Welcome Message

Javier Diéguez-Uribeondo
IAA21 Symposium organizer

On behalf of the International Association of Astacology (iz.carnegiemnh.org/crayfish/IAA/), it is with great pleasure that the organizing and scientific committees invite you to participate in the 21st IAA Symposium being held from September 5 – 8, this year at the Royal Botanical Garden, RBG, in Madrid, Spain.

The IAA symposium will take place for the first time in Spain, a country with strong traditions regarding crayfish fisheries. It has been almost 40 years since the crayfish plague struck the native population, which consequentially produced a dramatic change in the aquatic environment as well as in recreational and fishing activities in freshwater environments. The RBG, which belongs to the National Spanish Research Council (CSIC), has developed a line of research focused on the crayfish plague. The RBG is now a key center in Spain for the investigation of crayfish plague, the development of conservation plans, and the management of alien species.

Three crayfish species presently coexist in the Iberian Peninsula: one native, *Austropotamobius pallipes*, and two alien species from North America, *Procambarus clarkii* and *Pacifastacus leniusculus*. *Austropotamobius pallipes* is categorized as endangered and several conservation plans are being implemented in Spain for the species. The two alien species have been subjected to exploitation for both commercial and recreational purposes. The current decline of native crayfish species around the world, as well as the effect that globalization has on the management of invasive species, make Spain an ideal place to discuss current scientific problems regarding freshwater crayfish and aquatic ecosystems.

The RBG has a history spanning more than 260 years, and it is located in the historic center of Madrid within walking distance to the world-famous Prado, Tyssen, and Reina Sofia art museums, making possible to comfortably combine work and tourism. I hope that this symposium will facilitate crayfish scientists to share our experiences and projects and will contribute to enhance our global networking.

On behalf of the organizing committee, we welcome you to IAA21 in Madrid at the Real Jardín Botánico!
IAA21 International Symposium

Date: 5th (Monday) to 8th (Thursday) 20016
Venue: Lecture Hall, Real Jardín Botánico, Madrid (http://www.rjb.csic.es)

Organizing Committee
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Jesús Muñoz Fuente, Real Jardín Botánico, CSIC, Spain
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Laura Martín-Torrijos, Real Jardín Botánico, CSIC, Spain

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Trude Vrålstad, Norwegian Veterinary Institute, Norway

Student Auction Organizers
Laura Martín-Torrijos, Real Jardín Botánico, CSIC, Spain

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Real Jardín Botánico, CSIC, Spain
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PROGRAM

21st Symposium of the International Association of Astacology
Sunday 4

09.00: 17:30  Workshop: Conservation Plans for Endangered Species Facing the Threat of Emerging Diseases and Invasive Species [link]
Lecture Hall, Real Jardín Botánico

18.00: 20:00  Registration and Poster Set Up
INOFFICIAL GATHERING sponsored by Caramelos “El Caserio” and “Cerveza Alegria” Bonsai
Greenhouse, Real Jardín Botánico

Monday 5

08:00- 09:45  Registration and Poster Set Up
Bonsai Greenhouse, Real Jardín Botánico

09:45-10.00  Address of Welcome
Lecture Hall, Real Jardín Botánico

SUSAN B. ADAMS
President IAA

JESUS MUÑOZ
Director Real Jardín Botánico-CSIC, Madrid, Spain

10.00-10.30  Welcome Speech
Crayfish in Spain: past, present and future
JAVIER DIEGUEZ-URIBEONDO
IAA21 organizer, Real Jardín Botánico-CSIC, Madrid, Spain

10:30-11:00  COFFEE AND POSTERS
Bonsai Greenhouse Real Jardín Botánico

11:00-12:15  STURE ABRAHAMSSON MEMORIAL CONFERENCE
Melanization: an evolutionary conserved innate immune system
KENNETH SÖDERHÅLL
Uppsala University, Sweden

12:30  LUNCH
Comedor Delegación del Ministerio Cultura, Parque del Retiro (15 min walk)

CRAYFISH DISEASES SESSION
Lecture Hall, Real Jardín Botánico

14:00-14:30  KEY NOTE
Natural born killers: the Oomycetes as important pathogens of animals
GORDON BEAKES
Newcastle University, UK
Chair: JENNY MAKKONEN
14:30-14:50 Crayfish plague in Spain: origin and epidemiology
LAURA MARTÍN-TORRIJOS, Rezinciuc S, Kokko H, and J Diéguez-Uribeondo
Real Jardín Botánico-CSIC, Madrid, Spain

14:50-15:10 Pitfalls in the application of molecular methods for the diagnosis of crayfish plague
SATU VILJAMAA-DIRKS, Heinikainen S, Riva R, and S Pelkonen
The Finnish Food Safety Authority Evira, OIE, Finland

15:10-15:30 The crayfish plague pathogen, Aphanomyces astaci: pathways, vectors and potential consequences of its spread in the introduced ranges
Charles University in Prague, Czech Republic

15:30-15:50 Host-pathogen network analysis of Saprolegniales: host preference and specificity of Aphanomyces astaci
JOSE V SANDOVAL SIERRA, and J Diéguez-Uribeondo
Real Jardín Botánico-CSIC, Madrid, Spain

15:50 COFFEE AND POSTERS
Bonsai Greenhouse, Real Jardín Botánico

Chair: SATU VILJAMAA-DIRKS
16:20-16:40 Scenarios for Aphanomyces astaci adaptations to native and alien crayfish host in Europe
JAPPO JUSSILA, Kortet R, Vainikka A, Kokko H, and J Makkonen
University of Eastern Finland, Kuopio, Finland

16:40-17.00 Crayfish plague in Japan
Martin-Torrijos L, Sandoval-Sierra J, Makkonen J, Jussila J, Kokko H, Kawai T, and JAVIER DIÉGUEZ - URIBEONDO
Real Jardín Botánico-CSIC, Madrid, Spain

17.00-17:20 A survey of various wild crayfish populations in Germany and Austria reveals new insights into the spread and diversity of Aphanomyces astaci
JÖRN PANTELEIT, Keller NS, Jussila J, Kokko H, Makkonen J, Schulz R, Theissinger K, and A Schrimpf
University Koblenz-Landau, Landau, Germany

17.20-17.40 Identification of an Austropotamobius pallipes population with higher resistant to the crayfish plague pathogen, Aphanomyces astaci
LAURA MARTIN-TORRIJOS and J Diéguez-Uribeondo
Real Jardín Botánico-CSIC, Madrid, Spain

18.00 IAA Executive Board Meeting
Sala de Juntas, Real Jardín Botánico

Tuesday 6

CRAYFISH OMICS and eDNA SESSION
Lecture Hall, Real Jardín Botánico

09.00-09.40 KEY NOTE
Parametric models to trace the spatiotemporal evolution of populations and lineages
ISABEL SANMARTÍN
Real Jardín Botánico-CSIC, Madrid, Spain
Chair: HARRI KOKKO

09:40-10:00  
The complete genome sequence of the marbled crayfish  
FRANK J LYKO  
Cancer Research Center, Im Neuenheimer Feld 580, 69120 Heidelberg, Germany

10:00-10:20  
The transcriptome of noble crayfish Astacus astacus – an excellent tool and a reference for further gene expression studies  
The University of Eastern Finland, Kuopio, Finland

10:20-10:40  
Genomics-informed development of molecular markers for genotyping the crayfish plague pathogen Aphanomyces astaci  
DIANA MINARDI, Studholme DJ, van der Giezen M, and B Oidtmann  
University of Exeter, Exeter, UK

10:40-11:10  
COFFEE AND POSTERS  

Bonsai Greenhouse, Real Jardín Botánico

Chair: TRUDE VRÅLSTAD

11:10-11:30  
The annotation of two Aphanomyces mitochondrial genomes from A. astaci and A. invadans  
Makkonen J1, Vesterbacka A1, Martin F1, Jussila J1, Diéguez-Uribeondo J2, Kortet R1, and HARRI KOKKO1  
1The University of Eastern Finland, Kuopio, Finland  
2Real Jardín Botánico CSIC, Madrid, Spain

11:30-11:50  
How do invasive species cope with different environmental conditions? A proteomic study of two alien crayfish: Procambarus clarkii and Procambarus fallax f. virginalis.  
FRANCISCO J. OFICIALDEGUI, Roessink I, Biron D, Boyero L, Clavero M, Peeters ETHM, and MI Sánchez  
Estación Biológica de Doñana-CSIC, Sevilla, Spain

11:50-12:10  
Comparing classic crayfish cage surveillance with eDNA water monitoring during an on-going crayfish plague outbreak in Norway  
Norwegian Veterinary Institute, Oslo, Norway

12:30-13:30  
LUNCH  

Comedor Delegación del Ministerio Cultura, Parque del Retiro

CRAYFISH BIOLOGY & PHYSIOLOGY SESSION

Chair: PAVEL KOZÁK

14:00-14:20  
Physiological responses of freshwater crayfish (Cherax albidus Clark 1936) when exposed to various salinities of ocean water and inland saline water  
ANTHONY J COLE, Fotedar R, and TC Hoang  
Curtin University, Perth, Australia

14:20-14:40  
Survival, recovery and cardiac activity of three crayfish invaders under sub-zero temperature  
Yazicioglu B, KUKLINA IRYNA, Buřič M, Císař P, and P Kozák  
University of South Bohemia in České Budějovice, Vodňany, Czech Republic

15:00-15:20  
The mineral content of Cherax quadricarinatus in Southeast Queensland and Northeastern New South Wales  
LEYTON J. TIERNEY, Wild CH, and JM Furse  
Griffith University, Queensland, Australia
15:20-15:40 Return to crayfish high school: long-term monitoring of crayfish populations at the UCC outdoor school 2011 to 2016
PREMEK HAMR and E Wong
Upper Canada College, Toronto, Canada

15:40-16:00 Crayfish role in a canyon-shaped reservoir: case study from the Nýrsko, Czech Republic
VESELY L1, Bláha M1, Buřič M1, Fořt M1, Pešek V1, Kozák P1, Ruukonen TJ2, Ercoli P1, and A Kouba1
1University of South Bohemia in České Budějovice, Vodňany, Czech Republic
2University of Jyväskylä, FI-40014, Finland

16:00 COFFEE AND POSTERS
Bonsai Greenhouse, Real Jardín Botánico

Wednesday 7

CRAYFISH BIOLOGY & ECOLOGY SESSION
Lecture Hall, Real Jardín Botánico

09:00-09:40 KEY NOTE
Crayfishes and companions across the tree of life
PABLO VARGAS
Real Jardín Botánico-CSIC, Madrid, Spain

Chair: JAMES FURSE

09:40-10:00 Crayfish ecological diversity and conservation across a synthetic phylogeny
KEITH CRANDALL
The George Washington University, Ashburn, VA, US

10:00-10:20 Low local crayfish diversity and high species turnover in lowland streams may be influenced by a few widely distributed crayfish species
WILLIAM R. BUDNICK, Harlan AR, Pasco TF, Kelso WE, and MD Kaller
Louisiana State University, Baton Rouge, US

10:20-10:40 Range expansion of the signal crayfish (Pacifastacus leniusculus) in a recently invaded region in Croatia and potential for its control
SANDRA HUDINA, Galić N, Kutleša P, Duplić A, and I Maguire
University of Zagreb, Croatia

10:40-11:00 COFFEE AND POSTERS
Bonsai Greenhouse, Real Jardín Botánico

Chair: LENNART EDSMAN

11:00-11:20 Do you suffer from a lack of historic crayfish data?
SUSAN B ADAMS
USDA Forest Service, Oxford, Mississippi, USA

11:20-11:40 The first evidence of co-occurrence between native crab and crayfish in Italy
ELENA TRICARICO, Cianferoni F, Stasolla G, Inghilesi AF, Zoccola A, Innocenti G, and G Mazza
University of Florence, Firenze, Italy

11:40-12:00 Investigation of a localized decline in freshwater crayfish Paranephrops planifrons in the upper Waikato River, New Zealand
SUSAN J CLEARWATER, Quinn JM, and IA Kusabs
NIWA, National Institute of Water & Atmospheric Research, Hamilton, New Zealand 3251
University of South Bohemia in České Budějovice, Vodňany, Czech Republic
12:30-13.30  LUNCH  
Comedor Delegación del Ministerio Cultura, Parque del Retiro

Chair: ALVARO ANTÓN

14:00-14:20  The effects of the invasive weed Singapore daisy on the native Australian freshwater crayfish *Tenuibranchiurus glypticus* Riek  
JAMES M. FURSE, Houston AJ, and CH Wild  
Griffith University, Queensland, Australia

14:20-14:40  White-clawed crayfish (*Austropotamobius italicus*, Faxon) effects on macroinvertebrate communities from Mediterranean limestone mountain streams  
JUAN ANTONIO ARCE, Alonso F, Camacho A, and E Rico  
Junta de Comunidades de Castilla-La Mancha. Cuenca, Spain

14:40-15:00  Uptake and transfer of microcystins in noble crayfish in Lake Steinsfjorden, a cyanobacterial (Planktothrix) dominated lake  
JOHANNES RUSCH, Strand D, Haande S, Ballot A, Løvberg KE, Samdal IA, Miles CO, and T Vrålstad  
Norwegian Veterinary Institute, Oslo, Norway

15:00-15.20  Could crayfish care about safety of beer? - A long way from the research to the practical use  
University of South Bohemia in České Budějovice, Vodňany, Czech Republic

15:20  GENERAL ASSEMBLY  
Lecture Hall, Real Jardín Botánico

17.00  COFFEE & NATIONS BUFFET followed by IAA AUCTION  
Auctioneer: Gordon Beakes  
Bonsai Greenhouse, Real Jardín Botánico

Thursday 8

CRAYFISH AQUACULTURE AND FISHERIES SESSION  
Lecture Hall, Real Jardín Botánico

Chair: ELENA TRICARICO

09:10-09:30  Predicting harvest of the non-native signal crayfish in Swedish lakes – a role for changing climate?  
SLU, Institute of Freshwater Research, Drottningholm, Sweden

09:30-09:50  Influence of a relationship between selected trace elements and natural productivity on growth and yield of marron in a commercial farm  
SMITA S TULSANKAR and R Fotedar  
Curtin University, Perth, Australia

09:50-10:10  Polyphenols as feed additives: a new tool to prevent diseases in farmed crayfish?  
PAOLUCCI MARINA, Parrillo L, Sciscia E, Coccia E, Siano F, Pagliarulo G, Volpe MG, Jussila J, Makkonen J, and E Varricchio  
University of Sannio, Benevento, Italy

10:10-10:30  Harvesting New Zealand Freshwater Crayfish (*Paranephrops zealandicus*) – five years of harvest data and the implications for population dynamics and stock management  
JOHN HOLLOWS  
Ernslaw One Ltd., Mosgiel, New Zealand
10:30-11:00  COFFEE AND POSTERS
Bonsai Greenhouse, Real Jardín Botánico

Chair: MARINA PAOLUCCI

11:00-11:20  The use of a traditional māori harvesting method, the tau kōura, for monitoring of freshwater crayfish (kōura, Paranephrops planifrons) populations in the Te Arawa lakes, New Zealand
IAN A KUSABS
Tūwharetoa Māori Trust Board, Rotorua, New Zealand

11:20-11:40  New advances in astacid juvenile feeding research: development of practical diets
JOSÉ M CARRAL, Fuertes JB, Celada JD, and M Sáez-Royuela
Universidad de León, 24071 León, Spain

11:40-12:00 Identify factors influencing the variability of survivorship of juvenile redclaw crayfish Cherax quadricarinatus
DAMIAN RIGG
James Cook University, College of Marine and Environmental Sciences, Macgregor Road Smithfield, Queensland, Australia, 4878

12:30-13:30  LUNCH
Comedor Delegación del Ministerio Cultura, Parque del Retiro

CONSERVATION SESSION

Chair: CATHERINE SOUTY-GROSSET

14:00-14:30 KEY NOTE
Crayfish conservation in the: strategies and lessons learnt
LEOPOLD FÜREDER
University of Innsbruck, Austria

14:30-14:50 People's perception of crayfish
BRAM KOESE
Naturalis Biodiversity Center, Leiden, The Netherlands

14:50-15:10  Effects of flood-control impoundments on community assemblage of stream crayfish
ZANETHIA C. BARNETT
USDA Forest Service - Southern Research Station, Oxford, Mississippi, USA

15:10-15:30  The conservation of the white-clawed crayfish, Austropotamobius pallipes in South West England
JEN NIGHTINGALE
Bristol Zoological Society, Bristol, UK

15:30-15:50  Conserving white-clawed crayfish Austropotamobius pallipes in an upland catchment in Yorkshire, a case-study
STEPHANIE PEAY
AECOM and University of Leeds, Leeds, UK

