2nd International Seminar
Rearing of unionoid mussels

Clervaux, Luxembourg

Tuesday 24th November – Friday 27th November 2015

Book of abstracts

Restoration of Unio crassus rivers in the luxemburgish Ardennes
LIFE11 NAT/LU/857

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1 PREFACE

It is a great pleasure to announce our 2nd international seminar about the rearing of unionoid mussels. After a first successful seminar in 2008 in Heinerscheid/Luxembourg we decided to meet again to discuss the progress in the field of freshwater mussel culture in November 2015 in Clervaux/Luxembourg.

Freshwater mussels are one of the most imperiled taxonomic groups worldwide and many species are close to becoming extinct due to the pollution, degeneration and destruction of their habitats. To save these populations on the long run, the only possible way is by habitat restoration. Habitat restoration, however, is taking time and will not be completed before many local populations have become lost. One possibility to save the genetic diversity of many of these autochthonous populations could be to artificially breed young mussels to enhance the populations and pass the time until the restoration has been completed.

The aim of this seminar is thus to bring all experts working on the rearing of unionoid mussels, as well as people interested in this subject, together and to discuss the latest progress in this field. The exchange of knowledge and experience might help to improve and elaborate the rearing methods and hence save some species and populations from becoming extinct.

2 ORGANIZATION AND PROJECT PARTNERS

Organization:

natur & ëmwelt / Fondation Hëllef fir d'Natur
LIFE 11 NAT /LU/857
2, Kierchestrooss, L-9753 Heinerscheid, Luxembourg

www.naturemwelt.lu / www.unio.lu

Project Partners:

The European Commission

Luxembourg government:
Ministry for Sustainable Development and Infrastructures
Ministry of Agriculture

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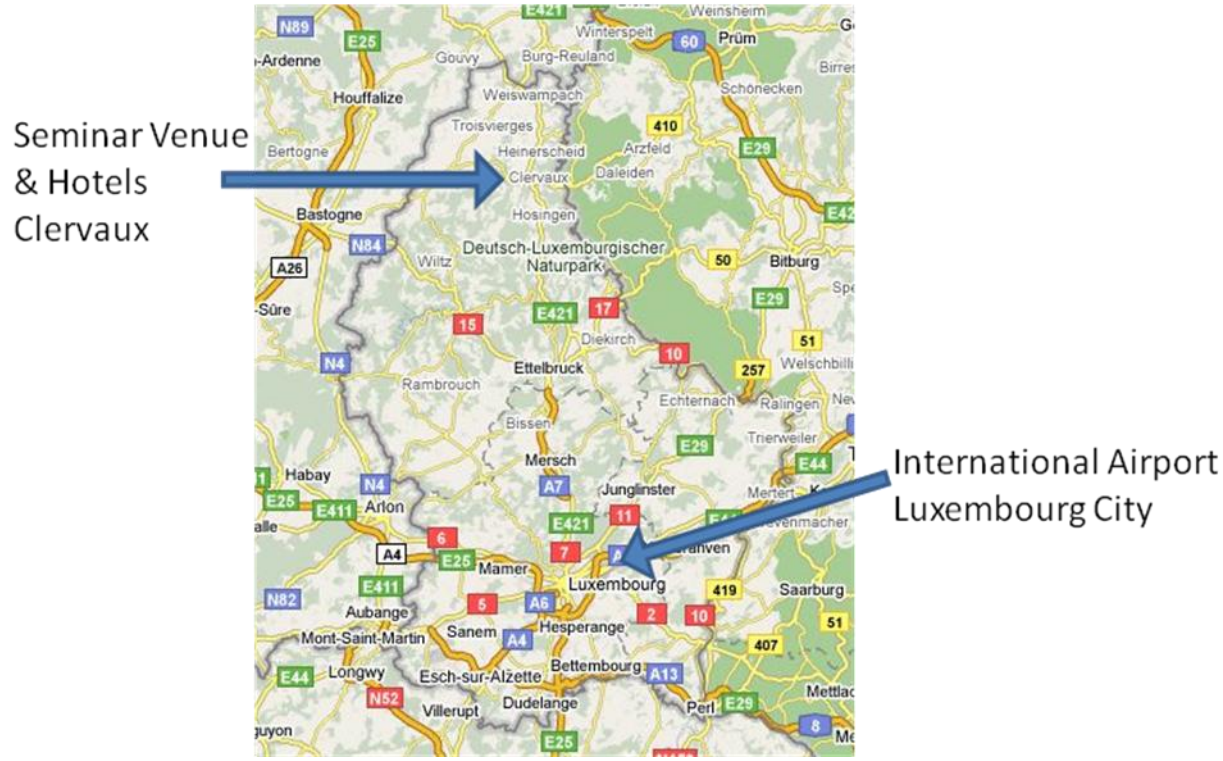
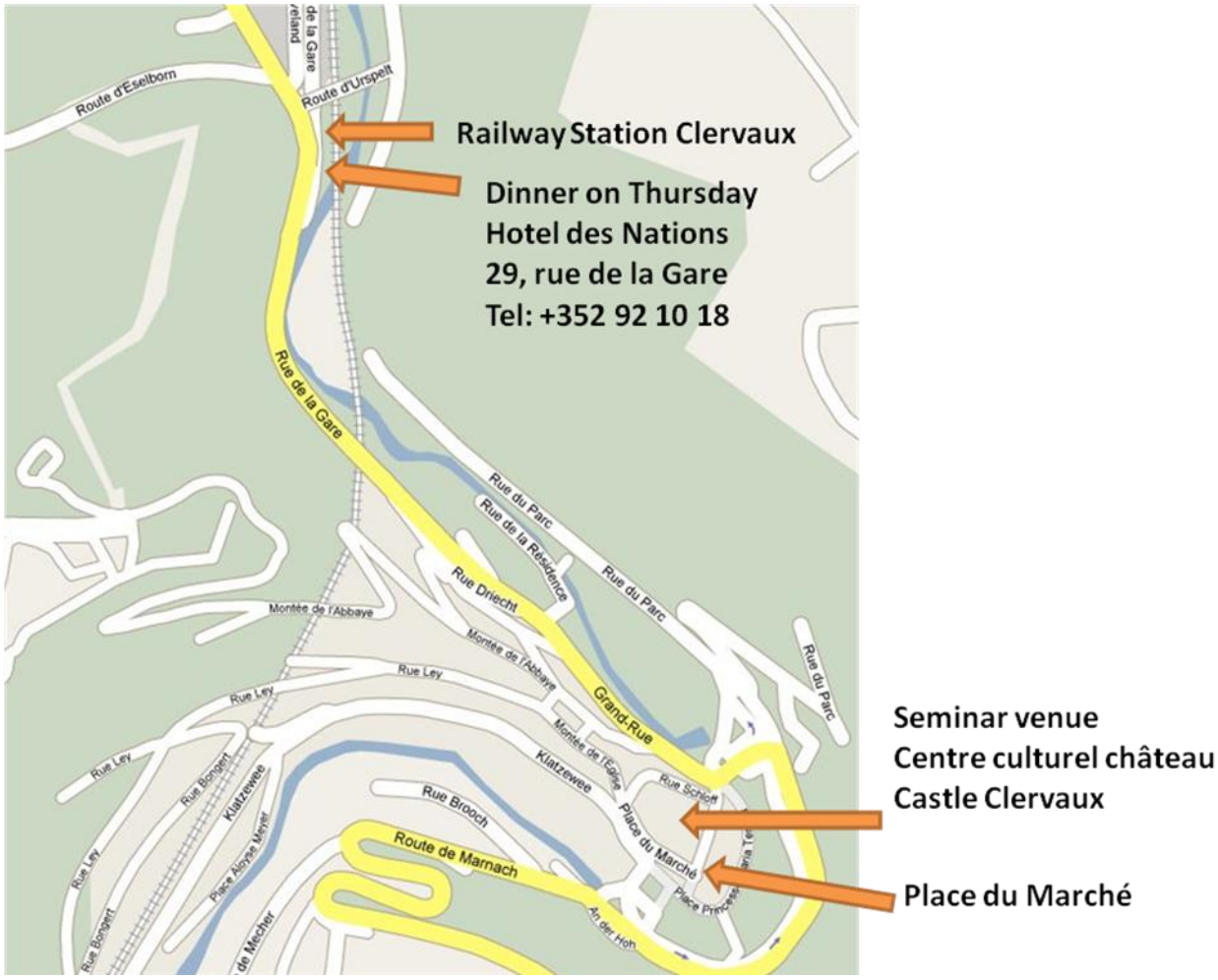
Chamber of Agriculture Luxembourg

3 MAPS



Overview of Luxembourg

Overview of Clervaux



Clervaux (Seminar venue, railway station, hotels and dinner location)

On Friday the bus for the fieldtrip will leave at the "Place du Marché" below the castle at 11:30. You will be back in Clervaux again at around16:30.

4 SEMINAR PROGRAM

Tuesday, 24th November 2015

19:30 Welcome reception + Registration at the Centre Culturel Château, Castle Clervaux

Greeting words from the Mayor Emil Eicher of the City Clervaux

Wednesday, 25th November 2015

08:00-09:00	Registration at the Centre Culturel Château, Castle Clervaux	
09:00-09:30		Opening and Greetings - Claude Meisch (Vice President of natur & ëmwelt / Fondation Hëllef fir d'Natur)
Session I	**Chair: Jürgen Geist**	
09:30-10:00	Jürgen Geist	*Aquatic Systems Biology Unit, Technical University of Munich, Germany* **Supportive breeding of freshwater mussels: Aspects to consider from a conservation biology viewpoint**
10:00-10:20	Mariana Hinzmann. *et al.*	*ICBAS UP—Abel Salazar Biomedical Sciences Institute, University of Porto & CIIMAR—Centre of Marine and Environmental Research, University of Porto, Portugal* **Reproductive cycle and glochidia maturation of Unionidae species from north of Portugal**
10:20-10:40	Jouni Taskinen *et al.*	*Department of Biological and Environmental Science, University of Jyväskylä, Finland* **Seasonal glochidium attachment/detachment cycle of *Margaritifera margaritifera***
10:40-11:10	**Coffee break**	
11:10-11:30	Janhavi Marwaha *et al.*	*Department of Biology, University of Bergen, Norway* **Prolonged time on gills benefits individual juvenile freshwater pearl mussels (*Margaritifera margaritifera*)**

11:30-11:50	Karel Douda	*Department of Zoology and Fisheries, Czech University of Life Sciences Prague, Czech Republic* **Host-dependent vitality of juvenile *Unio crassus* and *Anodonta anatina***
11:50-12:10	Phil Boon	*Scottish Natural Heritage, Edinburgh, Scotland* **How to publish in *Aquatic Conservation* – pearl mussel papers as examples of what to do and what to avoid**
12:10-13:30	**Lunch**	
Session II	**Chair: David Zanatta**	
13:30-14:00	David Zanatta	*Institute for Great Lakes Research, Biology Department, Central Michigan University, USA* **Genetic variation, structure and the complex histories of unionid mussel populations: A North American perspective on selecting source populations for propagation and augmentation**
14:00-14:20	Carles Feo-Quer *et al.*	*Consorci de l'Estany, Banyoles & Museo Nacional de Ciencias Naturales de Madrid-CSIC, Spain* **Experiments to improve the survival and growth of juvenile *Unio mancus* (Unionidae) during the first months of life**
14:20-14:40	Chris Barnhart *et al.*	*Biology Department, Missouri State University, USA* **Pulsed flow-through systems for the laboratory culture of early life stages of freshwater mussels**
14:40-15:10	**Coffee break**	
15:10-15:30	Louise Lavictoire *et al.*	*University of Cumbria, Ambleside & Freshwater Biological Association, Windermere, UK* **Effects of substrate size and cleaning regime on growth and survival of captive-bred juvenile freshwater pearl mussels, *Margaritifera margaritifera* (Linnaeus, 1758)**
15:30-15:50	Christian Scheder *et al.*	*Consultants in Aquatic Ecology and Engineering, Wels, Austria.* **Improvement of the rearing success of freshwater pearl mussels (*Margaritifera margaritifera*) by using different cultivation systems**
15:50- 18:00	**Poster Session with reception**	

