Welcome !

Dear Friends and colleagues,

The Laboratory Ecology, Evolution, Symbiosis is pleased to welcome you at the European congress “European Crayfish: food, flagships and ecosystem services”, taking place in Espace Mendes France, a friendly place in the town centre of Poitiers. The Espace organizes a series of exhibitions; as it is the International Year of Biodiversity, the current exhibition deals with “Areas and Species”.

In order to prepare the Meeting of the Ad Hoc Working Party on Crayfish of the European Inland Fisheries Advisory Commission (EIFAC), we have encouraged communications about:

1) Interactions of crayfish and fish in freshwater ecosystems (competition, predation, other ecological interactions).

2) Socio-economic aspects: –crayfish production, past and present, costs of fisheries; new ideas such as cultural values and ecosystem services for human well-being; new insights such as crayfish as flagship species, eco-tourism, linked with education of the public, and also with restoration of aquatic ecosystems. Recent projects such as the MEA and Biodiversity Plans are of equal importance with the production of food.

Of course other topics deal with the ongoing and increasing necessity to increase our Knowledge base of both indigenous crayfish and non indigenous crayfish for a better management of our heritage species. Thanks are due to our Keynote speakers Lennart Edsman, Leopold Füreder, Francesca Gherardi, Max Keller, Stephanie Peay and Julian Reynolds for greatly helping to update our knowledge and preparing the discussions conducted by Pavel Kozak and Adam Petrusek during roundtables and also by Markku Pursiainen during the EIFAC meeting.

Thanks are due also to the journal Knowledge & Management of Aquatic Ecosystems (Website: http://www.kmae-journal.org) for offering us the opportunity of a special issue about our discussions and findings. This is above all important because there is a place for opinion papers (from roundtables) and also because the numerous PhD students present during our meeting will be encouraged to publish among the astacologist community. The deadline for submitting your manuscript is mid January 2011.

Thanks are due to the sponsors for grant-aiding and facilitating the organization of the congress: our mother institutions as The University of Poitiers and the CNRS (Centre National Recherche Scientifique), The Regional Council of Poitou-Charentes, The General Council of Vienne, ONEMA (National Office of Water and Aquatic ecosystem and publisher of the journal KMAE), the Agence de l’eau (Water Agency) Loire Bretagne, the DREAL and CPIE for the field trip, the Espace Mendes France for devoted staff and the opportunity to offer one conference for Grand Public, and the Town Hall for the reception.

Finally, Thanks are due to all participants for their enthusiasm for the organization of the International Drinks!

Organizing Committee

Catherine Souty-Grosset
Frédéric Grandjean
Christelle Mirebeau
with the whole team UMR CNRS 6556 “Ecology, Evolution, Symbiosis”
University of Poitiers, France

Scientific Committee

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(UMR CNRS 6556 - University of Poitiers, France)
and in alphabetical order
Lennart Edsman (Swedish Board of Fisheries, Sweden)
Leopold Füreder (University of Innsbruck, Austria)
Francesca Gherardi (University of Firenze, Italy)
Pavel Kozak (Institute Fisheries, Vodnamy, Czech Republic)
Stephanie Peay (Scott Wilson, Leeds, Ecological Consultant, U.K)
Adam Petrusek (Charles University in Praga, Czech Republic)
Julian Reynolds (Trinity College Dublin, Ireland)

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PROGRAMME
Tusday 26 october 2010

08h45 : Welcome for participants in Espace Mendes France, Poitiers

First cup of coffee or tea !

10h00 : Opening of the congress

Session 1 : Interactions of crayfish and fish in the ecosystem
Chairman : **Pedro Anastácio**

10h15-11h00 : Keynote speaker **Julian Reynolds** :
Interactions between crayfish and fish, indigenous and introduced

11h00-11h20 : **Ramalho R.** and Anastácio P., Interactions between fish and crayfish: prey switching

11h20-11h40 : **Ruokonen T.**, Karjalainen J. and Hämäläinen H., Impacts of invasive crayfish on littoral fish in large boreal lakes

11h40-12h00 : **Gladman Z.**, Signal crayfish in Scotland

12h00-13h30 : Lunch

Session 2 : Knowledge base of indigenous crayfish and non indigenous crayfish
Chairman : **Ivana Maguire**

13h30-14h20 : Keynote speakers **Stephanie Peay** and **Leopold Füreder** :
Two indigenous crayfish under threat - how can we retain them in aquatic ecosystems for the future ?

14h20-14h40 : **Arce J.** and Alonso F., Factors with the presence of *Austropotamobius pallipes* in calcareous mountains rivers of Central Spain

14h40-15h00 : **Fea G.**, Ghia D., Bernini F., Nardi P.A., Comini B. and Tagliaferri A., White clawed crayfish (A*ustropotamobius pallipes*complex) reintroduction project in two Italian SCIs (Parco Alto garda Bresciano, Northern Italy)

15h00-15h20 : **Weinländer M.** and Füreder L., Are native and alien crayfish species different in their trophic role?

15h20-15h50 : **Zimmerman J. K. M.** and Palo T.R., Reliability of catch per unit effort (CPUE) for evaluation of reintroduction programs - A comparison of mark-recapture method with standardized trapping

15h50-16h10 : Coffee Break

Chairman : **Fernando Alonso**

16h10-16h30 : Alaranta A., **Laakkonen M.V.M.**, Partanen T., Sarajärvi K., Hupli H. and Ryyppö P., The conservation and management plan for noble crayfish (*Astacus astacus*) in state-owned areas of Finland

16h30-16h50 : **De Vaugelas J.**, Leyendecker V., Leca H., Luc P., Riva J.-C., Sabatier A. and Souty-Grosset C., Use of a smartphone (iPhone app) for the field identification of European Crayfish

16h50-17h10 : **Schmidt T.**, Hösler C. and Siewert W., Life history traits of *Pacifastacus leniusculus* explain its competitive success over noble crayfish, *Astacus astacus*. Preliminary results of an individual based model.

Poster Session
17h15- 18h30

18h30 : Continuing the poster session during Tasting regional gastronomy and International drinks

Free Evening

Wednesday 27 october 2010

Session 3 : Socio economics aspects
Chairman : **Jose Carral**

8h30-9h15 : Keynote speaker **Francesca Gherardi :**
Sustainable human use of crayfish

9h15-9h35 : **Paaver T.** and Hurt M., Noble crayfish *Astacus astacus* stock management, farming and conservation in Estonia

9h35-10h15 : Keynote speaker **Max Keller** :
Creating and cultivating genetical reservation pools for *Astacus astacus* in Bavaria

10h15-10h40 : Coffee Break

Chairman : **Tiit Paaver**

10h40-11h20 : Keynote speaker **Lennart Edsman :**
Pros and cons with the huge interest in crayfish- Implications for management and conservation in Scandinavia

11h20-11h40 : **Vos J.**, Preventive and mitigating measures against alien crayfish in the Netherlands: results of risk analysis and research

11h40-12h00 : **Ghia D.**, Fea G., Bernini F. and Nardi P.A., Reproduction experiment on *Austropotamobius pallipes* complex under controlled conditions : can hybrids be hatched ?

12h00-12h20 : **Arbačiauskas K.** and Rakauskas V., Crayfish in Lithuanian waters : current states and perspectives

12h20-13h30 : Lunch

Session 4 : Crayfish genetics
Chairman : **Frédéric Grandjean**

13h30-13h50 : **Schrimpf A.**, Schulz H.K., Polivka R., Dümpelmann C. and Schulz R., Phylogeographical patterns of the noble crayfish (*Astacus astacus*) and recommendations for a regional restocking program

13h50-14h10 : **Maguire I.**, Klobučar G.I.V., Jelić M., Franjević D., Futo M. and Grandjean F., Phylogenetic position of white-clawed crayfish from the east Adriatic coast

14h10-14h30 : Matallanas B., **Ochando M.D.**, Vivero A., Beroiz B., Alonso F. and Callejas C., The DNA sequence analysis of a COI region reveals eight haplotypes for the white-clawed crayfish in the Iberian Peninsula

14h30-14h50 : **Filipová L.**, Grandjean F., Crandall K.A., Kozubíková E., Sonntag M. and Petrusek A., Genetic variation in European populations of signal crayfish, *Pacifastacus leniusculus*

Session 5 : Crayfish Invasions and Crayfish plague
Chairman : **Catherine Souty-Grosset**

14h50-15h10 : **Kozák P.**, Buřič M., Kouba A., Musil M., Vích P. and Policar T., Spiny-cheek crayfish (*Orconectes limosus*) biology – the latest pieces of knowledge.

15h10-15h30 : Buřič M., Hulák M., Kouba A., **Petrusek A.** and Kozák P., Facultative parthenogenesis in spiny-cheek crayfish *Orconectes limosus*: a novel reproductive mode in decapod crustaceans

15h30-15h50 : **Kozubíková E.**, Koukol O., Martín M.P., Petrusek A. and Diéguez-Uribeondo J., What oomycetes can be found growing on crayfish and how to determine them ?

15h50-16h10 : **Delbecque J.-P.**, Bacqué-Cazenave J., Mini A. and Cattaert D., Control of aggressiveness and development by molting hormones (ecdysteroids) in the red swamp crayfish, *Procambarus clarkii*

16h10-16h30 : **Sandodden R.**, Eradication of signal crayfish (*Pacifastacus leniusculus*). Management and possible procedures to prevent further spreading

16h30-16h50 : Coffee Break

16h50-18h00 : Roundtables

Roundtable 1 :
Moderator : **Adam Petrusek**
Crayfish invasions and crayfish plague

Roundtable 2 :
Moderator **Pavel Kozak**
« Conservation of native crayfish, culture, restocking »

including the topics : «Developing Catchment-based conservation strategy for crayfish» and «Guidelines for evaluation of reintroduction»

18h10 : Departure for the town hall reception
18h25 : Photo in front of the Town Hall
18h30 : Reception at the town Hall

19h30 : Buffet-Dinner

20h30 Conférence Grand Public (in French)

Thursday 28 october 2010

FIELD TRIP
(See also Leaflet)

8h30 : Departure Field trip near Espace Mendès France
9h30 : Terrier du Fouilloux : Landscape view of the Auxances catchment
10h00 : Walk 3.4km discussing the management of a Natura 2000 site (Magot)

12h30 : Cocktail and lunch in CPIE

14h15-14h30 : Departure for the International Ornithological film festival of Ménigoute : the festival is considered as a national and even more significant event where associations come to meet a general public, local and public authorities open to engage in dialogue around environmental issues.

16h30-17h30 : Coming back to Poitiers

Banquet

20h00-2h00 : Restaurant Le TWISTER Poitiers

Friday 29 october 2010

9h00-12h00 : European Inland Fisheries Advisory Commission (EIFAC)
Meeting of Ad Hoc Working Party on Crayfish

Agenda

1. Opening of the meeting **Markku Pursiainen**

2. Participants, documentation, report of the meeting

3. Interactions of crayfish and fish in freshwater ecosystems
   a. Conclusions of the workshop Session 1 and discussions
   b. Other aspects on ecological interactions
   c. Messages and recommendations for EIFAC

4. Socio-economic matters of crayfish in inland waters (as a part of inland fisheries)
   a. Conclusions of the workshop Session 2 and discussions
   b. Other aspects on socio-economical matters
   c. Messages and recommendations for EIFAC

10h30 : Coffee Break

5. Native and introduced crayfish species in Europe
   a. distribution data
   b. number of populations
   c. capture and aquaculture production statistics

6. Future activities of the Working Party
   a. EIFAC Symposium and Session in Finland 2012
   b. Next meeting

7. Other matters

8. Closing of the EIFAC meeting

12h15-13h30 : Lunch

13h30-15h00 : Conclusions of the conference and the way ahead

15h00 : Last cup of coffee

Session 1 :

Interactions of crayfish and fish in the ecosystem

Chairman : Pedro ANASTACIO

Interactions between crayfish and fish, indigenous and introduced

Reynolds J.D.