15.50- 16.20 COFFEE AND POSTERS
Bonsai Greenhouse, Real Jardín Botánico

16:20-16:40 The future of endangered Austropotamobius torrentium (Schrank 1803) in the light of protected areas and habitat fragmentation: a case study from the Carpathians
LUCIAN PÂRVULESCU, Iorgu EI, Satmari A, Zaharia C, Drăguț I, Krapal AM, Popa OP, and LO Popa
West University of Timisoara, Timisoara, Romania
16:40-17:00  **Environmental education and awareness, fundamental tool in the conservation of the native crayfish**
NÚRIA VALLS, Llamas S, and O Comas
Associació de Defensa i Estudi de la Fauna i Flora Autòctona (ADEFFA), Barcelona

17:00-17:10  **An educational video produced as part of an awareness raising campaign of LIFE+ CrayMate project.**
JAPPO JUSSILA J
University of Eastern Finland, Kuopio, Finland

17:15  **COFFEE and Poster Removal**
Bonsai Greenhouse, Real Jardín Botánico

21:00  **BANQUET AND FAREWELL**

9-13th SEPTEMBER CRAYFISH FIELD EXCURSION GRANADA-SEVILLA
ABSTRACT STURE ABRAHAMSSON
MEMORIAL LECTURE
Melanization, an evolutionary conserved innate immune system
KENNETH SÖDERHÅLL
Department of Comparative Physiology, Uppsala University
Norbyvägen 18752 36 Uppsala, Sweden

Although invertebrates lack the complexity of the adaptive immune system and rely solely on innate immunity their amazing diversity, abundance and evolutionary success argue for a highly efficient defense system against infections. Innate immune responses include phagocytosis, synthesis of antimicrobial peptides (AMPs) and activation of proteolytic cascades that lead to melanization (the prophenoloxidase activating system, ProPO-system) and blood coagulation. The ProPO system is a proteolytic enzyme cascade, which is activated by minuscule amounts (picogram per l) of cell wall products from microorganisms (LPS, PGN and glucans) and subsequently generates immune factors such as for example peroxinectin. The proPO-cascade requires careful regulation by a spatial and temporal control to avoid dangerous side effects. All of the steps in the proPO-cascade are shared with the proteinase cascade leading to the activation of the Toll pathway for production of antimicrobial peptides. Recently we showed that caspase 1 is involved in release and regulation of proPO and that fragments from proPO are antibacterial and further that white spot syndrome virus inhibits the proPO-system and avoids the immune system and can replicate. I will in my talk give a history of our work on melanization.
ABSTRACTS KEYNOTE LECTURES

(in order of appearance in the program)
Natural born killers - oomycetes as important pathogens of animals
GORDON W BEAKES
School of Biology, Newcastle University, Newcastle upon Tyne, NE1 7RU, U.K.

The oomycete “fungi” belong to the Straminipile/Alveolate/Rhizaria superkingdom and are closely related to the golden-brown algae. They used to be thought of as predominantly freshwater saprotrophs or parasites of plants. Over past two decades molecular phylogeny has radically altered the way we view the oomycetes. There are a number of early diverging, non oogamously reproducing, clades that contain mostly marine parasites of nematodes, crustaceans and seaweeds, many of which have extra-ordinarily elaborate infection structures. The Haliphthorales are important pathogens of crustaceans, that can result in significant losses in marine aquaculture systems. The major divergence in the oomycete lineage came with the splitting off of the Peronosporalean and Saprolegnialean lines. The former are largely terrestrial, often soil born or plant pathogens and are characterised by their periplasmic oogenesis (which requires exogenous sterols) and often vesiculate discharge of zoospores. The latter, encompass most of the familiar water mould genera, and include the basal Leptomitales and the more familiar Saprolegniales, that contain general such as Aphanomyces, Achlya and Saprolegnia. The Aphanomyces clade is particularly interesting as it encompasses clades that are predominantly animal pathogens (including A. astaci), plant pathogens and saprotrophs. It also includes several genera that parasitize invertebrates such as rotifers (Aquastella) and nematodes (Sommerstorffia). The evolutionary equivalent in the Peronosporalean lineage appears to be the Pythiaceous clades that include pathogens of nematodes (Lagenidium, Myzocytiopsis), crustaceans (Salilagenidium) and vertebrates (Paralagenidium, as well as the more familiar saprotrophs and plant pathogens (Pythium spp.). The overall evolutionary significance of these findings will be discussed.

Parametric models to trace the spatiotemporal evolution of populations and lineages
ISABEL SANMARTÍN
Department of Biodiversity and Conservation, Real Jardín Botánico-CSIC, Madrid, Spain

Parsimony approaches were for many years the only option available for inferring the biogeographic history of populations, species, and lineages. The development in recent years of parametric approaches based on probabilistic models of range evolution has revolutionized the discipline, widening the range of questions that can be addressed with genetic and spatial data. These models, typically based on continuous-time discrete state Markov chain processes, are inspired by models used in phylogenetic studies to trace the evolution of a character in a phylogeny, but here the character states are the geographical ranges of the species. Unlike parsimony approaches, they allow integrating time and other sources of evidence (species' ecology, Earth history) and account for the uncertainty in phylogenetic and ancestral state reconstruction. Here, I review these approaches, their rapid growth, and current challenges. Some models, such as maximum-likelihood DEC and derivatives, provide detailed reconstructions of the history of individual lineages, including numerous types of biogeographic processes (extinction, range expansion, dispersal, speciation), at the expense of computational efficiency. Other class of models use Bayesian inference to jointly estimate phylogenetic relationships, divergence times, and ancestral states given molecular data and the geographic location of sequences. Because of their simple underlying biogeographic model, and the use of Bayesian MCMC to ease computational tractability, these models have become very popular in phylogeography to answer a wide range of questions, from routes of viral spread to historical patterns of gene flow across populations. In my talk, I will focus on the use of these models to test hypotheses in macroevolution and ecology using datasets of multiple lineages inhabiting the same region and a hierarchical Bayesian approach to account for species-specific differences. Finally, I will describe recent advances, including the development of time-heterogeneous models to incorporate the temporal dynamics of the dispersal process, partitioning the contribution of abiotic factors to migration rates, or the use of non-stationary models to model mass extinction events.
Crayfishes and companions across the tree of life

PABLO VARGAS
Department of Biodiversity and Conservation, Real Jardín Botánico-CSIC, Madrid, Spain

Crayfishes are not actually crustaceans. Well, the term Crustacea should not be used because crustaceans do not form a single evolutionary (monophyletic) group without insects. However, a more inclusive group of Hexapoda (mostly insects) + former Crustacea named Pancrustacea is currently recognised in the Tree of Life. In this presentation we will go across the natural classification of living organisms to find out where crayfishes and travel companions (pathogenic moulds, Rhizaria diseases, invasive angiosperms, toxic cyanobacteria blooms, humans) are placed in the Tree of Life.

Crayfish conservation in the Alps: strategies and lessons learnt

LEOPOLD FÜREDER
River Ecology and Conservation Research, Institute of Ecology, University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria

Crayfish have played a significant role in the social and cultural activities of Europe since the Middle Ages, today however, native populations have disappeared or are highly threatened. Also in the Alpine countries, the autochthonous crayfish Astacus astacus, Austropotamobius pallipes and A. torrentium have been exposed to various threats and their populations still are strongly decreasing, mostly as a consequence of human activities. Several species protection programs have been carried out in different areas of the Alps implementing measures to enhance the situation of the indigenous species. This study aimed at documenting these activities for the support of the three autochthonous and endangered crayfish species. As these measures are being applied on three different species, in several states with different legislation, we reviewed and compared the specific management plans for crayfish conservation. Most had in common a comprehensive survey of the species’ distribution in the regions, their populations’ phenotypical and genotypical characterization, their habitat conditions and threats as well as suggestions for their protection. Based on these data, species and country specific conservation measures where summarized and now, after several years of the implementation of these programs evaluated on their success and deficits.
ABSTRACTS ORAL PRESENTATIONS

(in order of appearance in the program)
Crayfish plague in Spain: origin and epidemiology
Martín-Torrijos L, Rezinciuc S, Kokko H and J Diéguez-Uribeondo
Department of Mycology, Real Jardín Botánico-CSIC, Madrid, Spain

The aphanomycosis is responsible for the decline of the 5 native species of crayfish in Europe. In the early 1970s, two North American crayfish species, i.e., the signal crayfish, *Pacifastacus leniusculus*, and the red swamp crayfish, *Procambarus clarkii*, were introduced into the Iberian Peninsula. Since then, countless cases of the crayfish plague have decimated the native populations of crayfish, *Austropotamobius pallipes*. In this study, we have analyzed clinical samples from more than 100 cases of aphanomycosis that have occurred in Spain since 1991 until today. We have used sequence analysis to identify the different genetic lineages of *A. astaci* responsible for the crayfish plague outbreaks in Spain. The results show a direct relationship of crayfish plague outbreaks with the presence of either signal or red swamp crayfish. Thus, in North Spain, where the signal crayfish is more abundant most samples tested positive for the genetic lineages PsI (specific signal crayfish), while in South, Central and Eastern regions where the red swamp crayfish is more abundant, the genetic lineage of *A. astaci* responsible for the crayfish plague was Pc (specific red swamp crayfish). The results demonstrate once more that these two North American crayfish species are responsible for native crayfish outbreaks in Spain and that their populations represents chronic focuses for spread of the pathogen *A. astaci*.

Pitfalls in the application of molecular methods for the diagnosis of crayfish plague
Viljamaa-Dirks S, Heinikainen S, Riva R, and S Pelkonen
The Finnish Food Safety Authority Evira, OIE Reference Laboratory for Crayfish Plague
P.O. Box 92, FIN-70701 Kuopio, Finland

Crayfish plague, a highly contagious disease of the European crayfish species, is caused by the oomycete *Aphanomyces astaci*. *Aphanomyces astaci* is a parasite of the North-American crayfish species. It resides in the cuticle of its host and spreads by zoospores. In the case of acute illness of the European crayfish, the production of zoospores is massive and the crayfish in the vicinity are effectively infected. However, the virulence of the *A. astaci* strains is variable and even a latent infection in the highly susceptible European host is possible. Before the development of molecular methods the diagnosis of crayfish plague relied on the cultivation of the causative oomycete, a complicated and often unsuccessful process. Specific PCR based methods improved the diagnostic results considerably even enabling the quantitation of the pathogen load and the further characterization of the strain. At the same time they present new challenges in interpreting the results. Standardized protocols especially concerning sampling schemes are lacking, as well as external quality controls. In addition, freshwater environment and the crayfish within can contain numerous unknown organisms that may interfere with the detection and characterization of *A. astaci*. A few examples are given concerning the problems arising from the insufficient validation of the molecular methods.
The crayfish plague pathogen, *Aphanomyces astaci*: pathways, vectors and potential consequences of its spread in the introduced ranges

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Introduction of the crayfish plague pathogen, *Aphanomyces astaci* to Europe is responsible for substantial declines in native European crayfish populations. Although the research on *A. astaci* dates back to the beginning of the 20th century, knowledge about its introduction pathways, vectors and reservoirs has still many gaps. The presentation will summarize some recent advances in *A. astaci* research, which resulted from projects undertaken during the first author's PhD studies. Several North American crayfish species, natural hosts of *A. astaci*, are used globally for stocking and aquaculture. Therefore, they may pose a threat to endemic crayfish diversity, as happened in Europe. As the first such documented case, we confirmed *A. astaci* infections in populations of the red swamp crayfish *Procambarus clarkii* and the signal crayfish *Pacifastacus leniusculus* in Japan where crayfish plague might have contributed to declines of the endemic crayfish *Cambaroides japonicus*. Apart from aquaculture, the aquarium trade constitutes a major introduction pathway of crayfish species to and within Europe, and may facilitate *A. astaci* spread if infected crayfish are released from household aquaria. After screening for pathogen presence in various non-European crayfish species from German ornamental trade, we confirmed that aquarium trade represents a source of *A. astaci*. Moreover, even ornamental crayfish of non-American origin may contribute to crayfish plague spread, if widely traded species exhibit elevated resistance. We experimentally demonstrated that the Australian yabby *Cherax destructor* seems indeed less susceptible to *A. astaci* than European crayfish species, and its release may result in formation of new pathogen reservoirs.

Host-pathogen network analysis of Saprolegniales: host preference and specificity of *Aphanomyces astaci*

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Ecological network analyses are allowing a better understanding of the biodiversity complexity. This type of analyses provides a systematic way of representing, characterizing, and comparing the complexity of the ecological communities and their interactions, where the species are represented by nodes and the interactions by links between nodes. The Saprolegniales (Oomycetes) comprise the crayfish plague pathogen *Aphanomyces astaci*, and their species have complex network interactions with their of hosts. In this study, we have applied ecological network analyses to a total of 1362 isolates corresponding to 51 species of Saprolegniales isolated from 107 species of different hosts, e.g., crustaceans, insects, amphibians, turtles, fish (both from embryonic and adult stages) as well as plants. The results showed that specialization indexes of Saprolegniales species (*d*) ranged from 0.51 to 1.00. The Shannon diversity (*t* = 58.502, *df* = 1962.4, *p* < 0), and niche overlap values (*t* = 192.05, *df* = 1421.5, *p* < 0) appear to indicate that some species posse high host specialization, e.g., *A. astaci*, which have a higher specialization index of 0.95 for freshwater crayfish species. The results also show that, in Saprolegniales, the preference and specificity for host species have been often overlooked due to the fact that the majority of the species also have a saprotrophic lifestyle.
Scenarios for *Aphanomyces astaci* adaptation to native and alien crayfish hosts in Europe

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We present here potential scenarios for *A. astaci* adaptation to native hosts. These are based on high, intermediate or low *A. astaci* virulence levels, which result in different mortality levels among the host crayfish and help understanding the main factors influencing virulence evolution. A high *A. astaci* virulence and the resulting high mortality level in the hosts allows high level of sporulation, i.e. transmission in *A. astaci*, but raises the risk of eradicating the whole host population. Intermediate *A. astaci* virulence and resulting on average intermediate, but strongly environment-dependent mortality level within the host population could either risk killing all hosts when mortality is intensified by a co-infection or unfavourable environmental conditions, or the parasite might sometimes have low possibilities for infecting new hosts in cases of low mortality. This could result in periods of failed reproduction or exhausting the host population, but turn out as the favoured strategy in a network of connected lakes. Low *A. astaci* virulence and the resulting low level mortality imposed to host population would increase the risk of cessation of infection as sporulation would likely be very low or even non-existent, as might be the case during latent infections carried by native crayfish. In the case of *A. astaci* infecting invasive crayfish in Europe, the adaptation scenarios are simpler and favor highly virulent *A. astaci*. The continuous selection for higher virulence occurring in *A. astaci* in alien crayfish hosts likely prevents any evolution towards lower virulence and adaptation to native European hosts as long as the alternative habitat, i.e. invasive crayfish, dominates in availability. Another potential outcome of adaptation is that the *A. astaci* will evolve towards two different species, one adapting to the native and one to the invasive hosts.

Crayfish plague in Japan

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Crayfish plague caused by the oomycetous pathogen *Aphanomyces astaci* is chronically carried by a number of North American species of freshwater crayfish, such as *Pacifastacus leniusculus* and *Procambarus clarkii*. These two species have been previously introduced into Japan. The only native freshwater crayfish species of Japan *Cambaroides japonicus* is highly susceptible to this pathogen and has experienced a continuous decline during last decades. So far crayfish plague outbreaks have only been reported in Europe but in other regions of the world where chronic carriers’ have been introduced. In this, work we report the first case of crayfish plague in *Cambaroides japonicus* in Japan, which constitutes the first crayfish plague case reported outside Europe. Preserved samples in ethanol from an outbreak occurred in Hokkaido Island were histologically studied and analyzed using disease diagnostic ITS and mitochondrial DNA based primers. Phylogenetic analyses of the obtained sequences indicated that the *A. astaci* strain causing the outbreak belong to the PC phylogenetic lineage, and was most likely transmitted from *Procambarus clarkii* in inhabiting in the near vicinity.
A survey of various wild crayfish populations in Germany and Austria reveals new insights into the spread and diversity of Aphanomyces astaci

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The crayfish plague agent, Aphanomyces astaci, is one of the main causes for the numerous losses of native European crayfish populations due to mass mortalities caused by the disease agent throughout Europe. Different genetic lineages of A. astaci have been introduced to Europe independently with repeated introductions of different American non-indigenous crayfish species (NICS). Some of these lineages have shown to be more detrimental to the indigenous crayfish species (ICS) than others. We studied crayfish from 26 locations (in total 427 samples) in Germany and Austria for possible A. astaci infection using quantitative real-time PCR. Additionally, we used microsatellite and sequence analysis to identify the different genetic lineages of A. astaci. We could identify A. astaci infections in 15 crayfish populations (a total of 26% of samples were showing A. astaci DNA). Aphanomyces astaci lineages of seven populations were also identified. In particular, three findings are of special interest: (i) the Up lineage, a recently discovered novel variant by Grandjean et al. (2014), was present in at least two crayfish populations in Austria. (ii) A population of stone crayfish (Austropotamobius torrentium) in Austria was infected by two genetic lineages of A. astaci (PsI and Up lineages), and (iii) the Pc lineage revealed mutations in the mitochondrial LSU-gene in a population of A. torrentium that lives in a stream which also contains a population of signal crayfish (Pacifastacus leniusculus). Our study provides insights into the current situation and diversity of A. astaci in the studied area.