Thursday, 26th November 2015

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Session III	**Chair: Chris Barnhart**	
09:00-09:30	Chris Barnhart	*Biology Department, Missouri State University, USA* **100 years of mussel propagation: What have we learned and where are we going?**
09:30-09:50	Christina Putz	*Landschaftspflegeverband Passau e.V., Germany* **15 years of experience in semi-natural rearing of freshwater pearl mussels**
9:50-10:10	Michael Lange *et al.*	*Planungsbüro Landes u. Denkmalpflege Vogtland, Plauen, Germany* **15 years of experience in rearing *Margaritifera*: practical suggestions on bioindication**
10:10-10:40	**Coffee break**	
10:40-11:00	Robert Vandré & Christine Schmidt.	*Schmidt & Partner GbR, Goldkronach, Germany* **Propagation of the pearl mussel *Margaritifera margaritifera* in its natural habitat**
11:00-11:20	Marie Capoulade *et al.*	*Bretagne Vivante, Brest, France* **Saving six freshwater pearl mussel *Margaritifera margaritifera* populations in the Armorican Massif (France)**
11:20-11:40	Megan Bradley *et al.*	*Aquatic Wildlife Conservation Center, Virginia Department of Game and Inland Fisheries, USA* **Innovations and advances in freshwater mussel propagation and recovery at the aquatic wildlife conservation center, Virginia**
11:40-13:30	**Lunch & Poster Session**	
Session IV	**Chair: Evelyn Moorkens**	
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14:00-14:20	Ian Killeen	*Malacological Services, Dublin, Republic of Ireland* **Identification of receptor sites for captive bred freshwater pearl mussels (*Margaritifera margaritifera*)**
14:20-14:40	Rebecca Kyle *et al.*	*Quercus, School of Biological Sciences, Queen's University Belfast, Northern Ireland, UK* **Development of an intermediate culture system for the release of the globally endangered freshwater pearl mussel, *Margaritifera margaritifera*.**
14:40-15:10	**Coffee break**	
15:10-15:30	Ivan Olsson *et al.*	*www.ucforlife.se, Sweden* **Bending rivers by mussels**

15:30-15:50	Lea Schneider *et al.*	*Department of Biology, Karlstad University, Sweden* **Local adaptation studies and conservation: The relationship of *Unio crassus* with two of its sympatric and allopatric host fish species**
15:50-16:10	Martin Österling *et al.*	*Department of Biology, Karlstad University, Sweden* **Rearing-related activities of *Unio crassus* – which host fish species to use?**
16:10-16:25	*www.ucforlife.se , Sweden* **Movie: The UC4LIFE-project at Fyleån**	
16:45	**Visit of the UNESCO Memory of the World Photo Exhibition** **The Family of Man by Edward Steichen** **Two guided tours: 16:45 and 17:00** **You have free choice to participate**  http://www.steichencollections.lu/en/the-family-of-man	
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Friday, 27 November 2015

Session V	**Chair: Karel Douda**	
09:00-09:20	Katharina Stöckl	*Aquatic Systems Biology Unit, Technical University of Munich, Germany* **New information on the habitat requirements of *Unio crassus* as a basis to optimize supportive breeding**
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09:40-10:00	Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia & Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Age, growth and demography of several populations of *Unio mancus* and *U. ravoisieri* in northeast of Catalonia**
10:00-10:10	Frankie Thielen *et al.*	natur & ëmwelt / Fondation Hëllef fir d'Natur, Heinerscheid, Luxembourg **The rearing facility at the mill of Kalborn. Some information before the visit**
10:10-10:40	Final Discussion	

11:30-16:30	**Visit of the rearing facility at the mill of Kalborn** Departure at the Place du Marché (Market Place) by BUS at 11:30 Lunch at the mill Back in Clervaux at the Market Place around 16:00 -16:30 

Poster Session

Wednesday, 25 November 2015 (15:50-18:00)
Thursday, 26 November 2015 (11:40-13:30)

Posters will be on display throughout the meeting

First Author	Title
Arturus Skute *et al.*	Institute of Life Sciences and Technology, Department of Ecology, Daugavpils University, Latvia **Perspectives of conservation and reintroduction of *Unio crassus* (Philipsson 1788) in Latvia**
Eloy Benito Reyes *et al.*	*Freshwater Biological Association, Windermere, UK* **Variability within pearl mussels populations and their lifecycle (in captivity)**
Karl M. Wantzen *et al.*	LIFE 13 BIO/FR/001162 & *Université François-Rabelais, Tours, France* **LIFE13BIO/FR/001162: Grande Mulette / Conservation of the giant pearl mussel in Europe**
Katarzyna Zając *et al.*	*Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland* **Habitat stochasticity and metapopulation structure as a problem for freshwater mussels conservation - the case of the *Unio crassus* in the Biała river (Poland)**
Katarzyna Zając *et al.*	*Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland* **Habitat requirements of *Unio crassus* in the Biała river (Poland): hydrology, substrate, chemistry and fish**
Keiko Nakamura Antonacci *et al.*	*Sociedad Aragonesa de Gestión Agroambiental (SARGA), Saragossa, Spain* **The first year of *Margaritifera auricularia* (Spengler, 1793). Breeding in captivity in Aragon, Spain**
Louise Lavictoire et al.	*University of Cumbria, Ambleside, UK & Freshwater Biological Association, Windermere, UK* **Investigations into feeding structures of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) through scanning electron microscopy**
Malgorzata Ozgo *et al.*	*Department of Evolutionary Biology, Kazimierz Wielki University, Bydgoszcz, Poland* **Risks associated with the maintenance of rivers outside protected areas – the case of *Unio crassus* in a tributary of the Biebrza river, north-eastern Poland**
Miquel Campos *et al.*	*Consorci de l'Estany, Banyoles, Spain* **Ex-situ breeding of native unionids in lake Banyoles (Spain) as part of a LIFE project**
Miquel Campos *et al.*	*Consorci de l'Estany, Banyoles, Spain* **Restocking of lake Banyoles (Girona, Spain) with juvenile naiad (Unionidae) born in captivity**
Monika Mioduchowska *et al.*	*Department of Genetics, Faculty of Biology, University of Gdańsk, Poland* **Male or female? Molecular sex identification of the thick-shelled river mussel *Unio crassus***

Pedro Castrillo *et al.*	*Department of Pathological Anatomy Faculty of Veterinary Medicine, University of Santiago de Compostela, Lugo, Spain* **Morphopathological changes in gills associated with experimental glochidiosis in Atlantic salmon (*Salmo salar* L.)**
Pierre Yves Pasco *et al.*	*Bretagne Vivante, Brest, France* **Experiments on reinforcement and in-situ rearing systems of the pearl mussel (*Margaritifera margaritifera*) in the Armorican Massif (France)**
Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia* **LIFE Potamo Fauna, a project for the recovery and conservation of endangered river fauna in the basins of the Ter, Fluvià and Muga rivers (Catalonia)**
Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia & Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Habitat preferences of *Unio mancus* and *U. ravoisieri* in northeast of Catalonia**
Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia & Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Naiads and fish, coupled destiny: the case of basins of north-eastern Catalonia**
Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia & Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Age, growth and demography of several populations of *Potomida littoralis* in northeast of Catalonia**
Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia & Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Age, growth and demography of *Anodonta anatina* in low Ter River flood plain (Catalonia)**
Quim Pou-Rovira *et al.*	*Consorci de l'Estany, Banyoles, Catalonia & Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Expansion of the exotic unionid *Sinanodonta woodiana* in low Ter River flood plain (Catalonia)**
Quim Pou-Rovira *et al.*	*Sorelló, Estudis al Medi Aquàtic SL, Girona, Catalonia* **Effect over naiad populations of flow drastic reduction in traditional irrigation channels in the low Ter River flood plain (Catalonia)**
Santtu Välilä *et al.*	*Department of Biological and Environmental Science, University of Jyväskylä, Finland* **River specific genetic diversity of *Margaritifera margaritifera* with respect to host fish and population size**
Tanja Eybe *et al.*	*natur & ëmwelt / Fondation Hëllef fir d'Natur, Heinerscheid, Luxembourg & University Duisburg-Essen, Fakultät für Biologie, Aquatische Ökologie, Essen, Germany* **Rearing of *Unio crassus* in Northern Luxembourg**

4 ABSTRACTS (ORAL PRESENTATIONS)

Supportive breeding of freshwater mussels: Aspects to consider from a conservation biology viewpoint

Geist J.*

Aquatic Systems Biology Unit, Technical University of Munich, Germany

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Freshwater mussels are considered target species in the conservation of stream ecosystems. Due to deficient natural recruitment in populations of many European species, supportive breeding and culturing have become important and widely applied conservation tools, particularly in the freshwater pearl mussel (*Margaritifera margaritifera*) and the thick-shelled river mussel (*Unio crassus*). However, to date, there is little awareness of how supportive breeding action can alter the genetic constitution of offspring compared to the source population, and which measures should be taken to minimize any potential negative effects due to the release of capture-reared mussels. The objective of this presentation is to highlight which aspects need to be considered from a conservation biology viewpoint to maximize retaining the genetic and evolutionary potential of freshwater mussel populations in supportive breeding, and how to minimize risk in such action. Key information required relates to the genetic constitution of source populations, to the identification of genetically diverse and distinct populations, as well as to the choice of suitable parent individuals that are representative for the source population. During the breeding, selection and genetic drift should be minimized, e.g. by a large enough number of parent individuals and by their exchange in different breeding years. Moreover, monitoring of the genetic constitution of the reared juvenile mussels compared to that from the source population should be applied. Also, the effects of host fish choice, including condition and holding temperature, as well as the rearing conditions in the post-parasitic phase need to be considered since they were found to be selection factors. From a practical point of view, risk spreading, e.g. by local separation of infested hosts, parent and juvenile mussels, needs to be taken into account. Tracking of released juvenile mussels and documentation of numbers and places of release are other key requirements of evidence-based conservation. Overall, an integrative strategy based on a strong collaboration between different rearing facilities is likely to be most successful. In any case, it also needs to be acknowledged that supportive breeding of endangered mussel species must run in parallel to the conservation and restoration of suitable habitats in the wild into which the mussels can then be successfully released.

Reproductive cycle and glochidia maturation of Unionidae species from north of Portugal

Hinzmann M.[1,2,*], Araújo S.[1,2], Lopes-Lima M.[2], Varandas S.[3], Teixeira A.[4], Sousa R. [2,5], Machado J.[1,2]

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The Freshwater mussels of the family Unionidae are among the most endangered species globally. Their particular life cycles, in which the larva (glochidia) needs a specific fish host and the constant threats to their natural habitats makes them highly vulnerable. Portuguese list of Unionidae is short and already encloses species with status considered as in endangered, *Margaritifera margaritifera* and another as vulnerable *Unio tumidiformis*, by the IUCN. Since knowing the main ecological and physiological traits of each species are essential for their conservation, the life cycles of *Anodonta cygnea*, *Anodonta anatina*, *Unio delphinus* and *Potomida littoralis* from North Portuguese rivers and Aveiro lagoons were fully studied by this team. The complete reproductive cycle was monthly analyzed for one year using histological observations of the gonads and gills. Morphometric and detailed microscopic study was made on the glochidia of each species since the larvae can be used in species identification and are key elements on infestation studies. *Anodonta* species seems to adapt their reproductive cycle to the environment, in the rivers they are mainly dioecious, on shallow lagoons tend to be more hermaphrodites, this in the case of *A. anatina*. *A. cygnea* can only be found in the lagoons and only females and hermaphrodite animals were found. The gonad maturation in gradual, presenting all stages of gonad maturation, and during the winter there is slight pause on oogenesis and spermatogenesis. The glochidia of these species are big with a large hook; the period of maturation is long (bradytictic breeders), but the spawning occurs once a year from the end of the autumn until beginning of the winter. *Unio delphinus* and *Potomida littoralis* are mainly dioecious species, with continuously reproductive cycles, oogenesis and spermatogenesis never ceases. The glochidia stay in the gills for a very short period; they are tachytictic species, with various spawning events during the year, being the main in the beginning of spring and summer. In *Unio delphinus* glochidia are released in clusters with a feather shape, while in *P. littoralis* the glochidia are release in a gradual form. These larvae are much smaller but still with characteristic ornamentations on the surface and hock apparatus.