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Crayfish and fish are both long-lived large members of freshwater communities. In many countries crayfish are used as bait for sport fishing, suggesting an unequal relationship, but the situation is more complex. Native crayfish and fish, both often keystone species, are in ecological balance, often involving mutual predation and sometimes habitat disturbance, but this balance is disrupted by range extensions of native fish and crayfish and translocations of non-natives of both. Salmonids have been translocated to South America and Australasia and percids across western Europe, with impacts on indigenous crayfish (ICS). Crayfish have also been translocated globally, and regional range expansions are shown by *Orconectes* species within North America and *Cherax* species in Australia. Competition between ICS and NICS may make the former susceptible to various mechanisms of interaction. NICS will impact on native fish, just as introduced fish impact on ICS.

In Europe, long-established non-indigenous crayfish (Old NICS - Signals, Spiny-cheek, Red swamp crayfish) tolerate warmer water and are more r-selected than indigenous European crayfish; they tend to be more fearless, aggressive and form greater densities than ICS, and show more interactions with fish. In addition to being vectors of crayfish plague, they can impact adversely on the aquatic environment due to their rapid growth, burrowing activities and dense populations, and will be further favoured by climate change.

Mechanisms of interaction between fish and crayfish include predation, competition, habitat modification, food-web, alteration etc., with greater or lesser involvement of bird and mammal predators, indigenous and introduced. Resultant changes in communities and ecosystems may be physical, chemical or biotal, and affect exploitation potential.

Keywords : Crayfish, freshwater fish, translocation, competition, predation, keystone species, exploitation

Interactions between Fish and Crayfish : Prey Switching

Ramalho R. and Anastácio P.

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Prey «switching» in predators which attack several prey species can potentially stabilize prey populations.

This study attempted to access predator-prey relationships between an invasive generalist predator, the largemouth black bass (*Micropterus salmoides*) and two invasive preys, the red swamp crayfish (*Procambarus clarkii*) and the mosquitofish (*Gambusia holbrooki*). The question we attempted to answer was: given that largemouth bass consume both preys, when presented alone, does it switch from a prey that becomes rare to another which becomes more abundant? To test an experiment was set up where largemouth bass (average weight: 22.9±1.4g) were individually exposed to five different proportions (0%, 30%, 50%, 70% and 100%) of juvenile crayfish and mosquitofish during one hour. At the end of the experimental trial the number of consumed prey was determined. Results suggest that experimental largemouth black bass present higher preference to consume mosquitofish than crayfish.

Assuming the preference of largemouth black bass for mosquitofish and also assuming a null hypothesis that - in case of no switching the proportion of prey in diet should not differ from the expected consumption - the results indicate that largemouth black bass “switched” its preference to crayfish when it became the most abundant prey. It is therefore our opinion that largemouth black bass has a potential for limiting crayfish populations.

Keywords : Micropterus salmoides, Procambarus clarkii, Gambusia holbrooki, predation; prey switching

Impacts of invasive crayfish on littoral fish in large boreal lakes

Ruokonen T., Karjalainen J. and Hämäläinen H.

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Invasive species are one of the main threats to the freshwater biodiversity and function. Freshwater crayfish have been widely spread by human beyond their native ranges around the world and their rapid invasion continues. As omnivores and powerful competitors alien crayfish can have strong direct or indirect impacts on native crayfish but also on other biota. Recent studies have frequently documented their negative impacts on macrophytes, macroinvertebrates and reptiles, whereas results on native fish species have been controversial. Crayfish and fish in lake littoral zone share the habitats and food resources which can potentially lead to competition between them.

In this study we investigate the impacts of invasive signal crayfish (*Pacifastacus leniusculus*) on littoral fish populations in Finnish large lakes. We compare the abundance and composition of fish communities between sites with dense crayfish population and sites without crayfish. In addition, using stable isotope analysis, we compare the width and variance of feeding niche and contribution of different food sources in two most common littoral fish species (bullhead, stone loach) between crayfish and non-crayfish sites.

Keywords : invasive species, littoral, fish, density, competition

Signal crayfish in Scotland

Gladman Z.

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Since its introduction to Scotland in the 1980s, the invasive signal crayfish (P*acifastacus leniusculus*) has become well established in the wild. Research into the distribution, impact and control of this species is urgently needed. In the summer of 2009, the impact of a large-scale trapping programme on the population of signal crayfish at Loch Ken in southern Scotland was evaluated using mark and recapture methods. The programme significantly reduced the number and size of males in the population but its effect on females was complicated by seasonal variation in trappability and the bias of traps towards males. Loch Ken contains crayfish at densities which are high compared with other lakes and the loss of biodiversity will considerable if no further action is taken. More recently, research has focussed on the impact of signal crayfish on species of high conservation or economic value, including the Atlantic salmon, which generates millions of pounds for the Scottish economy through recreational and commercial fisheries. The vulnerability of salmonid redds to predation or disturbance by crayfish was investigated experimentally. The results suggested that crayfish are unable to detect salmonid eggs buried at even shallow depths. In the wild, however, the vulnerability of redds to predation will vary according to a wide range of abiotic and biotic factors. The consequences of signal crayfish introductions for Scotland remain poorly understood but continued research will allow the threats posed to native biodiversity to be identified and the most appropriate mitigation or control measures to be implemented.

Keywords : signal crayfish, control, impact, scotland

Session 2 :

Knowledge base of indigenous crayfish and non indigenous crayfish

Chairmen : Ivana MAGUIRE, Fernando ALONSO

Two indigenous European crayfish under threat - how can we retain them in aquatic ecosystems for the future?

Peay S. and Füreder L.

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Indigenous species of crayfish are under threat and in decline in most countries in Europe. The three main causes are crayfish plague, which is carried by introduced American crayfish; competition from invasive non-indigenous crayfish species (NICS) and habitat degradation. The risks vary for different populations of the threatened indigenous crayfish species (ICS). We focus on two species, the white-clawed crayfish *Austropotamobius pallipes* and the stone crayfish *A. torrentium*. Research has been done on the ecology of these species and there is increasing understanding of the genetic resource, especially of white-clawed crayfish. Increasing efforts on surveys in many countries have helped identify remaining populations and shown the accelerating rate of loss in the late 20th and early 21st centuries. This loss has been concurrent with the rapid spread of NICS by human-assisted introductions and natural spread. Remaining populations of ICS are becoming increasingly fragmented. As plague-carrying populations of NICS become ever more extensive in Europe, it will become progressively harder to retain populations of European ICS.

Various degrees of legal protection have been introduced to protect European ICS, in particular the two *Austropotamobius* species, often too little and too late. Legal protection is not enough to protect either species from the three main threats. There has been some local effort on habitat protection and this may be aided by the new requirements for water quality under the Water Framework Directive. Eradication of NICS has only been achieved on a few small sites using a biocide. Other attempts at control of NICS by overharvesting have been ineffective and unsustainable and have risks associated with them. We recommend catchment-scale risk assessments, to identify 1) populations where loss is inevitable, 2) those at some risk of loss which can be made more secure by various measures and 3) isolated populations that can be kept biosecure. Resources can be targeted to achieve specific conservation goals. This is being developed in England for white-clawed crayfish conservation. Biosecure «ark sites» are likely to offer the best chance for long term survival of ICS, given the inexorable loss of most of their natural ranges to invading NICS. We discuss constraints and opportunities.

Keywords : crayfish, conservation, invasion, management, legislation

Factors related with the presence of *Austropotamobius pallipes* Lereboullet in calcareous mountain rivers of Central Spain

Arce J. and Alonso F.

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Alto Tajo Natural Park is a 105.000 Ha protected area located in central Spain. Its dense hydrographic net drains the higher Tajo basin, mainly over limestone country. This area has traditionally harbored abundant white-clawed crayfish populations. Nevertheless, its occurrence has been reduced during the last decades by different factors. To overcome the scarce information about the species status in the area, an extensive survey was carried during 2008 summer, exploring around 100 water bodies. Using a combination of sampling techniques, a total of 23 populations -showing different conservation degrees- were found within the Natural Park boundaries and its surroundings. They mainly inhabited headwater stretches less than 1 km in length, as well as small ponds with different human uses. A significant proportion of them could be related to previous stocking events. At the same time habitat characterization was carried out. Crayfish role as a bioindicator is discussed in relation to apparent preferences. The information obtained will be used to discriminate the most adequate localities for future stocking actions, hopefully improving the success of this management tool.

Keywords : Austropotamobius pallipes, distribution, habitat characterization, stocking.

White clawed crayfish (*Austropotamobius pallipes* complex) reintroduction project in two Italian SCIs (Parco Alto Garda Bresciano, Northern Italy)

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We carried out protective actions for the preservation of *Austropotamobius pallipes* from 2004 with the Life project «Riqualificazione della biocenosi in Valvestino Corno della Marogna 2». We monitored geographical distribution of *A. pallipes* in the 2 SCIs and sampled 29 sites in order to check the presence or absence of the species, water quality (total hardness, ph, conductivity, dissolved oxygen and temperature), as well as some habitat features about the rivers and their banks. Although the qualitative conditions of the waterbodies were good, only one crayfish population was found. In the autumn 2006 we started the rearing of the young crayfish according to 2 methods. In the first one we used 10 indoor tanks, with 30 males and 60 females taken from the area of «Parco Alto Garda Bresciano». In the second method we build an outdoor a pond (12 X 3 meters), with artificial shelters made with bricks and bundles of sticks, with 52 males and 131 females. We could compare 2 reproductive strategies. In September 2007 we obtained 328 young crayfish from tanks, while from the pond we got respectively 284 young crayfish in 2007 and 591 in 2008. After the reintroduction of these young crayfish in a SCI «Corno della Marogna» brook we have monitored the population until summer 2010 with positive results. These rearing techniques and reintroduction practices allowed us to improve the knowledge for native crayfish conservation.

Keywords : A. pallipes, breeding, reintroduction, monitoring, Life project, Italy.

Are native and alien crayfish species different in their trophic role ?

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Freshwater crayfish are directly and/or indirectly affecting their habitats and coexisting organisms, wherefore they are regarded as ecosystem engineers and keystone species. Especially non-indigenous crayfish species (NICS) are prominent for their negative impacts on indigenous crayfish species (ICS) and functioning of aquatic ecosystems within a short time after their arrival. While several studies have documented single crayfish species affecting algae, plants, leaf breakdown, fish and macroinvertebrates, little is known about species specific differences between ICS and NICS within freshwater ecosystems. We used two different types of enclosure experiments in Carinthian streams (Austria) to study the ecological effects of the native *Astacus astacus* and *Austropotamobius torrentium* and compared them to the alien *Pacifastacus leniusculus*. The first experimental setup with two different crayfish densities and a no-crayfish control examined the impacts of ICS and NICS on leaf breakdown, the production of coarse particulate organic matter (CPOM) and the accumulation of fine particulate matter (FPM). In the second part of the experiment the same individuals were used separately to investigate their predation on agile amphipods, slow moving trichopterans and the consumption of dead small arctic char. In both experiments ICS and NICS showed significant differences in their consumption, production and accumulation of the nutriments, which suggested distinct differences in their trophic role. Besides competition and the potential role as vectors for crayfish plague, NICS may show additional complex interactions within freshwater ecosystems.

Keywords : feeding experiments, trophic role, Astacus astacus, Austropotamobius torrentium, Pacifastacus leniusculus

Reliability of catch per unit effort (CPUE) for evaluation of reintroduction programs – A comparison of mark-recapture method with standardized trapping

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In 1999 the noble crayfish (Astacus astacus) population in the northern Swedish River Ljungan went extinct. A reintroduction program was set up by the local, regional and national authorities in cooperation with local fishermen. Since then over 75000 crayfish has been reintroduced to the river at more than 35 sites. Fishery of crayfish is banned in the river until the reintroduced crayfish population has developed and sustains fishery. In order to evaluate the success of the reintroduction program, local fishermen have trapped crayfish in a standardized way at the reintroduction sites for several years. Additionally, in order to estimate the population development a four-year mark and recapture study using PIT-tags, was performed at 5 of the sites. The results show that the reintroduction is successful so far. At all sites the trapping success has increased and catch per unit effort (CPUE) reflects the number of crayfish introduced with the conclusion that estimated population sizes have increased. Recapture data analyzed by the Jolly-Seber model imply about 50% mortality compared to the total crayfish reintroduced. The CPUE for evaluation of the reintroduction program is «rough», but seems to be a good predictor of the actual population increase. The results show that there is a correlation between the population sizes estimated from mark and recapture estimation and CPUE from the standardized fishery procedure used by the local fishermen. This indicates that the standardized way of trapping used in this program can be used for evaluation of the reintroduction program and of other reintroductions as well.