Identification of an Austropotamobius pallipes population with higher resistant to the crayfish plague pathogen, Aphanomyces astaci

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The crayfish plague pathogen Aphanomyces astaci is responsible for the decline of the native crayfish species of Europe. This pathogen is believed to be endemic of North America where it seems to have coevolved with its hosts. Thus, North American crayfish species exhibit a high resistance to this pathogen, while species from other regions of the world are highly susceptible. Recent field and laboratory observations appeared to indicate that some populations of the native species, Austropotamobius pallipes, of the Pyreneans might have a higher degree of resistance to A. astaci than others. Consequently, the objective of this study was to test their susceptibility by challenging 8 selected populations with zoospores of an A. astaci strain AP03 isolated from the North American species, Procambarus clarkii. The results show that there are significant differences in susceptibility to crayfish plague (P<0,001) among the selected A. pallipes populations. The majority of the populations showed high mortality rates. However, one population exhibited a 100% survival during the three months monitoring-period. Histological analyses revealed a high immune reaction in tissues examined, e.g., encapsulation and melanization of hyphae, similar to that found in North American resistant species. These results represent to our knowledge, the first observation of native European crayfish showing high resistance towards this pathogen. The identification of this population is of key importance for the management of these endangered species, and represents a crucial step forward towards the elucidation of the factors involved in the immune reaction against this devastating pathogen.
The complete genome sequence of the marbled crayfish
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The parthenogenetic all-female marbled crayfish (Procambarus virginalis) is a potent invader of freshwater ecosystems and also represents a novel research model. Marbled crayfish originated from sluggish crayfish (Procambarus fallax) through an evolutionary recent “macromutation” involving triploidization and concomitant (epi-)genetic changes. We have now used high-coverage Illumina sequencing to establish a first de novo draft assembly with a length-weighted median scaffold size (N50) of 40 kb. We determined the genome size at approximately 3.5 Gbp and identified >12,000 annotated genes. Further analyses initially focused on meiosis and DNA methylation factors to provide a mechanistic understanding of parthenogenetic reproduction and phenotypic variation, respectively. Comparative genome analysis of several individuals from diverse sampling sites demonstrated the clonality of the marbled crayfish population and confirmed autoploidy of the P. fallax genome as the underlying event for the triploidy of the P. virginalis genome. Finally, we also used whole-genome bisulfite sequencing to characterize the marbled crayfish epigenome at single-base resolution. The results demonstrate methylation specifically at CpG dinucleotides, consistent with a role in epigenetic gene regulation. DNA methylation was found at many genes and was particularly enriched in long and evolutionarily conserved genes, suggesting a role in the fine-tuning of gene expression programs. Our current activities focus on comparative methylome analyses for the identification of epigenetic variants that may underpin saltational speciation and rapid adaptation of marbled crayfish.

The transcriptome of noble crayfish Astacus astacus – an excellent tool and a reference for further gene expression studies
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The native noble crayfish (Astacus astacus Linné, 1758) is one of the keystone species in European freshwater ecosystems, which population trend is currently in decline due to invasive North American crayfish species and the deadly disease, crayfish plague (Aphanomyces astaci), carried and spread by them. We have recently published an annotated transcriptome of the noble crayfish from four combined tissues, including the abdominal muscle, hepatopancreas, ovaries and green glands. A total of 194 million 100 bp read pairs were generated in RNA-seq made on Illumina HiSeq. The transcriptome was assembled de novo using Trinity software, producing 158,649 non-redundant transcripts. Lowly expressed transcripts were filtered out leaving 45,415 transcripts of which 14,559 were containing open reading frames with predicted gene function. Of these, 13,770 transcripts were assigned at least one gene ontology term. The Transrate software realigned 91% of the total reads to the assembly and the BUSCO analysis then indicated the assembly being 64% complete. This first de novo transcriptome assembly is an important foundation for future genomic research on the noble crayfish. The RNA-sequencing analysis coupled with functional annotation and relative expression analysis will enable the comprehensive identification of genes that are up- or downregulated for example by different environmental stress factors or diseases.
Genomics-informed development of molecular markers for genotyping the crayfish plague pathogen <i>Aphanomyces astaci</i>

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<i>Aphanomyces astaci</i> is an animal parasite and causal agent of crayfish plague, a disease listed by the World Organisation for Animal Health. <i>Aphanomyces</i> spp. are water moulds belonging to the class Oomycota and this genus contains primary pathogens of plants and animals as well as opportunistic and saprotrophic species. <i>Aphanomyces astaci</i> was first introduced into Italy from the U.S. in the late 19th century and rapidly spread in Europe causing the decline of native crayfish. Random amplified polymorphic DNA PCR (RAPD-PCR) on isolates of <i>A. astaci</i> distinguished five genotypes (A, B, C, D and E). No discriminatory morphological or physiological characteristics are available and widely used markers such as ITS, LSU and COI failed to discriminate between genotypes. There are some practical drawbacks to genotyping by RAPD-PCR, not least the requirement for pure cultures. Therefore, we used whole genome sequencing (WGS) on multiple <i>A. astaci</i> isolates to catalogue DNA single nucleotide variants (SNVs) to be exploited as new diagnostic methods, in aid of detection and prevention of crayfish plague. By designing primers surrounding genotype-specific SNVs, amplifying the DNA fragment by PCR and exploiting enzymatic restriction digestion, we were successfully able to distinguish genotypes on pure cultures. This approach was subsequently used on historical crayfish samples available in our laboratories to validate the reliability of this method. Once tested and validated, this method offers a new tool for diagnostics and epidemiological studies aimed at understanding the history and spread of crayfish plague in Europe.

The annotation of two <i>Aphanomyces</i> mitochondrial genomes from <i>A. astaci</i> and <i>A. invadans</i>

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The genus <i>Aphanomyces</i> (Saprolegniales, Oomycetes) includes species in both aquatic and terrestrial environments. Two important pathogens and aquatic species are <i>Aphanomyces astaci</i>, the cause of crayfish plague and its close relative and <i>Aphanomyces invadans</i>, which causes the epizootic ulcerative syndrome on fish. In this study, we have assembled and annotated the first mitochondrial genomes of <i>Aphanomyces</i> from the whole genome shotgun sequence reads (PRJNA187372; PRJNA258292, respectively). The assemblies were generated from <i>A. astaci</i> Pc-genotype strain APO3 and <i>A. invadans</i> strain NJM9701. The sizes of these mtDNAs were 49,489 bp and 49,061 bp for <i>A. astaci</i> and <i>A. invadans</i>, respectively. The species shared similar genetic content and organization encoding 36 proteins, two ribosomal RNAs, three putative open reading frames and 33 transfer RNAs of 19 amino acids for peptide synthesis. Both species also had a large inverted repeat region (LIR) of approximately 12 kb, the LIR contained large and small ribosomal subunits and eight protein coding genes. These annotated mitochondrial genomes serve as a valuable genetic backbone for further development of diagnostic methods, genotyping and phylogenetic and migration studies of the parasitic species of <i>Aphanomyces</i>.
How do invasive species cope with different environmental conditions? A proteomic study of two alien crayfish: *Procambarus clarkii* and *Procambarus fallax f. virginalis*

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Invasive species are often able to cope with variety environmental conditions enabling them to expand to new habitats. Once introduced and successfully established those invasive species, often threaten native species in their ecosystems. New environmental conditions may act as selective pressures that drive organismal adaptation and evolution. However, alongside genetic adaptation, invasive organisms also show a high degree of plasticity in response to environmental challenge. To elucidate whether epigenetic mechanisms allow an organism to respond to the environment, we study how individuals of two invasive crayfish species respond to environmental challenges by means of a cutting-edge proteomic approach. Our objective is to investigate which types of proteins and how many of each type are expressed when individuals are exposed to different environmental conditions. Our target species are two invasive crayfish, i.e. the red swamp crayfish, *Procambarus clarkii*; and the parthenogenetic marbled crayfish, *Procambarus fallax f. virginalis*. The use of a parthenogenetic species will allow us to assess whether changes in protein expression are uniquely due to plasticity (as individuals are genetically identical). We conduct an experiment in which we expose the two crayfish species to treatments consisting of different combinations of temperature and water velocity for several days. After exposure, individuals will be collected and immediately subjected to tissue extraction from the muscle, gills and hepatopancreas to examine the proteome responses. Here, we will present the details of the experimental setup and hypotheses tested as well as any available results.

Comparing classic crayfish cage surveillance with eDNA water monitoring during an on-going crayfish plague outbreak in Norway

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The use of environmental DNA (eDNA) for detection and quantification of aquatic organisms is a rapidly growing field with a great potential for streamlined inventory- and monitoring purposes. The TARGET-project aims at implementing eDNA approaches for monitoring the red-listed *Astacus astacus* and its threats. In 2014-2016, we followed an outbreak of crayfish plague in the Norwegian Halden watercourse which had resulted from illegal transfer of *Pacifastacus leniusculus*. A surveillance program based on cages containing noble crayfish was already established, thus the spread of disease was monitored by observing and diagnosing mortalities in the cages, and water samples were collected regularly. Water (~5 L) was filtered on-site through glass fiber filters, and each sample was analysed using species specific qPCR assays for the crayfish plague pathogen *Aphanomyces astaci*, and the crayfish *A. astacus* and *P. leniusculus*. eDNA from all three species was successfully detected in the water samples. Crayfish plague spores was detectable in the water before the caged crayfish succumbed to the disease. The infection source (signal crayfish), representing a scarce *P. leniusculus* population (0.11 CPUE) in the southern part of the lake, was detected at trace levels. Furthermore, eDNA from noble crayfish was readily detected and increased in quantity during the mortalities, before decreasing to trace levels about 4-8 weeks after the outbreak. Our study demonstrates an efficient and non-invasive approach for combined eDNA monitoring of native crayfish and its threats (invasive crayfish and crayfish plague) from the same water samples.
Physiological responses of freshwater crayfish (*Cherax albidus* Clark 1936) when exposed to various salinities of ocean water and inland saline water

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The yabby (*Cherax albidus* Clark 1936) is a subject of aquaculture in Australia and has a considerable tolerance to salinity. Inland saline water shows far higher salinity fluctuations than freshwater, and is commonly low in potassium. Two independent experiments, each using a different water type, investigated the effects of different salinities, 0 (control), 2, 4, 8 and 16 ppt of two water types, ocean water and inland saline water, on stress physiology of yabbies over two weeks. 20 yabbies were stocked in 5 200L tanks with independent shelters and monitored for their physiological responses using haemolymph and tissue samples. Haemolymph osmolality of yabbies remained the same as control at low salinities. Osmolality increased in 16 ppt salinity after 256 hours in both experiments. The osmoregulatory capacity of yabbies decreased significantly as salinity increased. The yabbies were found to hyper-regulate their haemolymph until 8 ppt, while the haemolymph was isosmotic to the medium at 16.63 ppt in ocean water and 15.88 ppt in inland saline water. Health Condition was the same or better in inland saline water than in ocean water, in terms of hepatopancreatic energy content and organosomatic indices. This suggests that potassium deficiency was not present in *C. albidus* cultured in inland saline water. These results indicate that yabbies were tolerant to salinities up to 8 ppt and to inland saline water for 256 hours, but were intolerant to 16 ppt after 256 hours in both water types.

Survival, recovery and cardiac activity of three crayfish invaders under sub-zero temperature

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The effect of acute exposure to subzero temperature on the heart rate and recovering capacity of three invasive crayfish species (*Orconectes limosus*, *Pacifastacus leniusculus*, *Procambarus clarkii*) was investigated. These species are successful invaders in European freshwater ecosystems, in many respects due to adaptability to the wide range of environmental conditions including tolerance to temperature changes. The experiments were designed to evaluate cardiac activity (based on using of non-invasive sensor), survival and recovery of crayfish after short-term (30 min) and prolonged (60 min) exposure to sub-zero temperature (-18 ºC). All *O. limosus* and *P. leniusculus* died within following 12 hours after both short and longer exposure. In contrast to them, all *P. clarkii* successfully survived freezing, thawing and subsequently recovered after short-term exposure, while a quarter of the *P. clarkii* specimens successfully recovered even after prolonged exposure. In all tested species heartbeat was measurable several hours post exposure, although cardiac activity gradually declined in *O. limosus* and *P. leniusculus* after reaching the point of no return. The heart rate of *P. clarkii* decreased the most abruptly and the recovery to nearly pre-exposed state took longer time (= 35 min) than for *O. limosus* (= 25 min) and *P. leniusculus* (= 28 min). During freezing-thawing cycle both species demonstrated smoother heartbeat decline and faster heartbeat recovery to a non-return point, that can explain the mortality observed in *O. limosus* and *P. leniusculus*. Faster responsiveness to acute conditions and more gradual subsequent recovery of *P. clarkii*, as reflected in the heart rate course, seems to be more successful strategy for survival. Our findings can shift the knowledge in terms of understanding of wider ecological distribution of *P. clarkii* as well as insight into mechanisms of invasive crayfish adaptability.
The mineral content of *Cherax quadricarinatus* in Southeast Queensland and Northeastern New South Wales
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The freshwater crayfish *Cherax quadricarinatus*, is native to northern Queensland, the Northern Territory and southern New Guinea. Within Australia, the species has been translocated from its native habitats in the North to other parts of the Continent, but in particular throughout central-eastern Australia. In southeast Queensland and northeastern New South Wales, a number of feral populations of *C. quadricarinatus* have established in natural waterbodies and municipal reservoirs. The presence of these large and evidently self-sustaining populations, and the apparent continued spread of the species, has raised concerns about threats these feral populations might pose to other species and ecosystems. While *Cherax quadricarinatus* is the world’s most intensively studied species of freshwater crayfish, and there is a wealth of information available on aquaculture and physiology of the species, the wild biology and ecology of this species remains very poorly understood. As part of a broader study on the distribution and wild ecology of this species, *C. quadricarinatus* were collected from waterbodies of various types, with differing underlying substrates and physicochemical characteristics within southeast Queensland and the northeast of New South Wales. These collections permitted analysis of mineral content of 108 *C. quadricarinatus* from 5 different populations in the study area using the loss-by-ignition method. We will present the results of this study and discuss differences in mineral content between populations. We will also discuss the implications of these results in the context of this species as an aggressive and successful invader of non-native habitats in Australia and elsewhere.

Return to crayfish high school: long-term monitoring of crayfish populations at the UCC outdoor school 2011 to 2016.
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The life history of a population *Cambarus robustus* was studied as a part of on-going long-term monitoring at the Upper Canada College outdoor school in Norval, Ontario. Data on the synchrony of seasonal reproduction, population structure and age of maturity were obtained from regular spring sampling in the Credit River draining into Lake Ontario. This second report focuses on yearly samples from 2011 to 2016 and compares then to the first phase, which included samples from 2008 to 2010 (Hamr & Sit, 2011). A particular emphasis was placed on tracking the proportions of Form I and II males as well as females with and without glair glands in the population. Analysis of the data supports the findings of in the first phase of the study in terms of reproductive/maturity size, maturity, synchrony of breeding as well as yearly growth. As in the first phase, asynchronous individuals of both sexes were found in all spring/summer samples from 2011 to 2016. The percentage of asynchronous individuals (ie: females with no glair and males in From 2) was variable from year to year and ranged between 4 and 49% in males and 4 to 45% in females, however the percentage was similar in both sexes in a given year.
Crayfish role in a canyon-shaped reservoir: case study from the Nýrsko, Czech Republic

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Trophic webs in almost every ecosystem contain many units, which are linked together and in aquatic systems crayfish are one of the most important. They are considered keystone species as well as ecosystem engineers there and may represent up to 85 % of benthos biomass. Their omnivorous diet complicated evaluation of their position in the food chain. Using carbon and nitrogen stable isotopes we investigate the trophic role of noble crayfish (*Astacus astacus*) in canyon-shaped reservoir. We sampled all units of food chain (fish, zoobenthos, zooplankton, primary producers, and detritus) using wide range of methods such as manual collection, angling, trapping, and scuba diving. Our results revealed that role of crayfish changes during ontogeny. Also crayfish diet varied between season (spring, summer, autumn) and habitats (rubble slopes/beaches). Additionally, we found that crayfish are an important component in nutrient cycles due to their ability to connect different depth zones of reservoir.