Knowledge on their life cycle is fundamental if conservation measurements have to be made in the protection of these fundamental organisms for the equilibrium of the freshwater ecosystems.

We acknowledge the financial support by the Grant (SFRH/BD/76265/2011) and project (CONBI - PTDC/AAC-AMB/117688/2010) by the Portuguese Foundation of Science and Technology.

Seasonal glochidium attachment/detachment cycle of *Margaritifera margaritifera*

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Artificial rearing has been increasingly used in conservation of declining freshwater mussels, e.g. the endangered freshwater pearl mussel, *Margaritifera margaritifera* (FPM). This approach requires collection of glochidia – and knowledge on timing of glochidial release – to be able to infect the host fish. Glochidia release of FPM occurs, at least in the northern Europe, in autumn, being triggered by decreasing water temperature. Consequently, it can be assumed that (i) glochidia release would take place seasonally earlier in more northern, high latitude, colder regions. As the development of glochidia should also be lower in more northerly located areas due to a lower metabolic rate in low temperature, it is reasonable to assume that (ii) the detachment of glochidia from fish should become later by latitude. Finally, (iii) within a given geographic region, glochidia release should be earlier – and detachment later, in lower-temperature locations. The aim of this study was to investigate these hypotheses about FPM life cycle.

Host fish of FPM, brown trout (*Salmo trutta*) were collected by electrofishing with 3-weeks interval throughout summer from four rivers: River Matalusjoki, south, low-latitude (61°52' N), River Koivuoja and River Jukuanoja, mid-latitudes (65°46' and 65°85' N, respectively) and River Hanhioja, north, high latitude (68°60' N), representing south-north range of 850 km along Finland. Fish were examined microscopically for FPM glochidia.

As expected, glochidia attached to brown trout the earliest (late August-early September) and detached the latest (late July-early August) in the northernmost site, River Hanhioja – which was also the coldest river with max. water temperature of 16.9 ° C, only. Parasitic period of FPM in River Hanhioja, about 11 months, was 1.5 mo longer than in any other river. Of the two rivers at mid-latitudes, River Jukuanoja (max. 20.5 °C) was on the average 2.8 °C colder than River Koivuoja (max. 24.3 ° C). As expected, glochidia release (attachment to brown trout) was earlier, detachment from fish later, and total length of parasitic period longer in the colder river, River Jukuanoja, as compared to the warmer one. The southern river, River Matalusjoki (max. 18.1° C), was not the warmest, but even colder that the mid-latitude rivers by its temperature. Timing glochidia release (last half of August) and detachment from fish (late June-early July) in River Matalusjoki corresponded those in River Jukuanoja, mid-latitudes. Thus, local temperature condotions are probably more important determinants of the seasonal timing of glochidial cycle than the latitude.

Knowing the seasonal pattern and the factors affecting the timing of the glochidium release will be helpful when performing artificial rearing of FPM from different origins. Present results also give a baseline for monitoring of responses of FPM life cycle to increasing temperatures due to global warming in the future.

We acknowledge the financial support by EU Interreg IVA North Programme, Maj and Tor Nessling Foundation, Academy of Finland and Raija & Ossi Tuuliainen Foundation.

Prolonged time on gills benefits individual juvenile freshwater pearl mussels (*Margaritifera margaritifera*)

Marwaha J.[1,*], Jensen K.H. [1], Jakobsen P.J. [1], Geist J.[2]

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The unusual life cycle of the endangered freshwater pearl mussel (FPM) *Margaritifera margaritifera* involves an obligate parasitic stage on a fish host. A specialist parasite, it can only develop on the gills of salmon (*Salmo salar*), sea trout (*Salmo trutta f. trutta*) and brown trout (*Salmo trutta f. fario*). The parasitic glochidia remain attached to their salmon or trout host for a period of 7-9 months and grow 6-10 fold their original length. Once they excyst they remain buried in the river sediment for about five years and depend on stable substrate with good sediment quality and exchange of free-flowing and interstitial water. This post parasitic stage (juvenile mussels) is the most critical stage of the life cycle and a bottleneck in conservation projects. The length of the excystment period is highly variable and excystment periods lasting from 7 days up to 148 days have been reported. We have observed excystment periods of up to 60 days. However, the reason that some mussel populations have a prolonged excystment compared to others is not well understood. It has been observed that larger mussels excyst later during the excystment period. Larger juveniles are expected to have a better survival, but this remains to be validated. Neither the factors which govern excystment, nor the reasons or possible benefits associated with a prolonged time on gills have to our knowledge yet been described. The objective of our study has been to investigate the effect of time spent on the host gills (i.e., the time from when the first mussel excysts to the last one) on the survival and performance of juvenile pearl mussels. In particular, we hypothesized that there is a positive correlation between the time that a mussel spends on the host with mussel size and growth that has beneficial effects on survival and growth. In addition, we hypothesized that temperature has a strong positive effect on excystment rates. In order to do this we followed the excystment and post excystment growth of juvenile mussels from 8 rivers in southern Norway. Size at excystment and size after 20 days growth at 17 °C were recorded in order to calculate the growth rate. We also recorded the temperature throughout the excystment period. In line with our hypothesis, we found a strongly positive relationship between the duration of mussel encystment on gills and mussel mean size at excystment. In addition we observed a positive overall relationship between the time spent on the gills and growth rates. Moreover and in line with previous studies, temperature was identified as an important driver governing the numbers of dropped-off juveniles.

Host-dependent vitality of juvenile *Unio crassus* and *Anodonta anatina*

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Efforts to propagate and culture freshwater bivalves of the superfamily Unionoidea have expanded worldwide together with the increasing importance of the management of their host resources under field conditions. Because the life cycle of unionid mussels includes a larval stage (glochidium) using host fish to encapsulate and metamorphose into the juvenile stage, culturing protocols involve a controlled parasitic stage or its in vitro substitution using fish plasma. The criteria for host evaluation and selection are typically based on quantitative characteristics (transformation success rate), despite increasing evidence of nutrition derived by glochidia from the host fish during metamorphosis. The relationship between the quantitative and qualitative characteristics of the developmental success of unionid bivalves has not been studied in detail thus far. This study determines the possible differences in nutrition delivered to glochidia of two European unionid species with different levels of host specificity (*Unio crassus* and *Anodonta anatina*) developing on several potential host fish species. Juveniles were obtained, and the transformation success rate (the proportion of successfully developed juveniles) was determined after experimental infestations under laboratory conditions. The juvenile lipid reserves were assessed by an epifluorescence method (Nile Red staining) and compared among different host fish species and individuals. The juveniles of both species developing on different hosts significantly differed in their lipid reserves as indicated by fluorescence activity. Moreover, juvenile growth during the early post-parasitic stage was different among host fish species, congruently with the results of lipid quantification. These data indicate a positive association between the glochidia transformation success rate and juvenile vitality; nevertheless, a clear exception was also recorded. This study demonstrates that freshwater bivalves of the family Unionidae have not only a host-dependent survival rate during the parasitic stage but also highly host-specific juvenile performance. A more thorough incorporation of the qualitative measures of host fish values can lead to the further optimization of culturing protocols for unionid bivalves and to the improved management of their host resources.

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How to publish in *Aquatic Conservation* – pearl mussel papers as examples of what to do and what to avoid

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Aquatic Conservation: Marine and Freshwater Ecosystems (AQC) published its first issue in 1991. Now in its 25th year the size of the journal has increased from four issues per year, with an annual page budget of about 360, to six issues and 1210 pages. During that time the impact factor has risen from 0.5 to 2.14. The journal has an international readership; in 2014 papers were submitted from 55 countries around the world. During the lifetime of AQC we have published around 60 peer-reviewed papers on the conservation of freshwater mussels, most of them on bivalves, and many of those on *Margaritifera*. As with other leading journals in the field, competition for space is intense and for a paper to succeed in getting published several criteria need to be met. These include the quality of the science, relevance to conservation (clearly described), a discussion that recognises the application of the results beyond the confines of the study and the field setting, a standard of English appropriate to an international journal, a willingness to consider carefully the suggestions for revision made by the editor and the referees, and close adherence to the guidelines for presenting and formatting manuscripts. Although many species of freshwater molluscs are threatened by human impacts, and therefore clearly important to conservation, papers published in AQC will not merely describe another survey but will bring new insights to this important subject.

Genetic variation, structure and the complex histories of unionid mussel populations: A North American perspective on selecting source populations for propagation and augmentation.

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Freshwater mussels (Bivalvia: Unionida) are among the most endangered animals in North America. Understanding the genetic diversity of imperiled species across a large spatial scale is necessary for developing appropriate conservation strategies. The complex geologic and glacial history of North American river systems, makes this region ideal for understanding patterns of dispersal and evolution in unionid mussels. Mitochondrial sequences and/or microsatellite loci were used to analyze the population genetics of three common and widespread unionid species (*Lampsilis cardium*, *Lasmigona costata*, and *Quadrula quadrula*) from the Great Lakes and the historically connected Wabash, Illinois, and Wisconsin river drainages; hypothesized conduits for mussels from refugia south of the Pleistocene glacial maximum. Using standard methods, a fragment of the mitochondrial gene CO1 was sequenced and suites of microsatellite loci were genotyped. Mitochondrial DNA sequences resulted in 26 haplotypes for *Lam. cardium* and 18 haplotypes for *Las. costata*. Ten of the putative *L. cardium* haplotypes found in the Maumee (Great Lakes) and Wabash (Ohio River) drainages were revealed to be more similar to *Lam. ovata* sequences available on GenBank rather than *Lam. cardium*. *Lampsilis cardium* and *Lam. ovata* may be interbreeding in the drainage where they co-occur; analysis of microsatellite DNA genotypes may help to reveal if this is the case. Our findings suggest that both *Lam. cardium/ovata* and *Las. costata* entered the Great Lakes via multiple routes of entry, while Q. *quadrula* likely entered the Great Lakes only via a connection with the Illinois River. To conclude, a new project on the complex phylogeography and genetic structure of *Margaritifera margaritifera* in eastern North America has been recently initiated and preliminary results will be discussed. These results of studies are providing a greater understanding of large-scale genetic structure linked to the complex redistribution of mussels into new habitats following the dramatic climactic shift at the end of the Pleistocene and has important implications for recovering imperiled unionids.

We acknowledge the financial support by Fisheries and Oceans Canada, World Wildlife Fund Canada, and Central Michigan University.

