyton as an additional food source in indoor aquaculture of crayfish and show how its growth can be promoted by novel lighting techniques.

Keywords: artificial lighting, benthic algae, noble crayfish, irradiance,

Molecular systematics of *Procambarus* (*Austrocambarus*) in Yucatán Peninsula, Mexico: An aproximation to the subgenus diversification

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México is distinguished for its number of endemic crayfish species. Many interesting diversification processes resulted in a variety of forms but also, in some cases, in a problem to determine species. Subgenus *Austrocambarus* is an interesting cambarid group with a distribution range in the center and southeast of the country, dominating Yucatán Peninsula with the occurrence of three species: *Procambarus llamasi*, *Procambarus pilosimanus* and *Procambarus maya*. The complexity of the group lies in the wide variation of its forms, and the difficulty to distinguish intraspecific limits to determine species and distributional ranges. Using an integrative method, this study examines the cambarids species composition of Yucatán Peninsula to resolve the species identity, define their distributional ranges, and to contribute to increase the data base to the establishment of phylogenetic relationships of Mexican crayfish. We captured crayfishes from places previously recorded for cambarids and new places of occurrence. Environmental data were registered to determine types of habitat. Based on morphological and molecular data, the phylogenetic relationships were estimated. The results suggest the species composition of Yucatán Peninsula include at least three species additional to those previously recorded. Speciation processes and diversification hypothesis of the group are presented.

Keywords: Mexican crayfish, *Procambarus* (*Austrocambarus*), molecular systematics, Yucatán Peninsula

Biogeography of freshwater crayfish from Nautla river basin, Veracruz and Puebla, Mexico

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[2]quot;Decapodos, un mundo en diez patas" (Decapods, a world in ten legs" Museum

The Nautla river basin is an interesting water system, which, by its complex orographic structure presents many springs, streams and tributaries that harbor a high diversity of crayfish species, which has not yet been properly explored. Few studies known to this basin have been made in the region adjoining the states of Veracruz and Puebla, in the municipalities of Martínez de la Torre, Ver., Tlapacoyan, Pue. and Teziutlán, Pue., where three endemic species of crayfish, *Procambarus* (*Ortmannicus*) *gonopodocristatus*, *Procambarus* (*Villalobosus*) *tlapacoyanensis* and *P.* (*V.*) *teziutlanensis* are known. This work presents the results of a more comprehensive study of the region, showing new distribution records of the subgenus *Villalobosus* and new findings of the subgenera *Ortmannicus* and *Austrocambarus*.

Keywords: mexican crayfish, *Austrocambarus*, *Villalobosus*, *Ortmannicus*, biogeography of Nautla river basin

Differences in the virulence of crayfish plague (*Aphanomyces astaci*) strains confirmed with infection trials

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Several reports of European crayfish species carrying a latent infection of crayfish plague (*Aphanomyces astaci*) have been published lately. The discussion has concerned especially the virulence of As-genotype that seems to cause decreased mortality. The aim of this study was to compare different *A. astaci* strains with varying zoospore densities in controlled infection experiments. First, noble crayfish population from Lake Viitajärvi (Tervo) was infected with *A. astaci* strains from River Kemijoki, Lake Kivesjärvi (both As-genotype) and Lake Puujärvi (PsI-genotype). Second, Lake Mikitänjärvi (Hyrynsalmi) noble crayfish were infected with the *A. astaci* strains described above. Third, Lake Viitajärvi crayfish were infected with Lake Kivesjärvi and Lake Puujärvi *A. astaci* strains with different dosages (1, 10, 100 and 1000 zoospores ml^{-1}) of zoospores. The results confirm that PsI-genotype strain from Lake Puujärvi is highly virulent and kills the crayfish within few days. As-genotype strains caused the infection more slowly, and the mortality rate was often less than 100 %. Our results confirm the variance of virulence among *A. astaci* strains within the As-genotype. The mortality rate was also dose-dependent, i.e. dependent on the number of zoospores.

Keywords: Crayfish plague, *Aphanomyces astaci*, noble crayfish, genotype, virulence, zoospore density

Comparative karyological analyses of a parthenogenetic crayfish: The Marmorkrebs *Procambarus fallax* (Hagen, 1870) *f. virginalis* (Decapoda: Cambaridae) is a triploid organism

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The Marmorkrebs is the only obligate parthenogenetic decapod known to date. It was recently identified as a form of the slough crayfish *Procambarus fallax* from south eastern USA. Due to its reproduction mode Marmorkrebs has become a popular laboratory model organism. However, several aspects related to parthenogenesis are still unknown in this crayfish. Parthenogenesis is often linked with polyploidy. For this reason, we studied the number of chromosomes in Marmorkrebs, bisexual *P. fallax* and the closely related. *P. alleni*. During our investigation we faced two problems: (1) crayfish possess a large number of very small chromosomes, which are difficult to identify using light microscopy, (2) there is a lack of suitable tissue containing a high cell division rate in crayfish and the normally used testes are not available in the all-female Marmorkrebs. Nevertheless, we achieved good results by using Marmorkrebs embryos. As outcome we determined a diploid set of approximately 270 chromosomes in Marmorkrebs, whereas we found 92 chromosomes in the haploid gametes from testes of *P. fallax* and 94 of *P. alleni* males. This suggests that Marmorkrebs possesses a triple set of chromosomes. It has been shown in other animals that polyploidy can have a positive effect on fitness and adaptability. Hence, our results could be an explanation for the euryoecious nature of the Marmorkrebs.

Keywords: polyploidy, triple set of chromosomes, laboratory model organism, potential invader

Multiple population crashes in Swedish populations of signal crayfish

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To be able to give recommendations for either controlling or facilitating exotic crayfish it is important to understand the factors limiting these populations. A common problem is that long-term quantitative data documenting fluctuations and/or declines are very rare. Moreover, in most cases no conclusive explanations for rapid declines are suggested. In this study we used long-term data (catch per unit effort) from multiple lakes of signal crayfish in Sweden and then tested if crashes could be explained by any ecological and/or physicochemical characteristics in the lakes and their catchments. At this stage data from 27 lakes were included in the analysis (12 lakes with population crashes) and 12 habitat variables in the lake catchments (land use, air temperatures, and precipitation). A stepwise discriminant function analysis showed that 78% of the lakes were correctly classified (i.e. as lakes with or without crashes), and that lakes with population crashes had higher summer temperatures than lakes without crashes. Furthermore, some lakes with population crashes also had a higher percentage of agriculture in the catchment. In order to improve our understanding of the factors and the mechanisms affecting population crashes in signal crayfish, the next steps will be as follows. First, more lakes will be included in the analysis as well as data on water chemistry and biotic characteristics of the lakes. Then, the role of crayfish plague infection behind these crashes will be assessed. The results of these analyses will be presented and discussed in relation to the management of signal crayfish populations.

Keywords: exotic species, fisheries, signal crayfish, population crashes, crayfish plague

Genetic variability of *Austropotamobius torrentium* (Crustacea, Astacidae) in Romania

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Austropotamobius torrentium is a crayfish species distributed mainly in Central Europe, with the Eastern limit of its areal in the Carpathian Mountains in Romania. We studied 155 individuals of *A. torrentium* from 7 populations distributed along different rivers in Southern Carpathians. Procedures for DNA extraction, microsatellite amplification and genotyping at four microsatellite loci were performed as described in Iorgu et al. (2011). The average number of alleles per locus, the observed heterozygosity (HO) and the unbiased expected heterozygosity (HE) were computed using GENAlEX. Population differentiation was estimated from FST values which were calculated using Fstat ver. 2.9.3.2 software. Significance was estimated using 10 000 permutations. Migration rates (Nm) were calculated as Nm = 1/4((1/ FST)-1).

Observed heterozygosity ranged from 0.517 to 0.635, with an average of 0.600, while the number of alleles per locus was between 2.4 and 10.0, with an average of 5.4. No significant difference was observed between HO and HE. The mean FST value was 0.265, which indicates a very high genetic differentiation between the investigated populations, while the mean migration rate was 0.749.

The observed genetic differentiation is in agreement with the biogeographic characteristics of freshwater species for which watersheds (water divides) between river drainages represent un-crossable barriers. These species are therefore restricted to river drainage systems and show little capacity for trans-watershed dispersal.

Iorgu EI, Popa OP, Petrescu AM, Popa LO (2011) Cross-amplification of microsatellite loci in the endangered stone-crayfish *Austropotamobius torrentium* (Crustacea: Decapoda) Knowledge and Management of Aquatic Ecosystems 401: 8 p

Keywords: crayfish, microsatellite, population genetics, biogeography, freshwater

Predation of invasive signal crayfish on salmonid fish eggs during long winter incubation period in large boreal lakes

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Egg mortality of autumn-spawning salmonids in boreal freshwater systems during the long winter incubation period has been observed to be very high. In the worst case, only 1.5 ‰ of the all produced eggs of vendace population (*Coregonus albula*) may hatch and deliver viable larvae. However, the annual variation is high and in many cases the ultimate causes of the mortality remains open. Invasive species may cause dramatic changes in their new habitats. Introduced signal crayfish has been suspected to increase egg mortality of commercially important coregonid species. In this study, the potential of crayfish predation on the salmonid eggs were examined by lab experiments and bioenergetics modelling. The maximum food consumption rate and functional response of crayfish predation were tested at low temperatures (>6°C) typical during the salmonid egg incubation. Bioenergetics model construction was used to quantify the potential predation rates under lake conditions. The contribution of fish eggs on the diet of crayfish was studied using stable isotope analysis. Our results reveal that signal crayfish can consume considerable amounts of fish eggs. Temperature affected feeding rates significantly so that activity and consumption declined with lowering temperature. The potential population interactions between signal crayfish and coregonids were simulated in Lake Pyhäjärvi, SW Finland.

Keywords: Coregonid, egg predation, lake, *Pacifastacus leniusculus*

First report on the phylogenetic relationship between European and Asian populations of the *Astacus leptodactylus* species complex

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The taxonomy of narrow-clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823 species complex) is not yet resolved and is still in a state of flux. Results of recent morphological characteristics comparative analyses have shown that Asian populations of narrow-clawed crayfish differ significantly from European populations. The aim of this research was to study phylogenetic relationship between European (Croatia, Bulgaria, Poland, Turkey) and Asian (Armenia, Russia) populations of narrow-clawed crayfish. Mitochondrial 16S rRNA and COI genes were partially sequenced from 37 crayfish, sampled from seven localities. Phylogenetic relationships were reconstructed using different methods of phylogenetic inference on each gene region independently as well as on a concatenated data set. A total of nine different 16S rRNA and 30 COI haplotypes were obtained. The concatenated data set included 21 combined 16S/COI haplotypes. All three implemented criteria of phylogenetic reconstruction (MP, ML and BA) yielded congruent topologies, characterised by the presence of two main geographically localized phylogroups, corresponding to Asian and European populations, separated by pronounced genetic gap. The observed genetic divergence value between European and Asian narrow-clawed crayfish phylogroups ranged from 3.33% to 4.57% for 16S gene and from 4.22% to 8.25% for COI gene. Obtained results on phylogeny corroborate with results of morphological analyses, and indicate the presence of distinct evolutionary lineages within *A. leptodactylus* sp. complex.

Keywords: narrow-clawed crayfish, COI, 16S, phylogeny

Distribution pattern of the stone crayfish in Romania is driven by karst – a consequence of the Pleistocene glaciations?