Keywords : Noble Crayfish, reintroduction, evaluation, PIT-tags

The conservation and management plan for noble crayfish (*Astacus astacus*) in state-owned areas in Finland

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Metsähallitus administers state-owned land and water areas in Finland, including the majority of all conservation areas, such as national parks. Metsähallitus manages lakes and rivers, providing sustainable fishing opportunities and safeguarding viable fish and crayfish populations. Today there exist several viable populations of indigenous noble crayfish (*Astacus astacus*) in state-owned rivers and lakes, and these populations are either native in origin or stocked. The biggest threats to the noble crayfish in Finland are the alien species crayfish plague (*Aphanomyces astaci*) and signal crayfish (*Pacifastacus leniusculus*). In the year 2010 a crayfish plague destroyed one of the most productive noble crayfish populations existing in state-owned rivers in Finland. To improve the future of native crayfish, Metsähallitus has developed a conservation and management plan of noble crayfish (2011-2016). One of the most interesting aspects of this plan is to investigate the role of existing conservation areas (e.g., national parks) in the conservation of noble crayfish. Within conservation areas, fishing and other human activities are controlled or prohibited. Therefore, the spreading of crayfish plague due to human activity is restricted. Conservation areas could possibly conserve not only the existing native noble crayfish populations but also re-introduced or stocked populations. The conservation areas provide an interesting opportunity and great potential for noble crayfish conservation. However, conservation requires great financial effort and broad national co-operation with other stakeholders in the future.

Keywords : Conservation, management, Astacus astacus, Pacifastacus leniusculus

Use of a smartphone (iPhone app) for the field identification of European crayfish

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Identification keys (ID key) are the most precise and unambiguous tools to properly identify a specimen down to the species or infra-species level. This is especially true if the text of the key is richly illustrated with precise pictures or short videos, closely related to the taxonomic characters one has to check. New tools such as smartphones (iPhone, etc.), which can access through 3G to an unlimited and rapidly growing set of multimedia data (photos, videos, audios), are convenient to use in the field instead of traditional paper field-guides with a limited number of illustrations.

A student project at the University of Nice, France, proposes to adapt the «key to crayfish in Europe» from C. Souty-Grosset et al., 2006, to the iPhone interface and any other smartphone.

A prototype in English is ready to be discussed with taxonomy and field specialists in order to improve it. Then, a multilingual version could be designed, so that field specialists may use it in their native language. As the database underlying the project is wiki-compatible, the multilingual ID Key could be designed as a collaborative effort from the crayfish community. Data on each species (biology, ecology, distribution, etc.) could be added in a second phase, as a geolocalisation module is linked to the database. Then the identification of invasive species could be quickly related to maps, in order to alert the crayfish community.

Keywords : Identification keys, taxonomy, smartphone, iPhone.

Life History Traits of *Pacifastacus leniusculus* (DANA, 1852) Explain its Competitive Success over Noble Crayfish, Astacus astacus (LINNAEUS, 1758) - Preliminary Results of an Individual Based Model

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Invasive species of freshwater crayfish may threaten native species by competitive exclusion. Observations from the field propose, that the North American signal crayfish, *Pacifastacus leniusculus* (DANA, 1852), displaces the native noble crayfish, *Astacus astacus* (LINNAEUS, 1758), in Europe. Different life history strategies of these species may play a central role in this development. Furthermore, a suggested important process in the displacement is interference competition with agonistic behavior. Reproductive interference has been proposed to act as an Allee effect. We implemented an individual based simulation model to investigate these mechanisms and their effects on the dynamics of sympatric populations of the two crayfish species. Based on a scenario with an abundant population of *A. astacus* invaded by few specimens of *P. leniusculus* we tested two hypotheses: 1. Agonistic behavior inverts the relation of the size of the populations of the two species. 2. The Allee effect (reproductive interference) accelerates the decline of populations of *A. astacus*. Already in the control simulations, i. e. implementations of agonistic behavior and Allee effects not yet added, populations of *A. astacus* were driven to extinction with a probability bordering on certainty before 100 simulated years. Including the implementations of agonistic behavior and of the Allee effect had no effect on the probability of extinction, but shortened the time to extinction. These results indicate, that the known differences between the life history strategies of *A. astacus* and *P. leniusculus* suffice to explain the competitive success of P. leniusculus. Although agonistic behavior and reproductive interference accelerate species displacement, they are not the leading mechanisms.

Keywords : individual based model, competitive replacement, interference competition, agonistic behavior, Allee effects.

Session 3 :

Socio economics aspects

Chairmen : José CARRAL, Tiit PAVER

Sustainable human use of crayfish

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The various uses that man has made of crayfish are well documented since ancient times. In Europe, the value as food of species such as *Astacus astacus* is known since the Middle Ages particularly in Sweden, where still today crayfish are regarded as culinary icons. In the 19th century, the demand for crayfish increased and their commercial harvesting became an industry in Germany and Russia. The increased economic interest led to overharvesting and poaching crayfish populations which, along with the spread, since 1860, of the crayfish plague, caused a collapse of the European stocks, leading to the introduction of non-indigenous species, i.e. *Astacus leptodactylus* from Turkey, and *Pacifastacus leniusculus* (in the 1960s) and *Procambarus clarkii* (in the 1970s) from North America.

In USA, information about the original use of crayfish is scanty; the today most consumed crayfish of the world, *P. clarkii*, figured in lore of both the Attakapa and Houma tribes in Louisiana; the Houma used crayfish as their tribal symbols. There is mention of crayfish exploitation by European immigrants to Louisiana as early as the 1700s. In Louisiana, the catch of *P. clarkii* improved in the 1960s, reaching landings of over 30,000 tonnes in favorable years. Commercial crayfish farming developed in the 1950s: by 1986 more than 50,000 ha was in cultivation with an average annual harvest of 30,000-40,000 tonnes. However, the North American crayfish industry is today under threat from the competition in the market primarily by the People's Republic of China and secondarily by Spain.

Little is known about the crayfish use as a subsistence diet at a local scale, except for Madagascar, where in some villages, harvesting the indigenous Astacoides spp. contributes with an important sum to the local economy. Uses of crayfish other than gourmet food include their potential to control pests or diseases, their being blueprint for the synthesis of medicines, and their role in education and recreation. Crayfish key position in the food webs justifies their enormous ecological value, as prerequisites of the well functioning of ecosystems and of the multiple services they offer to man. Finally, their existence and bequest values, although difficult to quantify, may provide per se the rationale for protecting this taxon without the need of evoking images of cooked crayfish but under the general awareness of their role as «heritage» species.

Keywords : Human use, Values, Heritage species, Crayfish.

Noble crayfish *Astacus astacus* stock management, farming and conservation in Estonia

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Noble crayfish was the only decapod species in Estonia until 2008-2010, when two established populations of illegally introduced signal crayfish were found. Plague and habitat deterioration had caused serious decline of wild stock of noble crayfish. Management policy was concentrated on protection of native crayfish as a species. Limiting of size to 11 cm TL, fishing season with August and number of recreational fishing licenses with 500 were the main measures of regulating of the exploitation of wild stock. There is total ban of import of live alien crayfish (except aquarium trade). The legal recreational catch from natural waters is less than 10 th specimens. Catch of legal size crayfish per trap per night may exceed 20, but usually it is lower. There may be large not registered catch and sale of crayfish (50 th specimens yearly). Because of high prices and demand for noble crayfish on the markets of neighbouring countries (Finland, Sweden) crayfish farming was initiated in 1990s. The production of farmed crayfish for consumption is 2 tonnes. 15 farms are operating, but not all of them produce market size crayfish. Crayfish is reared in earthen ponds supplied with well or river water at density 2-4 specimens/m^2. Fluctuation of environmental conditions – high or low temperatures, drought, spread of plague from wild populations cause risks to intensive crayfish farming. Public image of crayfish catch and farming is positive, but awareness about differences between species is poor.

Keywords : noble crayfish, management, farming, Estonia.

Creating and Cultivating genetical Reservation Pools for Astacus astacus in Bavaria

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The unstoppable advance of invading American crayfish species in European waters threatens – as we know – all endemic crayfish especially by transmitting the fatal fungus disease *Aphanomyces astaci* (crayfish plague). **The noble crayfish** (*Astacus astacus*) too has meanwhile been extensively displaced from its original habitat, the middle and lower reaches of rivers and brooks as well as from the larger lakes, and is imminent danger of becoming extinct there. The, small and often isolated remainders will disappear on a long-term basis or possibly become genetically impoverished, at least in the absence of contacts with neighbouring populations.

As last refuges for this threatened crayfish species, which fortunately does not require a high water quality, plenty of larger sized and fairly isolated **substitute biotopes** are on offer - like fish ponds with a supply of spring water and especially the small lakes, sythetized to win gravel, of which there are many north of the Alps. When appropriate precautions are taken, the plague risk is essentially lower in such locations and the chance of survival much higher than in the original habitats.

In this way, for example, early in 1985, after a detailed examination of fundamental questions, the probably first private genetic reservation pool for *A. astacus* was established in a 3-hectare gravel pit near the Bavarian village of BRONNEN/Unterallgäu. To guarantee a stock likely to be free of parasites and diseases, the lake was initially stocked with 20,000 just hatched 2nd stage juveniles. The parent crayfish originated from different autochthonous populations from the rivers MINDEL, KAMMEL, SEMPT and WERTACH in the DANUBE river system.

Object in mind was primarily to preserve passably well the genes of A. astacus still regionally available and to utilize the new resources, above all to produce enduringly high-grade stocking material of an outstanding genetical variability.

Bavarian fishery laws regulate the catch of noble crayfish in natural waters, including gravel pits. The minimum size is 12 cm total length (TL), which corresponds to an idividual weight of approximately 80 g. For harvesting and trading smaller specimens, a spezial licence had to be obtained from the fishery and at first from the nature conservation authorities, too. The condition imposed was that the undersized crayfish caught in «Lake BRONNEN» were only to be used for restocking or breeding purposes by the First Bavarian Crayfish Hatchery.

Management of the new *A. astacus* population in the lake, which has meanwhile lasted more than 20 years now, has produced many valuable findings relating to growth, enduring yield opportunities, moulting phases and trapping, the stunting phenomenon in the event of high crayfish density with a natural food supply and, last not least, the ecological impact of noble crays on the occurrence of plants and animals. The results of this long term-study have been shown in tables and discussed in detail. Nevertheless it remains problematic to draw conclusions universally valid for other waterbodies, since special local conditions like water parameters, population genetics and density, shore and ground quality, shelter, food supply, predation, trapping intensity etc. usually differ considerably.

Pros and cons with the huge interest in crayfish – implications for management and conservation in Scandinavia.

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The widespread tradition to eat crayfish at crayfish parties (the so called «Kräftskiva») in Scandinavia is relatively recent and entirely a Scandinavian invention. The habit started to develop around a hundred years ago. In there worship of nature and pastoral life, the bourgeoisie in the cities initially started to have crayfish feast in connection to the romantic nature movement in literature and art. For people in rural areas, on the other hand, crayfish was traditionally not for eating but was so abundant that is was sometimes used as fertilizer. The interest from city people however created a market for crayfish, and people with access to lakes and rivers could start making good money by catching and selling crayfish. The custom gradually spread to the middle class and people in rural areas.