Experiments to improve the survival and growth of juvenile *Unio mancus* (Unionidae) during the first months of life

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The Naiad Breeding Laboratory of the *Consorci de l'Estany* was created in 2010 to restock *Unio mancus* and *U. ravoisieri* populations in Lake Banyoles. The laboratory has been funded by two LIFE projects (LIFE 08 NAT/ES/000078 and LIFE 12 NAT/ES/001091) that helped develop facilities for the semi-captive rearing of naiads. The infection of host fish with glochidia was a success and up to 200,000 metamorphosed into juveniles in 2015. Nevertheless, the survival of these juveniles during the first months of life varied greatly (from 0.7 to 77%) in the different seed and fattening systems used (sediment in channels, pools and tanks). Factors affecting survival in conditions of semi-captivity include the growth of algae biofilm, the formation of carbonate and the action of predators (*Salaria fluviatilis* and *Procambarus clarkii*).

At present, the main laboratory challenge is to improve first-year naiad survival since we found that mortality after the first year drops significantly. Although the initial aim of the laboratory was to use a natural diet (food from water and sediment) when raising the mussels, experiments involving an external food source were also conducted. Experiments were performed to improve juvenile naiad survival. *U. mancus* were fattened by adding algae and debris to containers inside the laboratory under controlled conditions. These systems allowed us to control predation and to carry out regular monitoring of growth and survival. In a first experiment in 2011, juveniles were fed with different compounds or combinations (lake water, marine algae, biofilm, leaf extract) in 400-ml containers. Survival rates of 3–12% and lengths of 1.6–2.2 mm were obtained after 12 months of feeding with marine algae and leaf extract.

A second experiment in 2015 used a container with a 200-micron mesh at the bottom suspended in a 70-litre tank, with periodical renewal of natural water and marine algae supplied every 48 hours. Three different water circulation systems within the tanks were tested. The only effective system obtained individuals up to 3 mm in length at 120 days with survival rates of 40–65%. These results demonstrate the potential of this new fattening system that will increase the size and number of juveniles available for restocking in the wild.

A summary of the results obtained with the different systems employed in the fattening is presented in this oral presentation.

Pulsed flow-through systems for the laboratory culture of early life stages of freshwater mussels.

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Newly metamorphosed freshwater mussels are very small and delicate, so that captive culture presents challenges for handling, for maintenance of suitable microhabitat, water quality, and food, and for the avoidance of competitors and predators in culture. Two commonly used approaches to laboratory culture are sediment-free recirculating systems (mucket buckets) and static sediment boxes (Hruska boxes). Both of these approaches can give good results with some species but also have drawbacks. A third approach was pioneered this year at the USGS Columbia Environmental Research Center in Columbia, Missouri, when flow-through diluter systems used for toxicology experiments (similar to Mount and Brungs, 1967, Water Research 1:21-22) were adapted to deliver water and food to culture mussels. These diluter systems periodically refresh the solutions in a series of screened beakers (0.25-1 liter) containing juvenile mussels and a 1-3 mm layer of sand substrate. The sand is sieved to smaller particle size than the juveniles being cultured. Flow of water is provided in gentle pulses of 2-3 minutes duration, at 1-2 hour intervals, with each pulse of flow replacing the volume of the beaker by overflow through a screened opening. At other times the water in the beakers is static. Substrate conditions are maintained by sieving the juveniles from the sand at 1-2 week intervals and transferring them to clean beakers and fresh sand. Food is cultured marine microalgae, kept ice-cold until just before mixing with culture water and delivery to the beakers. The combination of periodic renewal of water, fresh food, mainly static conditions, and the presence of clean substrate for burrowing, has proven to give excellent growth and survival with a variety of taxa including *Lampsilis*, *Margaritifera*, and *Anodonta*. Another major advantage is the separation of groups into independent replicate beakers, reducing the likelihood of disease or blooms of competitors or predators affecting the entire batch of mussels being cultured. The small beakers are also easily removed for observation or for subsampling juvenile mussels. The approach is adaptable and alternative designs are available.

Effects of substrate size and cleaning regime on growth and survival of captive-bred juvenile freshwater pearl mussels, *Margaritifera margaritifera* (Linnaeus, 1758)

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This study tested a culture system for rearing *Margaritifera margaritifera* at the Freshwater Pearl Mussel Ark in Windermere and investigated the effects of substrate size (0.25-1 mm and 1-2 mm) and cleaning regime (weekly and monthly) on survival and growth.

At 362 days (12 months) and 758 days (25 months), a total of 1207 and 518 juveniles were reared successfully in the system described here. After 362 days, survival was significantly higher in 1-2 mm substrate treatments cleaned monthly (55 ± 6%) and lowest in 0.25-1 mm substrate cleaned weekly (14 ± 3%). Growth was significantly higher in 1-2 mm substrates cleaned weekly (length = 1.15 ± 0.21 mm) and lowest in 0.25-1 mm substrates cleaned monthly (length = 0.83 ± 0.23 mm). Survival rates in this investigation were comparable to, if not better than, other published studies culturing *M. margaritifera* of this age.

Juveniles from most treatments did not display size-dependent over-winter survival, but a significant correlation was found between shell length and survival in the 0.25-1 mm weekly treatment which was the least suitable treatment.

Additionally we examined the effects of flow rate, dissolved oxygen concentration, ammonia concentration and biofilm as factors which may affect growth and survival of juveniles between July - September 2015. These results provide an insight into findings from the initial work and discussion is offered on the challenges for practically applying this method to captive rearing at the Ark in future.

Improvement of the rearing success of freshwater pearl mussels (*Margaritifera margaritifera*) by using different cultivation systems

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In Austria, freshwater pearl mussels (*Margaritifera margaritifera*) have been reared on a larger scale since 2011 on behalf of the Upper Austrian government. In a breeding station 100 adult mussels – originating from two river systems – are kept in flow-through channels in order to have appropriate host fish infected with glochidia. Every summer, juvenile mussels are collected from the tanks in which the host fish are kept. From the beginning, the maintenance of mussels and fish, the infection and hibernation as well as the collecting of the juveniles and the first weeks of rearing at the laboratory turned out to work without major difficulties, whereas suddenly dropping survival rates in midsummer proved to be the crucial point of the project in some years.

In 2014, for example, a total of 40,239 juveniles were collected and transferred to climate chambers in which they were attended to thoroughly: water and food replacement was performed once a week, in the course of which moribund or dead specimens were removed. Despite that time-consuming care, all juvenile mussels died within a few weeks after having dropped from their hosts, assumedly to either bad detritus quality or an undetermined infectious disease.

In order to avoid such an event in the future, risk reduction measures were taken: Out of the 48,452 juveniles collected in 2015 only about 10,000 were kept in the climate chambers according to the aforementioned procedure. Additionally, great importance was attached to germfree handling of the juveniles, for instance by using different pipettes for handling different mussel containers or by decocting the containers in the course of water and food replacement, in order to avoid spreading pathogenic organisms. Survival rates turned out to be constantly high (with 90–100 % in the majority of the containers); if high mortality rates occurred, they were always restricted to single containers and did not encroach on others.

The other mussels were only kept in the climate chambers for a maximum of four weeks and then relocated to various cultivation systems: More than 25,000 juveniles were transferred to sand-filled channels constantly supplied with brook water, about 7,000 were put into wooden or acrylic glass boxes situated in appropriate brooks, and the rest was kept in aerated aquaria where daily water replacement was performed. Survival rates were checked at regular intervals in the latter two.

Various cultivation systems are currently also being used for larger juveniles gained from previous collecting cycles, among them wooden boxes, adapted Buddensiek-cages with larger compartments, and hemispherical silos with transversal flow.

The results of the different cultivation systems for both recently collected and larger juveniles will be presented at the meeting in Luxembourg in November 2015.

We acknowledge the funding of the project by the Environmental Protection Department (Federal Government of Upper Austria) and the European Union.

100 years of mussel propagation: What have we learned and where are we going?

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A century ago, mussel propagation in the USA began as an effort to sustain mass harvests of shell for the pearl button industry. In contrast, propagation efforts in the past 25 years have aimed at preventing extinctions. Simple rearing systems and artificial diets enable efficient laboratory production of juveniles, and systems for holding in raceways, rivers and ponds allow grow-out with natural food. Continuing advances are improving survival and growth rates. Since the 1990s, at least 18 North American resource agencies, universities, and zoos have developed conservation-oriented mussel culture programs. To date, 122 species have been metamorphosed on fish hosts or *in vitro*, 79 species have been cultured to several months of age, and several to sexual maturity. 49 species have been stocked for population restoration or augmentation. Releases since 2010 totalled well over 100,000 individuals and current annual production estimates are well over 1 million. Equally important is the use of propagated mussels for research, particularly in toxicology. Studies of sensitivity to ammonia, metals, ions, and organic pollutants have informed regulatory agencies, leading to major settlements against polluters and tightening of federal and state water quality criteria. Many challenges remain. Particular needs include continued research on physical and dietary requirements of species and on the potential positive and negative genetic impacts of stocking programs. Laboratory toxicology results can be extended to the field, using experimental releases or caged juveniles. Many ecological questions can potentially be addressed with propagated mussels, including effects of habitat, predation, population density, and host relationships in limiting recruitment. Stream ecosystems are increasingly fragmented. As populations become smaller and more isolated, their vulnerability will increase. Impacts of pollution, invasive species, and climate change are unlikely to abate. The capacity of resource agencies to rear, stock, and track released mussels is a necessity to manage mussels and prevent further extinctions over coming decades.

15 years of experience in semi-natural rearing of freshwater pearl mussels

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The decline of freshwater pearl mussels and the lack of natural recruitment resulted in various conservation and breeding programs. Despite the establishment of a successful breeding method in the Czech Republic more than 30 years ago, there is still a need of rearing efficiency to produce high amounts of mussels and to optimize methods for local conditions. The aim of the Landschaftspflegeverband Passau e.V. was to further develop the Czech rearing method for *Margaritifera margaritifera* to elaborate a generally applicable scheme.

The process to develop the method took about 15 years, during it was constantly adjusted. At present, 1000 brown trout are infested per mussel population. After the infestation in August the brown trout overwinter in ponds in a hatchery until beginning of May. At this time they are transferred into circular-flow tanks with a permanent flow-through and air sparging. During the time of excystment, juvenile mussels sinking to the bottom are separated by a horizontal net from trout which stay up in the upper part of the tank. The trout are fed twice a week in external tanks. The sediment material at the bottom containing the juvenile mussels is controlled and sieved once a week. After the weekly checks the juvenile mussels are returned to the tanks with the trout, where they remain until the end of July without any extra feeding. Afterwards, the final material collection, and a few backup samples before, are transferred and kept in mussel boxes, which are placed in a natural river system fixed at the river bottom. During the summertime these boxes with the juvenile mussels are monitored every 5-6 weeks. After the first month in the hatchery, mussels measured between 0.8 and 1.1 mm. Until their second winter they grow up to about 2.0 to 4.0 mm and in the third year they are about 4.5 to 9.5 mm long.

Despite the lack of exact numbers or calculation of surviving rates, the results indicate that this semi-natural method is very effective to rear juvenile mussels in high numbers and the success especially of the last three years underlines the high importance of this system for future pearl mussel captive breeding. The breeding programne of the Landschaftspflegeverband Passau e.V. will be continued and extended in the recently started project ArKoNaVera.

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15 years of experience in rearing *Margaritifera*: practical suggestions on bioindication.

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The freshwater pearl mussel (*Margaritifera margaritifera*) is considered an indicator organism for high quality freshwaters in northern and central Europe. The Vogtland, an area in south-western Saxony, Germany, is well known for its long-lasting tradition in pearl fishing, but the local mussel populations suffered by multiple influences (pollution, river degradation, agriculture and climate change), resulting in their near-extinction.

In October 2000, the ´Anglerverband Südsachsen e.V.´ (AVS Chemnitz) started breeding activities of the first mussels, adapting the method originally described by Hruska (Nahrungsansprüche der Flussperlmuschel und deren halbnatürliche Aufzucht in der Tschechischen Republik, Heldia, Bd. 4 (6): 69-79). We soon realized the necessity of developing parameters for a successful growth at a local scale. Different methods have been used for this purpose, and some of the practical manuals, circumstances and problems are described herein. According to the different stages (age-size classes) of the mussels, we try to compare growth rates between populations and rivers, as well as with respect to historical data.