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The biogeographic distribution of a species can provide a useful insight into species history, thus extending the knowledge on ecological requirements for conservation. The stone crayfish (*Austropotamobius torrentium*) is one of the oldest freshwater crayfish in Europe. Romania is an ideal case to analyse a natural crayfish distribution, which has not been disturbed by translocations. We aim to understand the reasons that explain the restriction in the distribution of this species to the western part of the country. Randomly chosen upper river sectors were selected as sampling sites. The crayfish presence/absence and significant water and habitat parameters were recorded at 428 sites. A Geographic Information System (GIS) was used for spatial analysis. The distribution pattern was analyzed by means of a generalized boosted regression model. Our results showed that the best predictor of the species presence is the distance to the karst areas. These findings, combined with the spatial distribution data for the noble crayfish (*Astacus astacus*), enable us to formulate the hypothesis of the local karstic areas as providers of ecological niches during the Pleistocene glaciations. It is likely that the Pleistocene climate conditions led to the restriction of the stone crayfish populations to karst underground refuges, enabling species survival. After the last glaciations, the stone crayfish expanded from these refuges competing with the noble crayfish, which was also colonising most of the European river basins. Nowadays, a balance was reached, the stone crayfish being restricted to insular areas in Romania.

Keywords: *Austropotamobius torrentium*, Glacial refuge, Population history, Species distribution modelling, Boosted regression trees, GIS

Factors inducing invasive Red-Swamp-Crayfish (*Procambarus clarkii*) overland dispersion

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The ability for overland dispersion in some freshwater invertebrates can provide an important advantage over other species, when colonizing new water bodies. In this study the factors involved in red-swamp-crayfish (*Procambarus clarkii*), an exotic invasive crayfish species in Europe, overland dispersion were investigated. The numbers of individual *P. clarkii* moving out of water were monitored during a 12 month period in a rice cultivation system in Portugal. One of the main factors inducing crayfish overland dispersal was the drainage of the study area and the number of crayfish dispersing overland was inversely correlated with the water level in the rice pads. Overland dispersion was only observed immediately after the drainage of the study area and occurred until the area was reflooded due to heavy rain events. Other variables significantly affecting the overland dispersal of crayfish were the temperature, relative humidity and the period of the day. These results can be of great use for controlling and managing invasive crayfish population, especially in recently invaded areas.

Keywords: red swamp crayfish, *Procambarus clarkii*, overland dispersal

KEYNOTE LECTURE

Ecological notes on crayfish worldwide

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Decapod crustaceans play pivotal roles in some fresh waters, through their large size, mobility, omnivory and behaviour. In temperate fresh waters, dominant decapods are usually crayfish, while crabs and atyid shrimps become important in the tropics. Crayfish only extend into the tropics in North and Central America, in Madagascar and in Australasia, where they tend to live at higher altitudes than in the temperate zone. In addition to their large size, crayfish are often long-lived compared to other invertebrates, enhancing their significance in many freshwater systems. Crayfish are widespread, not just in well-oxygenated surface waters. The ecology of crayfish species also appears more individualistic than that of most other invertebrates and may be more akin to that of cold-blooded vertebrates. An organism in a new habitat must start with suitable adaptations, physical, physiological, behavioural or life-history, that allow its survival in a different biotope, and then refine these. All these specialisations have an ecological aspect. This keynote looks at four aspects:

(1) Morphological and biological features of crayfish that make them prime players in many freshwater ecosystems. Differences exist in crayfish morphology (the claws of decapods may be used for defense, digging, signalling, and in reproductive behaviour), physiology (some crayfish tolerate low levels of dissolved oxygen, breathing moist air; a few occur in brackish conditions) and life history (short or long-lived, different growth rates and final size, different reproductive strategies), all of which may allow penetration and dominance of different environments.

(2) Specific aspects of crayfish ecology as they relate to habitat and roles in the ecosystem: crayfish lack the possibility of moving to an alternate habitat for their adult stage, as do many insects. Therefore a heterogeneous habitat with refuges – safe hides for juveniles and burrows for refuge of larger forms – is important for the successful survival of different life stages of these long-lived and eventually large

invertebrates. Finally, their frequently omnivorous diet allows greater dominance of their habitat and invertebrate community.

(3) Aspects of crayfish ecology and habitat usage with a direct bearing on management and conservation of biodiversity: crayfish ancestors were probably warm-water dwelling and perhaps burrowing and their ecology had radiated out since. In Europe we associate crayfish with streams, lakes and ponds, but in other continents the group has many different biotopes and life history patterns. The major habitat groups are: cool, high quality water (streams and lakes); warm, high quality water; warm, lower quality wetlands; semi-terrestrial swamps and temporary wetlands (burrowers); and cave ecosystems. Each biotope demands different ecological and life-history approaches. In the first two, longevity and K selection may be strong drivers, while r selection may dominate the third. The fourth and fifth are habitats demanding specializations.

(4) Ecosystem roles and attributes of crayfish: these include bioindicators for communities or habitats; importance in trophic webs; keystone species; ecological engineers; indicators/surrogates for water quality. Further, they may act as umbrella species for the conservation of complex communities. Their ecology also includes threats to their well-being – one aspect of which is competition.

Finally, crayfish translocation has been widespread and successful, introducing various risks to native stocks and ecosystems. Other human mediated threats to crayfish survival include environmental degradation and climate change.

Keywords: bioindicators, decapods, ecosystem roles, freshwater crayfish, freshwater habitats, keystone species, morphological specializations, r and K life histories, translocation, umbrella species

Crayfish in Russia: a review of distribution and ecology

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Until today data about crayfish distribution in Russia is scattered or hardly available. This was obviously the reason for not including Russian data into the „Atlas of Crayfish in Europe". In Russia three native genera of crayfish are known (*Astacus*, *Pontastacus*, *Cambaroides*), additionally the alien species *Pacifastacus leniusculus* was introduced into the country. We review the knowledge about the distribution of the indigenous and non-indigenous crayfish species and summarize available ecological information. The results of this study were used to generate distribution maps for all Russian federal regions. The results of this study provide a baseline for conservation managers and policy makers for the future conservation and management of crayfish in Russia.

Keywords: Russia, distribution, maps, ecology, conservation

Crayfish plague pathogen detected in the Danube Delta – a potential threat to freshwater biodiversity in southeastern Europe

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The crayfish plague agent *Aphanomyces astaci* is the most serious threat to native European freshwater crayfish species. One of its hosts, the North American spiny-cheek crayfish (*Orconectes limosus*), extends its range in the river Danube. In a previous study we have shown that the spiny-cheek crayfish invasion front reached the territory of Romania and is slowly moving eastwards towards the Black Sea (15 km/year). Furthermore, we have shown that spiny-cheek crayfish as well as native narrow-clawed crayfish (*A. leptodactylus*) in the western Romanian Danube are carrier of *A. astaci*. The aim of the present study was to evaluate the *A. astaci* carrier status of narrow-clawed crayfish in the Danube Delta, about 970 km downstream of the current invasion front of American crayfish. Using highly sensitive *A. astaci*-specific real-time PCR we found that thirteen out of forty analysed crayfish tested positive. The pathogen seems to persist in this population as a latent infection, as no crayfish mass mortalities are known from the Danube Delta. *A. astaci* may have reached the Delta by long-range passive dispersal of infected hosts or pathogen spores, or by gradually infecting populations of native crayfish in upstream regions of the Danube in a stepping-stone manner. Alternatively, the crayfish plague may have persisted in the Danube Delta as chronic infection from an old plague wave in the nineteenth century. In any case, the presence of this pathogen in the lower Danube is a threat to freshwater biodiversity in southeastern Europe.

Keywords: *Aphanomyces astaci*, *Astacus leptodactylus*, Black Sea, Crayfish plague, Danube Delta

First large-scale genetic analysis of the vulnerable noble crayfish *Astacus astacus* in Europe

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Global changes, human impact and climatic oscillations had a severe impact onto the distribution and diversity of the vulnerable European noble crayfish *Astacus astacus*. The main aim of this study was to resolve the natural genetic structure of European noble crayfish populations and to estimate to what degree human translocations dissolved the natural genetic make-up. We sampled 500 individuals from 120 populations across Europe. We sequenced a 350 base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit I (COI) and a 500 bp fragment of 16s rRNA (16S). Furthermore, for 24 populations a microsatellite analysis was performed based on six polymorphic loci. Twenty-two COI-haplotypes and sixteen 16S-haplotypes were identified. Both genes exhibited one common haplotype across the whole study area. High numbers of private haplotypes suggested two separate glacial refugia on the western and central Balkan. In contrast, very low haplotype diversities in central Europe were detected, which could result from human translocations and/or founder effects due to postglacial re-colonization. Microsatellites exhibited strong genetic differentiation between central and eastern Europe as well as between the central and western Balkan, but little among central European populations. Despite human translocations a differentiation of noble crayfish populations in all major catchment areas was detectable. Our data thus support the establishment of distinct evolutionary significant units to protect the present-day genetic diversity of *A. astacus* in Europe.

Keywords: phylogeography, haplotype diversity, microsatellite analysis, human translocation, glacial refugia

Adaptive management of the noble crayfish (*Astacus astacus*) population in lake Steinsfjorden, Norway

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Lake Steinsfjorden is the most important noble crayfish locality in Norway. During 1979-81, a simple and non-expensive monitoring program for crayfish was developed. From the program, which includes data on total trap effort, catch statistics and population composition from test fishing before and after the fishing season, we have estimated yield and monitored population development since 1979. Based on data from the monitoring program, an adaptive management scheme was introduced for the crayfish population. It includes targeting objectives, trying out various measures to achieve these objectives, evaluate the effect of measures and replacing unsuccessful initiatives with new ones. The measures taken to achieve the objectives are hypotheses, rather than methods that lead to a specific final result. It is essential that measures are followed up by systematic observations and evaluations. The results from the monitoring program have been the basis for the County Environmental Administration when suggesting changes in the management scheme and revealing the effects.

From 1979 to 2011, total trap effort has been reduced by 80% from 182 000 trap nights to 37 000 and estimated yield has been reduced by 85% from 6.5 tons to 1 ton. Catch of legal sized crayfish per trap night during the first night has been reduced from 3.8 to 0.7. The fraction of legal sized crayfish varies between years and sex. Introduced Canadian pondweed (*Elodea canadensis*) combined with exploitation is a major cause for the overall decrease in crayfish population size and production. Management regulations have changed several times during the study period. The major changes involve reduced catching season and increased mesh size of the traps.

Keywords: adaptive management, noble crayfish, catching season, minimum size

Sampling juvenile *P. leniusculus* in the UK using refuge provision: The effect of aperture size and season on catch

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Juvenile crayfish are under-recorded using both active and passive sampling methods (e.g. electrofishing, seine netting, manual survey, night-torching and trapping). Novel passive refuge samplers used in this study have succeeded in retaining juvenile crayfish in the size range 5 to 37 mm post orbital carapace length. Materials used include perforated bricks (24 perforations: 9.83 ± 1.74 mm and 18 perforations: 17.24 mm ± 1.04 mm diameter), plastic roofing material (channel dimensions 8.9 x 8.9 and 6.1 x 8.2 mm) and invertebrate colonisation samplers/ mesh covered bundles of barley straw. Items were deployed individually (bricks) or on quadrat frames (all items) for a soak time of one month. In contrast to trap catch per unit effort (CPUE), where activity levels and catch are mediated by water temperature, juvenile brick sampler CPUE increased when water temperature decreased. Juvenile crayfish are extremely vulnerable to both predation and cannibalism and select apertures closely related to their body size in this study. The provision of appropriately sized refuges, appealing to their strongly thigmotactic nature, offers a new approach to sampling juvenile crayfish whose refuge dependency increases with falling water temperature. The use of passive refuge samplers avoids by-catch and obviates the need for bait whilst potentially tackling the problems associated with detection thresholds.