Crayfish ecological diversity and conservation across a synthetic phylogeny

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Habitat preference is an intricate component the ecology and biology of freshwater organisms, and likely has a central role in driving diversification dynamics and geographic distributions. Freshwater crayfish are highly valuable members of aquatic ecosystems around the world with diverse ecological requirements and habitat affinities. As an ecologically diverse and well-studied group of organisms, crayfish are an excellent system in which to explore the relationship among ecological and evolutionary variables, capitalizing on the phylogenetic synthesis approach. We test for a correlation between habitat-preference and lineage diversification rates, geographic range-size and current extinction risk. The evolutionary history of freshwater crayfish was marked by multiple radiations that may have been prompted by ecological opportunity. While we recovered a strong signal of correlation between habitat-type and geographic range size, we recovered only weak support for differential lineage diversification rates and current extinction risk. This phylogenetic synthesis framework has proven useful in testing evolutionary hypotheses in this group and will provide a platform for future studies in the systematics and evolution of other invertebrate lineages. Additionally, the synthetic phylogeny provides a framework for taxonomic revisions to adjust taxonomy to better reflect established phylogenetic relationships. We propose such a taxonomy, highlight important changes, and provide a comprehensive assessment of crayfish diversity.
Low local crayfish diversity and high species turnover in lowland streams may be influenced by a few widely distributed crayfish species

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Crayfish are a numerous and diverse group of decapods that occupy multiple niches in lowland streams. However, the diversity of Louisiana crayfishes (~39 species) is poorly understood and is based on sporadic and voluntary reporting. Thus, average local diversity (α-diversity) and compositional turnover among localities (β-diversity) have never been rigorously quantified or mapped. Herein, we quantify crayfish diversity using diversity indices, multiplicative hierarchical diversity partitioning, and variance in community composition among localities. Our data source from 59 wadeable streams in Central Louisiana sampled during summer 2013 and 2014 and span an area of approximately 24,000 km2 (~15% of state area), 5 major river drainages and 14 watersheds. Average α-diversity among all sites was characterized by low richness (typically 2-3 species) and extreme unevenness. Average α-diversity was typically lower than expected by random chance at drainage and watershed scales, whereas β-diversity was typically greater than expected at both scales. Species turnover was the predominant beta diversity pattern at all spatial scales, but species loss (nestedness) increased relative to turnover at drainage and watershed scales. Finally, most variance in community composition resulted from variation in relative abundances of Procambarus clarkii, Procambarus (Pennides) species, Orconectes lancifer, and Cambarellus puer. Our results suggest distributions and relative abundances of P. clarkii, O. lancifer and Procambarus (Pennides) species may play a deterministic role through competition and dispersal in diversity structuring of our study area. Future research will examine whether suppressed local diversity and enhanced turnover is a general feature of crayfish assemblages among lowland stream ecosystems.

Range expansion of the signal crayfish (Pacifastacus leniusculus) in a recently invaded region in Croatia and potential for its control

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The signal crayfish, Pacifastacus leniusculus has recently been introduced to the Korana River, a karstic river in the central part of Croatia, where it presents an alarming threat to its native crayfish diversity. In this study, we explored the dynamics of range expansion of the signal crayfish in the Korana River and developed an individual-based model (IBM) to explore different options for management of its populations. In 3 years, the invasive range of the signal crayfish increased 2.5 times, while dispersal rate was similar in both upstream and downstream direction. At former invasion fronts crayfish abundance increased 5 times and was translated into significant reduction of signal crayfish size (total length) in these populations. The IBM was based on the species basic life history and simulated multi-year population dynamics. We explored management scenarios for periods of 10 years that differed in catch per unit effort (CPUE), trapping period and frequency. Considering a catch effort of 10% of all individuals in the population > 60 mm, model simulations suggested that the most effective management option would be to harvest one week per month each year of the 10 year period. This would drive the population to 5% of the baseline, i.e. non-harvested abundances. The next best alternative is to harvest every year for a limited number of months (June – November). Both of these findings are conditional on a constant CPUE. We also discuss model results with a varying CPUE, and highlight the applicability of population models in invasive species management.
Do you suffer from a lack of historic crayfish data?
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Do you suspect that a crayfish species was extirpated from your study area but lack the historic data to prove it was ever there? Do you lay awake at night wondering if a crayfish species is native or introduced to your study area, or wondering how long an introduced crayfish has been there? The answers to your questions may be waiting in your friendly, neighborhood fish museum. Recent work I conducted with colleagues demonstrated that fish guts may provide valuable information about crayfishes in large water bodies. Furthermore, in the USA, fishes have been collected and curated in museums more intentionally over much longer periods than have crayfishes. Therefore, a gold mine of historic crayfish data should be sitting in museums…in the guts of preserved fishes. I examined crayfishes from fish guts in museums to examine historic crayfish distributions where data from direct crayfish sampling were lacking. I will share what I found, the fishes and sizes that were most informative, and discuss both potential uses and disadvantages of the method.

Keywords: crayfish, historic distributions, non-native crayfish, fish guts, indirect sampling

The first evidence of co-occurrence between native crab and crayfish in Italy
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In the Italian freshwater ecosystems, the crab Potamon fluviatile and the white-clawed crayfish Austropotamobius pallipes complex can live in sympatry in some streams, but do not usually occupy the same stream section or pond, suggesting a sharp segregation of the two species. The first instance of the co-occurrence of these two native crustaceans, sharing the same area, is here reported for Central Italy. No evidence of agonistic behavioral patterns has been observed. Co-occurrence may be favoured by the crayfish and crabs size, since in the shared area the size of both species is slightly smaller if compared with their size up and downstream, where the species singularly occur. Further observations are needed to better assess the stability of this cohabitation through time.
Investigation of a localized decline in freshwater crayfish *Paranephrops planifrons* in the upper Waikato River, New Zealand

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In 2014, we investigated a suspected localized decline of the native freshwater crayfish *Paranephrops planifrons* (or kōura) in an 80 km section of the upper Waikato River extending from Huka Falls to Ātiamuri dam. We used a combination of dive surveys, trapping, and literature review in an initial investigation of: a) crayfish abundance in the mainstem of the upper river, and; b) the local influence of increasing populations of two species of predatory fish on crayfish abundance. The surveyed section of the mainstem included three dams and associated lakes used for hydroelectricity generation. There are five more “hydro lakes” downstream. We established that only moderate to low densities of crayfish remain in the upper 4 km of the surveyed section. No crayfish were found in the remaining 76 km. A literature review of information on the eight Waikato River hydro lakes indicates that crayfish densities decreased around the mid to late 1990’s and are now low or absent. The decline in crayfish abundance was generally coincident with the establishment of catfish *Ameiurus nebulosus* (exotic species) in the early 1990’s and the stocking of native elvers (juvenile longfin eel *Anguilla dieffenbachi* and juvenile shortfin eels *A. australis*) in the hydro lakes from 1992 onwards. The present study was focussed on the potential impact of catfish and eels, however, other factors may be causing, or contributing to the decline of crayfish in the upper Waikato, for example disease, or loss of edge habitat due to flow-ramping for hydro power generation.

The effects of the invasive weed Singapore daisy on the native Australian freshwater crayfish *Tenuibranchiurus glypticus* Riek

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The native freshwater crayfish, *Tenuibranchiurus glypticus*, typically inhabits coastal Melaleuca swamps and is distributed from Maryborough (Queensland), to Wooli (New South Wales). *Tenuibranchiurus glypticus* is listed as “Endangered” on the IUCN Red List, primarily due to a highly fragmented distribution and habitat clearing/loss; specifically drainage of their swamps and development. It is therefore important to identify and understand any new or additional threats to allow effective management/conservation of this species in its remaining small, isolated habitat fragments. The invasive weed, Singapore daisy (*Sphagneticola trilobata* – Asteraceae), is successfully established in many parts of the world, including some of the seasonally inundated Melaleuca swamps which *T. glypticus* occupies in Southeast Queensland. The densely-matted nature of this weed is a potential problem for these small crayfish, smothering their habitat, possibly restricting access to burrows and limiting oxygen transfer at the air-water interface. The objectives of this study were to investigate if: (1) invasion of Melaleuca swamps by Singapore daisy renders them unsuitable habitat for *Tenuibranchiurus*, and (2) Singapore daisy infestations in Melaleuca swamps can be successfully controlled or eradicated using a primary control method of herbicide application. One hundred and fourteen paired plots of weed and no weed areas were surveyed for presence of *T. glypticus* across 2 sites in Southport, Queensland. We will present our findings and discuss any effects the weed is having on the presence and abundance of these crayfish. We will also discuss the effectiveness of herbicide as a control method for Singapore daisy in seasonally inundated Melaleuca swamps.
White-clawed crayfish (*Austropotamobius italicus* Faxon) effects on macroinvertebrate communities from Mediterranean limestone mountain streams
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The white-clawed-crayfish was well widespread over most of drain basins from the Iberian Peninsula in the past, but habitat destruction and exotic species-mediated diseases put it at risk of extinction during last decades, being remnant populations restricted to headwaters and small tributaries. Crayfish are generally recognized as omnivores, displaying an opportunistic foraging behavior on steam biota, among which macroinvertebrates play a main role. To better understand *A. italicus* interactions with benthic macroinvertebrate headwater communities we designed a field study in a permanent mountain stream from Central Spain, where three-month mesocosm-based experiments were carried involving crayfish densities usual in nature. Neither positive nor negative impacts of *A. italicus* on macroinvertebrates were evident in terms of richness, abundance and biomass, although some particular taxa resulted affected. Upstream recolonization, drift processes and particular evasiveness traits of many taxa were proposed as main compensation mechanisms displayed by macroinvertebrates to keep stable populations along time under theoretical crayfish predation. As a whole, and together with the absence of impacts on epilithic algae showed by previous studies, *A. italicus* should not be considered as a bioturbator agent in compositional, structural and functional terms for limestone headwaters. Therefore, restocking actions involving this species in suitable water courses are recommended, presumably not posing a risk on local benthic communities.

Uptake and transfer of microcystins in noble crayfish in Lake Steinsfjorden, a cyanobacterial (*Planktothrix*) dominated lake
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The Norwegian Lake Steinsfjorden, a major fishery-lake for noble crayfish, is often affected by cyanobacterial blooms caused by microcystin (MC) producing *Planktothrix*. A recent study demonstrated the presence of microcystins in noble crayfish originating from this lake. However, little is known about the impact of toxic cyanobacteria on crayfish health and crayfish as food-source. We have investigated the presence of MC in noble crayfish from Lake Steinsfjorden, and elucidated whether MCs are transferred and accumulated in vital organs and the edible parts. In 2015, crayfish were captured each month from June to October. Water samples were taken simultaneously. Tissue samples from tail muscle, intestine, stomach, and hepatopancreas were harvested for MC-analysis using an in-house method for enzyme-linked immunosorbent assay (ELISA). MC-analysis results for tissues and water samples were compared. Stomach, intestine and hepatopancreas contained the highest concentrations of MCs, respectively, suggesting that the crayfish acquire large amounts of MCs through diet, which is transferred to the hepatopancreas. The tail muscle contained very low MC-concentrations, with a tendency to decline towards the autumn where the measured MC-concentrations in the water dropped substantially. Results indicate that a normal portion of boiled crayfish tails (~100 g muscle) from Lake Steinsfjorden in 2015 was well below the tolerable daily intake (TDI) limit for MCs (0.04 µg/kg body weight) for adults. However, removal of the intestine from the tails seems a reasonable food safety precautionary measure for consumption of crayfish from cyanobacterial-dominated waterbodies, since the intestine would more than triple the MC-content if not removed.
Could crayfish care about safety of beer? - A long way from the research to the practical use
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Over the past decades, ecological status of the freshwater crayfish has been drastically changing from the sensitive indicator of aquatic environment to the tolerant species which can survive the wide range of unfavourable conditions, but despite all conventions on being or not being the proper bioindicators, crayfish are still regarded as essential aquatic community playing various key roles in the freshwater ecosystem. We developed a system for monitoring of etho-physiological status of crayfish combining analysis of the heart rate and detection of movement as basic parameters. Monitoring of cardiac activity is done with the aid of a non-invasive sensor and locomotion of crayfish is recorded by cameras enabling analysis of the complex data by a software developed particularly for this purpose. A range of stimuli where crayfish showed response to both adverse and common environments were successfully tested. We examined crayfish reactions to such chemicals as chloramine, chlorides, nitrites, as well as to the various natural stimuli (odours of food, conspecifics, predators and others). This monitoring technique is easy due to absence of long and complicated analyses, since measured parameters, locomotor and cardiac activity, are evaluated in a real time. The sophistication of such biomonitoring is consisted in reliable combination of behaviour and physiology that affords an opportunity of detection of individual animals reaction to environmental changes. After registration of the whole system as national patent, the first yield of its practical application was an agreement with the local South Bohemian brewery where the biomonitoring system is expected to guard the water used for production of beer.

Predicting harvest of the non-native signal crayfish in Swedish lakes – a role for changing climate?
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The North American signal crayfish (Pacifastacus leniusculus) was introduced to Sweden in the 1960ies for the fishery. It has had negative effects on native ecosystems by spreading the disease crayfish plague but, despite population collapses lately, it has also had a high commercial and recreational value. To better predict how climate warming will affect population dynamics of this cool-water crayfish, we explored the role of temperature and density dependence as explanatory factors of the subsequent years’ catch rates of commercially sized signal crayfish in four Swedish lakes. We found air temperatures to be good proxies for water temperatures in all lakes. We could only obtain water temperature data for one lake, and winter temperature data were therefore only included in the analysis of catch-per-unit-effort patterns in this lake. Our results show that increasing temperatures will potentially affect the population dynamics of cool-water freshwater crayfish species such as the signal crayfish. We found that the population dynamics of signal crayfish are lake-specific and could be affected by the temperature either during recruitment at the juvenile stage, the survival and growth of adults, or both. Increased fluctuations in water temperature during winter may potentially influence adult survival. To better predict the effects of global warming on the dynamics of cool-water crayfish populations, future studies should investigate recruitment in crayfish along temperature gradients and the influence of variations in water temperature on winter mortality.
Influence of a relationship between selected trace elements and natural productivity on growth and yield of marron in a commercial farm
Tulsankar SS and R Fotedar

The impact of the relationship among trace elements, natural productivity and the productivity of the aquacultured species is unknown. Data were collected from four marron ponds from an established commercial farm in Manjimup (34.2455°S, 116.1443°E), south-west of Western Australia. Approximately 155 kg of 90-130 g marron/pond were stocked for 12 months. The tri-monthly water samples were collected to investigate the role of 12 pre-selected trace elements viz. manganese, silicon, magnesium, calcium, iron, phosphorus, zinc, selenium, sulphur, copper, aluminium and cobalt on the natural productivity and marron yield. The results showed that phytoplankton density had a strong correlation with aluminium (R²= 0.927) but a weak correlation with iron (0.611). Manganese, magnesium and iron were the only three trace elements showing a strong correlation with zooplankton density. Phytoplankton diversity showed a strong correlation with manganese and iron but weak correlation with aluminium, whereas zooplankton diversity showed a strong correlation with zinc. A strong correlation was observed between wet weights of zooplankton and phytoplankton with aluminium and zinc respectively. Dry weight of zooplankton had a strong correlation with manganese, magnesium, and iron. Wet weight and dry weight of zooplankton showed a significant (P<0.05) correlation with phytoplankton density and diversity. A strong correlation was observed between marron yield at harvest and manganese, magnesium, iron, copper, phytoplankton density and species diversity, and zooplankton density, species diversity and dry weight. In conclusion, trace elements in the marron pond influenced the primary, secondary productivity of the ponds and in turn marron yield.