In general, we have to conclude that we have not yet found a single river or tributary providing the same high quality environment as we clearly had a century ago when *Margaritifera* was still prospering at a few of the historical sites.

Within the new national project "ArKoNaVera" which is intended to protect and support existing freshwater pearl mussel populations in Saxony and Bavaria, we will try to improve the populations in Saxony by the above described breeding method. These actions will be accompanied by research partners with the objective, among other things, to develop specific tools for identification and optimisation of mussel habitats at a wider scale. It is also intended to study and compare the ecology of the endangered Painter's mussel (*Unio pictorum*).

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Propagation of the pearl mussel *Margaritifera margaritifera* in its natural habitat

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Since Gerhard Bauer in 1988 proposed to introduce artificially infected host fish into pearl mussel streams, numerous attempts have been made to artificially increase the reproduction and propagation of pearl mussels. Following the reproductive cycle, we report measures and efforts to rejuvenate pearl mussel populations directly in their natural habitat.

The collection of glochidia from pregnant mussels in most streams is facilitated by a regular and simultaneous development of the larvae. However, if the adult mussels are under pressure from, e.g., constant turbidity or eutrophication, the development tends to become irregular and badly synchronised, and often larvae are released prematurely, making it very difficult to secure larvae suitable for artificial infestation of host fish. Due to the ongoing decline of the resident adult mussel population, the culturing efforts had to be abandoned altogether in several streams, when it was no longer possible to regularly collect glochidia.

If suitable glochidia have been collected, a controlled and satisfactory infestation of host fish can be achieved as a rule by bringing them together with the larvae in a tank for a short time with continuous monitoring. The most natural and cost-effective method is to catch resident brown trouts by electrofishing, to infest them with glochidia immediately and release them into their natural habitat. With this cost-effective measure young mussels were established in a stream and even propagated upstream.

Young mussels were also released directly after their development on cultured trouts infested on a fish farm. Presumably this „mussel seed“ contributed to an estimated overall number of 1.700 juveniles at an age up to 8 years. The measures were started only after habitat restoration and subsequent investigations provided excellent water and sediment quality. In several other pearl mussel streams with incomplete habitat restoration, however, infestation of resident fish as well as release of “mussel seed” have not been effective so far.

We conclude that habitat quality and restoration is the key factor for successful rejuvenation and propagation of pearl mussel populations. Since juvenile as well as adult mussels - and hence the glochidia - are impaired by habitat degradation, the time to be gained by the culturing of mussels without ongoing, forceful and target-oriented habitat improvement might be very limited.

Saving six freshwater pearl mussel *Margaritifera margaritifera* populations in the Armorican Massif (France)

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The freshwater pearl mussel populations of the Armorican Massif are old and their numbers are declining at an alarming rate. Without proper protection they will likely to go extinct within the next 10 years. Therefore, the conservation of this species using rearing stations is essential to preserve and support their population in the wild. The Fédération de pêche du Finistère [local fishing federation] is actively involved in the conservation research for the pearl mussel on the Favot fish-farming site at Brasparts, where a rearing station was established as part of the LIFE Mulette programme (LIFE09NATFR000583) and specifically dedicated to this species. The rearing protocol is constantly being improved based on communications with other projects, their feedback from their research and our own observations. Proper predictions of how each strain will behave under artificial rearing conditions are difficult to make, which in turn makes it difficult to develop a standard 'turnkey' protocol. In order to restore the habitat quality in rivers, local authorities and associations making every effort and mobilizing all possible means for action. We hope that these rivers regain their original potential to host dynamic freshwater pearl mussel populations that evolved naturally with their host fish, such as the salmonids in the rivers of the Armorican Massif.

Innovations and advances in freshwater mussel propagation and recovery at the aquatic wildlife conservation center, Virginia

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Freshwater mussels are some of the most threatened taxa in North America and more than 80 species were once found in Virginia with 42 extant and 7 extirpated species in the Tennessee River drainage in Virginia. Following a spill in the Clinch River in 1998 the Aquatic Wildlife Conservation Center was built to recover species lost or impacted in this drainage. Since its construction, the facility has endeavored to develop new techniques to first propagate then culture these rare species. Recent innovations include a move towards increased seasonal use of natural sources of food through ½ and ¼ acre pond systems. These include systems in the ponds (Floating Upweller Systems and baskets) and systems along the ponds, which employ filtration, that hold both newly transformed juveniles and larger sub-adults. These external systems include upwellers, downwellers and substrate-filled tanks. Juvenile culture has been significantly improved by the outdoor systems resulting in faster growth and improved survival. The systems accommodating newly transformed juveniles have resulted in the first successful culture of *D. dromas* and *L. rimosus* at AWCC as well as the first large-scale grow-out of *Cyprogenia stegaria* and *Lasmigona holstonia*. Survival and growth have also been improved through the transition from flow-through to recirculating RU (rearing unit) systems for the over-wintering of juvenile mussels.

Juvenile Augmentation – a short term breeding approach for *Margaritifera margaritifera*

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The Nore freshwater pearl mussel *Margaritfera durrovensis* is classed as a separate species under Annex II of the EU Habitats and Species Directive. Surveys undertaken since 1990 indicate that successful recruitment of young in the wild has not occurred for 40 years. A captive breeding programme has been undertaken since 2005 but juvenile survival beyond 2-3 years had not been successful enough to significantly assist in the rehabilitation of the population. Recent ecological studies have demonstrated the high near bed velocity requirements of *Margaritifera*, and redox potential studies have assisted in identifying river bed habitats in which juvenile mussels can survive. Thus key receptor sites offering the best survival opportunities for juvenile mussels can be targeted. In July 2013, 10 River Nore mussels were brought into captivity and kept in a tank with native brown trout *Salmo trutta*. In May 2014, the fish with encysted glochidia were transferred to a circular tank with plastic lining and a layer of clean local small gravel. In June 2014, the juvenile mussels dropped from the fish into these gravels. During the same period, a river survey identified 5 suitable receptor sites in the River Nore, Ireland. In July 2014, the juvenile mussels and gravel substrate mix was divided into seven bags and transferred to 7 excavated quadrats within the 5 areas that were identified as being suitable for juvenile survival. A sample from one receptor quadrat taken one year later (July 2015) contained live one year old juvenile mussels. In the long term, sustainable catchment management is required in order to provide conditions for long term natural levels of recruitment. This new system of assisted breeding is considered to have good potential application in the maintenance of *Margaritifera* populations where the catchment is not yet rehabilitated sufficiently to provide conditions for successful bankside encystment, but have some suitable juvenile habitat that can support young mussels in their early years.

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Identification of receptor sites for captive bred freshwater pearl mussels (*Margaritifera margaritifera*)

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Rearing the freshwater pearl mussel *Margaritifera margaritifera* in captivity is proving to be a high cost for low return enterprise. In many countries across Europe, considerable funding has been directed towards the provision of suitable infrastructure and man-management for these projects. The juvenile mussels produced in these rearing facilities are, therefore, extremely valuable, and there is often a reluctance to release captive-bred mussels back into the wild. It is imperative that these precious animals are given the best chance of survival when re-introduced and thus, identification of the best habitat which gives them the greatest chance is paramount. This is especially important in rivers where conditions have deteriorated to such an extent where suitable juvenile habitat is likely to be extremely restricted.

A new project has commenced in England to map out and identify juvenile habitat in a river with a large mussel population but with very little recruitment. Whilst still under development this method is also being piloted in Irish mussel rivers and has provided the receptor site selection criteria for Moorkens' successful juvenile augmentation work in the River Nore (ref. this seminar).

A series of transects are set-up and are assessed on a metre square basis, and each square is allocated a category for juvenile habitat presence and for juvenile habitat quality. Then assessments of fourteen parameters are made, twelve of which were visual, one a count (adult mussels) and one a measurement (depth). These reflected the overall assessment of physical habitat presence and condition as they all related in some to an assessment of the key combination of ideal juvenile habitat, which is the presence of coarse sand or fine gravel in a stable, wide combination of larger clast size range, with low quantities of fine sediment and low nutrients.

The results of the pilot studies have shown that transects needed to be relatively dense in order to provide sufficient accuracy for mapping. Higher mussel numbers, faster (but not very fast) velocity, wider substrate mix, and presence of sandy gravels in the lee of boulders were all positively associated with quadrats assessed as having juvenile habitat. Polygon outlines are drawn to show how the areas of good and potential juvenile habitat could be interpreted to allow for digitisation to GIS polygons and subsequent estimation of areas of each category.

Development of an intermediate culture system for the release of the globally endangered freshwater pearl mussel, *Margaritifera margaritifera*.

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The globally endangered freshwater pearl mussel, *Margaritifera margaritifera*, has been facing declines throughout much of its Holarctic range over the course of the 20th century due to habitat degradation and lack of suitable fish hosts. A number of *ex-situ* breeding facilities have been developed to try and reduce these dramatic declines. Ballinderry Rivers Trust has proved itself successful in rearing this species under semi natural conditions for nearly 20 years. Mussel silos (Barnhart et al., 2007) were trialled in the release of hatchery-reared juvenile mussels back into the Ballinderry River, examining the survival and growth rate between the different size classes, release sites and treatments. Size classes being used were class A 13.00-21.00 mm; class B 10.00-12.99 mm and; class C 4.00-9.00 mm. Two release sites were used, one on a small tributary (Site 1) and the other on the main Ballinderry channel (Site 2). Two treatment methods were also used; sediment present in the enclosed silo chamber (sediment) or the chamber was left empty (no sediment). Four individuals per size class were used in the silos (12 mussels per silo). 10 silos were deployed at each site; five with the sediment treatment and five with the no sediment treatment. This study has shown a clear difference between size of released mussels and survival, with the largest size class A having the highest survival (3%mortality) and the smallest size class C having the lowest (39% mortality). Release site played an important role in the survival and growth rate, with the highest survival and growth rate being found on Site 2. Growth rate was observed to be 4.3 times higher over the summer period than winter. Treatment was found to have no effect on the survival and growth of the mussels. High mortalities in the smallest size class C may be due the feeding phase the juvenile mussel is in, they may have not made the transition from pedal to filter feeding by this point and the mussel silos are limiting their ability to feed enough. This study reinforces the importance site selection plays with the site with the highest survival (Site 2) having the higher water temperatures and lower levels of siltation making conditions more suitable. This study would suggest this method isn't best suited to smaller mussels but could be useful when mussels are larger than 10 mm in an appropriate site.

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Bending rivers by mussels

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The rivers in the Baltic region have undergone drastic changes during the past centuries. Major consequences of anthropogenic disturbances (such as pollution, channelization, dredging and hydro power activities) result in habitat deprivation, fragmentation and loss of ecological connectivity, threatening aquatic biodiversity, water quality and ecosystem services, in freshwater environments, as well as in the Baltic Sea.

Here, we present results and achievements from a multidisciplinary LIFE-project aiming for improvements of habitat conditions and water quality, in twelve rivers and in the Baltic Sea, by using the thick shelled river mussel (*Unio crassus*) as the major tool. The presentation summarize main achievements related to river restoration measures by using *Unio crassus* as the preferred target and indicator, and serve as an introduction to the related two presentations dealing with local adaptation (L. Schneider) and rearing techniques (M. Österling) by *Unio crassus*. Finally, the "LIFE of crassus" movie will be shown for the first time ever.