Keywords: juvenile, passive, brick, refuge, temperature

Populations of native crayfish in Slovakia under siege

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All populations of native crayfish species (*Astacus astacus*, *Austropotamobius torrentium* and *Astacus leptodactylus*) are critically endangered by non-indigenous invasive crayfish species entering surface waters in Slovakia from Hungary, Austria and Czech Republic. NICS are taking advantage of an excellent migratory corridor formed by Danube and Morava/March rivers, where they already established stable and abundant populations. Colonization of tributaries at adjacent lowlands of western and southern Slovakia is taking places and eradication of native species is highly presumable.

Keywords: *Astacus astacus*, *Austropotamobius torrentium*, *Astacus leptodactylus*, NICS, Slovakia

Aphanomyces astaci spore dynamics in the ambient water of latent carrier crayfish revealed using qPCR

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The specialized crayfish parasite *Aphanomyces astaci* causes the devastating crayfish plague in European crayfish. Even though *A. astaci* sporulation has been thoroughly studied under pure culture conditions, little is known about the sporulation dynamic from its live host. Our purpose was to investigate the *A. astaci* spore dynamic in its native parasite-host relationship by monitoring the sporulation from carrier crayfish into the ambient water using agent specific qPCR. American signal crayfish (*Pacifastacus leniusculus*) with known positive carrier status were housed individually and communally in two experimental setups using multiple replicates and different temperatures. Water samples were collected weekly, and spore numbers were quantified. We demonstrate here that live latent carrier crayfish continuously released a moderate number of *A. astaci* spores (~2700 spores per crayfish/week) in the absence of death and moulting events. In contrast, a pronounced sporulation increase was seen already one week prior to death in moribund crayfish, suggesting a crayfish plague-like condition developing in weakened or stressed individuals. Significantly more spores were produced in 18 °C compared to 4 °C, while a negative correlation was detected between spore numbers and temperatures rising from 17 to 23 °C. This study is the first attempt to quantify the spore release from carrier crayfish on the basis of qPCR applied on water samples, and demonstrate that the approach successfully unravel *A. astaci* sporulation patterns. The results emphasize that carrier crayfish pose a constant infection risk to highly susceptible crayfish species regardless of crayfish life cycle state.

Keywords: Infectious disease, *Oomycetes*, pathogen, real-time PCR, *Saprolegniaceae*, quantification

Surveying noble crayfish (*Astacus astacus*) populations for the presence of crayfish plague infection

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Crayfish plague, caused by the oomycete *Aphanomyces astaci*, has affected the highly susceptible European crayfish species for over 150 years. Where ever the infection appeared, it seemed to wipe out the existing populations. This experience and the laboratory trials supported the hypothesis of a total mortality of the affected animals. However, in some locations the infection has co-existed with a highly susceptible species for years or even for decades. In this study 925 noble crayfish (*Astacus astacus*) individuals from ten water bodies were analyzed using a real time PCR method. The populations represented different crayfish plague backgrounds. Crayfish plague infection was identified in three populations. One represented an acute phase of the infection, one a slowly proceeding infection, and one population was presumably in a chronic state of the disease. Single positive PCR results were obtained from two other locations, but judged as unreliable in the lack of other evidence of the infection. In the acute case, more than 90 % of the sampled individuals were PCR positive, compared with the slowly progressing infection, where the prevalence was 5 %. In a lake with the chronic state of the disease the prevalence of 22 % was detected. Targeted sampling including crayfish with exosceletal injuries improved the probability of finding the infection with PCR. As there is no method for verifying a positive PCR as a true infection from a symptom free individual, repeated sampling in successive years may be necessary. Also the sampling method must be further standardized.

Keywords: crayfish plague, *Aphanomyces astaci*, surveillance, PCR, noble crayfish, *Astacus astacus*

Hunting the crayfish plague disease agent in water sources – challenges and possibilities

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The specialized North American crayfish parasite and oomycete *Aphanomyces astaci* causes crayfish plague in European crayfish. Infectious *A. astaci* zoospores are released from American carrier crayfish and from diseased or dead European crayfish. The *A. astaci* spore quantity in the ambient water of crayfish will therefore largely reflect disease outbreaks in European crayfish or carrier status in American crayfish. During the past few years, molecular methods detecting *A. astaci* directly from crayfish tissues has become widely accepted. Now, the first studies employing *A. astaci* specific qPCR directly from water samples are approaching. Monitoring waterways could facilitate early warning of *A. astaci* infections, improve risk assessments, and reveal of infection reservoirs. It might also serve as a supplementary tool for declaring crayfish stocks and water bodies infection free in nature and aquaculture. Over the past three years, we have worked on this topic in a Nordic collaborative project. Here, we present methodological experiences e.g. sampling volumes, filtration methods, and subsequent molecular analyses. Possibilities and challenges connected to the transition from laboratory, via aquaria models, to natural environments are discussed. Furthermore, unknown diversity of oomycetes resembling *A. astaci* in the target DNA motif challenges the reliability of qPCR results, both from crayfish and natural environmental samples. We have therefore identified means that increase the probability of distinguishing between possibly true and false positives. These include qPCR optimizations, and use of a decision support tool that evaluates the qPCR result reliability on the basis of linearity (R2) and amplification efficiency of the PCRs.

Keywords: *Aphanomyces astaci*, crayfish plague, desition support tool (DST), method optimization, qPCR, water analyses, ultrafiltration

Landscape analyses as a tool for managing native and alien crayfish

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Despite the knowledge on the mechanisms causing population declines in European crayfish stocks (e.g. crayfish plague, alien species, habitat loss) the assessment and quantification of their threats have received insufficient attention so far. Upon historical and current data of indigenous (ICS) and non-indigenous crayfish species (NICS) in Carinthia (Austria) we developed habitat suitability models assessing the potential range of native *Astacus astacus* and *Austropotamobius torrentium*, as well as alien *P. leniusculus*. Based on landscape elements we defined the level of fragmentation in catchments having ICS and NICS. This information together with spatial distances to potentially plague carrying NICS, roads and settlements was used to calculate a threat index evaluating the endangerment of each remaining ICS site (including *Austropotamobius pallipes*). We found *A. torrentium* having the widest distribution area, while the potential range of *A. astacus* and *P. leniusculus* was mainly overlapping. Riverine and lacustrine landscapes with ICS and NICS occurrence were highly fragmented and logistic regression models showed that extinct ICS populations were positively associated to settlements, while negatively to barriers. Still existing stocks from the genus *Austropotamobius* were less endangered than the ones of *A. astacus*, as these populations are located at higher elevations with lower human impacts. In contrast, *P. leniusculus* was related to human infrastructures in the lowlands. Finally the threat index identified the most endangered ICS populations. The herein applied tools and analyses at the landscape level are essential to establish catchment based conservation plans for threatened ICS and to prevent the further spread of NICS.

Keywords: *Astacus astacus*, *Austropotamobius* spp., *Pacifastacus leniusculus*, habitat suitability, fragmentation, threats

Local engagement – a prerequisite for successful reintroduction

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The reintroduction program for noble crayfish (*Astacus astacus*) in the River Ljungan, northern Sweden, has been running for 10 years. It has gone through several phases; including planning, introduction and assessment.

The stakeholders in the steering group shared the same goal, namely to regain the crayfish to the River Ljungan, but they had different motivations. Fishery, species conservation at both national and regional scale, as well as supporting and development of leisure activities and tourism were among the driving forces for the reintroduction program, which was pursued with State resources. The local inhabitants were informed about the actions of the project, by local newspapers, radio and television. The local stakeholders had an important role in the implementation, because of the voluntary work performed by the local fishery owners associations.

The program included reintroduction of 80,000 noble crayfish to more than 30 sites in the river, education of almost 200 people from the fishery owners associations and above 8000 hours worked by the voluntaries with reintroduction, restoration and monitoring.

The results from the program are a growing population of noble crayfish in the River Ljungan, immerse knowledge and awareness among local inhabitants about the noble crayfish and its sensitivity for disturbance and diseases, and a close collaboration between the fishery owners associations in terms of management and disease control.

Keywords: Reintroduction, Noble Crayfish, voluntaries, steering group

Where should you put the crayfish – Does the actual reintroduction site matter?

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Reintroduction is an important conservation measure for noble crayfish (*Astacus astacus*). Yet the knowledge of which factors that makes reintroduction of crayfish successful is far from complete. The aims of this study were to investigate if the numbers of reintroduced animals affect the population size and if the location of the actual reintroduction site does matter.

The noble crayfish (*Astacus astacus*) population in the River Ljungan was reintroduced after extinction in 1999. CPUE (catch per unit effort) was calculated in two sections of the river and assumed to reflect population size. The subpopulations were measured every 100th meter upstream and downstream of the reintroduction sites. At two sites crayfish were marked individually with PIT-tags during several years.

The results show that the sizes of the crayfish populations, some years after reintroduction are less than before the extinction, and that the population sizes were about 40% larger in the section with 3 times more introduced animals. The population sizes were independent of the distance to the reintroduction sites. However they were larger upstream (median CPUE=2.25) than downstream (median CPUE=1.75). 19 of 23 recaptured crayfish were found upstream and the most long-distant migrants travelled above 1000 meters.

The implication of these results is that the population development after reintroduction may be speeded if a large numbers of animals are introduced. Additionally, when good habitat is available upstream, the choice of reintroduction site can be chosen based on practical reasons, such as ease of access.

Keywords: Reintroduction, noble crayfish, upstream, migration

Abstracts for poster contributions

Establishing culture parameters for noble crayfish (*Astacus astacus*) in recirculating aquaculture systems

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Due to competition with invasive crayfish species and the crayfish plague, indigenous *Astacus astacus* populations have been eradicated from Belgian waters. Because the taste and quality of imported crayfish does not suit gastronomic demands, the consumption of crayfish is very low. Interviews with top chefs demonstrate a demand for a fresh and locally produced product.

Therefore, the University College KAHO-HUB started to investigate the potential of *A. astacus* as a commercial aquaculture species. Recirculating aquaculture systems (RAS) enable a controlled culture process, safe from crayfish plague and securing a stable product quality. Although capital investment and production costs are high, a high market price and the possibility to integrate crayfish culture in other activities could make the culture profitable and sustainable.

Because little is known about the culture of *A. astacus* in RAS, a 24 tank system (0.5 m² each), all connected to a RAS, was set up at Aqua-ERF (Aquaculture Education and Research Facilities). To obtain indication levels concerning optimal density, a trial with one-summer-old juveniles distributed over 12 tanks at three different densities (100-300-500.m^{-2}) was started. Results on growth, survival and agonistic behavior will be presented and discussed. A second density trial with 3- to 4-summer-old *A. astacus* is ongoing.

Future trials will investigate the effect of temperature, size and number of hiding places, photoperiod, light intensity and different feeds. A market and consumer study is ongoing which will, in combination with production characteristics and thus production cost, result in an economic evaluation of crayfish culture in Belgium.

Keywords: *A. astacus*, crayfish culture, diversification, RAS, sustainability

Conservation management in the area of conflict between legal constraints and intensive land-use: a case study on stone crayfish in Vorarlberg

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Stone crayfish (*Austropotamobius torrentium* Schrank, 1803) is one of three indigeneous freshwater crayfish species in Austria, together with noble crayfish (*Astacus astacus* Linnaeus, 1758) and white-clawed crayfish (*Austropotamobius pallipes* Lereboullet, 1858). For various reasons, mostly anthropogenic ones, in all over Central Europe the distribution of crayfish strongly declined during the last 150 years. Although stone crayfish is the least known species and its degree of threat still cannot be exactly determined, a dramatic decline and an ongoing negative tendency have to be assumed. Since several years it is protected by European Union and national legislation. Within this legal frame EU-member states are obliged to maintain or restore this species at favourable conservation status. In Vorarlberg, the westernmost federal state of Austria, the situation for stone crayfish has not been assessed in detail yet. To fill this gap a survey was performed including distributional range, habitat quality and data on population structure. Based on the results and against the background of legal constraints the conservation status of stone crayfish in Vorarlberg was estimated and management proposals were elaborated. Implementation of such plans needs consideration of ecological and genetic aspects and is confronted with practical difficulties: In a densely populated region of the Alps areas holding potential stone crayfish habitats face the problem of a strongly modified landscape and conservation plans compete with several land-use interests.