Polyphenols as feed additives: a new tool to prevent diseases in farmed crayfish?
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Polyphenols are a large class of chemical compounds with antioxidant properties, derived from plants. Epidemiological, clinical and nutritional studies strongly support the evidence that dietary phenolic compounds enhance human health by lowering the risk of most common degenerative and chronic diseases that are known to be caused by oxidative stress. Although some polyphenols are employed as feed additives to reduce free radicals and therefore to improve the oxidative stability and quality of the meat, their application in animal nutrition is a field largely unexplored. In this study, we present some encouraging preliminary data on the effects of polyphenols extracted from two agricultural wastes (olive mill waste water, chestnut skin) and from three different types of honey (chestnut, acacia and clover) tested in vitro against two pathogenic aquatic fungal isolates, Aphanomyces astaci and Fusarium avenaceum, that cause different diseases in native European crayfish, with a high mortality rate and severe economic repercussions. Moreover, olive mill waste water (OMWW) was tested in a long term feeding trial on the crayfish Astacus leptodactylus. Our results show in vitro inhibitory effects, of agricultural waste extracts on Aphanomyces astaci and Fusarium avenaceum. In vivo, OMWW enriched diets improved parameters of growth performance, feed utilization, survival and nutritional status, as well as immunological parameters (phenoloxidase activity, superoxide anion production) in crayfish. These results, although preliminary, encourage to promote their use in the prevention and treatment of fungal diseases. Our next step would be to test the efficacy of polyphenols on crayfish challenged with pathogens.
Harvesting New Zealand Freshwater Crayfish (Paranephrops zealandicus) – five years of harvest data and the implications for population dynamics and stock management

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A 102m² earthen pond was stocked with 65 ~35-gram freshwater crayfish (Paranephrops zealandicus) in 2010. The pond was harvested at the end of each summer growing season from 2012 to 2016. All crayfish >45 grams were measured and removed, while all smaller crayfish and berried females were measured and released back into the pond. The number of crayfish <45 grams captured on each harvest year increased from 11 (2012) to 232 (2016). The number of crayfish >45 grams captured each year ranged from 18 to 31 crayfish, with highest numbers caught in 2016. Similarly, the number of berried females caught each year ranged from four to ten, with the highest number caught in 2016. The size of berried females decreased from ~50 grams (2013 and 2014) to ~32 grams (2016). There was no difference in the crayfish sex ratio over all harvest years (~45 to 55%). The data suggest that the current harvest model has created a high density and stunted population of freshwater crayfish. This finding may be related to a lack of food resource, since the majority of ~32-gram crayfish sourced from other high density ponds, grew to >45 grams after one growth season in new ponds. Suggested management approaches to address this issue include the reduction in juvenile crayfish density and biomass annually, and increasing the food resource through the planting of aquatic plants.

The use of a traditional māori harvesting method, the tau kōura, for monitoring of freshwater crayfish (kōura, Paranephrops planifrons) populations in the te Arawa lakes, New Zealand

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Freshwater crayfish (Paranephrops planifrons) are endemic to New Zealand where they are known locally by the Māori name ‘kōura’. Kōura are an important component of lake food webs and support important customary fisheries for Māori in the Te Arawa lakes of New Zealand. Anecdotal evidence suggests that kōura populations have declined markedly since European settlement in the late 19th Century. Environmental factors implicated in this decline include, introductions of exotic fish and plants and eutrophication. Until recently, there was a lack of quantitative information on kōura abundance and ecology that made it difficult for tribal and government agencies to manage kōura populations in the lakes. However, development and use of the tau kōura, a traditional Māori harvesting method, has led to a resurgence of research and monitoring on lake-kōura populations. This method involves the placement of bracken fern bundles on the lake bed for kōura to take refuge in. It has advantages as a monitoring tool over conventional methods, as it samples all kōura size classes, can be used in turbid waters and at a wide range of depths, and does not require expensive equipment or specialised expertise. The tau kōura is now the principal method used to collect data on kōura populations in the Te Arawa and Taupō lakes. It has been used to determine environmental factors influencing kōura abundance and distribution, in the development of sustainable fisheries regulations, and in resource management decision-making.
New advances in astacid juvenile feeding research: development of practical diets
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The drastic reduction of wild populations during the last five decades due to crayfish plague, loss of habitat and pollution, has led to growing interest on development of astacid crayfish culture in Europe. Production of juvenile crayfish under controlled conditions would increase the possibilities of success in both restocking programs and crayfish supply for human consumption. Considering that the first period of exogenous feeding is the most critical factor for further survival and growth, our research group has formulated and developed a specific practical diet for juvenile astacid, which allows for acceptable results. Drawing on this diet, experiments to determine the optimal protein content were carried out.

Fish meal is the main protein ingredient used in aquaculture but it is widely recognized that fisheries pressure on wild stocks to cover its increasing demand is unsustainable. For this reason, the possibilities of replacement of fishmeal by alternative protein sources (soybean protein, pea protein concentrate, poultry byproduct meal and feather meal). After periods between 80 and 100 days from the onset of exogenous feeding, good survival rates (mean around 74% in grouped animals) and growth were obtained.

Identify factors influencing the variability of survivorship of juvenile redclaw crayfish Cherax quadricarinatus
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Aquaculture production of redclaw has shown great promise for a number of years but has failed to live up to its promise due to high variability in survival and growth and therefore inconsistency in commercial outcomes and yields. Recently there has been some work done to intensify aspects of the industry and develop a hatchery system to produce consistent high quality craylings for stocking out into ponds. We now need to look at the techniques and procedures that will provide reliable production of hatchery-reared craylings, that in turn support predictable yields and survivorship through grow out. My PhD research will focus on the hatchery production of craylings and subsequent nursery phase to produce advanced juveniles. I will examine: dietary requirements, feeding husbandry, feeding morphology, temperature, stocking density, and intraspecific predation factors. In this first study the aim was to identify the dietary requirements which optimise the numbers of juvenile crayfish produced in a hatchery environment and their subsequent survivorship. Feeding redclaw from the 2nd post-hatch moult (crayling) is thought to promote growth and better survivorship through the subsequent juvenile stages. We tested a manufactured diet developed by CSIRO based on the broad body of nutrient requirement information in the literature, against three other commonly used crayling feeds i.e. blood worms, frozen Artemia and commercial post larval shrimp feed. Data and analyses of survival and growth over 4 weeks are presented.
People's perception of crayfish
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Different audiences in The Netherlands were invited to ‘draw a crayfish’ at the beginning of a lecture. On average, people mentally reproduce crayfish with 7.04 legs (range: 0-14) and 1.67 clawed legs (range: 0-8). However, there seems to be consistent variation in perception between different groups of people (e.g. children, men and women). Various concepts of a crayfish will be discussed. Other than acquiring drawings and data, asking people to draw crayfish can significantly increase the interest in your lecture.

Effects of flood-control impoundments on community assemblage of stream crayfish
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Over half of the world’s rivers have seen changes in the magnitude and timing of flows due to water regulation and increased water usage, affecting the diversity and abundance of stream organisms. I tested whether flood-control impoundments altered crayfish assemblage up and downstream of impoundments in two impounded creeks (Little Bear and Cedar creeks) and one unimpounded creek (Rock Creek) northern Alabama, USA. Crayfish and fish were sampled and physiochemical variables were measured at 6 - 8 sites along each creek in 2015. Crayfish abundance and diversity (species richness) differed between impounded and unimpounded creeks, as well as up and downstream of impoundments. Catch-per-unit-effort (CPUE) of *Orconectes validus* was higher in Rock Creek than impounded creeks in both the spring and fall. Upstream of impoundments also showed higher CPUE of *Orconectes erichsonianus* than sites downstream. In Rock Creek, crayfish diversity at the site furthest downstream was significantly greater than at the three furthest upstream sites. There was no difference in the diversity at similar distances up and downstream of impounded creeks, except the furthest up and downstream sites in Little Bear Creek (p<0.05), with diversity being greater downstream. Fish predators of crayfish were significantly greater in impounded than unimpounded creeks. Water temperatures and percent dissolved oxygen were higher in impounded than unimpounded creeks. Turbidity was higher downstream than upstream of impoundments. Crayfish diversity and abundance in thousands of stream kilometers are being affected by dams, which will influence freshwater and riparian ecosystems by impacting the important functions that crayfish provide.
The conservation of the white-clawed crayfish, *Austropotamobius pallipes*, in South West England

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The white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet 1858) has suffered severe declines within the South West of England. One of the main threats is due to the invasive signal crayfish *Pacifastacus leniusculus* (Dana 1852), which was introduced into UK crayfish farms in the mid 1970’s. In response to this decline, The South West Crayfish Partnership (SWCP) was formed in 2008; comprising Bristol Zoological Society, Buglife, Centre for Environment, Fisheries and Aquaculture Science (Cefas), the Environment Agency and Wildlife Trusts. The SWCP implements landscape scale, strategic conservation for *A. pallipes*, in an attempt to safeguard the future of this species in South West England. The conservation effort has four main strands: (1) Ark sites: safe refuges established throughout the South West, for translocation of a proportion of the most highly threatened white-clawed crayfish populations; (2) Crayfish captive breeding facility: established at Bristol Zoo, which provides plague-free *A. pallipes* brood stock for ark site release, reintroductions, research and outreach; (3) Communication strategy: running in tandem with the other two elements, targeting key audiences such as anglers, restaurants, students, school children and zoo visitors; and (4) Invasive crayfish control: Cefas have spent the past two decades developing a variety of techniques to control the invasive crayfish species within the UK. This presentation will cover the key elements of the conservation programme, evaluating its success to date and the impact it has made to preserving white-clawed crayfish within South West England.

Conserving white-clawed crayfish *Austropotamobius pallipes* in an upland catchment in Yorkshire, a case-study

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White-clawed crayfish were abundant throughout the catchment of the River Ribble in northwest England until an outbreak of crayfish plague caused the loss of the population in most of the catchment. Some relict fragments of population survived in semi-isolated areas and show signs of recovering. Restocking was carried out successfully in one tributary and another stocking carried out to establish an ‘ark site’ population in an isolated part of the catchment. The reintroduced population expanded to approximately 4 km range in six years, with an expanding peak abundance detected by trapping four years after stocking. In another tributary initial stocking too high up in the headwaters was unsuccessful and necessitated restocking further downstream in a stretch with lower gradient and more flow. Future constraints and opportunities are discussed.
The future of endangered *Austropotamobius torrentium* (Schrank 1803) in the light of protected areas and habitat fragmentation: a case study from the Carpathians

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Fragmentation is one of the most challenging issues in conservation ecology. The aim of this work fits within the goal to find reliable methods for its assessment in freshwater habitats through a case study on the endangered stone crayfish, *Austropotamobius torrentium* (Schrank 1803). We focused on 17 populations in the Danube basin, from which 297 individuals were sampled and genotyped. Genetic variation and genetic structure were analysed based on 5 polymorphic DNA microsatellite loci. By following rivers linearity, the connectivity between the populations was assessed by determining the geographical distance. Next, based on ecological data from the known populations in Romanian Carpathians, we built an ecological model in order to find the most suitable predictors describing the species distribution. We developed a Random Forest model with catchment CORINE land-cover (as a surrogate for habitat quality), slope, altitude and mean multiannual temperature as predictors. We used this model to predict the species occurrence to the entire river network connecting the target populations, thus obtaining a third type of distance, an ecological one. Only one population showed significant deviation from Hardy-Weinberg equilibrium. Genetic variation, expressed by mean expected heterozygosity (HE) ranged between 0.422 and 0.752. AMOVA analyses show that the diversity between populations corresponds to 44% of total variability. High levels of genetic differentiation among populations were confirmed by high average FST and RST values (0.2896 and 0.4856 respectively). We found the best explanation of the inadvertences between molecular and geographical distances was provided by the ecological component, thus highlighting the importance of ecological approaches in the assessment of fragmentation. This case study points out a practical method for the assessment of ecological fragmentation, and also suggests that the communication pathways for freshwater organisms are of high importance for the preservation of diversity in populations.

Environmental education and awareness, fundamental tool in the conservation of the native crayfish

Valls N, Llamas S and O Comas

Associació de Defensa i Estudi de la Fauna i Flora Autòctona (ADEFFA). MasiaCamadoca, s/n. Santa, Maria de Merlès, Barcelona.

The Association of Defence and Study of Native Fauna & Flora (ADEFFA) runs a conservation program of the native crayfish in the Llobregat river basin since 2005, including the study of populations, captive breeding, repopulation, removing invasive species, land custody contracts, habitat preservation and environmental education. All activities within the conservation program run from the Generalitat de Catalunya. ADEFFA believes that environmental education is an essential tool in the conservation of the native crayfish, especially in the fight against the spread of invasive crayfish species. The main objective of ADEFFAs environmental education program is to present the risk factors that threat native crayfish populations and how people can contribute to their conservation. The activities are focused to general public, schools, and specific groups such as fishermen, hikers, etc. A highlight of the activities that has been under development for 5 years is the “Day of the invading crab catch at Merlès River. Has a main purpose, which is the environmental awareness of the participants related to the introduction of invasive crayfish species. Explaining how this species affect native crayfish, how invasive species deteriorate river ecosystems and the need for public to collaborate not expanding invasive species are the main goals of the date. Two or three dates are held annually. The activity takes place overnight and it is aimed at all ages. The result is a great success of participation, more than 200 persons per event, a high disclosure in nearby villages and around 25000 crabs caught (mainly American signal crayfish) since 2010. A pioneering experience that is worth to be known.
ABSTRACTS POSTERS

(in order of number assigned)
1. Adaptive response to early dominance of an acute invader? A case study of native crayfish populations recovery in the Lower Danube
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Successful invasive species compete for the same existing resources with related native species, frequently driving the latter to the cusp of extinction because of a lack of adaptive response. In this paper we analysed the behavioural relationships between two species of crayfish, the native *Astacus leptodactylus* and the invasive *Orconectes limosus* in an ongoing invasion process in the Lower Danube. We tested the species’ ability to acquire food and shelter in laboratory experiments in both intra- and interspecific confrontations. Moreover, we extended the investigation in the field by collecting biometrical data from crayfish individuals in old, new and non-invaded Danube sectors in order to compare the crayfish body fitness. The innate aggressive behaviour of the invasive crayfish reveals a pattern directed even towards its congeners, while the native species displays a more tolerant conspecific behaviour. With respect to interspecific confrontation, the invasive crayfish males and females exhibited prone sex-specific dominance behaviour regarding shelters. A roughly balanced behaviour was noticed for intersexual confrontations, larger specimens being slightly more dominant. The results of this study also highlight that the occupancy of a shelter is more disputed than food resources, which appear to be opportunistically achieved. Field data revealed better body fitness indexes in non-invaded sites but also in growing populations in old invaded Danube sector. Considering those results, we hypothesise that the stress caused by dominance might lead to acute decrease of the invaded native populations biological quality and thus increasing the incidence of the crayfish plague which consequently resulted in the declines. Further investigations may reveal if there is any hope for recovery of the native species.

2. Crayfish epibionts *Branchiobdella sp.* on the stone crayfish in the Czech Republic
Vlach P* and L Šrámková

This contribution evaluates the occurrence of crayfish epibionts *Branchiobdella* inhabiting stone crayfish in the Czech Republic. The study was conducted in 8 localities (from app. 40 known localities), respecting its natural range in the country. In total, we determined 723 individuals of 4 species: *Brychiobdella pentodonta*, *B. hexodonta*, *B. parasita* and *B. astaci*. *B. pentodonta* dominated (403 ind.) in the whole sample, and also *B. parasite* occurred frequently (316 ind.). On the other hand, *B. hexodonta* was recorded only three times and *B. astaci* only once in the left tributary of Novosedlský brook in Upper Palatine Forest. The most variable locality was Huníkove brook with 3 recorded Branchiobdella species - *B. parasita*, *B. pentodonta* and *B. hexodonta*, whose occurrence was recorded only in this stream. *B. parasita* was mostly the dominant species, or occurred equally to *B. pentodonta* in the stream Chýlava. On the contrary, in Zubřina, the abundance of *B. pentodonta* prevailed. The highest abundance of *Branchiobdella sp.* was recorded in Zubřina (17 ind. per one crayfish on average), conversely the lowest number in Medvědí brook was observed (0.3 ind. per crayfish). Moreover, we found out that *B. pentodonta* significantly preferred its position on crayfish chelae, whereas *B. parasita* inhabited the whole crayfish body equally, except for chelae. The last findings could correspond with interspecific competition between both species.
3. White-clawed crayfish (*Austropotamobius pallipes*) endangered species sampling methods: efficiency and disruptions in the balance
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The distribution of *Austropotamobius pallipes* in Biscay (Basque Country) is being increasingly restricted. After crayfish plague and the effects of many further threats, this species is found in small headwater creeks away from the main streams. The species inhabits in small populations, generally isolated and fluctuating number of individuals. The main characteristic of these river stretches with a little flow is that they do not allow trap sampling methodology and so, other methods based on night viewing are used to get data to calculate population estimates. Two small rivers were sampled for 5 years. In this study, we have compared the values obtained by night viewing from river-bank (CPUEs) with manual searching on one pass (CPUEc). We have also correlated these methods with the estimate population size obtained by Removal method (ABUNDANCE). Both indirect estimates were highly correlated with ABUNDANCE, being stronger CPUEs-ABUNDANCE relationship (p=0.80; R² = 0.64) than CPUEc-ABUNDANCE one (p=0.67; R² = 0.46). Night viewing is an acceptable strategy for population estimates without a direct interaction with the individuals. This strategy may optimize sampling, as it lets increasing the efficiency in effort and time and minimizing the perturbation generated with catching activity.