Crayfish now is the main ingredient in the almost ritual crayfish parties, which takes place in the dark but moonlit and mellow nights of August. The larger part of the human population take part in these parties. The annual wholesale value of the crayfish that is fished from lakes and rivers in Sweden amounts to approximately 30 million EURO. If the value of the hats, bibs and lanterns, and the dill, cheese, whine, beer and aquavit that is consumed during the crayfish parties is included, the total annual value probably amounts to around 100 million EURO. A 170 km long crayfish party along the «Göta channel» in middle Sweden was organized in 2010. In Finland crayfish is usually sold not by kilos but by numbers and a premium sized noble crayfish may cost up to 5 EURO on sale in the most prestigious fish shop in Helsinki. For people with fishing rights it is also possible to get a considerable additional income by selling fishing licenses or recreation and experience activity products. In Norwegian lakes people are willing to pay up to 1200 EURO for the possibility to catch crayfish along a 1 km shoreline, no other services included. A «Crayfishing event» with catching, and crayfish party, may fetch a price of 120 EURO per person, drinks not included.

Apart from its ecological significance crayfish thus has a traditional, cultural, social and recreational value and hence a high economical value. Poaching and illegal introductions of the plague-carrying signal crayfish are common place. Everybody knows what crayfish is and, consequently, also have strong beliefs and opinions on crayfish biology and crayfish plague biology, on how to fish, on the best bait, on why the catches increase/decrease, on the effect of the fishery and on the reasons for declines. Often these opinions differ greatly between persons and they are usually not based on current knowledge and facts. Examples on how this facilitates and hampers management and conservation work will be presented.

Keywords : Traditions, culture, economy, management, conservation.

Preventative and mitigating measures against alien crayfish in the Netherlands: results of risk analysis and research

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The first alien crayfish in the Netherlands were observed in the 1960's (Orconectus limosus), which were followed by several other species. At present at least seven alien crayfish species have been reported. The Dutch authorities have commissioned an independent market research office to carry out a risk assessment for the species present and for species which may enter the Netherlands in due time. The risk analysis has indicated that the ecological effects caused by alien crayfish differ between species.

On basis of the risk assessment, the Dutch Invasive Alien Species Team of the Ministry of Agriculture, Nature and Food Quality has identified knowledge gaps and possible measures. Presently, the distribution of the individual crayfish species is inventoried. On basis of the inventory will be decided if preventive measures would be effective. Especially vulnerable species in isolated areas may profit from preventive measures.

For areas in which crayfish are broadly distributed, fishing may be a suitable measure to reduce crayfish densities. Alien crayfish may be an interesting alternative source of income for fishermen. However, the effectiveness of fishing to reduce crayfish numbers is questionable. Recently, alien crayfish are legally allowed to be caught, but artificial restocking is prohibited.

Most literature suggests fishing in combination with restocking with predatory fish as suitable measure. This measure is studied in artificial ditches at present. Preliminary results will be presented.

Keywords : policy, measure, fishing, predatory fish, inventory.

Reproduction experiment on *Austropotamobius pallipes* complex under controlled conditions: can hybrids be hatched ?

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The *A. pallipes* complex is formed by the two species *A. pallipes* and *A. italicus*, recently defined only on genetic basis, without valid morphological features for a positive identification. Dealing with this problem, we have conducted a breeding experiment between these two presumed species under controlled conditions, in order to investigate the possible existence of reproductive barriers. We have performed four series of experiments, using on the whole 80 breeders collected from genetically checked populations (56 females and 24 males): Fita x M_{pal} and F_{pal} x M_{ita} interspecific; F_{ita} x M_{ita} and F_{pal} x M_{pal} intraspecific as blank samples. We have monitored only the main phases of the reproduction in order to avoid excessive interference for the crayfish. In December 2009 the 42.9% of females having intraspecific matings, as well as the 78.6% of females having interspecific matings produced eggs. In June 2010 the eggs from the 28.6% of females having intraspecific matings, and those from the 39.3% of females having interspecific matings did hatch. We emphasize the hatching of hybrids and their vitality during the early months of life.

Keywords : Reproduction, hybrids, Austropotamobius pallipes complex.

Crayfish in Lithuanian waters: current state and perspectives

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Currently, the four crayfish species inhabit the inland waters of Lithuania. The only native species is the noble crayfish *Astacus astacus* (Linnaeus, 1758). The narrow-clawed crayfish *Astacus leptodactylus* Eschscholtz, 1823 was introduced to Lithuanian waters during the end of 19th century from adjacent countries, and still inhabit around 11 lakes located mostly in the eastern part of the country. Two American species invaded Lithuanian waters during the 20th century. The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) was translocated from Sweden in 1972. Some closed lakes were known to harbour it, however, recently the invasion of signal crayfish to medium sized river (the Žeimena river) was observed. The last alien crayfish, the spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) for the first time was recorded in 1994, and since then its expansion is ongoing. This crayfish invaded Lithuanian waters from Poland by natural dispersal, however, its spread across inland waters is strongly facilitated by human-mediated vectors. In ten years, the spiny-cheek crayfish managed to reach Latvian waters probably by natural dispersal though waterways connecting the catchments of Nemunas and Lielupe rivers.

The current survey of populations of noble crayfish indicated that although still healthy populations of this valuable invertebrate exist in Lithuania, the general situation in the country is going worse. The program of noble crayfish breeding and restocking, which is still performed by the Ministry of Agriculture, probably would be not sufficient to sustain the native crayfish. Present threats and perspectives for protection of the noble crayfish will be analysed.

Keywords : native, invasive, distribution, dispersal, threats.

Session 4 :

Crayfish genetics

Chairman : Frédéric GRANDJEAN

Phylogeographical patterns of the noble crayfish (*Astacus astacus*) and recommendations for a regional restocking program

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Genetic diversity could be a crucial factor for the long-term survival of endangered species. However, restocking measurements are often conducted without taking into account genetic differences between populations. To obtain a picture of large-scale differences and to reconstruct the phylogeography of the endangered European crayfish species Astacus astacus, we analysed partial sequences of two mitochondrial genes (16s rRNA, COI) from specimens of the main European river basins. Among 60 crayfish stocks and 312 specimens 9 alleles of 16s rRNA and 14 alleles of COI could be identified. One allele of each fragment was dominant whereas all other alleles were less frequent in the studied populations. Resulting phylogeographical patterns were in accordance with following expectations:

1. A glacial refuge in the Balkans served as source for postglacial recolonisation of central Europe.

2. A north- and westward spread after the last ice age took place along the Danube basin.

3. Due to repeated founder effects, haplotype diversity was reduced from southern to northern Europe.

On a regional scale, mitochondrial haplotypes were identified and additionally population genetic diversity estimated by microsatellite analysis for more than 20 stocks within one river basin (Lahn). Resulting genetic patterns form a knowledge basis for a conservation project in Hessen, a federal state in Germany, to identify suitable donor populations for restocking.

Keywords : Astacus astacus, phylogeography, management, conservation genetics, restocking.

Phylogenetic position of white-clawed crayfish from the east Adriatic coast

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The white-clawed crayfish (*Austropotamobius pallipes* (Lereboullet)) is a native European freshwater crayfish inhabiting Croatian freshwater habitats. It is naturally distributed in the Adriatic Sea drainage, while in Europe it is found in its southern and western parts, including British Isles. It has been frequently studied and it presents a taxon that is highly structured, so the latest suggestion is to consider *A. pallipes* as a species complex.

The number of populations is continuously diminishing so it is treated as endangered species and it protected by Croatian and international laws.

The purpose of this research was to study the phylogenetic position of Croatian populations in comparison to already studied populations of this species in the rest of Europe utilizing broad range of phylogentic methods on two mitochondrial genetic markers. Results of phylogenetic analysis are discussed in details.

Keywords : 16S, COI, Austropotamobius pallipes complex, Croatia.

The DNA sequence analysis of a COI region reveals eight haplotypes for the white-clawed crayfish in the Iberian Peninsula

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Austropotamobius italicus distribution, abundance and habitat range has been reduced in Europe during the last thirty years. The Iberian Peninsula is probably the most severely affected regression area. Only around 600 small Spanish populations now remain, occupying marginal areas or short stretches of watercourses. Consequently the species is now listed in different regions and protected in the national legislation. In this context, there is an urgent need to know the levels and patterns of distribution of genetic diversity in this threatened species.

We have conducted a comprehensive study on the genetic diversity present in *A. italicus* throughout its entire distribution range in Spain. This work reports the data of the analysis of a mtDNA COI region encompassing 1184 bp -the longest for this species reported to date-. The results permitted us to describe eight haplotypes for the white-clawed crayfish in the Iberian Peninsula (accession numbers FJ897840 - FJ897845). Four of the haplotypes were present at intermediate frequencies and the four remaining at low frequencies.

The outcomes of this study indicate, first, the existence of genetic diversity in this species in Spain, and second, a geographical pattern of this diversity. The strategies for future conservation programmes should take these relevant results into account to preserve this valuable species in our freshwater ecosystems.

Keywords : Austropotamobius italicus, genetic diversity, COI mtDNA haplotypes.

Genetic variation in European populations of signal crayfish, *Pacifastacus leniusculus*

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The invasive North American signal crayfish, *Pacifastacus leniusculus*, has been introduced to Europe since 1959 several times, in large numbers (overall more than 10,000 individuals). Secondary introductions followed, resulting in its current presence in at least 22 European countries. In North America, three signal crayfish subspecies are known, overlapping in their morphology as well as range: *P. l. leniusculus*, *P. l. klamathensis* and *P. l. trowbridgii*. Based on allozyme analyses, it has been suggested that more than one of these subspecies are present in Europe. Our aim was to analyse genetic variation and taxonomic status of European signal crayfish populations. We obtained partial sequences of the mitochondrial gene for the cytochrome c oxidase I (COI) of 327 signal crayfish from 16 European countries, and compared the patterns with the variation of 201 individuals from the Klamath River Basin (California and Oregon, USA), where all three subspecies are native. Based on the available data, the subspecies seem to be reciprocally monophyletic at mtDNA level (with average between-group divergences ranging from 4 to 5.3 %), but show different level of intra-group variation (*P. l. leniusculus* is substantially more diversified than the other two subspecies). All analysed European individuals belonged to *P. leniusculus leniusculus*. The geographic distribution of 26 haplotypes found in Europe did not show any clear pattern, which corresponds to numerous secondary introductions within the continent. If the relatively high divergence among signal crayfish subspecies is confirmed in a wider geographic area, it may warrant a revision of their taxonomic status.

Keywords : Pacifastacus leniusculus, COI variation, subspecies, Europe, North America.

Session 5 :

Crayfish Invasions and Crayfish plague

Chairman : Catherine SOUTY-GROSSET

Spiny-cheek crayfish (*Orconectes limosus*) biology – the latest pieces of knowledge

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Spiny-cheek crayfish (*Orconectes limosus*) belongs to the group of the most important freshwater invaders in European freshwater ecosystems and it is continuously spreading, naturally as well as by assistance of man, to the new areas. Nevertheless, some parts of the life history patterns of this large macroinvertebrate were still unknown. Studies published recently make clearer some of these unrecognized parts of its functional and reproductive biology, ethology and ecology. Presented contribution summarizes the knowledge with spiny-cheek crayfish obtained in the last five years by our team. The large emphasis is put on spiny-cheek crayfish continuous spreading, its reproductive cycle and potential, growth patterns, chelae regeneration, form alternation in males and females, diurnal and nocturnal activity, behaviour, intra- and inter-specific interactions, habitat preferences and spatial as well as temporal migrations. These topics should help us to comprehend spiny-cheek crayfish life history and strategy which can allow us to better understand its invasive spreading.

Keywords : invasive species, interaction, fertility, migration, moulting.