Keywords: Aquatic conservation, freshwater management, invertebrate ecology, species action plan, legislation

Phylogeography of white-clawed crayfish *Austropotamobius pallipes* complex in Italy: a focus on Lombardy Alpine foothills and Northern-Central Apennines

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White-clawed crayfish *Austropotamobius pallipes* complex is widely distributed in Italy, but many populations are isolated and threatened by habitat fragmentation, invasion of alien crayfishes and climate change. Previous studies conducted on white-clawed crayfish in Italy using 16S mtDNA and a portion of COI mtDNA as molecular markers showed a complex phylogeographic structure, with the presence of 4 different subspecies of *Austropotamobius italicus*. As genetic diversity is considered a key factor to ensure long-term survival of endangered species we found necessary to obtain new information about the phylogeographic pattern of distribution of white-clawed crayfish in Northern and Central Italy, providing a useful knowledge basis for future management and conservation projects. In order to analyse the phylogenetic relationships between crayfish populations of Lombardy Alpine foothills and of Northern and Central Apennines, tissue samples were taken from over 230 individuals collected in 53 streams and a 1178 base-pair region of COI mtDNA gene was selectively amplified and sequenced using two primers couples designed for this study. Part of the samples from Central Apennines and Lombardy Alpine foothills were analysed in the context of the Life+ Project "Conservation and Recovery of Austropotamobius pallipes in Italian Natura 2000 Sites" (Life+08 NAT/IT/000352 – CRAINat).

Keywords: *Austropotamobius pallipes* complex, COI mtDNA, genetic diversity, conservation

Morphometric analysis of *Procambarus* (*Austrocambarus*) from southern Yucatán Peninsula, Mexico

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In order to determine the intraspecific and interspecific variation limits of crayfish from the southeast of Yucatán Peninsula, a morphometrical análisis was made. It consisted in the measurment of somathic characters and the obtaining of statistical proportions from crayfish collected in previously recorded places and new places of ocurrence. Particularly the total lenght/cefalotórax lenght, broad/lenght of palm and total lenght/firs pereiopod lenght helped to determine the presence of at least three populations different to that of *Procambarus llamasi*, in the southeast of Yucatán Peninsula.

Keywords: Mexican crayfish, *Procambarus* (*Austrocambarus*), morphometric analysis, Yucatán Peninsula

Crayfish Species Distribution in Francis Marion National Forest, South Carolina, USA

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A distributional survey of epigean crayfishes was conducted in the Francis Marion National Forest (FMNF) in Berkeley and Charleston counties, South Carolina, in 2011. Five species were collected at 72 of 84 sites sampled: *Fallicambarus fodiens*, *Procambarus acutus*, *P. ancylus*, *P. enoplosternum*, and *P. troglodytes*. Statistically significant differences were found in collection site elevations for two species pairs: *P. enoplosternum* + *P. troglodytes* and *P. acutus* + *P. ancylus*. *Procambarus acutus* occurred in ephemeral habitats, and was co-distributed with *P. ancylus* in the interior, higher elevation sites sampled in the FMNF. *Procambarus ancylus* also collected from habitats where surface water persists throughout the year. *Procambarus enoplosternum* and *P. troglodytes* were found in perennial aquatic habitats and were conspicuously absent in collections from ephemeral habitats and the interior of the FMNF. *Fallicambarus fodiens* was found in each of the habitat types. Because *P. acutus* and *P. troglodytes* are considered ecological generalists, phenology and hydrologic regime possibly influence their distributions in the FMNF.

Keywords: survey, spatial distribution, landscape scale

Reproductive efficiency of *Austropotamobius pallipes* complex in experimental rearing centers, in relation to some individual measurements, to their original watercourses and to the reproduction year

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Our research aimed to collect data about fertility of *Austropotamobius pallipes* in order to evaluate its reproduction efficiency. From 2008 to 2012 two rearing centers formed by outdoor pools were used as experimental farms and young crayfishes produced were used for reintroduction according to LIFE project CRAINat (LIFE08NAT/IT/000352). Between September and October we caught 407 reproductive females (activated glair glands). They originated from 17 watercourses of the Lombardy pre-alpine zone. We have measured all the animals as concerns total length (from the tip of the rostrum to the end of telson; average 77.07±8.38 mm) and weight (average 13.15±4.26 g). They were individually marked. The whole rearing cycle (mating, egg-bearing, hatching of juveniles) was conducted in semi-natural conditions in the outdoor pools. In all the months of May we checked the egg-berried females. In the five years period we have obtained 40.8% of berried females. We tested if reproductive efficiency varied between females, rearing centers, the original watercourses and the rearing years. To do this, we used a GLMM (generalized linear mixed model) with binomial error in which the present or absent of egg-berried was the response variable, the total length and the original watercourses were the fixed component, while the rearing years are the random one. The rearing year was the only variable significantly affecting the reproductive efficiency of females (LR-χ^2 = 6.543, df = 1, P = 0.010; σ^2 = 0.23±0.48), since the probability to produce fertile eggs resulted higher in 2008 and 2011 while the lowest occurred in 2009.

Keywords: Reproductive efficiency, *Austropotamobius pallipes* complex, Italy

Prevalence of the crayfish plague *Aphanomyces astaci* in populations of signal crayfish *Pacifastacus leniusculus* in France

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The pathogen of the crayfish plague *Aphanomyces astaci* first appeared in Europe in 1859 and nowadays it still causes mass mortalities of native European crayfish. Its spread across the continent is facilitated especially by invasive North American crayfish species which serve as its reservoir. In France, multiple cases of native crayfish mortalities were suggested to be connected with the presence of the signal crayfish *Pacifastacus leniusculus*, which is highly abundant in the country. It shares similar habitat as the native white-clawed crayfish, *Austropotamobius pallipes* and, if infected, the signal crayfish might therefore easily transmit the pathogen to the native species. The aim of our study was to investigate the prevalence of *A. astaci* in French signal crayfish populations and thus to evaluate the danger they represent for local populations of native crayfish. Using the quantitative TaqMan minor groove binder (MGB) real-time PCR, we have analysed 513 individuals of *Pacifastacus leniusculus* from 45 French populations. Altogether, 20% of these crayfish were found to be infected, and the pathogen was detected in more than a half of studied populations. Local prevalence varied significantly, ranging from 0% up to 80%. Our results confirm that the widespread signal crayfish serves as a reservoir of *Aphanomyces astaci* in France and represents therefore a serious danger for native crayfish species, especially the white-clawed crayfish. Additional analysis of several individuals of other non-indigenous crayfish species (*Orconectes limosus*, *O. immunis* and *Procambarus clarkii*) revealed infections among two of these, *O. immunis* and *P. clarkii*. Prevalence in other introduced crayfish should therefore be investigated as they may also contribute to the transmission of the pathogen in the country.

Keywords: crayfish plague, *Aphanomyces astaci*, signal crayfish, France

The functional role of symbiotic branchiobdellida – are they grazers, omnivors or parasites?

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Branchiobdellida (Annelida: Clitellata) are mostly found as epi-commensals adhering to the external surfaces or the gills of crayfish and have been reported associated with crayfish and other crustaceans from North America, Europe and East Asia including Japan. Up to now, only few records exist on branchiobdellid species in Europe and no published information is available on the functional role of individual species. Considering the lack of knowledge on the ecology of these symbiotic worms, we looked at crayfish-branchiobdellid associations from three indigenous crayfish host species and studied i) the distribution of individual branchiobdellida species, ii) their body and jaw morphology and iii) the pontial difference in food consumption and assimilation by applying stable isotope ($\delta^{13}C$, $\delta^{15}N$) analyses. Five branchiobdellid species where found on the studied crayfish, usually one up to three species co-occurring in the individual crayfish populations. The most abundant species was *Branchiobdella pentodonta*, followed by *B. balcanica*, whereas *B. hexodonta*, *B. parasita* and *B. italica* showed lower densities. The density and number of branchiobdellid species on crayfish (individuals and populations) differed. Some branchiobdellid species were found to occur on all body parts, whereas others showed a tendency for certain locations (e.g. gills). The identified body regions with branchiobdellids were not found to differ in annelid densities however some species were host specific. Our results on branchiobdellid's preferred location on the crayfish, their jaw morphologies combined with the stable isotope signals were used to provide a first picture of the functional categorisation of the Branchiobdella species.

Keywords: Annelida, commensals, indigenous crayfish, stable isotope analysis, feeding types

Are symbiotic Branchiobdellida (Annelida: Clitellata) adequate indicators for explaining crayfish populations' origin and condition?

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Branchiobdellid annelids are usually found as commensal symbionts associated with crayfish populations, but knowledge of their dispersion and ecology in Europe is generally scarce and the potential relationship of crayfish population characteristics, water quality and occurring branchiobdellid species has never been explored. We looked at crayfish-branchiobdellid associations from 25 sites and present results on i) the distribution, species number and abundance of branchiobdellids in selected crayfish populations in Tyrol and Italy (South Tyrol), of the three autochthonous species, *Astacus astacus*, *Austropotamobius pallipes* and *A. torrentium*, ii) the relation of these findings with crayfish population characteristics and environmental conditions of the water bodies, in order iii) to explore the strength of the indicator role of Branchiobdellida to explain the origin and condition of indigenous crayfish populations. Five branchiobdellid species where found on the studied crayfish, usually one up to three species co-occurring in the individual crayfish populations. The branchiobdellid assemblage corresponded generally to the geographical distribution of their crayfish hosts' locations but also confirmed previous assumptions of crayfish translocations. When crayfish population parameters and environmental conditions were considered, branchiobdellid species, their densities and biomass followed distinct patterns. This study clearly demonstrates that these symbiotic annelids are suitable to obtain important information on the biogeography of crayfish, crayfish population condition and the ecological status of freshwaters.

Keywords: annelid worms, indigenous crayfish, biogeography, water quality

Modelling environmental niche for the endangered freshwater crayfish species *Austropotamobius pallipes* complex in Northern and Central Italy.

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The potential distribution of endangered species is a necessary step to assess species conservation status and manage reintroduction plans. In the context of the Life project CRAINat (LIFE+ 08 NAT/IT/000352) on the endangered *Austropotamobius pallipes* complex, we modelled the environmental niche of the species in two large areas of Northern (Lombardy, 43 records) and Central Italy (Abruzzo, Province of Isernia, Gran Sasso e Monti della Laga National Park; 69 records).

Suitability models were built by using the maximum entropy approach as implemented in the MaxEnt software (vers. 3.3), which predict the occurrence of a species using presence-only data. The occurrence of *A. pallipes* was modelled using six variables: altitude, slope, aspect, anthropic disturbance (3 classes derived from Corine Land Cover), mean temperature of warmest quarter (from www.worldclim.org) and distance from stream system (as control variable accounting for small streams not solved in the digital map of rivers within studied areas). Each study area was modelled independently.

Both potential distribution obtained high performance scores as measured by the AUC index (N-Italy: 0.92; C-Italy: 0.88). In both areas, altitude, slope and distance achieved the greatest predictive power. Moreover, in C-Italy the mean temperature of warmest quarter was also significant in predicting species occurrence.

The white-clawed crayfish prefer habitat next to stream system and with low slope; in N-Italy the species select the altitude interval of 400-600 m, while in the C-Italy it occur preferentially within 800-1200 m, due to the increase of temperature.