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Local adaptation studies and conservation: The relationship of *Unio crassus* with two of its sympatric and allopatric host fish species

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The study of host-parasite systems offers interesting opportunities to explore coevolutionary dynamics. Antagonistic interactions driven by reciprocal selection pressures often result in parasitic local adaptation. In theory, parasites are predicted to have advantages in adapting to their local hosts if they have shorter generation times, larger population sizes and higher migration rates. As a result, parasites often show higher infection success on sympatric hosts than on allopatric hosts. Freshwater unionoid mussels have a life cycle including a larval parasitic stage on fish, but surprisingly, local adaptation in this host-parasite system has barely been studied. Unionoids are relatively long lived compared to their host fish. Still, local adaptation to sympatric host fish species, resulting in large numbers of juvenile mussels hatching from fish, would be important for growth and survival of mussel populations. Since many of the unioinoid mussels species are highly threatened, increased knowledge of local adaptation patterns may be helpful for conservation, particularly when reintroducing mussels or their host fish species. *Unio crassus* is Europe's most threatened unionoid mussel species and considered to be highly valuable for conservation. To investigate patterns of local adaptation of *U. crassus*, we conducted a common garden experiment where we cross-infested the European minnow *Phoxinus phoxinus* and the bullhead *Cottus gobio* from two rivers, with their sympatric and allopatric mussel larvae. Interestingly, juvenile excystment from *P. phoxinus* was highest in a sympatric mussel-fish combination in one river, but not in the other. For *C. gobio*, juvenile excystment was highest when infested with allopatric mussels and was two-fold higher than found in all other mussel-fish combinations. The results thus do not meet the assumptions of local adaptation theory for *U. crassus* using the host *P. phoxinus* and there are maladaptive signs to *C. gobio*. Thus, our results show that patterns of juvenile excystment can vary between rivers and species. Our study therefore suggests that conservation incentives, such as juvenile production and reintroduction of *U. crassus* gains from studies of host fish suitability and local adaptation.

Rearing-related activities of *Unio crassus* – which host fish species to use?

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One aim within the EU life project "*Unio crassus* brings life back to rivers" is to re-introduce mussels into rivers where they are now extinct. Juvenile mussel rearing with the aim to produce high numbers of juvenile mussels is a common measure that is used for many mussel species. Host fish is then artificially infested with mussel larvae, whereupon the fish is kept in aquaria until the juvenile mussels release from the fish. Here, we tested many fish species, and compared their suitability as mussel hosts at several rearing-related activities. We compared methods to catch fish in the field, and in the lab we measured encapsulation and juvenile excystment rates, handling efforts, and fish survival. Using these measurements helped us to develop a tool to discriminate among host fish species. The results can be used when planning re-introduction activities in relation to the fish community composition in rivers where mussels are to be re-introduced.

New information on the habitat requirements of *Unio crassus* as a basis to optimize supportive breeding

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The thick shelled river mussel *Unio crassus* is a highly endangered freshwater mussel species in Central Europe. As a result of dramatic population declines during the 20th century, it is estimated that the species has lost 50 % of its original distribution. Currently applied conservation strategies often include both restoration of habitats as well as supportive breeding, despite a lack of information on the autecological and synecological requirements of the species. Herein, we present an integrative approach that identified key habitat parameters of *U. crassus* including flow regime, substrate composition and fine sediment deposition, water chemistry as well as host fish suitability and availability. Shear stress, flow velocity and penetration resistance of the stream substrate were examined in relation to mussel presence/absence data in functional streams. Physicochemical substratum and water quality was measured in a stream with recent recovery and recruitment. Host fish suitability was assessed in artificial infestation experiments in combination with fish community analyses to test host availability under natural conditions. The results of this study show that densely colonized stream patches were characterized by low flow velocities (< 0.3 ms-1), low mean penetration resistances (0.36 ± 0.52 kg cm-2) as well as by low bottom shear stress compared to non-colonized sites. Mussel density was significantly negatively correlated with bottom shear bed forces (Ncm-2). At sites with juvenile recruitment, fine sediment deposition was high with 19.4 kg/m²/month. Results of chemical water analyses indicated high nitrogen loads in a range of 4.1 to 6.5 mg NO3-N/l. In terms of host fish analyses, the laboratory infestations identified seven host fish species for *U. crassus*, with host specific glochidial development times. In natural *U. crassus* streams, sixteen fish species were found and fish community structure and densities were highly variable. Our results indicate that – despite its conservation status *U. crassus* has a broad niche width for habitat concerning hydrological and substrate characteristics of the stream. Interestingly, high mussel densities and mussel banks were predominantly found in areas with low flow velocities. These areas are presumably most crucial during the reproductive cycle, where gravid mussels change their location towards the stream bank to release their glochidia and thus have a higher risk of being displaced at high flows. The greater tolerance of *U. crassus* to adverse substratum and water chemical conditions should also be considered in supportive breeding and propagation programs.

Conservation of threatened fish and mussel populations in northern rivers of Portugal (Douro Basin)

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The Mediterranean areas are globally identified as ecosystems with higher biodiversity. Among them, aquatic ecosystems are reported to be particularly fragile and several autochthonous species are threatened and in extinction risk due to negative human activities. In two northeastern watercourses of Portugal, Sabor and Tua rivers (Douro Basin), large areas are suffering a big reduction in the natural lotic habitats, namely after the construction of two big dams. For this reason it is crucial to include mitigation and conservation measures on the areas outside the influence of those dams. This would include the rehabilitation of the disturbed lotic reaches of these rivers and the protection of habitats and ecosystems with good ecological integrity, as well as the development of monitoring and applied conservation programs for those organisms. The national Project "SOS (Save Our Species): threatened fish and bivalve populations of northeastern Portugal" was designed to: 1) promote in-situ conservation plans for mussel and fish species; 2) define the adequate methodologies to the ex-situ reproduction of the target species, and 3) develop environmental education in an Aquaculture Station, belonging to the Forest and Environmental National Services (Posto Aquícola de Castrelos - ICNF). The selected target species of this project were two Iberian endemic cyprinids, *Squalius alburnoides* and *Achondrostoma arcasii*, and 4 autochthonous mussel species (Unionoida) *Margaritifera margaritifera*, *Potomida littoralis*, *Unio delphinus* and *Anodonta anatina*.

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Age, growth and demography of several populations of *Unio mancus* and *U. ravoisieri* in northeast of Catalonia.

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In northeast of Catalonia, several isolated populations of *Unio mancus* and *U. ravoisieri* remains, despite the wide and big decline that these species have suffered during the second half of the XX century. Main known populations occur in the rivers Ter and Fluvià basins. Along last three years, we have surveyed eight of these populations, occurring in rivers, diverting channels and Banyoles Lake. We compare age, growth and demography to analyse the implications for the conservation of both species. Age has been determined by means of lectures of annual marks on the shell, combining an external identification of youngest marks, and an internal counting over a sectioned shell for the older marks on big shells. This method allows more reliable age determinations for both species. Growth is described on the basis of adjustments of the Von Bertalanffy Growth Model (VBGM). Parameters estimations obtained by means of these adjustments are used to compare growth patterns of the analysed populations. Demography is analyzed over the age structure of each population. Mortality rates for the well represented fraction on the samples are also obtained. We approximate the present recruitment pattern by the relative abundance on samples of young and medium aged individuals.

Maximum longevity observed for the whole area is 27 and 29 years for *U. mancus* and *U. ravoisieri*, respectively. Growth rates (*K* parameter from VBGM) range from 0.11 to 0.22 $year^{-1}$ in *U. mancus*. However, in low land channels they have faster growth respect the rest of populations situated in medium high mountains, despite they achieve lower maximum ages. Maximum length is always higher in channel populations for this species, regardless of the situation of these channels (low land plain medium high mountains), often achieving the maximum length described for both species (11cm). The only known river population of *U. ravoisieri*, show an estimated growth rate (*K*) of 0.10 $year^{-1}$. For both species there's a strong evidence of the existence of a negative relation between longevity and growth rate.

In relation with demography, both *U. mancus* and *U. ravoisieri* show similar general patterns. Best preserved populations in some small rivers have a stable population structure, with evidence of regular recruitment. In contrast, populations situated in channels and in the Banyoles lake are aged, with a total lack of recent recruitment or at most a very sporadic and low recruitment. Thus, most of these populations are doomed to vanish. However, meanwhile some of this channel or lake populations show lowest mortality rates of old fraction, and so oldest registered individuals for the overall study area. That seems a clear effect of habitat high stability, due to stable water level, and also water conditions, and also due to the absence of natural big disturbances as river floods. Now, situation in Banyoles Lake has improved substantially thanks to the release of individuals coming from a captive reproduction program, among other conservation measures, all them initiated in the context of a project LIFE (LIFE08 NAT/E/000078).

These results emerge from several surveys and monitorings performed in the context of LIFE Potamo Fauna (LIFE12 NAT/ES/001091), and other conservation projects funded by "Diputació de Girona".

5 ABSTRACTS (POSTER PRESENTATIONS)

Perspectives of conservation and reintroduction of *Unio crassus* (Philipsson 1788) in Latvia

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Unio crassus is distributed over the territory of Latvia sporadically, but very irregularly and data about size and age structure of the population are insufficient. Data of the project EMERALD showed that only in 23 of 674 protected areas of Latvia *U. crassus* was found, all other populations occur outside of protected areas. This makes the research of distribution, state of population and habitats and possibility of reintroduction of the *U. crassus* in Latvia actual. The research of distribution of the *U. crassus* and state of its habitats was made together with I.Čakare, M.Rudzītis, I.Miķelsone, E.Parele within the framework of development of the Action Plan (Rudzite et al, 2010) and as its implementation until 2015. The parameters studied in field were: distribution, sizes, population density, and the condition of water and of the habitats. In the result of the research and for coordination of protection of the *U. crassus* in Latvia the Action Plan (Rudzite M *et al,* 2010) was developed in 2010, which describes threats for the species and its habitats and recommends conservation activities. The main negative anthropogenic factors for habitats are: pollution of rivers by waste waters; transformation of habitats in the result of building and work of small water power plants, which fragment and isolate the populations. The main negative natural factor is activity of *Castor fiber*, which overlaps small rivers by damps. Therefore the Plan suggests restoration of habitats of these small rivers and limiting of quantity of beavers in the habitats, enforcement of population. In the aquaculture laboratories of Daugavpils University the possibility of establishment of the *U. crassus* aquaculture was evaluated. *U. crassus* reintroduction using animals from aquaculture is offered in the result of the study. The aquaculture requires scientific, veterinary, and technical support and developing the zooculture of fishes – hosts. As material resources for the aquaculture laboratories of Daugavpils University will serve. It's planned to release the *U. crassus* within 6 – 10 years both at the stage of parasitizing glochidium and after it. As potential places of the first releasing are chosen small rivers, side streams of the river Daugava, which are located on the territory Natura 2000 Augsdaugava in the south-east part of Latvia.

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Variability within pearl mussels populations and their lifecycle (in captivity)

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The Freshwater Biological Association is the only organisation in England to maintain and study adult and captively-reared juvenile freshwater pearl mussels (FWPM) over the last 8 years. At the FBA facilities in Windermere, six populations of *Margaritifera margaritifera* are held in an ARK. Their home rivers are spread over a wide range of the UK with different water quality and chemistry as well as habitat conditions. The mussels at FBA are provided with water from Lake Windermere. Maintenance has been successful since they have managed to complete the whole reproductive cycle and viable juveniles have been collected every year. Over eight years, each population showed distinct differences from each other in its lifecycle; including the preference for host fish, mussel spawning times and encysted glochidial growth rates.

These marked differences between FWPM populations have been examined and our initial results are discussed in the poster. Further study is required but it is already clear that many differences need to be considered and better understood in culture and introduction of mussels or when restoring their habitats, in order to maximize the effectiveness of the actions taken to protect the species.

This work highlights the importance of the ARK; not only as a mechanism to safeguard the FWPM species but to preserve/retain the different existing populations which are currently under acute stress in their natural habitats, prior to introduction to their restored, ancestral rivers.