Facultative parthenogenesis in spiny-cheek crayfish *Orconectes limosus* : a novel reproductive mode in decapod crustaceans

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North American crayfish species are prominent invaders in freshwaters, defying the «tens rule» which states that only a minority of species introduced to new regions become established, and a minority of those become invasive and pests. So far, the success of invasive crayfish of the family Cambaridae has largely been attributed to their rapid maturation, high reproductive output, aggressiveness towards native species, and tolerance to pollution. We provide experimental evidence that females of the spiny-cheek crayfish *Orconectes limosus* collected from a Czech population are capable of facultative apomictic parthenogenesis. Over 25 females, which were kept during the mating season physically separated from males but were able to detect them by chemical cues, produced offspring genetically identical to mothers (as proven by analysis of nine microsatellite loci). The genetic uniformity of juvenile individuals and their identity to mothers, which were often heterozygous at several loci, rules our alternative explanations than asexual reproduction such as long-term sperm storage by females. Females mated to males in the same experiment produced genetically diverse offspring in Hardy-Weinberg equilibrium, with Mendelian inheritance of microsatellite alleles. Facultative parthenogenesis is a reproductive mode never before been recognized in decapods. Such reproductive plasticity of non-mated females may be possibly present in other cambarid crayfish species, and may contribute to their overwhelming invasive success.

Keywords : Orconectes limosus, asexual reproduction, reproductive plasticity, microsatellite analysis.

What oomycetes can be found growing on crayfish and how to determine them ?

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Numerous oomycete species may grow in or on crayfish cuticle, especially after crayfish death. Knowledge on their diversity may help us in developing diagnostic tools and make easier isolation of the crayfish plague pathogen *Aphanomyces astaci*. In a study originally attempting to isolate *A. astaci* from noble crayfish infected by crayfish plague, and from the pathogen carrier spiny-cheek crayfish, soft cuticle, melanized areas in the cuticle and crayfish exuviae were used as inoculum. Altogether, 75 laboratory cultures of various oomycetes (predominantly from the family Saprolegniaceae) were obtained. The isolates were characterized morphologically and by sequencing of the ITS region of the nuclear rDNA. Morphological determination of this group is based on the characters on their asexual (zoosporangia) and sexual structures (oogonia and antheridia). However, induction of sexual structures production necessary for species determination was successful only in about 20 % of isolates. Only one type of oogonia, typical for *Saprolegnia ferax*, was observed. Sequencing uncovered genetic diversity even in morphologically identical isolates. Combining both approaches, five species of the genera *Aphanomyces*, *Saprolegnia* and *Phytopthora* and *Pythium* sp. were determined. The most frequent isolates belonged to the species *S. ferax*, *S. parasitica* and *S. australis*. Sequences of six isolates were affiliated with the Saprolegniaceae family but did not match any publicly available oomycete sequence. These may represent new species or species hitherto not sequenced. Our results show that molecular approaches are likely to reveal substantial cryptic diversity within the oomycetes.

Keywords : Saprolegniaceae, morphological determination, sequencing, ITS rDNA.

This study was supported by the EU program SYNTHESYS.

Control of aggressiveness and development by molting hormones (ecdysteroids) in the red swamp crayfish, *Procambarus clarkii*

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The red swamp crayfish, *Procambarus clarkii*, is a very aggressive species that is invading many water places in Europe, making severe damages, as observed in the South-West of France near Bordeaux. We have investigated the changes in aggressiveness and in agonistic interactions during the molt cycle of this species and analyzed more particularly the role of ecdysteroids, which are major hormones controlling molts and reproduction. Aggressive acts generally decrease in intensity during pre-molt period and progressively recover their initial levels during post-molt. Dominant animals generally keep their social status until molt, but avoid fighting after molt and behave as subordinate animals until the intermolt period. Injections of ecdysteroids induce similar sequences of events as those observed in naturally molting animals. Our results also demonstrate that ecdysteroid effects on behavior involve changes in neuromodulation by serotonin, a well-known regulator of aggression and of many other behaviors. These data will be discussed in terms of possible tools for the control of expansion of this invasive species.

Keywords : Molt, Agonistic behavior, Ecdysone, Serotonin.

Eradication of signal crayfish (*Pacifastacus leniusculus*). Management and possible procedures to prevent further spreading

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During October 2006, the first documented discovery of signal crayfish (*Pacifastacus leniusculus*) in Norway was reported. The signal crayfish were suffering from crayfish plague.

In 2007, the Norwegian National Veterinary Institute produced a feasibility study for eradication of signal crayfish, including a review of suitable methodology. This resulted in recommended use of the pharmaceutical BETAMAX VET.®, a cypermethrin-based pharmaceutical developed for treatment of salmon lice (*Lepeophtherius salmonis*), infestation of farmed Atlantic salmon (*Salmo salar*), followed by pond drainage.

Upon request from the Norwegian environmental authorities, attempted eradication was performed at two separate locations during August 2008 and during October 2009. Both eradications projects consisted of two separate treatments. Draining was performed before the onset of winter.

During and after the second treatments and draining of the ponds in both eradication projects, no live signal crayfish have been found. It is too early to conclude whether the treatments have led to the complete eradication, but the results so far are promising.

Norwegian Food Safety Authority and Directorate for Nature Management are currently finishing a management plan which presents the objectives of noble crayfish management. Special attention is given to the introduction of crayfish plague and other plague carrying species. Efforts to limit or eradicate populations of signal crayfish are not discussed in this plan. This led the Directorate for Nature Management to order a procedure which in detail describes what to do if new populations of signal crayfish are discovered in Norway. Our hope is that this procedure will give us a powerful tool to prevent the spread of signal crayfish in Norway, and safeguard the noble crayfish in Norway.

Keywords : Signal crayfish, crayfish plague, eradication.

Posters

Session 1 :

Interactions of crayfish and fish in the ecosystem

Naïve fish learning abilities: how does learning period affects the capture rate of a new prey item ?

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The learning abilities of an invasive fish (largemouth black bass: *Micropterus salmoides*, Lacepède 1802) when dealing with a new crustacean prey item (red swamp crayfish: *Procambarus clarkii*, Girard 1852) were studied. Both predator and prey are invasive species in Iberian Peninsula and were naïve to one another at the beginning of the experiment. Largemouth Black Bass (LBB) were submitted to four different periods of contact with juvenile crayfishes and prey consumption was recorded.

We found significant differences between learning periods both for consumed crayfish fresh weight and for number of individuals. LBB submitted to four days of learning period preyed more crayfish than the control group (5.75±2.50 vs 0.40±0.54 crayfishes respectively). Our results indicate that LBB have a four days learning period when switching to new food items. These results showed that naïve predacious fishes, such as largemouth black bass, have a fast learning curve having the potential to easily adapt to new preys in a recently invaded habitat. We consider the results are relevant both for management and modelling of LBB and crayfish populations.

Keywords : learning, Micropterus salmoides, Procambarus clarkii, naïve fish, predation success

Session 2 :

Knowledge base of ingenous crayfish and non indigenous crayfish

Distribution of *Austropotamobius torrentium* Shrank, (1803) crayfish, in the drainage of river Bosna (Bosnia and Herzegovina)

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The paper is the result of diffusion research of crayfish in the upper and middle course of the Bosna River Basin in the period 2000. - 2010. The populations of the researched species in the area of Sarajevo (River Rail, White, Koševsko stream) have been determined, and in the area of the middle course, numerous populations have been determined in river basin Gostovic and streams in the area Banovic. The area in which they the named populations appear, spreads over on altitude from 500 to 900 m. Crayfish was found, also, in the Neretva river basin, Una, waterfalls (Trožic-Borovac, 2008). For the stream of Bosna, it is named the widespreadness of *Astacus leptodactylus* (Eschscholtz, 1823) in the upper stream of river Miljacka near the city of Sarajevo. In the future, data on mitochondrial DNA variation in the populations studied will be presented.

Keywords : crayfish, Bosnia and Herzegovina.

Update on the distribution of freshwater crayfish in Croatia

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Four native European and two non-native American freshwater crayfish species inhabit Croatian freshwater habitats. In general, noble crayfish, narrow-clawed crayfish and stone crayfish are distributed in the water bodies belonging to the Black Sea drainage, while white-clawed crayfish inhabits rivers belonging to the Adriatic Sea drainage. Non-native species, namely signal crayfish and spiny-cheek crayfish, entered Croatia through the Mura River and the Danube River respectively, and so are distributed in the water bodies of the Black Sea drainage.

Recently, the distribution of Croatian crayfish populations has been studied more intensely within the frame of Natura 2000, and in this paper we present results of field work that was conducted from 2008 till 2010, and compare them whit previous data.

Approximately 400 sites were investigated. Results indicate that NICS are spreading and, expectedly, displacing noble and narrow-clawed crayfish populations. No mass mortalities in the contact zone have been reported. This survey also revealed spreading of narrow-clawed crayfish westwards and southwards displacing noble and stone crayfish from their original habitats. Stone and white-clawed crayfish populations undergo biggest decline in their numbers. Main reasons for this decline are disturbance of their habitats (water bodies' construction-canalization, riverside vegetation removement etc.) and intensive droughts that are pronounced in the last few years.

Collected data are helpful as a starting point for development of management strategies by state institutions dealing with protection of endangered native crayfish species.

Keywords : ICS, NICS, distribution, protection, Croatia.

The status of the endangered species *Austropotamobius torrentium* (Schrank 1803) in Romania

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The present paper aims to analyze the distribution of EC priority species *Austropotamobius torrentium* in Romania in relation to water quality, as given by several physico-chemical indicators. The physico-chemical analysis consists of measurements of several general water quality indicators such as pH, dissolved oxygen, total hardness, cyanide, tannin + lignin, dissolved inorganic nitrogen forms (N-ammonium, N-nitrite, N-nitrate) and soluble reactive phosphorous. The statistical techniques are used in order to find differences between the parameters of the habitats in which crayfish were present and the parameters of those stations in which crayfish were not discovered. This paper also tries to show the optimal conditions in which this crayfish species lives within the limits of the measured parameters.

Keywords : Austropotamobius torrentium, optimal conditions, water quality, physico-chemical indicators.

Study funded by CNCSIS-Exploratory research projects PCE-4 1019/2008 «The stone crayfish (*Austropotamobius torrentium*), distribution in Romanian habitats, ecology and genetics of populations».

The assessment of the habitat and water quality requirements of the stone crayfish (*Austropotamobius torrentium*) and noble crayfish (*Astacus astacus*) species in the rivers from the Anina Mountains (SW Romania)

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The species *Austropotamobius torrentium* and *Astacus astacus* are two EU acknowledged species that do require a deeper understanding of their autecologic requirements before any sustainable conservation effort can be successfully applied. Therefore, the paper aims to analyze the presence of these two species of crayfish in the Anina Mountains (Romania) in relation to several physic-chemical indicators measured on site. The results suggest that the anthropogenic impacts registered in some of the sampling sites might have influenced the disappearance of both species. Both species suggest similar ecologic requirements, suggesting that the species A. astacus might be slighter more resistant to pollution as compared to *A. torrentium*. Nevertheless, the lack of cohabitation of the populations of both species is discussed as well.

Keywords : Astacus astacus, Austropotamobius torrentium, crayfish, distribution

Study funded by CNCSIS-Exploratory research projects PCE-4 1019/2008 «The stone crayfish (*Austropotamobius torrentium*), distribution in Romanian habitats, ecology and genetics of populations»

Comparison among different approaches to model chemical-physical water requirements of white-clawed crayfish (*Austropotamobius pallipes*) in North Western Italy

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In Piedmont (NW Italy) the environmental changes due to human impact have deeply altered the white-clawed crayfish habitat and native populations have decreased markedly. The evaluation of the chemical-physical factors determining its presence can contribute to its conservation.

The study system consisted of 175 sites and *Austropotamobius pallipes* was recorded in 98. Two different approaches were used to assess its presence: Logistic Regression (LR) and Decision Tree (DT) models.

The data were normalized proportionally (between 0-1) before a data set was used to build different models. Attributes were selected through the Information Gain method and a subset of 9 inputs (NH4+, NO3-, PO43-, SO42-, Ca2+, Mg2+, water hardness, pH, SpO2) was obtained from the starting one of 12. The performances of LR were estimated from a leave-one-out jack knifing involving a holdout procedure repeated 10 times using a model derived from a calibration set of 80% of the sites.

In building DT models, the J48 algorithm was used with a binary split and the tree-pruning optimization method was applied. DT training and validation were based on stratified 10-fold cross-validation and to estimate a reliable error of the models, experiments were repeated 10 times.