Keywords: environmental niche model, MaxEnt, *Austropotamobius pallipes* complex

Recent data on crayfish (Decapoda: Astacidae) distribution in Bulgaria

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Three native crayfish species occur in Bulgaria: *Astacus astacus*, *Astacus leptodactylus* and *Austropotamobius torrentium*. All of them are protected by the Biological Diversity Act (2002) and Law on Fisheries and Aquaculture (2001). Data on their distribution were published in 1961 and 1998. Recently, studies on the assessment of conservation status of EU protected habitats and species including *A. torrentium* were undertaken. Our goal was to summarize data on crayfish distribution, population status and potential threats in terms of river basin and conservation management as required by the EU HD and EU WFD.

Samples were collected in the period 2006-2012. *A. torrentium* was recorded at over 50 sites in the Danube River basin (rivers in the West- and Central Balkan Mountains and Vitosha Mountains), and the Aegean Sea basin (Struma and Mesta river basins). It occurred at altitudes in the range from 190 to 1180 m a.s.l. (reported from 100 to 1700 m a.s.l. in Bulgaria). *A. astacus* was much rarer in our samples, it was found only at 7 sites in the Danube River basin at altitudes from 20 to 320 m a.s.l. (reported in the range from 0 to 1600 m a.s.l. in Bulgaria). *A. leptodactylus* was most frequently found in the Danube River, rivers and reservoirs in the Danube River basin and Aegean Sea basin, as well as in the Black Sea coastal lakes and river basins. It appeared at altitudes from 0 to 500 m a.s.l. The data were compared with previous range of distribution. Potential threats to native crayfish were identified, such as: water pollution, hydrotechnical construction, the competition with *Potamon ibericum* in some regions, and the impact of invasive alien species. Necessary conservation measures, especially within Natura 2000 protected sites, were discussed.

The study was funded by the National Science Fund (Project DO 283/2008) and Ministry of Environment and Water of Bulgaria.

Key-words: *Astacus astacus*, *Astacus leptodactylus*, *Austropotamobius torrentium*, geographical distribution, Danube River, Black Sea basin, Aegean Sea basin, threats, conservation

Changes in population characteristics and structure of the signal crayfish at the edge of its invasive range in a European river

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The ability for rapid range expansion is one of the key determinants of invasive species success. In order to investigate potential drivers behind the rapid spread of invasive species, we explored changes in population characteristics and structure along the invasion pathway of a successful invader in European freshwaters, the signal crayfish (*Pacifastacus leniusculus*). Diverse population parameters such as relative population abundance, size and sex structure, differences in morphometry and frequency of injuries were compared between signal crayfish population samples at three uniformly distributed segments (approximately 40 km apart) in the lower section of the Mura River, which differed in time since invasion. Examined signal crayfish populations exhibited notable differences, with more recently established populations towards invasion front characterized by lower abundance and male biased sex ratios, which highlighted males as initial dispersers. We also recorded significant increase in the relative claw size, a competitively advantageous and allometric trait for males, in more recently established populations away from source population. The recorded differences in population structure and male morphometry along the invasion pathway could lead to important clues about dynamics of range expansion and population establishment, highlighting the traits that promote dispersal and better response to local conditions in new habitats. Established differences can also provide insights for the development of targeted management responses aimed at invasive species control.

Keywords: invasive species, range expansion, time since establishment, population characteristics, crayfish

Recent invasion of karst river systems in Croatia by the signal crayfish

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Signal crayfish (*Pacifastacus leniusculus*) is one of the most successful crayfish invaders of European freshwaters. Until 2012, its distribution in Croatia was limited to the Drava River basin where it exhibits extremely high rates of downstream dispersal. In 2012 its presence was recorded in the karstic river of the Sava river Basin, the Korana River, where it has been deliberately introduced. This area hosts numerous populations of native crayfish (*Astacus leptodactylus*, *A. astacus* and *Austropotamobius torrentium*). In order to estimate the extent of this recent invasion and its potential threat to existing populations of native crayfish, we examined signal crayfish distribution in the Korana River and its tributaries and analyzed size and structure of recorded populations.

Preliminary research showed that the current signal crayfish distribution in the Korana River is localized within 20 kilometers of its lower reach. The downstream end of its distribution range is less than 20 kilometers upstream from the confluence with Mrežnica River. Thus, recorded signal crayfish invasion has a high potential to spread on a vast majority of surrounding water bodies. Recorded populations differed in relative population abundance, with the downstream populations exhibiting a higher catch per unit effort when compared to the populations at the upstream end of the distribution. Based on obtained data, we identified and prioritized native crayfish populations which are under immediate threat from signal crayfish spread. All obtained results are discussed in the context of potential management activities aimed at conservation of invaded karstic rivers and their native crayfish fauna.

Keywords: freshwater invasion, karst rivers, signal crayfish, distribution, population size

The congruence between stream morphology and the distribution of native and invasive crayfish in the Czech Republic

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Stream morphology is besides water quality and species interactions one of the most important factor influencing crayfish distribution. The differences in habitat preferences between indigenous and non-indigenous crayfish species have not been thoroughly studied till today although they can be helpful in predicting the spread of non-indigenous crayfish species (thus also of a crayfish plaque pathogen) and for the assessment of the vulnerability of threatened species. The preferences of stream morphology characteristics were studied for three crayfish species: indigenous *Astacus astacus*, *Austropotamobius torrentium* and non-indigenous *Orconecets limosus*. In 2004–2008 we searched 6.768 sites of running waters within the whole Czech Republic for crayfish occurrence and also for stream morphology characteristics. Crayfish were caught by hand in 100 m sections of streams. The character of stream basin, the type of flow, meanders, the substrate type, the type of potential shelters, the width of the stream, the maximal depth of the stream, the surroundings of the stream, the effect of catchment area or altitude were recorded. The significant differences between native and non-native crayfish were found in these characteristics: water depth, altitude, the width of the stream, mud in bottom substrate, type of stream, meandering, built-up-area in the surroundings and flow speed of the streaming water. Orconectes limosus inhabited generally the type of sites nearby the large rivers with slow flow in the built-up-areas, whereas *A. astacus* preferred sites with stones in the riverbed.

Keywords: Central Europe, habitat, indigenous, running water, invertebrate

Marbled crayfish (*Procambarus fallax f. virginalis*) resistance and survival rates at low (under 5°C) temperatures during winter period

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According to the Nature Conservation Act, it is prohibited to introduce non-native species into wild. It is also prohibited to bring live specimens of the signal crayfish, narrow-clawed crayfish and spiny cheek crayfish into Estonia or conduct transactions with live specimens of these species. In recent years, many aquarium shops in Estonia have sold marbled crayfish to aquarists as pet species. Now there is a risk that marbled crayfish is released into nature, as it has already happened in many European countries. In Estonia, marbled crayfish have not been found from nature so far and were not known, if marbled crayfish could survive Estonian winter conditions or endanger noble crayfish. Therefore had to be tested whether it is necessary to include marbled crayfish to the Estonian Nature Conversation Act as a potentially dangerous species to noble crayfish. To estimate the low temperature effect to marbled crayfish survival and growth rate, behavior and reproduction we carried out the experiment with 50 marbled crayfish of various sizes (27-51 mm TL) at Estonian Univeristy of Life Sciences from September 9th 2011 to April 18th 2012 in two 1 m^3 tanks in outdoor conditions. The experiment results indicate that marbled crayfish can live and moult at temperatures under 10 degrees for several months and even survive at temperatures 0.5 degrees. The highest survival rate was observed in the first tank (60%) and lower in the second tank (8%). This data shows that marbled crayfish have good resistance for to Estonian climate conditions.

Keywords: Marbled crayfish, *Procambarus fallax f. virginalis*, survival rate, low temperature

Acute toxicity of peracetic acid to the signal crayfish (*Pacifastacus leniusculus*): possible therapeutic treatments in crayfish culture?

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Peracetic acid (PAA) is successfully applied for number of occasions in the current aquaculture, especially for treating a wide variety of serious fish diseases. If knowledge on PAA toxicity to fish is limited, even worse situation occurs in case of crayfish, where these issues have never been investigated. In this study, we conducted acute toxicity tests of Persteril 36, a commercial PAA-containing product, on three age/size classes of signal crayfish, *Pacifastacus leniusculus*. We found high tolerance of adults to this product (96 h LC50 = 77.3±1.0 mg L^{-1} PAA) and slightly higher toxicity to 9 months old juveniles (96 h LC50 = 69.5±0.4 mg L^{-1} PAA) with only low post-treatment mortality. Shortened 1-day test on the second developmental stage confirmed 24 h LC50 to be 14.3±1.1 mg L^{-1} PAA. As PAA concentrations successfully applied in fish aquaculture are generally 2.0 mg L^{-1} or lower, PAA can be recommended for use in astaciculture. Possible uses might include e.g. limiting spread of crayfish plague, treating saprolegniosis on artificially incubated crayfish eggs, or avoiding translocations of crayfish-related non-native species when introducing crayfish or stocking closed culture systems.

Keywords: Acute toxicity, *Aphanomyces astaci*, *Saprolegnia* sp., Invasive species

Electrostimulation of spermatophore extrusion in signal crayfish *Pacifastacus leniusculus*

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The main aim of present study was to determine whether the spermatophores of crayfish can be stripped by means of an electrical stimulation. Signal crayfish (*Pacifastacus leniusculus*) was used as a model organism. The transformer type AC250K2D was used as an electrical device in this experiment. In order to find proper voltage for electrical stimulation, two groups of crayfish, each containing 6 males were tested by 10 and 15 volts, respectively. There was not any significant difference in response of animals to different voltages (both groups 67% success). After experiment, no mortality or obvious disorder was observed implying the method as non-invasive. The results of this trial suggest, that the method of electro-stimulation could be used for obtaining spermatophores from mature crayfish males. Findings from a subsequent electron microscopy of sperm morphology confirmed the harmlessness of electrostimulation on the ultrastructure of sperm cells and spermatophores. The spermatophores obtained by this method can be possibly used in process of artificial fertilisation of crayfish eggs in the future; however, more in-depth studies focusing sperm capacitation and process of fertilisation have to be done.

Keywords: crayfish, electrostimulation, spermatophore, transformer, voltage

Narrow-clawed crayfish as real-time bio-indicator: can it detect overdosed disinfection compounds in drinking water chloramination?

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In order to establish whether the narrow-clawed crayfish (*Astacus leptodactylus*) are sensitive enough to be used as bio-indicators in drinking water quality monitoring, adult males were exposed to widely used disinfecting agent, chloramine-T (Cl-T), in concentrations recommended in industry and aquaculture, i.e.10 mg/l and 50 mg/l respectively. Parameter of cardiac activity, heart rate (HR) was chosen as main factor for assessment of crayfish sensitivity to disinfecting compounds, as well as stress index (SI) was chosen as secondary parameter for evaluation of HR variability. As a result, the narrow-clawed crayfish was found as well-reacting species on dissolved in water Cl-T, since value of the HR increased during exposure compare to pretreatment state was proved as significant. Crayfish cardiac reaction to 10 mg/l was evidently lower than to 50 mg/l, while established stress state under impact of 50 mg/l became evident later than under lower concentration, and was deeper. At the same time, crayfish have shown high tolerance to impact of this chemical, and returned to the normal state within short time after exposure was finished. Considering such crayfish reaction as rapid increasing the HR, obtained results let us suppose a good possibility of their use as control species in monitoring of the water quality.