4. PIT tagging effect on noble crayfish (*Astacus astacus* L.) survival
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A laboratory experiment was conducted to assess the potential impacts of 1.5 X 7 mm passive integrated transponder (PIT) tags on survival of noble crayfish (*Astacus astacus* L.). 30 noble crayfish (mean TL 96.9 ± 4.4 mm, 26 females and 4 males) were internally implanted with PIT tags and were kept together with untagged (as control) 30 crayfish (mean TL 96.0 ± 5.4 mm, 23 females and 7 males) in three 1 m³ tanks (10 tagged and 10 untagged crayfish in each) and maintained for 219 days at room temperature with a light regime of 16 h: 8h LD. 31 noble crayfish originated from a natural lake and 29 noble crayfish originated from a crayfish farm. 90% of tagged crayfish and 77% of untagged crayfish died during the experiment. The highest survival rate (30%) was observed in the third tank where the mean TL (93.4 ± 4.9 mm) was smaller than in the first (97.6 ± 3.0 mm) and in the second tank (98.5 ± 5.0 mm) where the survival rates were 10%. All survived crayfish originated from the crayfish farm. We can assume that smaller crayfish tolerate tagging better and the farmed crayfish survive better than wild ones.
5. Mate choice in spiny-cheek crayfish (Orconectes limosus, Rafinesque 1817): females prefer males from the same population, males not
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Investigating of different effects on mate choice belong to major principles in behavioural and ecological studies. The mate choice is often based on recognition of conspecifics and social experiences (e.g. hierarchy, aggression or fighting), which play important roles in decision-making. In crayfish, it is known that females, which have large energy cost, are more choosy than males. We analysed mate selection of the spiny-cheek crayfish (Orconectes limosus) in the laboratory conditions. Our objective was to explore the effect of different origin of individuals (three geographically separated populations) on preference in mate choice. Both sexes were divided into triad groups (male with a familiar and unfamiliar female; female with a familiar and unfamiliar male). Our results suggest that spiny-cheek crayfish females choose mates from the familiar population, whereas males do not exhibit any specific preference between conspecifics from all. Our study offers information around reproductive behaviour that previous experience enables invertebrate females to easier decision making. While the males confirmed hypothesis about lesser selectivity. This study has the potential for future research on the largely overlooked field of mate choice in invertebrates.

6. Epifauna on freshwater crayfish (Crustacea: Decapoda) in Croatia
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The crustacean exoskeleton is well known substratum for associate species - epibionts. During 2014 and 2015, we have studied epifauna on six different freshwater crayfish species collected from continental and mediterranean regions of Croatia (Europe). Four of them are autochthonous (Astacus astacus, Astacus leptodactylus, Austropotamobius pallipes, Austropotamobius torrentium) and two allochthonous invasive species (Pacifastacus leniusculus and Procambarus fallax f. virginalis). The epibiont samples were collected from the exoskeleton surface and from the crayfish gill cavity. The aims of this research were: (i) to analyse epifaunal assemblage on different freshwater crayfish and (ii) to compare epifauna among different crayfish populations, especially between autochthonous and allochthonous species. A total of 44 different epifaunal taxa were recorded. The most abundant group was Ciliophora, with Vorticella campanula and Epistylis sp. as the most frequent taxa. Rotifera was the most diverse group and 16 different rotifer species were identified. Four rotifer species (Lepadella astacicola, L. branchiola, L. parasitica and Dicranophorus hauerianus) were determined as specific inhabitants of certain crayfish species and these rotifers were found exclusively on autochthonous species. Branchiobdellidans were constant crayfish epibionts, and for the first time the North American species Xirogiton victoriensis has been recorded in Croatia. Representatives from Catenulida, Gastrotricha, Nematoda, Bivalvia, Hirudinea, Tardigrada, Crustacea, Hydrachnidia and Chironomidae were also recorded as epibionts on freshwater crayfish. Results of this study suggested the separation of epifaunal assemblage between autochthonous and allochthonous crayfish, as well as the separation of epifauna among autochthonous species. Our results indicate highly diverse, complex and specific epifaunal assemblage on different crayfish populations. Thus, multiple relationships between crayfish host and epibionts will be the topic of further studies.
7. The stone crayfish in the czech republic: lost-and-found in last seven years
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Within the period 2012-2015, an intensive research concerning: 1. mapping the occurrence of stone crayfish; 2. monitoring of presently known populations; 3. an evaluation of ecological demands of this species, was carried out. This contribution focuses on newly described localities, losses of some populations, and populations affected by a dramatic decrease in population densities in some localities. Moreover, the contribution introduces a hypothesis or particular reasons for that decrease or extinction. The extinction in Úpořbrook and Hýskovskýbrook has already been published; recently we have recorded the following losses: Zákolanskýbrook, Bertínskýbrook, Vlčíbrook, Kornatíckýbrook, Hrádeckýbrook, and Medvědíbrook. Whereas the combination of crayfish plague and (probably) low water quality caused the total mortality in lower part of the Zákolanskýbrook, crayfish plague outbreak killed crayfish in Kornatícký and Hrádeckýbrook in 2015. Conversely, there was a lethal concentration of BOD5 in Vlčíbrook. Also dramatic droughts in 2015 influenced the population densities in Chocenický and Přešínskýbrook. The mass mortality in Klabava was caused by a combination of stream acidification and toxic metal accumulation. The reasons for other losses are mostly speculative. On the other hand, within the mapping campaign, we found more than 10 new populations of stone crayfish. Nevertheless, the newly recorded occurrences correspond with a present range of this species in the Czech Republic.

8. Fine structure of the spermatozoon in three Species of (Arthropoda: Crustacea: Decapoda) Cambarus robustus, Orconectes propinquus and Orconectes rusticus: a comparative biometrical study
Yazıcıoğlu B1*, Hamr P2, Kozák P1, Kouba A1 and H Niksirat1

The ultrastructure of spermatozoa in three species of cambarid crayfish, including Cambarus robustus, Orconectes propinquus, and Orconectes rusticus were studied and compared with eight previously studied species from different crayfish families using morphological features and biometrical data. The ultrastructure of spermatozoa show a generally conserved pattern including an acrosome and nucleus in the anterior and posterior parts of the cell, respectively, radial arms that wrap around the nucleus, and the whole cell is enclosed by an extracellular capsule. The most outstanding morphological feature in spermatozoa of three studied cambarid crayfish is the crest-like protrusions in the anterior part of the acrosome that can be used as one of the features for distinguishing the members of this family. Results of biometrical data reveal that acrosome size in the representatives of Parastacidae are the smallest, while representatives of Astacidae show the biggest acrosome. The acrosome size in species belonging to Cambaridae occupy an intermediate position between the two other families of freshwater crayfish. In conclusion, a combination of morphological features and biometrical data of spermatozoa can provide an effective tool to distinguish different species of the freshwater crayfish.
9. Metabolic rate of *Cambarellus montezumae* (crustacea: cambaridae): effects of size and seasonal changes  
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The measurement of metabolic rate is a key element of a species energy balance. The evaluation of respiratory metabolism in natural conditions, allows us to know the energy requirement of the animal for different processes including growth, activity, reproduction, among others. The objective of this study was to know the element (R) of the energy budget equation: C = P+R+F+U (IBP, 1968) in *Cambarellus montezumae*, during hot rainy season (May – August) and transitional months (September – October) in the area of study. Recently collected samples of crayfish (n=15) representing small, medium and large sizes of both sexes were employed in respirometry experiments, using a closed respirometer. The temperature and water quality were similar to the conditions of the sampling site. Two measurement periods were used: (noon and evening). Average routine metabolic rate was used for comparisons (QO₂ : mg O₂ /g. dry weight/h) and was converted to cal/g.dw/day using the Qox = 3.31 cal/mgO₂  (Brafield & Solomon, 1972). Comparisons for size and month were made by ANOVA factorial (3 X 6) and Tukey test post hoc (p<0.05). Significant differences were obtained by month and size. QO₂ was similar between the months of the rainy season, with a temperature average range of (22.3 ± 1.6°C) and had a significant decrease (p<0.05) in October. Relationships of QO₂ – WW were calculated for all months using a potential model. We discuss results in relation to energy requirements of the species for growth and reproduction and its potential use for intensive management.  
Keywords: *Cambarellus montezumae*, Xochimilco, metabolic rate, Mexico.

10. The impact of streetlights on an aquatic invasive species: artificial light at night alters signal crayfish behaviour  
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Artificial light at night (ALAN) can alter the behaviour, communication and orientation of animals, and could interact with other stressors to affect biodiversity. Invasive, non-native species are one of the largest threats to freshwater biodiversity; however, the impact of ALAN on such species is unknown. This study assessed the effects of ALAN at ecologically relevant levels on the behaviour of a globally widespread invasive species, the signal crayfish (*Pacifastacus leniusculus*). Crayfish were exposed to periods of daylight, control (<0.1 lx) and street-lit nights to test two hypotheses: (1) signal crayfish under natural conditions are nocturnal animals and (2) ALAN reduces the duration of crayfish activity and intraspecific interactions, whilst increasing their propensity to use shelter. Our results confirm that signal crayfish are largely nocturnal, showing peak activity and interaction levels during control nights, whilst taking refuge during daylight hours. However, when exposed to ecologically relevant simulated light pollution from a streetlight at night, activity and interactions with conspecifics were significantly reduced, whilst time spent sheltering increased. Global anthropogenic changes such as ALAN may alter the life history traits and behaviour of invasive species and ultimately influence their impact on invaded ecosystems. The results of the present study suggest that ALAN could reduce the success of signal crayfish in urban areas, by drastically reducing their nocturnal activity. This study is the first to show an impact of ALAN on the behaviour of an invasive, non-native species, and provides information for the management of invasive crayfish in areas where ALAN is prevalent.
11. Life history and population ecology of signal crayfish, the new invader in Northern Italy
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The occurrence of the signal crayfish *Pacifastacus leniusculus* in the Valla stream, north-western Italy, was one of the first reports of this invasive alien species in Italy. Since 2009 signal crayfish was spreading upstream from the artificial lake formed by a dam for hydroelectric purpose. We studied the signal crayfish population in this novel environment by trapping sessions during a year. Five sampling sites were located along the invaded range of the Valla stream, along more than 10 km. At each site, six baited traps were set once a month for two days, from April 2015 to March 2016. All signal crayfish were removed from the watercourse. Crayfish were sexed, weighted and the following morphometric parameters were measured: total length (TL), and cephalothorax length (CL). We trapped overall 433 females (mean TL: 84.7 mm) and 428 males (mean TL: 85.5 mm). Crayfish activity was correlated with water temperature. Egg-bearing females occurred from early November and hatching in late May. Sex ratio was about 1:1 in all sites except the most downstream one, where females were more abundant; and more females were caught during summer months. Crayfish TL and condition factor fluctuated during the year, but both decreased significantly moving upstream. Management actions should be promoted to limit its spreading overall within watercourses potentially suitable.

12. Analysis of abundance, fecundity and allometric relationships from *Cambarellus montezumae* females during an annual cycle
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*Cambarellus montezumae* population at Xochimilco, Mexico City, is in serious problems of disappearance. This species was highly appreciated and it was regularly consumed as part of the diet of the Xochimilcas and Mexican people. Nowadays, crayfish is very scarce in this area. As a part of a collaboration research, UNAM and UAM universities have undertaken the project of assessment and rescue of native species in the area of Xochimilco. In this study, we analyze the female population of *C. montezumae* during an annual cycle. 42 berried females were collected in ten months of sampling (January – October). Abundance varied significantly by sampling month (p<0.05). 76.2% of females were collected during hot rainy season (April – August). Description and analysis of these females were made through: total and cephalothorax length, (TL and CTL). Large and width of abdomen, wet weight, ovigerous mass weight and number of eggs. Minimum, maximum and average values for TL, WW and number of eggs were: (28.31, 43.21, 34.5) mm, (0.58, 1.91, 0.99) g, and 13, 150 and 46.9 eggs respectively. Distributions of TL and WW were positive skewness. Allometric relationships were calculated for WW – TL (potential model, r²=0.93), CTL – TL (linear model, r²=0.86). Fecundity – TL was positive correlated (r=0.68, p<0.05). Also positive and significant correlations (p<0.05), were obtained for abdomen width and length – TL and female abundance – temperature. Ovigerous mass weight was higher on April and number of eggs per female was bigger on September. We discuss results in relation to scarcity of berried females, the decrease in the number of eggs per female, the size of first maturity, and anthropogenic impacts in the study area.
13. Some aspects of the dynamic population of a Mexican crayfish species, *Cambarellus montezumae* (Saussure) from Xochimilco
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At Xochimilco’s lake, still remains a wild population of *Cambarellus montezumae* crayfish. This population has been declining in the last decades, because of fragmentation and loss of habitat, overexploitation, pollution, competition and predation by alien species. In this study, we analyze dynamic population of this species through indicators of abundance, meristic indexes (wet weight, WW; total length, TL; and cephalothorax length, CTL); frequency of TL distributions, cohort’s growth rate, sex ratio and allometric relationships in crayfishes of both sexes. A total of 1345 *C. montezumae* crayfish, were collected during a twelve-month sampling, at Xochimilco channels in 2011 annual cycle. WW varied between (.01 – 1.53) g, and TL (6.47 – 41.21) mm, significant differences (p<0.05) were found by sex and month. Abundance was higher during hot rainy season (April – August). Significant differences were detected in total sex ratio population (Female:Male) being almost (2:1). Allometric relationships: WW- TL and WW - CTL - were better adjusted by a potential model, and CTL – TL by a linear model. Two cohorts were identified through the annual cycle (the one at April and second in August), and growth rate was calculated for both of them using TL frequency distributions. We discuss dynamic population of this species in relation to habitat changes and anthropogenic disturbances, which have led almost to its collapse and disappearance.

14. Detection of invasive crayfish populations by environmental DNA in fishponds from the natural park of Brenne
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Environmental DNA (eDNA) is a powerful method for assessing the presence and the distribution of invasive aquatic species. We used this tool in order to detect and monitor several invasive crayfishes *Procambarus clarkii*, *Orconectes limosus* and *Pacifastacus leniusculus* present or likely to invade the fishponds of the Natural Regional Park of Brenne, one of the most important wetland in France and listed as an International RAMSAR wetland zone since 1991 We designed specific primers for each crayfish species, and set up an experimental aquarium approach to confirm the specificity of the primers and the sampling protocol. We analysed samples taken in the Natural Regional Park of Brenne in 2014 and 2015. The field experiment has proven the reliability of the eDNA detection method. Both experiments confirm that qPCR using SybrGreen protocol with the same primers give better reliable results that with TaqMan protocol. After optimization of the eDNA detection in water samples, it is concluded that sampling must be made during the main period of activity of crayfish, i.e. in summer. This method is a powerful tool for establishing the presence or absence of invasive species in the numerous ponds (more than 2000) in the National Regional Park of Brenne.
15. Reproductive cycle of the marble crayfish from an established population in Croatia
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The marble crayfish (Procambarus fallax f. virginalis) is a relatively new non-indigenous crayfish species that has established populations in Europe. Until today, it is the only known decapod species with parthenogenetic reproduction. Established population of marble crayfish was recently discovered in a gravel pit in Northwest Croatia. Apart from parthenogenetic reproduction, the invasion success of this species stems from its early maturation, and higher fecundity compared to other crayfish species. Therefore, the aim of this study was to examine the year cycle of the marble crayfish in Croatia, and its potential (number of ovarian eggs) and realized fecundity (number of pleopodal eggs and juveniles). Our results show that all 140 examined individuals were reproductively active during the whole sampling period. Peaks of reproduction activity were recorded from September to November, when both individuals with pleopodal eggs or juveniles as well as individuals with ripe ovarian eggs were recorded. The smallest reproductively active female was 40.81 mm TL, while reproductive output increased with size (TL). The average number of ovarian eggs was 297. The average number of pleopodal eggs was 15% lower than the number of ovarian eggs, while number of juveniles attached to pleopods was reduced by 50% from ovarian egg count. This represents a lower brood loss compared to other crayfish species. Due to almost constant reproduction, early maturation and relatively high potential and realized fecundity in an established population in Croatia, the marble crayfish represents a major threat to the surrounding waterbodies and their native crayfish fauna.