The percentage of correctly classified instances was: LR = 67.30% (sensitivity = 82.81%, specificity = 47.21%), DT = 63.76% (sensitivity = 75.06%, specificity = 49.71%). The Mann-Whitney U test showed that the LR performed better than the DT. The concentration of Ca2+, water hardness, and BOD5 were the most important inputs used in building LR models.

Keywords : Austropotamobius, habitat requirements, multivariate statistics.

Crayfish as trophic agents: effect of *Austropotamobius torrentium* on zoobenthos structure and function in small forest streams

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Crayfish are among the largest and most threatened invertebrates in freshwater habitats. Due to their size, behaviour and feeding activity they may affect structure and function of aquatic ecosystems and their organisms. Despite their importance in many freshwaters and available information on their ecology for several species little is known about the European crayfish *Austropotamobius torrentium*. In order to evaluate the potential effects of indigenous crayfish presence on the structural and functional composition of the zoobenthic community, we measured population size and densities of three *A. torrentium* populations and compared macroinvertebrate assemblages and physicochemical parameters in three streams with and three without crayfish. The experimental setup considered crayfish effects at a large scale in defined reaches of pristine headwaters in association with the whole benthic fauna under natural conditions. Presence of *A. torrentium* significantly affected zoobenthic abundance, diversity and the relative proportions of functional feeding groups. In crayfish streams, especially Trichoptera and collector-gatherers were more abundant and diverse, while sites without crayfish had significantly higher abundances and diversities of shredders and wood feeders. Our study provided strong evidence that the presence of the indigenous crayfish *A. torrentium* had important effects on the trophic cascades of headwater stream communities.

Keywords : indigenous invertebrate, ecosystem engineer, headwaters, aquatic ecosystem, functional feeding guilds.

Migration of Noble crayfish (*Astacus astacus*) and Stone crayfish (*Austropotamobius torrentium*) during three vegetation seasons in the Křivoklátsko Landscape Protected Area, Czech Republic

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Crayfish are the most mobile invertebrate animals in central European streams. Their migratory ability is quite high and concrete knowledge can influence the conservation management and the number of crayfish plaque outbreaks.

We present results of our crayfish migration study situated in the Stroupinský stream river catchment in the Křivoklátsko Landscape Protected Area, central Bohemia, Czech Republic. Migratory ability of two native European species of crayfish was studied by recaptures of marked individuals of *Astacus astacus* and *Austropotamobius torrentium* for three years (2008–2010) in August and September. Study area is a 220 m long part of stream with its tributary divided into 16 segments. Between these segments the migration of crayfish was observed. Crayfish were catched by hand and permanently marked using visible implant elastomers. On the basis of recaptures, the type of movement, size distribution of population, sex ratio, and the size of population were obtained for both crayfish species.

In 2010, further detailed description of habitat (number of big stones, water velocity, water depth, type of substrate, insolation) of our site will be studied in detail for each segment. In 2008 and 2009, altogether 1139 specimens of Au. torrentium and 470 specimens of *As. astacus* were marked. The recapture ratios were 10.4% for Au. torrentium and 5.5% for *As. astacus*.

The preliminary results from 2008 and 2009 indicate following facts:

- There is a tendency of As. astacus to move up the stream and a tendency of Au. torrentium to stay in the same segment of the stream.
- The probability of catching male or female of both crayfish species is the same.
- The type of movement (up the stream, down the stream, staying in the same segment) is the same for males and females for both crayfish species.
- No tendency of migration of both crayfish species from stream to its tributary or from tributary to the stream was observed.
- The longest distance travelled by a crayfish is 133 m down the stream in 55 days (female of Au. torrentium) and 151 m up the stream in 36 days (female of *As. astacus*).

Mentioned preliminary results will be influenced by data from the third year of migration study, which is going to be held in August and September 2010. On the presented poster results from all three years will be presented.

Keywords : migration, movement, Stone crayfish, Noble crayfish, Crustacea, Decapoda, Astacidae, visible implant elastomer, habitat selection, Křivoklátsko Landscape Protected Area.

Indigenous crayfish management in Central Italy : the case study of *Austropotamobius pallipes* complex in the National Park of the «Foreste Casentinesi, Monte Falterona e Campigna»

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The indigenous crayfish *Austropotamobius pallipes* complex is threatened but our knowledge about its status in Italy is still limited. This study aims at analyzing the distribution of populations of this species in a protected area of Central Italy, the National Park of the «Foreste Casentinesi, Monte Falterona, Campigna» (Tuscany and Emilia-Romagna). During 2008 and 2009, we monitored eight streams. In three of them previous researches had shown the presence of the species. For each stream, we analyzed a number of physico-chemical and biotic parameters. Crayfish were found in four of the eight streams analyzed but of the three old reports, only one was confirmed, while the species appeared to be extinct from the other two. All the streams are characterized by good quality of both water and soil, as indicated by the high values of IBE (Extended Biotic Index), IFF (Fluvial Functionality Index), and QBS-ar index (Soil Biology Quality Index). Non-indigenous crayfish were never recorded in the study area. Among the several causes of *A. pallipes'* disappearance, drought, pollution from small domestic discharges, introduction of fish predators, and over-exploitation through illegal fishing seem to be the more likely.

Keywords : Austropotamobius pallipes complex, monitoring, management, protected area, Central Italy.

Condition index of stone crayfish throughout a year

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The stone crayfish (*Austropotamobius torrentium*) is one of four native European freshwater crayfish species inhabiting Croatian freshwater habitats. It can be found at higher altitudes in streams and rivers belonging to the Black Sea and the Adriatic Sea drainages. Since it was never of an economic importance, it was not frequently studied so the results presented in this paper are contribution to the knowledge on stone crayfish condition, as a possible tool for evaluating protection and restocking measures of this threatened species. The research included three populations in three streams within the Nature Park "Medvednica", north-west Croatia, and it was conducted during the 18 months period. Crayfish condition was determined for both sexes, separately for juvenile and adult crayfish, using condition factor (CF), Fulton's condition factor (FCF) and crayfish constant (CC). Condition indices varied throughout a year (between seasons), and there were significant differences in recorded values between females and males, as well as between juveniles and adults. Obtained results for all three indices are compared between populations and discussed in details.

Keywords : condition indices, Austropotamobius torrentium, Croatia.

Evaluation of body appendage injuries to juvenile signal crayfish (*Pacifastacus leniusculus*): relationships and consequences

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Aggressive behaviour occurs frequently in crayfish and commonly results in injuries to body appendages. This study aimed to evaluate injuries to antennae, chelae, and walking legs of juvenile signal crayfish after 7 months of rearing at high stocking density. We suggest that the high incidence of antennae injuries (66.8%) is related to their delicate structure and exposed position, which makes them vulnerable to damage. Chelae were more frequently injured (45.5%) than walking legs (7.8–23.6%). Considering the robustness of these structures and the scarcity of animals with both chelae missing and/or regenerating (4.9%), it seemed that injured animals were often killed by intact ones. Antennae of crayfish with a single injured chela were more frequently injured on the side of the body with the damaged chela, and a similar pattern was observed for walking legs. Expanding on previous research reporting a negative relationship only between incidence of chela injury and crayfish size, we found this relationship to be significant for all evaluated appendages. We hypothesize that any injury and accompanying regeneration may have significant impact on subsequent injuries, overall growth, and reproductive success, and may result in death through cannibalism.

Keywords : antennae, autotomy, intraspecific interaction, regeneration, walking leg

Life+ Project «Conservation and Recovery of *Austropotamobius pallipes* in Italian Natura 2000 Sites – CRAINat» : preliminary results in Northern Italy

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The Life+ Project is taking place in 47 Italian Natura 2000 Sites in the regions of Lombardy and Abruzzo, the provinces of Chieti and Isernia, and in the Gran Sasso e Monti della Laga National Park. The aims of this project for the native crayfish are: protecting and increasing the presence of *Austropotamobius pallipes* through the release of juvenile and mature crayfish by creation/adjustment of new reproductive plants, habitat restoring and controlling the poaching and the spreading of introduced alien crayfish. During Summer 2010 we assessed the present distribution of the species in 14 Natura 2000 Sites in Lombardy and the condition of potential reintroduction/restocking sites by estimating the consistency, state of health and genetics of the resident native populations. We found *A. pallipes* in eight SCIs, *Procambarus clarkii* in one and no crayfish in the other five. Catch-per-unit-effort (CPUE) at night time shows a wide range from 0.0002 to 0.0425 crayfish/estimated sampling area/effort. These results are compared with habitat features in order to establish the causes of decreasing.

Keywords : Life+ project, Austropotamobius pallipes, conservation, Northern Italy.

Patterning and predicting the white-clawed crayfish presence using artificial neural network as a tool for biodiversity management

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Fragmentation of natural ecosystems is generally seen to be one of the most important threats to biodiversity (Saunders et al. 1991, Miller et al. 1995). Fragmentation due to human activities greatly modified matrix of the natural ecosystem, and especially in freshwater ecosystem. Because of its status as a 'sentinel of good water quality' and as a threatened species, several action plans have been proposed to preserve this species mainly in Europe. Among the main measures to conserve this species, restocking attempts in suitable habitats are proposed to try to raise its natural range. However, this action required a good knowledge about the ecological requirements of A. pallipes to limit the risks of failure. Several studies have been performed using both physical and chemical criteria. They suggested that an increase of organic matter was a discriminant factor for the presence or absence of *A. pallipes*. If this descriptive analysis gave doubt about its status of bioindicator, it seems to be of limited interest for applied issue as the selection of brooks to restocking attempts. Traditionally, conventional multivariate analyses have been applied to solve these problems. This task, however, is not easy to achieve as nonlinear, complex interactions occur in the dataset consisting of many species and sampling areas. To respect the natural nonlinearity of ecological data, artificial intelligence methods could be preferred. This study evaluates the potential use of artificial neural network (ANN) modelling the freshwater crayfish conservation from water chemistry data based on 19 physical and chemical parameters measured in three years (2000-2002) with twice monthly sampling at 10 sites in Poitou Charentes region. The interpretation of resulting sensitivity curves may reveal impacts of environmental conditions on the occurrence of the white-clawed crayfish. The aim is to predict the occurrence of the white-clawed crayfish at a site from a minimum set of predictor variables and then select some potential brooks for restocking attempts.

Keywords : white-clawed crayfish, ecological requirements, physicochemical parameters, Artificial neural network, species prediction.

Session 3 :

Socio economics aspects

Invaders for sale: Does the ornamental freshwater crayfish trade constitute an actual and overlooked risk ?

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The trade of live ornamental freshwater crayfish has grown rapidly in the last decade and became the major pathway of new non-indigenous crayfish species (NICS) introductions into Europe. In the present study I report on the German ornamental crayfish trade, which is the main importer of non-indigenous crayfish into Europe. In total, 123 NICS have been available as ornamental aquarium species. One hundred and seven species originate from North or Central America and are therefore suspected to be crayfish plague vectors. The annual import rate since 2005 was estimated at six new species. A significant correlation between species´ availability and the probability to occur in the wild became evident. In 2009, at least 37 species were offered by 16 online shops. The risk and invasiveness (assessed in FI-ISK), availability, likelihood of intentional release (`nuisance´) and price of the offered species were used to predict their invasive state (not introduced, introduced, established, invasive). Stepwise linear regression analysis showed that availability, FI-ISK score and `nuisance´ were significant predictors. A further disturbing finding was that especially high risk species were likely to be released intentionally. Subsequent survival and colonization success was therefore considerable higher than expected by the three tens rule of Williamson (1996). The development of proactive management strategies, e.g. a cutback of the availability of high risk species, is clearly needed.

Keywords : live animal trade, invasion pathway, alien crayfish, aquarium discards.