Keywords: water disinfection, chloramine-T, real-time monitoring

Genetic characterisation of Astacidae from Greece: Preliminary results

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Four different species of crayfish exist in Greece: three indigenous (*Astacus astacus*, *Astacus leptodactylus* and *Austropotamobius torrentium*) and one of North American origin (*Pacifastacus leniusculus*). For the first time an extensive sampling effort is carried out on the Astacidae of continental Greece, with a total of 158 sampling sites all over the country. Seventy five individuals were genetically analysed using the 16S rDNA genetic marker. The length of the aligned sequences in the final dataset was 356 base pairs (homologous sequences from *Homarus americanus* and *Cambaroides japonicus* were used as outgroups). BLAST analysis confirmed that the individuals from Greece belonged to the *Astacus astacus*, *Austropotamobius torrentium* and *Pacifastacus leniusculus* species. Dendrograms produced by maximum likelihood and parsimony methods showed that *Astacus* astacus from Greece constitute a distinct group from *A. astacus* originated from Northern European countries; this might be an indication of different subspecies as pointed by morphological studies. Within *Austropotamobius torrentium* group, four clusters were formed, indicating a subsequent genetic structure. It is also showed that *Pacifastacus leniusculus* individuals formed a uniform cluster, reinforcing their introduced origin from North America. The average genetic distance values of the Greek Astacidae to those from other areas were 0.012 for *Astacus astacus*, 0.017 for *Austropotamobius torrentium* and 0.006 for *Pacifastacus leniusculus*. Further laboratory work has to be conducted using different genetic markers to assess the degree of population divergence.

Keywords: Astacidae, continental Greece, genetic characterisation

Species complex and ecological relationship between cambarids and gastropods of hydrological region of Balancán, Tabasco, Mexico

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This study was aimed to contribute to the knowledge about freshwater crayfish and gastropods complex species and their ecological relationship in the Hydrological Region of Balancán, Mexico. These groups of organisms are very important economical, social, and ecologically but also for biomedical research, so there is an interest in those which can adapt to different environmental conditions, such as Balancán region, where hydrological dynamic keeps populations in a changing condition stress, and about what there is no previous information. Both cambarids and gastropods have been found inhabiting the same variety of habitats but there is no certain about distributional patterns converging. With this work we present the taxonomical status of cambarid and gastropod populations in Balancán hydrological region and the scenes for ecological relationships.

Keywords: *Cambaridae*, *Gastropoda*, ecological relationships, taxonomy

Scannig electron microscop (SEM) analysis of *Procambarus* (*Austrocambarus*) in Mexico

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For a long time, cambarids species description was based on the observation of somatic characters, particularly gonopods using a stereoscopic microscope. The drawings from such observations, while supporting the descriptions over time have hindered the identification of species, especially when dealing with groups of wide variation within populations. The use of scanning electron microscope for viewing of the apical processes of the male gonopod and annulus ventralis of the female has revealed differences and similarities that establish limits on intra-and interspecific variation, facilitating both the identification of species such as new forms. The analysis presented here is an example of how the SEM-based morphological analysis has helped to clarify the specific status of crayfish subgenus *Austrocambarus* in Mexico.

Keywords: Mexican crayfish, *Procambarus* (*Austrocambarus*), SEM, Mexico

Distribution and habitat requirements of the Stone Crayfish (*Austropotamobius torrentium* Schrank) in the Nature Park Medvednica, Croatia

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The stone crayfish (*Austropotamobius torrentium* Schrank) is one of four native crayfish species in Croatia that usually inhabits mountain streams with fast current and stony bottom. Although its populations are relatively well preserved, a decline in the number of stone crayfish populations throughout Croatia has been recently recorded, mostly due to anthropogenic habitat disturbance and droughts. This research aimed to investigate the stone crayfish distribution in the Natural Park Medvednica, where its populations are still very abundant, and to establish how altered hydromorphology of some of the streams in the Park affects its distribution and population size. Altogether 52 streams were selected for the study on the basis of detailed habitat characterization (including indicators of habitat heterogeneity and level of hydrological alteration) and measurement of physical and chemical parameters. During 6 monhts, baited traps were set up at altogether 71 locations in the 52 streams. Stone crayfish relative population abundance was estimated using catch per unit effort (CPUE) and divided in three categories: large > 5; medium 2 – 5; small < 2. Species was found in 35 (67%) streams at 62 % of examined locations. At 20 % locations stone crayfish had large to medium population size. Heterogeneity of substratum was positively correlated with stone crayfish population size, while the increased level of hydromorphological alteration, in the form of dikes, embankments, small dams etc. that reduced substrate heterogeneity, was negatively correlated with CPUE Based on collected data, stream management activities were porposed and conducted in the Nature Park Medvednica.

Keywords: stone crayfish, Medvednica, habitat, hydromorphology

Is it possible to detect narrow-clawed and noble crayfish probable hybrids using morphometric MDA?

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Hybrids between *Astacus leptodactylus* and *Astacus astacus* have been obtained in laboratory conditions. Even though their occurrence within natural habitats has been sporadically mentioned, to our knowledge, no detailed study has been conducted so far to confirm their existence. In Croatia both *A. astacus* and *A. leptodactylus* are native, with the latter expanding west- and southwards and slowly displacing *A. astacus*. During a distribution survey on the Mrežnica River (central Croatia) both species were recorded; with *A. astacus* distributed upstream, *A. leptodactylus* downstream, and specimens of morphological appearance that are indecisive in term of species determination, in-between the two. Therefore the aim of this research was to measure 28 morphometrical characteristics from a large number of specimens caught from all three parts of the Mrežnica River and to analyse them using multivariate discriminant analysis (MDA). Additionally, extra *A. astacus* and *A. leptodactylus* specimens from populations whose determinations are undisputable, according to morphological characteristics, were also included into analyses. The results showed a clear separation between *A. astacus* and *A. leptodactylus* with a high percentage of correct classification. Specimens of peculiar morphological appearance were positioned in-between the two. The analyses showed that the carapace width had the highest discriminant value for both sexes, followed by carapace height and claw palm length (in males) or claw length (in females). Therefore, it is possible to detect separation between studied specimens using MDA, but for final decision on the possible hybrid existence in the natural habitat further molecular analyses should be applied.

Keywords: *Astacus astacus*, *Astacus leptodactylus* sp. complex, discriminate analysis, natural populations

First report of crayfish plague in *Cherax quadricarinatus* (von Martens, 1868) reared in Sicily

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Sporadic mortalities of redclaw crayfish *Cherax quadricarinatus* were registered in a Sicilian crayfish farming facility in October 2011. Red swamp crayfish *Procambarus clarkii* reared in separate tanks at the same facility did not experience any losses. Twelve moribund specimens of *C. quadricarinatus* with evident signs of infection were sent to the CISS of the University of Messina, for diagnostic investigations. At necropsy diseased crayfish showed loss of appendices with brownish pigmentation of stumps and melanized ulcers on the exoskeleton. After gross examination, fresh tissue samples from eight crayfish were obtained for microscopic evaluation and histology. Four specimens (2 frozen and 2 fixed in formalin) were sent to the IZS delle Venezie for histological and bio-molecular analyses. The observation of fresh samples showed hyphae with several round zoosporangia. In histological sections serpiginous lesions were evident in the cuticle, and aseptate hyphae in the hypodermis were associated with hemocytic infiltration and melanin deposition. PCR and sequencing analyses confirmed the suspicion of crayfish plague, identifying the oomycetes as *Aphanomyces astaci*. According to the literature this is the first report of aphanomycosis in farmed redclaw. The role of *P. clarkii* as asymptomatic carrier of the disease must be seriously taken into account. After the epidemic plague incidence, the farm owner decided to wipe out all crayfish present in the facility, carefully disinfect the tanks, before restarting the production of *P. clarkii*.

Keywords: *Cherax quadricarinatus*, redclaw crayfish, *Aphanomyces astaci*, crayfish plague, Sicily

Digestive enzymes in the crayfish *Cherax albidus*: polymorphism and partial characterization in response to different feeding regimes

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The activity of digestive enzymes (amylase, pectinase, alginase, chitinase, lipases and proteases) along the digestive tract of juvenile freshwater crayfish *Cherax albidus* in response to different diets containing an equal percentage of nutrients but different polysaccharides (pectin, alginate and chitosan) as binders is reported. Gastric juice, hepatopancreas and intestine were sampled for enzyme analysis. Digestive enzyme activities did not show statistically significant differences along the digestive tract except for amylase activity that was significantly higher in the intestine compared to the gastric juice and hepatopancreas of control animals and animals fed feed pellets containing pectin as binder. Proteases were characterized by employing specific protease inhibitors. Both trypsin and chymotrypsin activities were detected. The majority of protease activity was ascribable to trypsin. Several isotrypsin and isochymotrypsin were identified by gel electrophoresis, with minor differences among experimental groups. The effect of different diets was tested on growth. Crayfish fed pectin containing pellets exhibited an average weight gain significantly higher than the other groups. This work provided basic information to study the digestive abilities of crayfish in relation to diet composition.

Keywords: *Cherax*, digestive enzymes, biopolymers

Genetic characterization of Belgian noble crayfish populations (*Astacus astacus*)

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One goal in conservation biology is to conserve the genetic diversity of endangered species to protect their adaptive potential. Therefore, it is essential to explore the genetic composition of natural populations and to define evolutionary significant units (ESU) as basis for modern conservation management plans. In Europe the genetic structure of noble crayfish (*Astacus astacus*) is highly influenced by human translocations. The main aim of this study was to resolve the genetic structure of Belgian natural noble crayfish populations. We sequenced a 350 base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit I (COI) and a 500 bp fragment of 16s rRNA (16S) from ten individuals per population from 18 populations in Belgium. For 20 individuals per population a microsatellite analysis was performed based on six polymorphic loci. Haplotypes and genotypes were compared with about 500 samples from over 100 sampling sites in Europe to estimate the genetic differentiation among the Belgian populations and European reference populations. Preliminary results indicate a relatively low genetic diversity in Belgian noble crayfish populations that is consistent with other central European populations. Strong genetic differentiation was found between Belgian and southwestern European populations, but little between Belgian and central European populations. Our findings could imply that human translocations dissolved the natural genetic structure. Future conservation strategies in Belgium should therefore, manage populations of noble crayfish as distinct ESUs.

Keywords: population genetics, microsatellite analysis, sequencing analysis, evolutionary significant units, restocking management plans

Co-infection by a yeast-like organism in *Thelohania*-infected white-clawed crayfish *Austropotamobius pallipes* (complex) from Lombardy (Northern Italy)

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During September 2011 an outbreak of the microsporidian parasite *Thelohania contejeani* (Henneguy,1892) was reported in a population of *Austropotamobius pallipes* in Lombardy (Northern Italy). Macroscopically affected white-clawed crayfish collected from Agna creek, tributary of the river Chiese, in the province of Brescia, were analyzed using histological and bio-molecular techniques. Microscopic examination of the abdominal and cardiac striated muscle fibres revealed microsporidia in several developmental stages, with abundance of sporophorus vesicles and free mature spores. PCR and DNA sequencing referred the parasite to the species *T. contejeani*.

Nearly half of the specimens affected by thelohaniasis also harboured a systemic infection by a yeast-like organism. Budding yeast-like cells were observed intracellularly in circulating haemocytes and free in the host haemolymph, particularly evident in the haemal sinuses of the hepatopancreas. These elliptical narrow base budding yeast cells (2,1-2,8 x 3,2-5,7 μm), stained positively with silver and periodic acid-Schiff (PAS) reactions. No hyphae or pseudohyphae were recorded.

Yeast proliferation in the haemolymphatic system of crustaceans has been described from the order Isopoda, Amphipoda, Calanoida, Anostraca and Decapoda frequently associated with other pathogens. The co-infection of microsporidia and yeasts in *A. pallipes* should be further investigated to assess if yeasts could act as primary pathogens or are mere opportunistic.