LIFE13 BIO/FR/001162: Grande Mulette /Conservation of the giant pearl mussel in Europe

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Since July 2014, the University of Tours and the General Departemental of Charente Martime, France, cooperate with partners in France, Spain and Germany to preserve the last populations of the "big sister" of the pearl mussel. Less sensitive to water pollution than the smaller species, *Margaritifera auricularia* lives in potamon major streams and rivers. This population, however, suffered considerable losses due to habitat degradation and death of its host fish, including the European sturgeon. Our project seeks to overcome the bottlenecks for conservation, namely a high mortality of juvenile mussels in the anoxic sludge buildup on the site harboring the largest population in the Charente, and to seek alternative hosts fish species.

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Habitat stochasticity and metapopulation structure as a problem for freshwater mussels conservation - the case of the *Unio crassus* in the Biała river (Poland)

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The Biała river harbors one of the most important populations of *Unio crassus* in Poland. The distribution of the species within the river is related to the channel slope - the highest density and continuous distribution of population overlap with the river section of the smallest slope, whereas the reaches of higher slope contain fragmented mussels' sub-populations, which frequently disappear. Upper part of the river, was isolated in the past by dams and *U. crassus* died out there, due to water pollution. In 2012 we restored the species occurrence there, in a series of stepping stones, in order to speed up re-colonization of the upper reach after rebuilding of the dams. For the next years we observed the process of young dispersal and dynamics of adults populations introduced into the stepping stones. At present, almost all these stepping stones are destroyed, whereas the species persisted due to juveniles dispersal to new microhabitats.

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Habitat requirements of Unio crassus in the Biała river (Poland): Hydrology, substrate, chemistry and fish

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The Biała river flows in S-Poland, from the Beskid Niski Mts. to their forehills (N Carpathians). It harbours the largest estimated population of *U. crassus* in Poland (200 000 ind.). In the past it occupied much larger river section, but almost half of the population died out due to water pollution and river fragmentation by dams. In a longitudinal hydrological profile, the present population occupies the flattest part of the channel, where the clean tributaries join the river. The population number correlates with occurrence of fine sediments, and high pH values. It occurs outside the range of the most suitable fish hosts. In a cross sectional profile, individual mussels inhabit only still water margins. The young recruitment varies between years, showing no correlation with local population number. Numerous individuals occupy also the regulated reaches of the river, although are sensitive to water level changes.

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The first year of *Margaritifera auricularia* (Spengler, 1793). Breeding in captivity in Aragon, Spain

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The objectives of this work were to find out the best rearing conditions for *Margaritifera auricularia* juveniles in order to obtain a size at which their survival in the wild will be more likely.
For the breeding in captivity we obtain glochidium from several adults, collected from the Canal Imperial de Aragón (Ebro river basin). Once the juveniles were obtained, the technique of "plastic boxes" was implemented. This original procedure was developed in Luxembourg, which works with the species *M. margaritifera* (Eybe *et al*., 2013, Aquatic Conservation: Marine and Freshwater Ecosystems, 23-6: 964–975).
Multifactorial experiment was arranged to test the effect of: substrate, commercial algae, extra oxygen and detritus. The best rearing results were achieved with a combination of river water, substrate between 0.4 and 2 mm., detritus obtained by river's edge; and commercial algae: Shellfish Diet1800® and *Nannochloropsis* sp. 200 newborns were placed inside each container. Each month the amount of algae was increased. The cultures were kept in dark conditions under controlled temperatures between 18 and 20° C. Weekly culture progress was checked. The ammonium and nitrite levels were controlled by replacing the river's water at the end of each week.
Cultures were set up in May 2014; although the survival rate was low at the end of the year, these young individuals have reached maximum measurements of 3.5 mm. long and 2 mm. high, in a year-long observation period, representing a growth of over 2000% since birth.

Investigations into feeding structures of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) through scanning electron microscopy

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Very little is known about the morphological changes juvenile freshwater mussels undergo during their first few years and how these affect growth and survival and therefore success in culture systems. Of particular interest is the stage when juveniles transform from pedal- to filter-feeding and the morphological changes of the gills and associated feeding apparatus at this time. This investigation provides a novel insight into biological development in three different age classes of the freshwater pearl mussel, *Margaritifera margaritifera*: newly excysted (pre-transformation), 10-20 months old (around the time of transformation) and 3 years old (post-transformation).

Using scanning electron micrographs the ultrastructural features of juveniles is described at these different developmental stages, focussing particularly on features of the gills such as: overall structure of lamellae/gill buds, formation of the inner and outer demibranchs, development of cilia and cirri, gill reflection and development of the oral groove. Structure of the mouth, labial palps, foot and mantle were also considered.

More information on the timing and type of morphological changes during this period is required to understand not just the basic biology of juvenile mussels but also how changing morphology can affect dietary requirements, juvenile growth and survival and ultimately how we deliver captive rearing activities for conservation of this mussel species.

Risks associated with the maintenance of rivers outside protected areas – the case of *Unio crassus* in a tributary of the Biebrza river, north-eastern Poland

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In recent years, in response to the possibility of obtaining funds for the so-called flood protection and river maintenance, works on deepening of riverbeds have intensified. In areas not included in any form of nature protection, rarely there are any doubts about the legitimacy of such treatments and their possible negative impact on the biota of the watercourses. In 2015, in the area adjacent to the Biebrza National Park, we carried out a malacological survey of three tributaries of the Biebrza River: the section of the Sidra River in which the deepening of the riverbed had been planned, and the Kosódka and Brzozówka Rivers, which were deepened in 2014. In the dug sections of the rivers, in most of the surveyed sites we have not found any unionoid mussels; only in the river Brzozówka few individuals of *Unio pictorum* and *Anodonta anatina* were present; we found one *Unio crassus* individual. In the undamaged section of the Sidra River, *U. crassus* was abundant; all other unionoid mussels occurring in Poland were also present: *A. anatina*, *A. cygnea*, *Pseudanodonta complanata*, *U. tumidus* and *U. pictorum*. *U. crassus* occurred on sandy-gravel bottom in places with fast water flow, reaching the density of 0.5 - 7 ind. / m^2. The average length of the shell (± SD) was 60.8 ± 9.5 mm, and the average weight of live animals (± SD) was 36.9 ± 17.4 g (n = 182). The presence of individuals with large body size (maximum shell length - 81 mm) and the presence of juveniles indicate a good condition of this population. In response to our report of this survey submitted to the Regional Directorate for Environmental Protection, the planned deepening of the riverbed has been suspended giving a chance to save these mussel populations. However, throughout the country, the pressure to carry out such works is very high and a lot of effort to protect natural mussel habitats is needed.

Ex-situ breeding of native unionids in Lake Banyoles (Spain) as part of a LIFE project

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Lake Banyoles is the second largest natural lake in the Iberian Peninsula and is one of the first places in the Iberian Peninsula where massive planned fish introductions have taken place. The proliferation of alien fish species explains the current scarcity of the native species that are the natural hosts of the parasitic larvae of unionids. This situation has led to a dramatic regression in the four native unionid species found in this lake (*Unio mancus*, *U. ravoisieri*, *Potomida littoralis* and *Anodonta anatina*).

As a result of management geared towards the preservation of natural heritage, the ecological quality of this site has improved in recent decades. However, the main challenge for the management of the lake and its surrounding areas is now posed by invasive alien species. Projecte Estany (LIFE 08/NAT/E/000078) was a four-year project (2010–2013) whose main aim was to design and implement a large-scale intervention actions that would combat, slow down and revert the decline in species and habitats of Community Interest via (1) the control of invasive alien species, (2) the strengthening of populations of seriously threatened native species, and (3) the restoration of key patches of riparian habitats. As part of the project to recover populations of two Unio species, a breeding laboratory was set up. During the first years of the project, several methodologies were tested to obtain mussel glochidia (larvae), optimize fish infection and design a methodology for fattening juveniles. Up to three different native fish species were tested as hosts for local Unio larvae: *Barbus meridionalis*, *Squalius laietanus* and *Salaria fluviatilis*. All demonstrated their capacity to act as hosts and release viable juveniles; however, due to their larger size, only the first two species are used for intensive mussel breeding, along with *Luciobarbus graellsii*. Between 2011 and 2013, a total of 108,875 *U. mancus* and 27,423 *U. ravoisieri* juveniles were produced via the artificial infection of host fish with larvae grown in a number of semi-natural sequential breeding systems fed by water and sediment from their natural habitats, pools, plastic outdoor channels, and/or cages. In the tested systems, *U. mancus* reached a mean length of 9.7 mm (SD_1.53) in one year and 12.4 mm (SD_1.55) in two years; for U. ravoisieri these values were 15.8 (SD_0.76) and 21.2 mm (SD_2.45).

In the context of the further four years (2014-17) allotted to the project (LIFE Potamo Fauna, LIFE12 NAT/ES/001091), efforts to breed Unio species in this laboratory, which has been enlarged, will continue. We will describe the results of the different juveniles' collection systems and the facilities created to improve their growth over the past six years. The protocols implemented, the current installations and the results of the captive breeding of Unio species are shown here.

Restocking of Lake Banyoles (Girona, Spain) with juvenile naiad (Unionidae) born in captivity

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The Naiad Breeding Laboratory belonging to the Consorci de l'Estany, funded by two consecutive LIFE projects (LIFE08 NAT/E/000078 and LIFE12 NAT/ES/001091), was created in 2010 as part of the project to restore populations of *Unio mancus* and *U. ravoisieri* in Lake Banyoles. Its facilities are used for semi-captive breeding of naiads. The main goal of this laboratory is to obtain juveniles for restocking suitable habitats in the basins of the rivers Ter and Fluvià, as well as Lake Banyoles.

From 2011 onwards, three different systems were used to release juveniles of Unio into the lake: 1) small juveniles (<0.3 mm) were directly seeded in cages with natural sediment that were then placed in the lake and its outflow channels; 2) one-year-old naiads (10–12 mm) were seeded using the same system; 3) two-year-old naiads (25–30 mm) were directly seeded into the natural sediment at the bottom of the lake and its outflow channels. The two-year-old mussels were individually marked with a code. Using the first system, over 6000 juveniles were seeded; however, only 100 and 502 young naiads were seeded using the second and third systems, respectively. The results of the first system were wholly negative in the lake, although some live individuals were recovered two years later in the outflow channels. All the naiads released using the second system were predated, probably by carp (*Cyprinus carpio*) and pumpkinseed (*Lepomis gibbosus*). Finally, the two-year-old naiads (third system) had differing survival rates depending on the area in which they were released. We only recovered individuals in two out of six seeding sites – in 2015, two years after release – but they had notably increased in length. We conclude that there is important predation pressure on young naiads, probably from species such as Louisiana crayfish (*Procambarus clarkii*), freshwater blenny (*Salaria fluviatilis*) and carp, or even certain aquatic birds. Thus, in places with a stable presence of carp or other potential predators of molluscs, we recommend that restocking is carried out with naiads over 40 mm in length or, alternatively, that they be released into habitats with natural refuges (rocks, vegetation, etc.). Given that, if they survive, juveniles grow faster whenever directly seeded into natural habitats, we are now experimenting by retaining them temporarily in cages with protective nets.

The release of fish infested with glochidia into the wild is another system that is used for restocking natural systems. Although not as such a restocking system using juveniles bred in captivity, it is still a widely used method. This system has been especially successful in the lake's outflow channels, where huge increases in juvenile recruitment have been achieved. This poster shows the results obtained by these three different systems.