Combination of sodium chloride antifungal bath and removal of dead eggs; an effective antifungal treatment of artificial egg incubation in *Austropotamobius pallipes*

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This study was carried out to investigate the effectiveness of two chemicals as an antifungal bath during the artificial egg incubation of *Austropotamobius pallipes*. These treatments were namely; sodium chloride "S" with three different concentrations: 30 000ppm "30", 60 000ppm "60" and 90 000ppm "90" and formaldehyde with one concentration: 3000ppm "F3", as a control. Each chemical treatment was applied once per three days with an exposure time of 15 minutes. Each chemical treatment was applied with ("R-") or without ("C-") removal of dead eggs. Removal of dead eggs occurred with identical frequency as antifungal bath. Two groups were treated without chemicals with "R" and without "C" removal of dead eggs as control groups for each chemical treatment. In total, 10 different treatments were tested in triplicates: R-; R-S30; R-S60; R-S90; R-F3 and C-; C-S30; C-S60; C-S90; C-F3 during 29 days of artificial incubation. This artificial incubation was carried out in thirty 1 liter polyethylene jars (surface area 15.7cm2), where in total 3 300 stripped eggs at embryonic phase XII were stocked at an egg density of 7 eggs.cm-2, at the beginning of this study.

After hatching and first moult of juveniles the survival levels were recorded. The highest survival was found in treatments R-F3; R-S90; R-S60 and C-F3 (stage1: 89.6-92.3% and stage2: 88.8-85.5%). The lowest production of juveniles was recorded in treatment C- (stage1: 46.5±8.2% and stage2: 45.0± 2.5%). Treatments C-S60; R-; C-S30 showed survival of stage1: 70.2-60.5% and stage2: 67.2-56.0%.

Our results showed that the combination of an antifungal bath with a high concentration of sodium chloride (concentrations: 60 000ppm and 90 000ppm) accompanied with the removal of dead eggs, has the potential to act as an environmental friendly treatments and an alternative to environmental unfriendly formaldehyde treatments.

Keywords : White-clawed crayfish, fungi, hatching, survival, stage2.

Proposal of a practical reference diet for juvenile astacid crayfish studies from the onset of exogenous feeding under controlled conditions

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Considering the knowledge of freshwater crayfish nutritive requirements and other cultured crustaceans, a practical extruded diet was formulated with the aim to be used as reference for juvenile astacid studies from the onset of exogenous feeding. According to a bifactorial design, the practical diet was compared with the diet which has allowed the best results so far (control: feedstuff formulated for trout combined with *Artemia* cysts) in groups (100 juvenile /m2) and isolated animals in an 80-day experiment. The highest survival rate (mean: 93%), carapace length (mean: 13.2 mm) and weight (mean: 578.1 mg) were reached with the practical diet without significant differences from the animals fed the control diet. Survival rates did not show significant differences neither among crayfish maintained in groups (86 % for the practical diet and 81.7% for the control diet) nor among isolated crayfish (100% of survival). Isolated crayfish had significantly higher growth (14.3 mm carapace length and 760 mg weight) than the grouped crayfish (11.5 mm carapace length and 354 mg weight). Results show the feasibility of the proposed practical diet as reference for further studies on astacid crayfish.

Keywords : Astacid crayfish, juveniles, practical diet.

Effects of the additional supply of decapsulated Artemia cysts for various periods from the onset of exogenous feeding in intensive rearing of juvenile crayfish (*Pacifastacus leniusculus*, *Astacidae*) at two stocking densities.

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In intensive astacid crayfish rearing from the onset of exogenous feeding, the dry diets must be supplemented with live feeds or Artemia cysts. However, the use of these supplements involves several drawbacks which advise to reduce both amounts and periods of supply. A 100-day experiment was carried out with stage 2 *Pacifastacus leniusculus* to evaluate the effects of different administration periods of cysts. According to a bifactorial design, six treatments differing in the time in which the cysts were withdrawn (after 20, 30 or 50 days of experiment) and the stocking density (100 and 200 crayfish m-2) were conducted. Survival rates did not show significant differences among groups, with final values averaging 81%. The longest period of *Artemia* cysts supply (50 days) at both densities (100 or 200 crayfish m-2) supported significantly higher growth (around 14,20 mm carapace length and 625 mg weight) than the rest of treatments, whose values did not differ significantly among them. This study shows that a short period of *Artemia* cysts supply is feasible even at higher stocking density tested (200 crayfish m-2) with no effects on survival and that different feeding options can be adopted depending on the growth-cost ratio.

Keywords : Astacid crayfish, feeding, Artemia cysts.

Session 4 :

Crayfish genetics

Complex population genetic structure of the white-clawed crayfish *A. pallipes* from Alpine region (Haute-Savoie, France): balance between natural presence and human-mediated transfers

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Many ecosystems are highly threatened by human impact resulting in fragmentation and degradation of habitats with great consequences on size and structure of *A. pallipes* populations. Decreasing numbers of individuals can lead to a loss of genetic variability and their ability to react to changing environment. The knowledge of the evolutionary history and genetic status of the species is deemed critical for the success of *ex situ* and *in situ* conservation programmes, because it allows the definition of management units and the design of management strategies aimed at minimizing genetic erosion while preserving sub specific distinctiveness.

We have analysed the patterns of genetic variability and differentiation of 24 crayfish populations (480 individuals) from Haute-Savoie (closed to Italian and Swiss borders) with 16S RNA and COI mtDNA sequences and microsatellites. Results from mtDNA clearly revealed the presence of several species: *A. pallipes*, *A. italicus* and *A. torrentium*. Among the 24 populations, 17 harboured *A. pallipe*s exclusively, 5 only *A. italicus*, one both species and one *A. torrentium* species. Microsatellites analysis- performed only on A. pallipes populations due to the presence of many nul alleles in the two other species- revealed the presence of moderate genetic structure due to the recent fragmentation of populations. The Tenalles population, naturally isolated from the others, showed a clear differentiation. These data are important not only for delineating the units of conservation in this Alpine region but also for a better understanding of the contemporary and historical factors that shape the population structure of the species.

Keywords : Genetic variability, species complex, conservation units, Alpine region

Identification of autochthonous and genetically diverse noble crayfish stocks (*Astacus astacus*) using microsatellite analysis

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Genetic diversity could be a crucial factor for the long-term survival of endangered species. The genetic structure of a population or species has implications for conservation management and the design of restocking programs. The Convention on Biological Diversity (CBD), negotiated at the United Nations Conference on Environment and Development (the Rio «Earth Summit») in 1992, highlighted the diversity within species as one of three levels of the biological diversity. However, restocking measurements are often conducted without knowledge of the genetic structure within and between populations.

For more than 2000 years the noble crayfish has been an object of commerce and trade sometimes over large distances. The translocations could have resulted in a mixture of autochthonous and allochthonous populations, thus in a «genetic contamination» of local stocks. Genetic analysis could be a tool to identify autochthonous and genetically diverse populations which are most suitable for a restocking program. For a conservation project in Hessen, a federal state in Germany, mitochondrial haplotypes (16s rRNA, COI) were identified and additionally population genetic diversity estimated by microsatellite analysis for 15 stocks within one river basin (Lahn). Resulting genetic patterns form a knowledge basis to identify suitable donor populations for restocking.

Keywords : Astacus astacus, management, conservation genetics, restocking

ISSR variability in *Austropotamobius italicus* from Spanish populations

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The distribution of genetic variability can reveal not only the history of populations but also the direction and patterns of their evolution. An understanding of the within- and between- populations genetic variation is crucial for defining appropriate strategies in ecosystems conservation.

Thus, we performed an extensive survey to assess the genetic diversity present in Spanish populations of *Austropotamobius italicus*. This species faces a critical situation due to crayfish plague, spread of red swamp and signal crayfishes, habitat loss, as well as other anthropogenic impacts. Its populations show a very strong decline in Europe over the last decades, being particularly dramatic in Spain.

In the present work, PCR amplification of inter-simple sequence repeats (ISSR technique) was applied for the first time to this species. Thirteen populations (eleven from Spain, one from France and one from Italy) were studied to determine the degree and pattern of their genetic variability. Five primers were used and a total of 108 markers were found in the 246 crayfishes studied. Amplified bands ranged from 300 to 2400 base pairs.

The results show low levels of genetic variability in some populations and a considerable level of polymorphism in some others. Analysis of Molecular Variance evidenced that observed variability is mainly found inside of the populations. It was not found a clear relationship between geographical (or hydrographical) and genetic structures.

Keywords : Austropotamobius italicus, genetic variability, ISSR marker, population genetic structure.

Session 5 :

Crayfish Invasions and Crayfish plague

Limiting the red swamp crayfish *Procambarus clarkii* in Brenne (France).

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Identified for the first time in 2007 in the "Parc naturel regional de la Brenne" (Indre), the red swamp crayfish (*Procambarus clarkii*) is colonizing significantly and very quickly all aquatic environments. This species causes real imbalances in aquatic ecosystems. Eight colonized sites are now identified in the park territory including two new sites discovered very recently (2010). It is therefore very urgent to limit its spread and to fight actively against this species. This study aims to establish a protocol suitable for estimating the population size of *P. clarkii* in fish-ponds. For this, a Capture-Mark-Recapture (CMR) technical was performed to investigate different sites. A biometric study was also performed in order to obtain additional information about population structure. This first approach will allow following eventual changes in populations due to intensive trapping campaigns and other methods of control settled by the Park.

Keywords : Red swamp crayfish, invasion, Capture-mark-recapture, control

Interactions between Indigenous and Non Indigenous Species: Waterbird-Mediated Crayfish *Procambarus clarkii* Dispersal.

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The red swamp crayfish (*Procambarus clarkii*) is an invasive species currently present in five continents. Once established this crayfish disperses rapidly to new areas, widening its invasive range. Both human transportation and the autonomous dispersal capabilities of this species are usually referred to potentiate this rapid spread. Here we investigate the possible role of a third component in the spread of this crayfish: dispersal by animal vectors, more specifically waterbirds. Concerning this purpose we performed several experiments to test the survival of this species to air transportation for which we used both trained pigeons and transportation outside a moving vehicle. Further, we also quantified the probability of passive external transport (ectozoochory) of juvenile crayfish on duck's feathers. Results showed that this crayfish was able to survive distances up to 150 km in simulations of bird transport using a car and up to 62 km when transported by birds. We also found that passive transportation on bird feathers is possible despite of a low probability of occurrence. This is the first study to address the dispersal of this invader by animal vectors. Our results indicate that this possibility should also be taken into account to explain the vigorous spread of this species and as such further research should be conducted.

Keywords : Procambarus clarkii, non indigenous species, waterbird-mediated dispersal

Colonization and dispersal rates of two non-native crayfish species (*Pacifastacus leniusculus and Procambarus clarkii*) in NE Portugal

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In Europe, a generalised regression of the native crayfish was observed during the past decades. Simultaneously, several non-native species spread in most countries. In Portugal, the red swamp crayfish, *Procambarus clarkii*, is now occurring in most inland waters.

The NE Portugal is one of the most isolated and low populated regions of Western Europe. Most rivers in this region present low levels of human impact and some are actually pristine. Recently, two American crayfish species reached the area: signal crayfish (*Pacifastacus leniusculus*) coming from Spain and red swamp crayfish. The isolation and the natural conditions of these rivers make them particularly valuable for the study of crayfish colonization and spreading.

The colonization by both species was followed throughout the last decade in the Maçãs river (Douro catchment). Sampling was undertaken every year with baited traps.

The average dispersal rate of signal crayfish was approximately 2 km/year, although a great variability among sectors was observed. Coexistence of signal and red swamp crayfish was detected in a large part of the river system. Signal crayfish developed well adapted and persistent populations. The lower abundance of red swamp crayfish is apparently caused by the low winter temperatures.

Keywords : Pacifastacus leniusculus, Procambarus clarkii, dispersial, coexistence

Crayfish (*Procambarus clarkii*) survival time out of water and its implications for overland dispersion

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The red-swamp-crayfish (*Procambarus clarkii*) is a worldwide invasive species and it spread rapidly in many of these new ranges. *P. clarkii* is known to survive drought periods and overland migrations have also been regularly reported. These characteristics potentiate both active and passive dispersal and may help to explain both the rapid spread of this invader and its ability to colonize isolated and remote water bodies. In this context, our study measured the survival time of different sized individuals at different values of air temperature and relative humidity. Results ranged from 150 minutes, with juveniles, to 990 minutes, with a large adult at 16ºC and 53% relative humidity. At 24ºC and 44% relative humidity, survival time ranged from 150 minutes (juveniles) to 480 minutes (large adults). Regression analysis on log transformed data demonstrated that survival time increases with crayfish size. In spite of this, an ANCOVA lead us to conclude that the relationship between survival and crayfish size is altered when environmental conditions such as temperature and relative humidity are modified. Overall, our results testify the ability of this crayfish to survive large periods of time out of water. These results support that the study of the active and passive overland dispersal mechanisms is fundamental for a better understanding of the dispersal of this species.