Keywords: *Thelohania contejeani*, *Austropotamobius*, yeast, co-infection, microsporidia

First occurrence of *Aphanomyces astaci* epidemic infection in cultured yabby *Cherax destructor* (Clark, 1936) in Northern Italy

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In the winter of 2009 an epizootic mortality occurred in cultured yabby *Cherax destructor* on a semi-intensive crayfish farm in the Veneto region (Northern Italy). The death of crayfish of all ages and sizes was observed over one month with a 70-100% cumulative mortality. During the following winter season in 2011 a new, more severe episode of the disease affected the surviving yabbies. Moribund and dead crayfish were found in ponds often with loss of appendages. The clinical signs observed were poor limb coordination with crayfish lying on their dorsal surface unable to upright themselves.

Affected crayfish (total number =107) were collected for laboratory examination. Erosive and melanised ulcerative lesions were recorded in the intersternal soft abdominal cuticle, the joints of pereiopods and telson. Histological examination showed infiltration of aseptate branching hyphae in correspondence to epicuticular ulcers in the abdominal segments. Hyphae in the cuticle and in the hypodermis were surrounded by deposits of melanin and haemocyte infiltration. Subepidermal granulomas and cuticular bacterial ulcers with heavy melanisation were also present. *Aeromonas hydrophila*, *A. sobria*, *Hafnia alvei*, *Citrobacter brakii*, *Pseudomonas anguilliseptica* and *Shewanella putrefaciens* were isolated from the haemolymph of some specimens.

Molecular examination of melanised abdominal cuticle was performed by PCR and DNA sequencing. Positive samples were homologous to published sequences of *Aphanomyces astaci*.

Under field conditions, *C. destructor* seems to exhibit incomplete resistance to crayfish plague as previously shown experimentally by Unestam (1975). In this particular episode (2010 to 2011) the disease course has been influenced by stressful environmental conditions (e.g. high stocking density, and low temperature) which also promoted secondary bacterial infections. The probable route of infection is discussed.

Keywords: *Cherax destructor*, *Aphanomyces astaci*, crayfish plague

Managing the white-clawed crayfish in Ireland

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Austropotamobius pallipes (Lereboullet) occurs in much of western and southern Europe. The still widespread Irish populations of *A. pallipes* have been attributed to introductions from Britain or the Continent. In 2000, genetic examination of crayfish from 5 catchments across Ireland showed stocks to be very homogeneous, with some loss in heterogeneity going from south to north. This was explained as indicative of an introduction in the south of the country, probably from France, followed by translocations from catchment to catchment further north, with consequent population bottlenecks. However, no stocks were then examined from Northern Ireland, and the close trading and transport ties within the U.K. indicated a high possibility of introduction of crayfish stocks to Northern Ireland from Great Britain, with its suggested different genetic origin of stocks. Thus until the genetics are known, Northern Ireland stocks cannot safely be used to restock systems in the Republic of Ireland, or vice versa, and more importantly, are not being considered for restoring the depleted *A. pallipes* stocks in Great Britain. Samples were therefore sourced from the Moneycarragh Crayfish Hatchery, Newcastle, Co. Down, and from the Erne (Upper and Lower Erne systems) and Bann (Ballingarry and Black-water systems) catchments. Their molecular analysis provides evidence of the genetic structure of Northern Ireland crayfish stocks, their relationships within the European range of this species, and the implications for conservation management.

Keywords: indigenous crayfish, translocations, trans-border catchments, mitochondrial genetics, white-clawed crayfish, *Austropotamobius pallipes*

Absence of the crayfish plague agent may explain coexisting populations of European and American crayfish in central Europe

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All North American crayfish species were under suspicion to be carrier of the causative agent of the crayfish plague *Aphanomyces astaci*, being lethal for European crayfish species. The existence of coexisting populations of European and American crayfish species is therefore regarded almost impossible. Nevertheless, some coexisting populations have been observed. The aim of this study was to resolve whether the coexistence is possible due to reduced virulence in local *A. astaci* strains, increased immunity in susceptible crayfish species, or a complete absence of the pathogen in some American crayfish populations. Crayfish were collected from nine standing waters in central Europe with a coexistence of introduced spiny-cheek crayfish (*Orconectes limosus*) and native noble crayfish (*Astacus astacus*). We used highly sensitive *A. astaci*-specific real-time PCR to find out if *A. astaci* is present in these populations, and we conducted exposure experiments to further validate the molecular results. No *A. astaci* positive individuals were detected among the 523 tested crayfish (490 spiny-cheek crayfish, 33 noble crayfish). Exposure experiments approved these results: No abnormal mortality or behavioral changes were seen in noble crayfish kept together with American crayfish from the coexisting populations. Results of a statistical test show that disease absence can be declared in eight of the nine coexisting populations (98% confidence interval). Therefore, a consistent absence of *A. astaci* most likely explains the coexisting populations in central Europe. These findings reveal new perspectives for the protection on native crayfish species and restocking programs, even in areas where American crayfish species are present.

Keywords: Crayfish plague, Coexistence, Real-time PCR, Exposure experiment, *Aphanomyces astaci*

Ten years after – a story of successful reintroduction of *Astacus astacus*

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A small lake in East Tyrol, the "Tristacher See" and its outlet the "Tristacher Seebach" were known to hold a population of *Astacus astacus* since the Middle Ages. The crayfish in this water bodies became extinct in the late 1990s due to unknown reasons. Among the local residents the wish arose soon to restore this population of historical significance as it was already mentioned in the "Hunting and Fishing Book" of Emperor Maximilian I in 1504. Thus a stream restoration and crayfish conservation programme was started.

To make the small brook more favourable for crayfish, its banks were renaturated and several types of artificial and semi-natural shelter were provided to reduce the migration habit of adult crayfish when transferred to their new habitat. From 2001 to 2003 adult egg bearing females from a nearby fish pond were transferred to a crayfish hatchery where they were kept over summer. In autumn the females were stocked in the outlet together with males and the juveniles of the year to fund a new population of the noble crayfish (Sint & Füreder 2004).

In May 2012 the success of this reintroduction measure was evaluated by exposing baited traps and night searching the stretches of the stream where the crayfish were stocked ten years before. The investigation revealed that all the effort to bring the noble crayfish back was not done for nothing, but achieved a new population that reconquered its ancestral habitat.

Sint, D. & Füreder, L. (2004) Reintroduction of *Astacus astacus* L. in East Tyrol, Austria. Bulletin Français de la Pêche et de la Pisciculture, 372-373, 301-314.

Evaluation of the functional state of crayfish *Cherax quadricarinatus* used as test-organisms in bioelectronics systems bioindication of water quality

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The functional state of crayfish *Cherax quadricarinatus* cultured in the laboratory was assessed by the characteristics of cardiac activity before and after prolonged exposure to biologically treated sewage (BTS). Heart rate (HR) of six crayfish was registered non-invasive method using fiber-optic sensors that are connected to the system of registration and analysis of cardiac activity. After a period of adaptation, which can be described by three consecutive phases of adaptive reactions which are reflected in the dynamics of HR, the functional state of crayfish has stabilized and was characterized by expressed circadian rhythm. This rhythm has the following characteristics: regular nocturnal activity more than 8 hours with HR in the range of 100-180 beats / min., and at rest HR is maintained at 50 - 60 beats / min. To determine the energy status of the organism used a specially designed the test suspension. A typical reaction of crayfish who is in good physiological state at the short-term exercise, is the rapid increase in heart rate (100% or more), maintaining this level during exercise and in the rapid (within 30-60 min) decrease to the initial level. All test organisms showed a similar reaction to the suspension. This study of crayfish cardiac activity showed that its characteristics after prolonged exposure to BTS match those observed in pure water laboratory. BTS not significantly affect the functional status of crayfish, as evidenced by the preserved ability to adapt to the quality of the crayfish habitat and an adequate response to functional load requirements.

Keywords: *Cherax quadricarinatus*, functional state, heart rate, treated sewage

Status of the emerging invader, the white river crayfish, in Dutch waters

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White river crayfish, the *Procambarus acutus/zonangulus* species complex, important in aquaculture and fisheries in the southern United States, are emerging invaders in Europe. Their identification and taxonomy remains unclear, as recent genetic analyses uncovered not only presence of two divergent mitochondrial lineages in the introduced population in the Netherlands but also substantial variation in the samples originating from the USA. White river crayfish have been spreading in the Dutch waters since at least 2005, and at present form viable and commercially exploited population. On the poster, we will summarize data on spread, present distribution, and genetic structure of *P. acutus/zonangulus* in the Netherlands, and highlight potential future directions of research on these invasive crayfish.

Keywords: New NICS, *Procambarus acutus/zonangulus*, distribution, genetic variation

Porcelain disease in the white-clawed crayfish *Austropotamobius pallipes* populations from Western Europe: implications for restocking

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Conservation of the European indigenous white-clawed crayfish cannot be undertaken without sanitary controls of populations selected for reintroduction because restocking plans can be a way for pathogen dispersion. Control measures must include careful examination of a representative sample from the population chosen and estimation of the prevalence of pathogens estimated with molecular tools. Our study concerns the pathogenic microsporidian (*Thelohania contejeani*, Henneguy 1892), responsible for porcelain disease infecting muscles leading to a decrease of locomotive activity and nutritive capacity until death. The aim of our study is to describe the infection status of French populations of the white clawed crayfish (*Austropamobius pallipes*). The sampling concerns mainly two basins (Loire and Rhône) and also reared populations for restocking. For comparison, populations of *Austropotamobius italicus* were also analysed. The diagnosis is based on amplification of the subunit RNAr 16S. When *T. contejeani* is present, the prevalence observed is from 6 to 29 %. Only *A. pallipes* are infected and populations from the Allier watershed (Loire) are the more infected. Populations reared for restocking have the same genetic diversity but one of them was particularly infected and consequently must be avoided for reintroduction plan.

Keywords: indigenous crayfish, conservation, microsporidia, thelohaniosis, restocking

Assessment of burrowing activity of the red swamp crayfish, *Procambarus clarkii*, in fish-ponds of La Brenne

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In 2007, the invasive crayfish *Procambarus clarkii* was first identified in the "Parc Naturel Régional de la Brenne" located in the southwestern area of Indre department (Centre region, France). Ten infestation sites have been listed in the park, with 86 fish-ponds colonized and 170 000 crayfish trapped by the stakeholders. In the park, there is a need to better understand the current situation of the invasion including information about what species are being impacted by the crayfish as well as how the structure of the habitats (fish-ponds) could be affected. The objective was to test a methodology suitable for monitoring recently drained fish-ponds on a weekly basis to follow the progression of crayfish burrowing behaviour. We aimed to quantify such impacts by identifying the factor(s) inducing burrowing activity and which habitat features make a fish-pond more susceptible to damage by burrowing. Weekly inspections of burrowing activity, density, occupation, location with respect to the banks, and micro-habitat features were carried out in dry fish-ponds for 2 months in spring. *P. clarkii* seek soil humidity in a dry pond and prefer burrowing on silt substrates. The analysis showed no statistical difference between sites but difference among weeks independently of the sites. Given the duration of the study and the number of fish-ponds surveyed, conclusions drawn provide a first record that can be used as a starting point for future studies, and it gives an idea of how other colonized fish-ponds may be affected.

Keywords: Invasive crayfish, burrowing, fish-ponds, control

The indigenous crayfish in the Plitvice Lakes National Park (Croatia)

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The Plitvice Lakes National Park is the oldest national park in Southeast Europe and the largest national park in Croatia, situated in the mountainous karst area of Central Croatia. Its interconnected lakes are arranged in cascades separated by natural travertine dams, and due to their specificity NP is placed to the UNESCO World Heritage list. Crayfish research within the NP borders was sporadic in the past and there is no reliable data on the state of astaco fauna. Continuous crayfish research within the NP was conducted between 2002 and 2005. Research was dedicated to the distribution, ecology and dynamics of crayfish present in the water bodies of the NP. Two out of five European crayfish species were recorded, namely *Astacus astacus* and *Austropotamobius torrentium*. *A. astacus* was recorded on 17 localities mainly in lotic habitats on the travertine dams, while it was never recorded within the lentic habitats. *A. torrentium* was recorded only within five localities, in the small streams feeding lakes. In addition, on three localities mixed populations of the two species were recorded, presumably *A. astacus* presence is of anthropogenic origin. All of the obtained results on the distribution, ecology and dynamics of *A. astacus* and *A. torrentium* are valuable contribution to the knowledge of the biodiversity and specificity of Croatian and European astaco fauna. The obtained data are also fundamental for the development of management strategies with the aim of better crayfish protection in the circumstances of increasing anthropogenic pressure onto the different habitats within the NP.