16. Assembly and annotation of the marbled crayfish genome
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Marbled crayfish (Procambarus virginalis) are the only freshwater crayfish known to reproduce by cloning (apomictic parthenogenesis). Notably, among genetically identical offspring raised in the same environment distinct phenotypic differences can be observed. These characteristics render the marbled crayfish an interesting laboratory model. A prerequisite for introducing this triploid arthropod as a new model organism and focus of this thesis is the identification of its complete genome sequence. We experimentally determined the genome size at approximately 3.5 Gbp by k-mer analysis and flow cytometry. High coverage sequencing data (~70X) of one individual female was used for a first de novo draft assembly with a length weighted median scaffold size (N50) of 40 kb. Assessing genome completeness using the benchmarking software BUSCO we were able to identify 56% complete and 21% fragmented (out of 2675) conserved single-copy arthropod orthologs. Single nucleotide variations (SNP) analyses of four additionally sequenced individuals from different strains confirmed clonal reproduction and enabled us to describe genomic characteristics such as triploidity and common genotypes. By interspecies comparisons to the closest relative, the sexually reproducing Procambarus fallax, and preliminary automatic genome annotation of about 15,000 protein coding transcripts we found potential alterations in meiosis related genes. These findings provide new insights into mechanisms of parthenogenesis. Genomic data and manual curation services are, after registration, publicly accessible at our Marmorkrebs webserver (http://marmorkrebs.dkfz.de).
17. North American Branchiobdellida (Annelida: Clitellata) or crayfish worms in France: the greatest diversity of these alien ectosymbionts in Europe
Parpet JF and S R. Gelder
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Over the last five years, biomonitoring of freshwater bodies resulted in alien crayfishes being collected from the Garonne, Loire, Seine and Rhône basins in France. These crayfishes (*Pacifastacus leniusculus* (Dana, 1852), *Procambarus clarkii* (Girard, 1952), *Orconectes limosus* (Rafinesque, 1817)) from North America also carried ectosymbionts of the order Branchiobdellida (Annelida, Clitellata) or crayfish worms. Initially we reported (Gelder et al., 2012) the distribution in France of *Xironogiton victoriensis* Gelder and Hall, 1990, *Cambarincola gracilis* Robinson, 1954 and *Cambarincola okadai* Yamaguchi, 1933 on the signal crayfish, *P. leniusculus*, indigenous to the Pacific Northwest of North America; additional information is included here. Signal crayfish found in the Seine basin also carried *Triannulata magna* Goodnight, 1940, and this is the first record of this species in Europe. Louisiana red swamp crayfish, *P. clarkii*, were collected in the Adour basin along with their endemic, *Cambarincola mesochoreus* Hoffman, 1963. This is the first report of both host and branchiobdellidan in France. A unique host/branchiobdellidan combination was discovered when western North American *X. victoriensis* was observed on eastern North American spiny cheek crayfish, *Orconectes limosus* (Rafinesque, 1817). Although France has the most reported alien branchiobdellidan species in Europe, a number of other countries also have established North American branchiobdellidan populations. The impact of these alien species on the endemic *Branchiobdella* spp. is unknown, but warrants concern and further study, besides investigating their impact on other invertebrates in the freshwater ecosystem.

18. No species recognition between two alien crayfish species?
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Crayfish rely on odours for finding a resource, detecting predators and recognizing species. Species that are not co-evolved could not chemically recognize each other. The red swamp crayfish *Procambarus clarkii* and the spinycheek crayfish *Orconectes limosus*, both native to North America where they occupy different areas, have coexisted in the “Parc Naturel Régional de la Brenne” (Centre Region, France) since 2007 but in different ponds. Behavioural observations were conducted in October 2014 and April 2015 to assess if *P. clarkii* and *O. limosus* are able to chemically recognize individuals of both sexes belonging to the same or other species. In experimental individual aquarium, records of behaviour of 20 *Procambarus* males and 20 *Orconectes* males were made of 3-min observation bouts for each of two sequential phases: (a) the “water” phase, following the introduction of 10 mL of well water, (b) the “smell” phase, following the introduction of 10 mL of well water conditioned by (1) *P. clarkii* male, (2) *P. clarkii* female, (3) *O. limosus* male and (4) *O. limosus* female odour. The time spent by each crayfish in locomotion and other activities (feeding, cleaning), and the time spent in one of three postures (raised, intermediate, or lowered) were recorded every 15 s. Crayfish seem able to recognize the conspecific of the same sex (males), and not the heterospecific, underlying the absence of species recognition in these two species that separately evolved.
19. Invader showdown: interference competition between *Orconectes immunis* and *Pacifastacus leniusculus*

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Alien crayfish are among the most damaging invaders of European inland waters and changes in the crayfish fauna should be monitored prudently. Here, we investigated for the first time direct aggressive interactions and competition for shelter between alien calico crayfish, *Orconectes immunis*, and signal crayfish, *Pacifastacus leniusculus*, which are highly invasive in Central Europe. Specifically, we monitored bouts of interspecific 1:1 combinations in laboratory experiments and offered limited shelters in a similar setting. In size- and sex-matched combinations, *O. immunis* won significantly more interactions than *P. leniusculus*; however, *O. immunis* females were inferior to size-matched *P. leniusculus* males. Similarly, *P. leniusculus* males were dominant over 4 mm smaller *O. immunis* males in chelae size-matched bouts. Both species showed a similar affinity to the provided shelter, but *O. immunis* was dominant in the shelter competition experiments in both sexes. The dominance of *O. immunis* males in size-matched combinations may be related to their greater ‘resource holding potential’ due to larger chelae size than *P. leniusculus* males. *Orconectes immunis* females, however, featured similar sized chelae than size-matched *P. leniusculus* females, and their dominance can best be explained by higher inherent aggression of *O. immunis*. Based on these results, *O. immunis* can be expected to outcompete similar sized *P. leniusculus*. However, since the latter invader grows considerably larger than *O. immunis*, and gained dominance at a size advantage of 4 mm, outcomes of interactions in the field may be much more complex, presumably also due to contrasting life-history strategies.

20. Novel microsporidian infection in the Japanese endemic crayfish *Cambaroides japonicus*

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The endemic crayfish, *Cambaroides japonicus* (de Haan, 1841) is the only native crayfish species in Japan. The rapid decreasing of its native range, which comprises Hokkaido and the Northern Honshu, led the Environmental Agency in Japan to consider *C. japonicus* as an endangered species. During a monitoring survey on the distribution of *C. japonicus* in Hokkaido in 2011, a specimen with atypical whitish appearance of the abdominal musculature were observed in Lake Toyoni and porcelain disease (*Thelohania* sp.) has been suspected. A focused survey conducted in Lake Toyoni in 2013 revealed the presence of macroscopically affected crayfish with a prevalence of 6.3%. Ethanol fixed *C. japonicus* were analyzed and muscle samples for molecular analysis were taken from 8 affected specimens. The small subunit ribosomal DNA (SSU rDNA), amplified with generic microsporidian primers (Weiss and Vossbrinck, 1998), was sequenced and compared with other marine, freshwater and terrestrial Microsporida. Histological analysis of the abdomen revealed high densities of ovoid single spores, not contained inside a sporophorous vescicle, in striated muscle cells of the pleonal extensor and flexor muscles. No spores were recorded in the intestinal musculature nor in the ventral ganglia. The affected muscle fibers were frequently surrounded by haemocytic infiltration and sporadic melanization. The phylogenetic analysis of the SSU rDNA placed this undescribed microsporidium between members of the class Marinosporida (Vossbrinck and Debrunner-Vossbrinck, 2005), distant from the microsporidia of other European and Australian crayfish. Further studies are needed to describe the developmental stages and the ultrastructural features of this microsporidium.
21. Genetic diversity and differentiation of wild populations and captive stocks of the noble crayfish (*Astacus astacus* L.) in Estonia

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There are about 20 crayfish farms in Estonia which produce and sell noble crayfish for restocking and enhancement of natural waterbodies and/or for human consumption. So far, the origin of captive stocks and their genetic characteristics have not been studied and likewise, there is lack of information about genetic diversity and population structure in a wild. The aim of this study was to evaluate genetic diversity and population genetic structure of captive stocks and wild populations of the noble crayfish in Estonia. A total of 1923 individuals from six crayfish farms and 38 natural lakes and rivers in Estonia were analyzed using 19 polymorphic tetranucleotide repeat microsatellite markers. For comparison, two populations from Czech Republic were included. The results showed that Estonian noble crayfish populations were on average less variable than the Czech populations and formed two clear genetic clusters according to their geographic origin (Island of Saaremaa and mainland Estonia). The captive stocks were genetically similar to the wild populations of the same region and displayed no significant loss of genetic variability. However, two captive stocks possessed increased levels of inbreeding. The results of the study will be used for improving conservation and management plans of the noble crayfish in Estonia.

22. Positive selection of beautiful invasives: long-term persistence and bio-invasion risk of ornamental crayfish

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Following a distinct peak interest to keep freshwater crayfish in home aquaria in the mid-2000s, the aquarium trade has become a novel introduction pathway for alien crayfish species in Central Europe. Here, we provide an update on the German ornamental crayfish trade approximately one decade after the ‘crayfish hype’ to explore the long-term implications in terms of bio-invasion risk. Specifically, species’ availability and potential invasiveness, as well as the determinants of availability were assessed. In July 2015, a total of 31 online shops offered 28 crayfish species, which represents a decline of 24% in species diversity compared to the late 2000s. In addition, the estimated rate of import of new species has considerably flattened and approaches pre-hype values (< 1 species ∙ y⁻¹). However, the risk associated with the offered species, as assessed by a risk screening tool (FI-ISK), has not decreased compared to the late 2000s. Long-term availability in the trade (covering one decade) was primarily determined by bright coloration, the ability to reproduce under warm aquarium conditions, and a preference for lentic habitats. Species featuring such traits are likely to persist in the aquarium trade and include four high-risk species, most notably invasive and crayfish plague-carrying red swamp crayfish (*Procambarus clarkii*) and Marmorkrebs (*Procambarus fallax f. virginalis*). Persistent propagule pressure from aquaria has substantially contributed to the establishment of both species in Central Europe, stressing the need for effective pathway management.
23. Presence of Branchiobdellida in five population of native crayfish in Northern Italy: preliminary results
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Branchiobdellid annelids are commensal symbionts associated with crayfish populations, knowledge of their dispersion and distribution is generally scarce. The aim of this research was to identify the species of Branchiobdella isolated on the crayfish in different populations, and the prevalence among the species. We collected white-clawed crayfish *Austropotamobius pallipes* complex during the autumn 2013 from five creeks in Northern Italy. Worms were removed by inserting one-sidedly crayfish into a container with salty water (35 ppt): 1-minute each for claw, abdomen and cephalothorax; then branchiobdellids were placed into separate vials. The branchiobdellids identified, were *Branchiobdella italica*, *Branchiobdella parasita*, *Branchiobdella astaci*, *Branchiobdella hexodonta* and *Branchiobdella pentodonta*. We also found coexistence of two or more species of Branchiobdella on crayfish at the same location. 46% of worms were present on abdomen and 40% cephalothorax while 14 % were on claw. In all the populations studied at least two species of worms were detected and one crayfish was found to support four species of Branchiobdella.

24. Effect of pharmaceuticals on crayfish
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The release of human pharmaceuticals and personal care products into aquatic ecosystems continues to be a serious environmental problem. There is a staggering list of pharmaceuticals that have been detected in surface water, groundwater and tap water. These compounds typically modify the physiology or behavior, of the intended target system, without lethal effect. The wide range of entry points into freshwater systems reaffirms that animals living within those habitats face continuous exposure to pharmaceuticals, even if only at low doses. Crayfish are a crucial invertebrate in freshwater ecosystems. They are omnivorous, and occupy a key position in the trophic web as both predator and prey. With the importance of crayfish to their ecosystems, the introduction of pollutants such as pharmaceuticals, could have negative effects to crayfish populations. For example, pharmaceuticals can cause a mediating aggressive behavior. It has been demonstrated that crayfish injected with serotonin (antidepressant) fights lasted considerably longer. Also, the lasting impact of pharmaceuticals leads to immune system function decline, which greatly increases the mortality rate. Moreover, they can slow down synthesize and release of hormones from the X-organ sinus complex, and thus influence molting, gonad development, water balance, blood glucose etc.. However, despite all data the impact of pharmaceuticals on particular developmental stages, mortality, growth rate and postembryonic development of crayfish had not yet been fully explored. Therefore, we provide an overview about the known effects of pharmaceuticals on crayfish and demonstrate that these effects could be quite multifaceted.
25. Photoperiod affects light/dark preference and exploratory behaviour in noble crayfish (Astacus astacus)
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Artificial day-night cycles are known to affect crayfish growth, behaviour and physiological stress levels in aquaculture. Based on the protocol by Fossat et al. (2015), who validated decreased exploratory behaviour and raised photophobia as stress-induced anxiety-like behaviours in Procambarus clarkii, we evaluated the effect of different photoperiods on noble crayfish activity and light/dark preference in an aquatic plus maze. We kept 135 two-summer-old crayfish in a recirculating aquaculture system and exposed them to five different photoperiods: hours light/dark (L:D) 0:24, 8:16, 12:12, 16:8 and 24:0. All animals had access to brushes and PVC pipes as shelters. After 144 days, the crayfish were submitted to the plus maze test. During a ten minute period, each individual's location was scored every five seconds. Exploratory behaviour was assessed by quantifying the number of movements between different locations in the maze. Light/dark preference was determined by the time spent in the dark or lit arms. A linear mixed model for the different outcomes was fitted using tank as a random intercept and treatment as a fixed effect. Crayfish kept in 24L showed a higher amount of movements (43.6±4.3 mean±stdev) than individuals from all other treatments (ranging from 30.9±2.4 in L16:D8 down to 23.3±3.3 in L12:D12, p=0.0004). They also spent more time in the lit arms of the maze (43.40±1.74%) than animals from the 24D treatment (25.07±4.22%) (p=0.040). These results show that continuous light stimulates exploratory behaviour and continuous darkness causes more neophobia towards lit areas.

26. The River Barle Signal Crayfish Project: assessing the potential of male sterilisation as a signal crayfish control technique
Green N1, Stebbing P2, Bentley M3, Andreou D4 and M-R Lane5,
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4 Environmental Science, Bournemouth University, Fern Barrow, Talbot Campus, Poole, Dorset BH12 BB, UK
5 Biodiversity Technical Specialist, Environment Agency Devon Team, Manley House, Kestrel Way, Exeter EX2 7LQ, UK

This poster will present the methods and results to date of the River Barle Signal Crayfish Project which aims to test the effectiveness of a Sterile Male Release Technique in controlling signal crayfish on a 1.5km stretch of the River Barle Site of Special Scientific Interest, south west England, UK, between 2015 and 2017. The methodology consists of the intensive trapping and removal of all crayfish apart from dominant males (above 40mm carapace length) which are manually sterilised (vasectomised) and returned to the river. Two types of trap are used, standard funnel traps and novel artificial refuge traps which have proved far more effective and less biased than funnel traps. Detailed sampling of crayfish density, fish and invertebrates is taking place in the study area and a control site throughout and beyond the trial period. Laboratory experiments are investigating various aspects of mating behaviour including mate choice and female monandry. The results feed into a PhD study guided by Bournemouth University, the Centre for Environment, Fisheries Aquaculture Science (CEFAS) and the Environment Agency. Trapping is being carried out by a group of 40 volunteers, many of them local people with an interest in the river. If the method is successful, the project aims to pass ownership on to local stakeholders so it can be continued over the long term without relying on external funding - a trial of this approach is also underway.
27. Eradicating signal crayfish with a biocide: what worked, what didn’t
PEAY, Stephanie
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In its introduced range in Europe signal crayfish *Pacifastacus leniusculus* has become invasive, with impacts on indigenous crayfish and other fauna. Where a new population is detected while it is still localised there is the potential to carry out a biocide treatment to try to eradicate the population. During 2004 to 2012 six treatments with natural pyrethrum have been carried out in Scotland and England, followed by intensive monitoring for five years to determine outcome. Results show there were two confirmed successes and one probable (after three years monitoring, ongoing) and three sites where complete eradication was not achieved. Treatments and relevant factors are outlined and key lessons learned.

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The native white-clawed crayfish (*Austropotamobius pallipes*) was a species commonly found in most watercourses in the basins of the rivers Fluvià, Ter and Muga until the end of the 1970s, when the crayfish plague *Aphanomyces astaci* arrived, a water mould that led to the disappearance of almost all the populations in these river basins. The white-clawed crayfish populations are currently found in second-order or larger seasonal rivers and fast-flowing streams where, due to some kind of natural barrier (drought in the lower reaches of the watercourse, cliffs, etc.), the fungus was unable to grow. Moreover, during the 1990s, the red swamp crayfish (*Procambarus clarkii*) population expanded rapidly in the basin and the situation became far worse, since the North American species is both a carrier of the crayfish plague and resistant to this fungal disease. Later, the signal crayfish (*Pacifastacus leniusculus*) arrived in the Ter River and the spinycheek crayfish (*Orconectes limosus*) arrived in the Muga River Basin, both species being carriers of the pathogen *Aphanomyces astaci*. This situation means relict populations of the white-clawed crayfish cannot expand and are forced to survive in the most inaccessible seasonal rivers and fast-flowing streams, facing the danger of a new outbreak of crayfish plague.
Conservation Plans for Endangered Species Facing the Threat of Emerging Diseases and Invasive Species

Emerging diseases and invasive species are one of the major problems currently faced by conservation plans for endangered species. Recent investigations indicate that both emerging diseases and invasive species are on the rise due to the synergistic effects of climate change and habitat destruction. Their impact on ecosystem services is increasingly recognized as a worldwide threat to natural ecosystems and biodiversity.