Keywords : Procambarus clarkii, biological invasions, desiccation, dispersal

Interactions between exotic crayfish and native river shrimp

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In the south of Portugal there are only two species of strictly freshwater decapods: the native freshwater shrimp *Atyaephyra desmarestii* and the exotic crayfish *Procambarus clarkii*. The aim of this study is to understand if the exotic crayfish acts as river shrimp predator and if both species share the same type of microhabitat. We conducted laboratory experiments to study predation and field work in the river Sorraia to verify the microhabitat distribution of both species.

Laboratory experiments demonstrated significant predation on river shrimp for crayfish with a cephalothorax length (CT) over 24 mm. The average daily consumption increases, with crayfish CT, to a maximum of 0.8 shrimps at 44 mm CT and decreases for larger crayfish size classes.

Field work confirmed that both species share the same microhabitats although with some slight differences e.g. on the water quality parameters such as conductivity and the percentage of oxygen saturation. These microhabitats are shallow pools with abundant aquatic vegetation with no preference for the type of substrate. In the field, crayfish density and its cephalotorax length are negatively correlated with shrimp densities.

In conclusion, *P. clarkii* can predate *A. desmarestii* and the major impacts are expected at high densities of large sized crayfish.

Keywords : Interactions, Athyaephyra desmarestii, Procambarus clarkii, predation, habitat use

Invasibility of a pond network by the red-swamp crayfish, *Procambarus clarkii*, is negatively related to the degree of isolation from a large source population

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The red-swamp crayfish, *Procambarus clarkii*, is probably the alien species that has most quickly invaded most of French large-sized inland marshes during the last 20 years. However, the ability of P. clarkii to colonize ponds is poorly documented despite the high conservation value of such ecosystems. Here, we studied the spatial distribution of red-swamp crayfish in a network of ponds in the vicinity of the Brière marsh (France) where *P. clarkii* was introduced 25 years ago. More particularly, we investigated whether the occurrence of *P. clarkii* is explained by distance to the marsh or by two landscape variables: number of ponds in a radius of 500 m (NP) and distance to the nearest streams (DS). Sixty eight ponds were sampled in spring 2010 using baited traps. The size of pond ranged 40-3100 m2 and ponds were located at 200-5000 m from the marsh. *P. clarkii* was recorded in 23% of ponds. Data analysis (GLIM models) showed that the presence of *P. clarkii* decreased with distance to the marsh and with NP. The presence of *P. clarkii* was not correlated to DS. These preliminary findings suggest that the colonization pattern of the pond network depends primarily on the proximity to the well-developed population in the marsh, but a step-by-step colonization is not excluded and might increase the colonization risk of isolated ponds.

Keywords : colonization, connectivity, fragmentation, GIS analysis, landscape

Evaluating various trap designs to quantify crayfish population characteristics across multiple freshwater habitats

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The ecological importance of the red-swamp crayfish (*Procambarus clarkii*) in the functioning of freshwater aquatic ecosystems is becoming more evident. Because accurate determination of population characteristics is a requirement for predicting the ecological success and potential impacts of *P. clarkii*, it is important to know the limitations of sampling methods. In the current study, we addressed the question of gear efficiency by comparing population characteristics associated with 8 trap devices (varying in the number and position of entrances, mesh size, size and materials of the frame) across 3 habitat types (reed/grassland/pool) in a French marsh in spring 2010. Based on a large collection of *P. clarkii* (n = 251, 586 and 5774 respectively in reed/grassland/pool), we found that galvanized steel wire traps (GT, two horizontal entrances, 5.5-mm mesh) were the most efficient trap in any habitats in terms of catch probability (97-100% compared to 10-90% according to habitats and trap types) and catch-per-unit effort (CPUE: 9.3, 5.3 and 20.6 crayfish per trap compared to 2.4, 1.6 and 0.2-10.2 crayfish per trap in the other gear types respectively in reed/grassland/pool). GT was also most effective for sampling all age classes, especially small individuals. CPUE of small crayfish (carapace length ≤ 26 mm) were 1.6, 2.6 and 2.9-12.3 times higher in GT than in the other devices according to habitats. Based on these findings, it is now possible to test different sampling efforts using only GT as sampling gear that would ultimately allow appropriate comparisons of population structure and habitat use among different *P. clarkii* populations.

Keywords : catch-per-unit effort, Procambarus clarkii, sampling method, size-frequency analysis, trap efficiency

Thelohaniasis in white-clawed Crayfish *Austropotamobius pallipes* complex from Belluno province (North Eastern Italy)

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From 2004 to 2006, during summer and early autumn, a parasitological survey aimed at detecting the microsporidian parasite *Thelohania contejeani* Henneguy, responsible for Porcelain Disease or Thelohaniasis, was performed on 177 wild white-clawed crayfish *Austropotamobius pallipes* complex captured in six streams and rivers, in particular: 16 in Gresal, 1 in Morol, 20 in delle Tose Lake (Lagole), 90 in Valturcana, 27 in Ardo and 23 in Vena d'oro watercourses. Gross examination of each specimen was performed and only a small number of subjects, in relation to the population consistency, was sacrificed to evaluate the health status. Parasitological surveys were performed by microscopical exam of skeletal muscles; histopathological analysis was carried out by different histochemical stains on full transverse and sagittal sections of cephalothorax and abdomen. Transmission electron microscopy (TEM) observations have been also conducted on the parasites recovered during the survey. The presence of *Thelohania contejeani* (Microsporidia, Thelohaniidae) was recorded only in one crayfish from the Vena d'oro population. The examination of skeletal muscle from the positive subject revealed the presence of several developmental stages of the parasite, with mature spores as the most common stage. Sporophorous vescicles were also present. At the histological examination the skeletal, cardiac and intestinal muscles showed the fibers heavily filled with spores. Melanine infiltrations were focally present in infected striated muscles. The gill phagocytotic nephrocytes were engulfed with small masses of spores. This survey permitted to report for the first time the sporadic presence of T. contejeani in white-clawed crayfish populations from Northeastern Italy.

Keywords : White-clawed crayfish, Austropotamobius pallipes complex, Thelohaniasis, Italy

Temporal variation in the prevalence of the crayfish plague pathogen (*Aphanomyces astaci*) in three Czech spiny-cheek crayfish populations

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The crayfish plague pathogen *Aphanomyces astaci* is highly adapted for living in the crayfish cuticle. Natural hosts of this pathogen in Europe are non-native North American crayfish species. The spiny-cheek crayfish *Orconectes limosus*, widespread throughout the continent, is the main reservoir of *A. astaci* in the Czech Republic. We tested if prevalence of infected individuals (i.e., proportion of individuals in which the pathogen is detected by a molecular method) varies in spiny-cheek populations among seasons and years. We hypothesized that percentage of crayfish detected as infected could depend on their stage of moulting, as crayfish may get rid of the parasite with exuviae. Crayfish were sampled from three populations shown previously to be infected to a different extent (highly, intermediately and lowly) two to three times a year in two consecutive years. Presence of *A. astaci* in the soft abdominal cuticle of 10 to 30 individuals per sampling was tested by a specific amplification of the pathogen DNA. We did not observe any temporal variation in infection prevalence in the highly and lowly infected populations. However, the percentage of individuals detected to carry *A. astaci* in the intermediately infected population (stream Pšovka) varied, ranging between 0 and 50% of analysed individuals. No clear seasonal trend was observed, and infection prevalence in the two summers strongly differed. The temporal variation may be related to the fact that the stream population is open, with fluctuating density, while the other two populations are closed with no possibility for crayfish migration.

Keywords : crayfish plague, Orconectes limosus, Aphanomyces astaci, infection prevalence

Molecular detection of the crayfish plague pathogen (*Aphanomyces astaci*) in Lake Eğirdir (Turkey)

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The presence of the crayfish plague pathogen *Aphanomyces astaci* in Turkey has been assumed since the mid-1980s when many local populations of the narrow-clawed crayfish *Astacus leptodactylus* drastically declined. This was also the case of Lake Eğirdir (Isparta province) where the crayfish stock collapsed in 1986 but some crayfish still survive there in lower numbers. *A. leptodactylus* is considered sensitive to crayfish plague. Several hypotheses may explain its continuing presence at affected localities: 1) misidentification of the pathogen with some yet unrecognized pathogenic oomycete; 2) lower virulence of A. astaci strain present in Turkey; 3) increased resistance of the crayfish hosts to the disease; 4) recolonization by crayfish after disappearance of the pathogen from the locality; 5) high concentrations of Mg2+ preventing the disease transmission; 6) host population density not reaching a threshold necessary for development of massive plague outbreak and elimination of all crayfish. As the identity of the pathogen has never been confirmed by molecular methods, we tested whether *A. astaci* indeed coexists with *A. leptodactylus* in Lake Eğirdir. 34 crayfish were analysed for the infection by species-specific amplification of the pathogen DNA (ITS region), and subsequent sequencing of PCR products. The detection was positive for five individuals (15%); the resulting sequences were identical with those of known *A. astaci* strains. We therefore unambiguously confirm that the crayfish plague pathogen is common in Lake Eğirdir. Given the importance of *A. leptodactylus* for aquaculture, further research should focus on the mechanisms allowing its long-term coexistence with the plague pathogen.

Keywords : Astacus leptodactylus, Aphanomyces astaci, coexistence, ITS sequencing

Is it possible to locally stop the spread of crayfish plague ?

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In August 2009, fishermen observed a massive mortality of white clawed crayfish (Austropotamobius pallipes) in the Grozon tributary of Doux (St Barthélemy, Grozon, France). Several managers (Saules et Eaux -T. Duperray-, FDPPMA 07, ONEMA 07) confirmed these observations without any other aquatic organisms' mortality. Fresh dead crayfish are located upstream with also individuals are at their last gasp. Two nocturnal investigations from upstream to downstream showed that crayfish were trying to go upstream an consequently contaminated the population situated in head waters. PCR analyses having confirmed the presence of Aphonomyces astaci, it was attempted to settle a physical barrier avoiding their swimming upstream and fragmenting the population. This type of barrier was established mi August on the two tributaries still harbouring healthy white clawed crayfish and situated at more of 300 meters of the outbreak event. In May 2010, i. e. nine months after, one site was well secured. We analyzed differences between both sites for explanation of the success about only one.

Keywords : Austropotamobius pallipes, fighting against Aphanomycosis, physical barrier

Eradication of the signal crayfish by mechanical sterilization of males.

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Pacifastacus leniusculus distribution is extending continuously since its introduction in France forty years ago. This species extirpated populations of the indigenous crayfish and particularly Austropotamobius pallipes by outcompeting and because of carrying crayfish plague. In front of no existing solutions T. DUPERRAY (with his society «Saules et Eaux») has developed an eradication protocol by sterilization (no chemical) of males in the field where sites are very important. The different phases are: Capture of the greatest number of individuals, destruction of females and small males (immature stage), and release of adult males after sterilization. Consequently these males are capable to find hidden females and mate, avoiding their reproductive ability for the forthcoming year. Trials are performed in tanks since 5 years and in streams since 2 years. Current trials are successful in tanks with obtaining 33% of berried females with control males and only 4.4% with sterilized males (Besnard 2009). An experiment in the field with the Parc National des Cévennes and scientific investigations from CNRS started since 2009 in a stream harbouring both species (*A.pallipes* and *P. leniusculus*) including population size measurement (Capture Mark Recapture method) twice per year and the beginning of sterilization in 2010 following by a 4 years plan of controlling *Pacifastacus* by PIT tags.

Keywords : Pacifastacus leniusculus, control, no chemical, sterilization

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