Keywords: noble crayfish, stone crayfish, distribution, dynamic, management

Native crayfish restocking programme in Slovakia to launch

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The restocking programme of native crayfish species (*Astacus astacus* and *Austropotamobius torrentium*) was launched in Western Slovakia as a result of cooperation between Comenius University, Bratislava and Volkswagen Slovakia. Subjective of the cooperation is to support a research and protection of biodiversity (with crayfish as the flagship species) by construction of crayfish hatcheries for noble crayfish as well as for stone crayfish, support inventory and monitoring research of native and invasive species as well as assure establishing of crayfish at carefully selected places.

Key words: crayfish restocking programme, *Astacus astacus*, *Austropotamobius torrentium*, Slovakia

Contribution to knowlegde of the recent Decapoda distribution in Balkan region

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Several sampling trips were carried out by the Slovak crayfish research team during last five years to diverse regions and countries of Balkan Peninsula. There were found very abundant populations of stone crayfish, *Austropotamobius torrentium* in Serbia and Monte Negro, confirmed occurrence in Trakya (Turkey), Romania, Bulgaria and Greece. We found white-clawed crayfish, *Austropotamobius pallipes* in Bosnia. No noble crayfish was found during our research trips; however we did not sample lakes and large rivers there. Freshwater crabs, *Potamon ibericum* was found in Bulgaria and Turkey. Non-indigenous species *Orconectes limosus* was reported in river Tamis in Serbia, which opens the gate for invasion to large catchment in northern Romania.

Key words: Decapoda, crayfish, Balkan

Fresh-water crayfish distribution and commercial stocks

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That report is based on materials of the researches, carried out last decade by scientific workers of FGBNU "GosNIORKH" and its regional sections.

Among fresh-water crayfishes in the European part of Russia *Astacus astacus* L. and some species, subspecies and morphs of genus *Pontastacus* (Starobogatov taxonomic division, 1995), including the most widespread – *P. leptodactylus* Esch, have commercial value.

The geographic range of the most valuable *A. astacus* is limited by Russia's Northwest territories in the vicinity of the Baltic Sea. Species of genus *Pontastacus* are extended practically in all territory of the European part of Russia.

The main crayfishery regions are the Lower Volga with Volgo-Ahtubinskaja watermeadow, the Azovo-Black Sea basin, reservoirs of Average Volga, waterbodies of Northwest region.

Stable commercial stocks of *A. astacus* exist only in three regions: Pskov -140 t, Leningrad - 65 t and Novgorod - 3,5 t (according to data of 2008).

Southern areas of Russia possess the greatest stocks of crayfishes. For example, in 2007 and 2011 crayfishes commercial stocks in reservoirs of the Lower Volga constituted: in Saratov reservoir - 140 and 168 t, in Volgograd reservoir - 172 and 160 t, accordingly. In 2011 commercial catch in these two reservoirs constituted 67 t in the sum.

The volume and character of crayfish stocks distribution are substantially defined by natural factors. However, the role of anthropogenous factors (waterbodies pollution, illegal fishery and others) has increased now.

The most rational way of maintenance and increase of crayfish stocks - artificial cultivation.

Keywords: fresh-water crayfishes, distribution, commercial stocks

Temporal dynamics of spore release of the crayfish plague pathogen from its American vector

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The crayfish plague pathogen, *Aphanomyces astaci*, is one of the most serious threats to indigenous European crayfish species. In Central Europe, the spiny-cheek crayfish (*Orconectes limosus*), an invasive species of North American origin, seems to be an important source of this pathogen because of its widespread distribution, frequently high prevalence of the parasite, and ability to host it over a long period. Some literature sources suggested that the pathogen dispersal from American vectors is more intensive in periods of moulting or when the host crayfish dies. However, direct evidence for such hypotheses was lacking. The aim of our work was to test these predictions by experiments investigating *A. astaci* transmission from infected spiny-cheek crayfish to non-infected noble crayfish (*Astacus astacus*). We filtered defined volumes of water regularly to quantify spore concentration, and sampled the tissues of all crayfish at the end of the experiment. The filters, as well as crayfish tissues, were tested for presence and quantity of *A. astaci* DNA by species-specific real-time PCR. The experiments supported the evidence that *A. astaci* can be transmitted to susceptible crayfish even if the American host is neither moulting nor dying. However, the concentrations of the pathogen spores substantially varied during the experiments, and significantly higher amount of spores were detected during moulting of the infected host. Therefore, while probability of crayfish plague transmission by water transfer seems to be highest when hosts are moulting, other time periods cannot be proclaimed safe.

Keywords: *Orconectes limosus*, *Aphanomyces astaci*, *Astacus astacus*, transmission, real-time PCR

Distribution of crayfish populations (*Astacus astacus* and *Pontastacus* (*A.*) *leptodactylus*) in the lakes of Leningrad region (according to the research of 2009-2011)

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In the lakes of Leningrad region two species of crayfish inhabit *Pontastacus* (*A.*) *leptodactylus* (Esch.) and in the main – *Astacus astacus* L. In some water-bodies only of *P* (*A.*) *leptodactylus* inhabit, or the cohabitation of two species is noted.

The aim of the research is to commercial crayfish stocks in the lakes of the Leningrad region and to determine the possibility of their catch.

Materials and methods. The 20 lakes, located in two different geomorphologic characteristics the areas of the Leningrad region (the Karelian isthmus and the Tikhvin area) were studied in the period of 2009 - 2011.

The standard rectangular folding creels (20×20×40cm) were used for the crayfishing.

Results. Abiotic conditions are near the borders of the best values for crayfish (especially for *A. astacus*) in the studied lakes of Leningrad region. The density of crayfish is at a low level in most lakes (from 0,025 to 0,21 ind./m^2). To the category of medium - and high-yield water-bodies it may be attributed not more than 25% of the studied lakes. *A. astacus* are found individually or in form of accumulation in small lokales. A number of lakes in which of *P* (*A.*) *leptodactylus* and *A. astacus* are found together, is increased (of the investigated - 2).

Keywords: freshwater crayfish, commercial stock crayfish

Phylogeography of *Procambarus* (*Austrocambarus*) in the northern of Yucatán Peninsula

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The study of the crayfish species diversity in Yucatán Peninsula has required an exhaustive examination and review of populations throughout its wide geographical spread. This work is part of a study of the entire peninsula, and presents a phylogenetic relationships analysis of the populations found in the northern region and its distribution, made in order to clarify their identity. Our results support the populations found do not correspond to *Procambarus* (*Austrocambarus*) *llamasi*, as stated in previous studies and we suggest they should be treated as different forms.

Keywords: Mexican crayfish, *Procambarus* (*Austrocambarus*), phylogeography, Yucatán Peninsula

Breeding and introduction of threatened *Austropotamobius torrentium* and *Astacus astacus* populations in Tyrol

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Autochthonous crayfish of the Alpine countries (*Austropotamobius torrentium, Austropotamobius pallipes* and *Astacus astacus*) have been exposed to various threats and their populations still are strongly decreasing. Like in many endangered species, the decrease of native freshwater crayfish as well as their acute threat has been portrayed as a consequence of human activities. In ongoing species protection programs carried out in the Austrian and Italian Tyrol, we undertake measures to enhance the situation of all three indigenous species. This study aimed at documenting measures to support the two autochthonous and endangered stone crayfish and noble crayfish. One *A. torrentium* population was studied, female and male individuals held in breeding tanks for ten months, and monitored for the whole breeding period. Two *A. astacus* populations were taken from the wild and were also used for breeding and introduction activities. Data were collected for crayfish activities, fertility, size and structure of the individual populations. The comparison of population characteristics showed no significant differences between egg carrying females in the tanks and in the wild. We found significant differences concerning condition, injuries, activities and brood care of the crayfish populations, due to a difference in water temperature, structure and food supply in the different water bodies. This documentation of population parameters and investigations of the fecundity of crayfish during breeding and the comparison with wild individuals brought important information for the success of breeding and reintroduction activities and provides essential knowledge for the further implementation of crayfish conservation measures.

Keywords: crayfish, aquatic conservation, management, environmental protection, fecundity


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Austropotamobius pallipes


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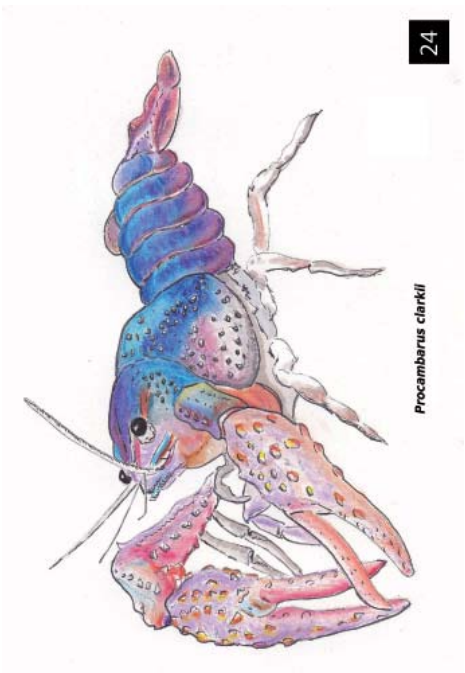
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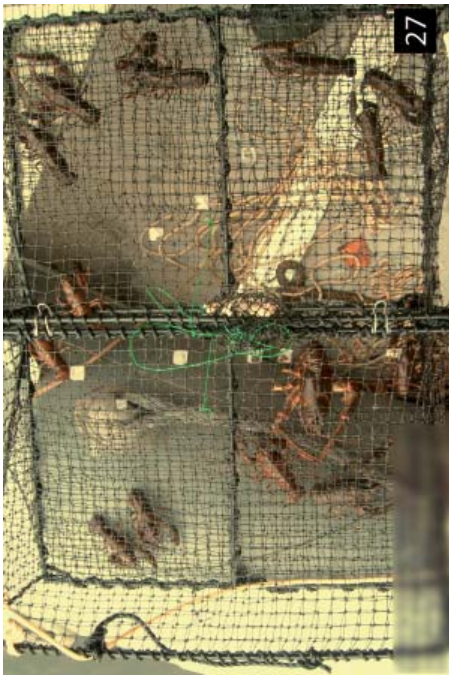
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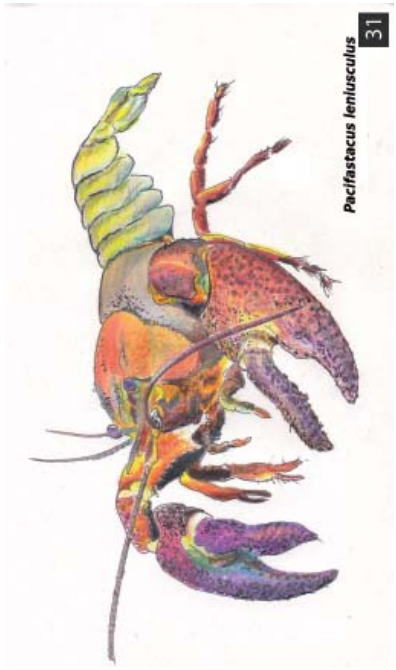
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Pacifastacus leniusculus


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