Successful management for these two factors is a key aspect for the development and implementation of conservation plans, especially, in aquatic ecosystems. The course will be given by specialists in main emerging diseases and invasive species and will consist on 10-30 min lectures providing examples on how to deal with these two problems. Crucial aspects of biology, epidemiology, early detection, identification of routes of introduction and pathways of dispersal, and development of efficient control measures will be discussed. The lectures will also deal with main pathogens on diverse animal species such as reptiles (e.g., Fusarium spp on sea turtles), amphibians (e.g., Saprolegnia spp), crustaceans (e.g., crayfish plague pathogen Aphanomyces astaci), bees (e.g., Nosema spp), corals (e.g., Aspergillus sydowii), as well as with main invasive species of the world such as the Asian predatory wasp (Vespa velutina), the rainbow trout (Oncorhynchus mykiss), the red-swamp and signal crayfish (Procambarus clarkii and Pacifastacus leniusculus), and the invasive plants.

The titles of the presentations will be: Crayfish and crayfish plague: two invasive species for the price of one; Biological invasions: problems and possible solutions; The invasive species situation in Australia; Rainbow trout threatening amphibians species of The Andes; The Asian predatory wasp and the small hive beetle: invasive species in Europe; The invasive plants: problems around the world; Fungi and the problem of emergence in virulence; Fusarium egg-disease: an emerging disease in sea turtles; The fungus Aspergillus sydowii in Corals; The Oomycetes. Who do you think they are?; The Saprolegnia threat on amphibians; Nosema ceranae (Microsporidia): an obligate intracellular parasite of bees; Evolutionary applications for the identification of disease threats to biodiversity; Emerging diseases and immune system; eDNA-monitoring of the crayfish plague Aphanomyces astaci; Crayfisheries management Finnish style: ignore warnings and implement on personal desires, The decline of the invasive signal crayfish in Scandinavia; The need of a conservation plan for native crayfish species of far-east Asia; followed by a Round table discussion on how to deal with both emerging and invasive species.
WORKSHOP PROGRAM
09.00. Presentation. Conservation Plans for Endangered Species Facing the Threat of Emerging Diseases and Invasive Species
Javier Diéguez-Uribeondo and Tadashi Kawai

Invasive species
09.10. Biological invasions: problems and possible solutions
Elena Tricarico, Università degli Studi di Firenze, Italia
09.30. The invasive species situation in Australia
James Purse, Griffith University, Queensland, Australia
09.50. An overview of aquatic invasive species in the US: from Nile crocodiles to Mysis shrimp
Susan B. Adams. Southern Research Station Asheville. USDA. US
10.10. Rainbow trout threatening amphibians of The Andes
Laura Martin Torrijos. RJB-CSIC, Spain
10.20. The Asian predatory wasp and the small hive beetle: invasive species in Europe
Raquel Martín-Hernández, Centro Agrario de Marchamalo (IRIAF), Guadalajara, Spain

10.40. COFFEE

Emerging Diseases
11.00. Insights into the origin of virulence from model organisms
Arturo Casadevall, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, US
11.30. Fusarium egg-disease: an emerging disease in sea turtles
Melissa Sarmiento RJB-CSIC, Spain
11.40. The fungus Aspergillus sydowii in Corals
Maria del Mar Soler MNCN-CSIC, Spain
11.50. Nosema ceranae (Microsporidia): an obligate intracellular parasite of bees
Raquel Martín-Hernández, Centro Agrario de Marchamalo (IRIAF), Guadalajara, Spain
12.10. The white spot virus in crustaceans: a serious concern
Kenneth Söderhäll, Universidad de Uppsala, Sweden
12.30. Evolutionary applications for the identification of disease threats to biodiversity
Javier Pérez-Tris, Universidad Complutense, Madrid, Spain

12.50 LUNCH (on your own)

Dealing with invasive species and emerging diseases: the crayfish experience
14.30. Invasive crayfish and crayfish plague: two for the price of one
Javier Diéguez Uribeondo. RJB-CSIC, Spain
15.00. eDNA-monitoring of the crayfish plague Aphanomyces astaci
Trude Vrålstad. Veterinary Institute, Norway
15.15. Active spreading of an invasive species challenges ecosystem-based management of crayfisheries
Japo Jussila, The University of Eastern Finland
15.30. Dealing with invasive species and diseases: the crayfish experience in Sweden?
Lennart Edsman, SLU Stockholm, Sweden
15.45. The need of a conservation plan for native crayfish species of far-east Asia
Tadashi Kawai. Wakkanai Fisheries Research Institution. Japan

16.00. COFFEE

16.30- 17.30. ROUND TABLE. How to deal with both emerging and invasive species
WORKSHOP ABSTRACTS

(in order of appearance)
Biological invasions: problems and possible solutions
Elena Tricarico
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Biological invasions cause severe negative impacts on the world’s ecosystems, human wellbeing and economy. Up to date, Europe is hosting more than 14,000 alien species (DAISIE 2009 and updates) with a growth of 76% in the last thirty years, and with several invasive species exerting relevant ecological and economic impacts. To face the phenomenon, Europe promoted several projects on invasive alien species to increase the knowledge (and provide some useful tools) and a new dedicated Regulation (EU 1143/2014) that has recently entered into force. The Regulation is based on the three-stage hierarchical approach recommended by the Convention on Biological Diversity (prevention, early detection and rapid eradication, and management) in order to protect native biodiversity and ecosystem services, and to minimize and mitigate the human health or economic impacts that these species can have. A list of EU concern invasive alien species has also been adopted, and it is now mandatory to manage the species in the list. Finally, together with the Regulation, awareness of the general public should be raised to address this increasing threat.

The invasive species situation in Australia
James Furse. Griffith University, Queensland, Australia
Environmental Futures Research Institute, Gold Coast campus, Griffith University, Queensland 4222, Australia, and Miyazaki International College, 1405 Kano-hei, Kiyotake-cho, Miyazaki-shi, Miyazaki, 889-1605, Japan.

The Australian Continent is geographically isolated and is accepted as being physically isolated at various times over geological timescales. More recently, the Continent is thought to have been physically isolated from Asia for at least 6000 years. The previously outlined conditions have permitted a great deal of speciation, and has led to typically high levels of endemism (from 24% in fishes and insects, through 83% in mammals, to 99% in freshwater crayfish). Obviously, the natural isolation of Australia limited the opportunities for natural immigration of species to the Continent and subsequent colonization. This situation changed with arrival of the first humans (at least 40,000 years ago), the subsequent arrival of Europeans in the 1700s, and the advent of widespread International trade and travel. More recently, social media has added a new and worrying dimension. Australia is now experiencing a large number of invasive species challenges, and compounding the existing challenges is the uncertain future under the scenarios of increasing environmental temperatures and changing rainfall patterns. Australia is one of the two Global centres of diversity for freshwater crayfish, with 151 species of freshwater crayfish in 10 genera: 150 of those species are endemic to Australia. The vast majority of Australian species have isolated and highly restricted distributions, many species are considered Endangered. This talk will outline the current Invasive Species situation in Australia with a particular focus on threats posed by invasive species, from various groups (plant and animal), to the endemic freshwater crayfish of the Australian Continent.
An overview of aquatic invasive species in the US: from Nile crocodiles to Mysis shrimp
Susan B. Adams
USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research

The United States has an estimated 50,000 non-native species, including plants, animals, and microbes. In addition, many more species have been introduced from one region of the country to another. Summary statistics for non-native aquatic species are difficult to find, but I will present some numbers relating to non-native aquatic species, focusing on fishes. I will then discuss pathways of introductions and likelihood of establishment and spread in Florida and in the Laurentian Great Lakes. To give a better idea of the range of issues we face in thinking about invasive species impacts on aquatic systems, I will give four examples: reptiles in the Everglades National Park, Florida; rusty crayfish (Orconectes rusticus); opossum shrimp (Mysis diluviana) in Flathead Lake, MT; and diseases of “foundation” tree species in the eastern US. If time allows, I will discuss some economic impacts of non-native species.

Rainbow trout threatening amphibians of The Andes
Laura Martin Torrijos. RJB-CSIC, Spain
Real Jardín Botánico (RJB-CSIC), Madrid, Spain.

Amphibian populations are declining due to a myriad of abiotic and biotic factors, including invasive species and pathogens. In temperate freshwater ecosystems, for example, amphibian populations are threatened by the predation of introduced salmonids. Salmonids not only directly predate upon amphibian eggs and larvae but may also transport deadly pathogens into freshwater systems. Though most research has focused on temperate systems, much less is known about the effects of introduced species in Neotropical streams. We conducted two experiments to investigate the impacts of rainbow trout (Oncorhynchus mykiss) in two Neotropical anurans. First, we assessed the effect of the rainbow trout on tadpole survivorship and morphology (phenotypic plasticity depending on different types of chemical cues) in Nymphargus grandisonae, a glassfrog species endemic of the Andes. Results showed that the presence of rainbow trout affects the larval morphology of this glassfrog. In the second experiment, we test whether rainbow trout is a vector of the pathogenic freshwater mold Saprolegnia diclina (Oomycetes). Results showed high mortality rates on Engystomops petersi eggs exposed to trout infected with S. diclina. This represents the first evidence that rainbow trout may have a direct negative effect on Neotropical amphibian populations, and thus should be considered a threat. Management programs should be implemented to eradicate trout from Andean rivers, especially in areas with high number of endangered amphibians.
Insights into the origin of virulence from model organisms
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The germ theory of disease was a landmark moment in human progress because it catalyzed progress that greatly reduced mortality from infectious diseases. However, the germ theory left unanswered two major questions that have preoccupied scientists for the past century: 1) why are some microbes pathogenic and others not? 2) why are some hosts susceptible and others not?. To these questions can be added the deeper question: how does the capacity for virulence emerge in some microbes? For microbes acquired from other hosts virulence, which includes many common pathogenic microbes, disease often results from host-microbe interactions that perturb host homeostasis. However, for the set of pathogenic microbes that are acquired directly from the environment, the origin of virulence is less clear, since those microbes have no need for animal virulence for their survival. Among the best candidates to study these problems are pathogenic fungi, which provide clear example of pathogenic microbes acquired from other hosts and directly from the environment. Studies with the fungus *Cryptococcus neoformans* have provided insight in how virulence can emerge in the environment through pressures that have no relation to the final host. *C. neoformans* is often found in the same environmental niches as amoeba, and fungal-amoeba interactions have been proposed to select for traits that also allow it to survive in mammalian hosts, in a process that has been called accidental virulence. A comparison of the interaction between amoeba and mammalian phagocytic cells reveals remarkable similarities in intracellular survival strategy despite the enormous phylogenetic distances for these two cellular hosts. Many of the virulence factors that are needed for *C. neoformans* virulence in mammals are also needed for survival against amoeba predation. The experience with *C. neoformans* has now been corroborated for several other pathogenic fungi. The environmental predatory selection hypothesis can also explain the non-specific nature of environmental fungal pathogens. Furthermore consideration of host susceptibility to fungal pathogens provides a fertile ground for re-thinking evolutionary processes including great mammalian radiation and the end of the age of reptiles after the events at the Cretaceous-Tertiary boundary.

*Fusarium* egg-disease: an emerging disease in sea turtles
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Sea turtle are one of the most endangered groups of animals. Only seven species have survived to date, and the majority of them have experienced recent drastic population declines as results of habitat loss, fishing and hunting, or diseases. Here, we described two novel pathogenic species of the *Fusarium solani* species complex, i.e., *Fusarium testudinum* sp. nov. and *Fusarium falciforme* that are globally distributed in main nesting areas and implicated in low hatch success. The preferred temperatures of these fungi overlap with the optimal incubation temperature for turtle eggs, and they are able to kill up to 90% of the embryos in experimental trials. Environmental forcing, e.g., tidal inundation and clay/silt content of nesting sites, seem to be key aspects in disease development. Thus, both *F. testudinum* and *F. falciforme*, constitute a major threat to sea turtle nests experiencing environmental stressors such as tidal inundation and reduced gas exchange due to unsuitable substrate content. These findings have serious implications for the survival of endangered sea turtle populations and the success of conservation programs worldwide.
The fungus *Aspergillus sydowii* in Corals

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Emerging fungal diseases are threatening ecosystems and have increased in recent decades. In corals, the prevalence and consequences of these infections have also increased in frequency and severity. Coral reefs are affected by an emerging fungal disease named aspergillosis, caused by *Aspergillus sydowii*. This disease and its pathogen have been reported along the Caribbean and Pacific coasts of Colombia. Despite this, an important number of coral reefs worldwide have not been investigated for the presence of this pathogen. In this work, we carried out the surveillance of the main coral reef of the Ecuadorian Pacific with a focus on the two most abundant and cosmopolitan species of this ecosystem, *Leptogorgia* sp. and *Leptogorgia obscura*. We collected 59 isolates and obtained the corresponding sequences of the Internal Transcribed Spacers (ITS) of the ribosomal DNA. These were phylogenetically analyzed using MrBayes, which indicated the presence of two isolates of the coral reef pathogen *A. sydowii*, as well as 16 additional species that are potentially pathogenic to corals. Although the analyzed gorgonian specimens appeared healthy, the presence of these pathogens, especially of *A. sydowii*, alert us to the potential risk to the health and future survival of the Pacific Ecuadorian coral ecosystem under the current scenario of increasing threats and stressors to coral reefs, such as habitat alterations by humans and global climate change.

Evolutionary applications for the identification of disease threats to biodiversity

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Emerging diseases are a recognized threat to many life forms, yet we hardly ever know which pathogens will turn into agents of emerging disease, where they are going to cause problems, or which species will be affected. Although disease emergence has a great stochastic component, disease emergence often comes along with pathogen invasions, and several patterns may allow us to sketch the perfect invasive pathogen. In these circumstances, biogeography, phylogenetic analysis, and host-pathogen networks may prove valuable tools to predict disease emergence risks. We need to be ready for new conservation challenges associated with the spread of pathogens. Learning how to recognize those that are most likely to develop into emerging diseases may be a critical first step to prevent them from spreading.

Invasive crayfish and crayfish plague: two for the price of one.

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Crayfish plague is an infectious disease of freshwater crayfish that is caused by the oomycete species *Aphanomyces astaci*. Crayfish plague is one of the most devastating diseases ever reported in animals and is included among the 100 world's worst invasive species list. This pathogen is endemic of North America and is chronically carried by their native freshwater crayfish species. Some of them have been introduced into Europe and behave as invasive, i.e., *Procambarus clarkii*, *Pacifastacus leniusculus*, or *Orconectes limosus*. As consequence, the pathogen was also introduced and dispersed causing the destruction of the majority of the native populations of freshwater crayfish in Europe. Because of this dramatic population decline, the European native species of crayfish are listed in the Red List of Threatened Species of the IUCN. Except for North American, the native crayfish species of other continents are also susceptible to this disease, and globally, this invasive pathogen and their invasive carriers are currently threatening more than 300 native species not only in Europe but also in Asia, Madagascar, Australasia and South America.
eDNA-monitoring of the crayfish plague *Aphanomyces astaci*
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European freshwater crayfish are threatened by crayfish plague (*Aphanomyces astaci*) carried by North American crayfish. During the past decade, crayfish plague diagnostics has moved from culture-based methods with low detection success towards molecular methods with high detection success. Now, use of environmental DNA (eDNA) detection of aquatic organisms is a rapidly growing field with a promising potential for streamlined monitoring purposes both for threatened and invasive species, as well as serious aquatic wildlife diseases. We have during the past years expanded the use of molecular *A. astaci* diagnostic methods to also involve eDNA monitoring of pathogen propagules in waters from experimental facilities and natural lakes with either carrier signal crayfish or diseased/dead noble crayfish. Here, efficient sampling that optimizes the probability of detection is particularly challenging in waters where the target organism is rare and/or the content of PCR inhibitors high. This lecture will briefly summarize method development, sample procedures and example results. After years of research and development, eDNA monitoring has for the first time in 2016 been included in the national surveillance program for crayfish plague in Norway, and also been used to confirm crayfish plague infection in a river system where all crayfish recently have vanished.

Active spreading of an invasive species challenges ecosystem-based management of crayfisheries
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We describe the impact of the alien crayfish introduction on the Finnish inland fisheries. The decision to introduce the alien signal crayfish was made to solve the problem of collapsing native crayfish stocks and crayfisheries due to the crayfish plague epidemics. The original idea was founded on overly optimistic expectations, partially by ignoring the available warnings. The outcome of the massive stockings conducted during the last four decades now shows that the alien crayfish has not performed as expected and might even end up as being a failure. The alien crayfish is a permanent reservoir of the crayfish plague disease agent, *Aphanomyces astaci*, spreads it efficiently and has been shown to experience elevated mortality when infected. In spite of this, the official policy in Finland was until recently still driving towards spreading of the alien crayfish threatening original aquatic ecosystem functions and slowly leading in the elimination of the native crayfish. The policy includes national alien species strategy and national crayfisheries strategy, both being currently revised, since they were contradicting EU alien species policy and knowledge on the behavior of people who tend to illegally transfer signal crayfish. We will focus on the loss of the native crayfish and its cumulative effects in ecosystems and society.
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