Male or female? Molecular sex identification of the thick-shelled river mussel *Unio crassus*

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Assessment of an individual's sex is usually difficult in species with little sexual dimorphism or with internal gonads, as in mussels. However, information on sex and sex ratios is often critical for any efforts towards conservation and culturing of freshwater mussels. Nowadays, molecular techniques offer a relatively simple and reliable alternative to morphological sex identification. We developed the molecular sex identification method for *Unio crassus* using male-specific M-cox1 mitochondrial marker. Appropriate primers were designed. Total DNA was extracted from foot tissue, which can be sampled by biopsy. The sex of individuals was verified using microscopic examination of gonadal tissues This method resulted in roughly 100% assignment of 40 specimens examined, and thus, facilitate non-invasive and reliable sex identification.

Morphopathological changes in gills associated with experimental glochidiosis in Atlantic salmon (*Salmo salar* L.)

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Margaritifera margaritifera (Linneo, 1758) is considered one of the most imperilled freshwater mussels since its population has been declining dramatically across its range. Therefore, in the last years a large number of captive breeding programs have been launched in Europe relying on the artificial infestation of salmonid hosts. As a consequence, most studies have focused on the survival and production of the juvenile mussels or on the susceptibility of different fish species. However, the development of the glochidia in the fish received relatively little attention. It is assumed that the glochidia are a temporal parasitic burden in the host gills. In this sense, histopathological studies represent a useful tool to enhance the knowledge of host-parasite relationship which may contribute to successful conservation programs.

The purpose of this study was to evaluate the morphopathological changes in the gills of Atlantic salmon (*Salmo salar* L.) parr due to an experimental infestation with *M. margaritifera* larvae. Glochidia load was set at 6000 per fish. A total of 128 salmon were collected in a time frame ranging from a few hours to six months after infestation. Fish were selected taking into account the highest degree of infestation. After euthanasia, gills were fixed in Bouin fluid and processed for histopathological procedures. The routine staining hematoxylin and eosin, and PAS were performed.

In general, gills showed multiple glochidia distributed over the filaments, some of which were twisted and presented an anomalous orientation. At microscopic level, affected filaments showed an increased thickness and sometimes were blunted and fused. Glochidia were evident, within a cystic cavity surrounded by proliferating and hypertrophic epithelial cells from lamellae, which were often fused. Sporadically, mononuclear inflammatory infiltrates were also present, surrounding glochidia and within the filament blood vessels underneath this reaction. In later stages of infestation, gills apparently displayed less but more developed glochidia. Nevertheless, some degenerated-like cysts were perceived showing an irregular surface and loss of the larva inside. These empty cysts were often associated to an evident inflammatory reaction of the lamellae. Our preliminary findings suggest that salmon gills respond to the presence of glochidia through branchitis of diverse degrees of severity. It remains uncertain whether the host immune cells reaction may be related to the sloughing of the parasite in the final phase of infestation. Further research is required on the mechanisms involved in host-parasite interaction, which play an important role in this crucial stage of development of *M. margaritifera*.

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Experiments on reinforcement and in-situ rearing systems of the pearl mussel (*Margaritifera margaritifera*) in the Armorican Massif (France)

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The objective of the LIFE Mulette program (LIFE09NATFR000583) is the conservation the six major pearl mussel populations in the Armorican Massif. One of the problems identified here is the lack of juvenile recruitment. A rearing station has been built and habitat restoration activities started on rivers. The first population reinforcements began in 2012. We used cylindrical tubes of stainless steel mesh to test the survival and growth of some of the young mussels released in 2014. In each stream, 12 mesh tubes (with a mesh size of 0.42 or 0.8 mm) each containing five one-year-old mussels were installed for 3 months (60 mussels / stream). The survival rate varied from 6.67% to 95%. The size of the mesh of the tubes could have an influence on mussel survival. The average growth was about 0.5 mm for the rivers in Brittany. For rivers in Lower Normandy, very few measurements could be made because of the low survival rates.

It is possible that these results could have been skewed because: (i) the tube installation method was different from those in Brittany and Normandy, and (ii) the mussels used on rivers in Lower Normandy came from a Breton stock whereas in Brittany we were able to use young mussels collected from the mussel populations of the very same rivers in which they were released. However, the use of this technique seems to be of interest in the context of a population reinforcement program to measure the survival and growth of young mussels.

LIFE Potamo Fauna, a project for the recovery and conservation of endangered river fauna in the basins of the Ter, Fluvià and Muga rivers (Catalonia)

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In January 2014 has started Potamo Fauna LIFE project: "Conservation of river fauna of European interest in the Natura 2000 Network of the basins of the Ter, Muga and Fluvia rivers" (LIFE12 NAT/ES/001091). With a total budget of 1.9 million euros, involve six partners (Consorci de l'Estany, Consorci del Ter, Generalitat de Catalunya, Associació d'Amics de la Tortuga de l'Albera, Forestal Catalana y Universidad del País Vasco) and 6 cofinancers, apart from the European Union, which provides 50% of the overall budget. The overall objective of this project is the recovery and long-term preservation of 12 endangered native species of aquatic fauna, including 3 species endangered in Catalonia and Spain, through a wide range of measures: captive breeding, population reinforcements, habitat improvement, control of exotic species, and dissemination and research on the status of these species and the value of river and lake systems.

Among the planned conservation action, the main lines of action planned are:

Conservation and recovery of riverine populations of three threatened species of aquatic fauna, mainly with specimens coming from captive breeding centers: *Unio elongatulus*, the native crayfish (*Austropotamobius pallipes)* and European Pond Turtle (*Emys orbicularis*).

Conservation and recovery of populations of three endangered species of aquatic fauna, mainly through translocations of individuals coming from healthy populations into each basin: *Vertigo moulinsiana* and *V. angustior*, and Mediterranean Barbel (*Barbus meridionalis*).

Improvement of populations of an aquatic turtle and of 5 amphibians in the Ter river, by creating micro wetlands: Mediterranean Turtle (*Mauremys leprosa*), Marbled Newt (*Triturus marmoratus*), Common Midwife Toad (*Alytes obstetricans*), Western Spadefoot (*Pelobates cultripes*), Natterjack Toad (*Bufo calamita*) and Mediterranean Tree Frog (*Hyla meridionalis*)

Fight against various invasive alien species of crabs, fish, and freshwater mollusks, to mitigate its negative effects on aquatic fauna and their habitats, through a battery of different actions: population control in specific sectors, experiments against aphanomycosis, prevention of penetration, and other.

Plans and milestones of this project are presented.

Habitat preferences of Unio mancus and U. ravoisieri in northeast of Catalonia

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In northeast of Catalonia, several isolated populations of *Unio mancus* and *U. ravoisieri* remains, despite the wide and big decline that these species have suffered during the second half of the XX century. Main known populations occur in the rivers Ter and Fluvià basins. Along last five years, we have surveyed eight of these populations, occurring in rivers, diverting channels and Banyoles Lake. Joint to mussel surveys we performed a general habitat characterization on the basis of nearby thirty environmental variables, mainly related with the state and the structure of the habitat at different scales (microhabitat and mesohobitats).

We show the mains results about selection of habitat for both species. In rivers, they show a very similar pattern of habitat selection, they occupy preferably shores on best preserved fluvial stretches with a stable structure, excellent riparian forest and a good hydromorphological status. The more you move away from these optimal habitats, densities of Unio mussels decrease and the population structure become more irregular, often due to an irregular and low recruitment. On channels, in contrast, *U. mancus* occupy all the bed, including the central part, probably due to the lack of extreme flow disturbances, i.e. torrential floods. *U. ravoisieri* has not been detected on channels, but is present in Banyoles lake, so in a very singular habitat, were on the other hand *U. mancus* is not present nowadays. For both species relative densities of both species are correlated positively with a structural index of fluvial habitat (IHF), derived from habitat measures.

These results emerge from several conservation projects: Projecte Estany (LIFE08 NAT/E/000078), LIFE Potamo Fauna (LIFE12 NAT/ES/001091), and other small projects funded by two public institutions ("Diputació de Girona" and "Generalitat de Catalunya").

Naiads and fish, coupled destiny: the case of basins of north-eastern Catalonia

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Native unionids are among the most threatened groups, as a whole, of the fauna of the Iberian continental waters. Most species are currently in regression, and in fact many of these are cataloged at some level of threat, whether international, national or regional level. Several factors explain this situation, including the destruction or alteration of river habitats and declining water quality, but also the degradation of fish communities.

In north-eastern Catalonia four species of naiads are considered native: *Potomida littoralis*, *Unio mancus*, *Unio ravoisieri* and *Anodonta anatina*. The conservation status of these species in Catalonia is uneven, but generally we can consider all of them highly threatened, given the clear regression observed in their distribution. Much of the historical citations are probably missing at present, or have become residual populations with very low population densities and often on the verge of local extinction due to lack of recent recruitment. In north-eastern Catalonia *P. littoralis* is in the worst situation, with only four known small populations, with a few observed living animals in each site. On the other hand, recently has been detected an exotic naiad, *Sinanodonta woodiana*, which is currently expanding.

We give a general revision of the known populations of naiads on the area, on the basis of their current structure, and in relation with the recent evolution of local fish communities. In general, it is confirmed that the few native unionid populations with current regular recruitment are placed on river stretches with not severely modified fish communities. These fish communities are characterized by a stable presence of at least one native fish species, and also in general by the absence of exotic fish species, or at most a not stable presence. In contrast, the exotic *S. woodiana* is now expanding in areas where original fish communities are nowadays strongly altered, often without native fish species.

These results emerge from several surveys and monitoring performed in the context of Projecte Estany (LIFE08 NAT/E/000078), LIFE Potamo Fauna (LIFE12 NAT/ES/001091), and other small local projects.

Age, growth and demography of several populations of *Potomida littoralis* in northeast of Catalonia

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In north-eastern Catalonia *P. littoralis* is in the worst situation, with only four known small populations, with a few observed living animals in each site. These populations are placed in small sections of Llémena and Fluvià Rivers, in Banyoles Lake, and also in an irrigation channel of the low Ter River flood plain.

Along last ten years, we have surveyed these populations. We compare age, growth and demography to analyse the implications for the conservation of this endangered species. Age has been determined by means of lectures of internal annual marks on the shell. Growth is described on the basis of adjustments of the Von Bertalanffy Growth Model (VBGM). Parameters estimations obtained by means of these adjustments are used to compare growth patterns of the analysed populations. Demography is analyzed over the age structure of each population. We approximate the present recruitment pattern by the relative abundance on samples of young and medium aged individuals.

Maximum longevity observed for the whole area is 49 years, in lake Banyoles. Although in the low Ter River flood plain maximum age registered is 22 years, there are strong evidences of possible underestimation of real age on older shells. Here, estimated growth rate (K parameter from VBGM) is low, 0.07 $year^{-1}$. Growth is actually very slow; directly observed growth increments in marked individuals are near zero, for a period of 1,5 years. In all populations there's a complete lack of effective reproduction, or at most they have a very sporadic and token recruitment. So, these populations nowadays are clearly on the brink of an imminent extinction. The only reason for its persistence till now is the great longevity of the species.

These results emerge from several conservation projects: Projecte Estany (LIFE08 NAT/E/000078), LIFE Potamo Fauna (LIFE12 NAT/ES/001091), and other small projects funded by two public institutions ("Diputació de Girona" and "Generalitat de Catalunya").

Age, growth and demography of *Anodonta anatina* in low Ter River flood plain (Catalonia)

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In northeast of Catalonia, some remaining populations of *Anodonta anatina* are located in irrigation channels associated with the traditional system of agricultural irrigation, placed in lowland floodplains, mainly in river Ter basin.

Along last three years, we have surveyed three of these populations. We compare age, growth and demography to analyse the implications for the conservation of this species. Age has been determined by means of lectures of external annual marks on the shell. Growth is described on the basis of adjustments of the Von Bertalanffy Growth Model (VBGM). Parameters estimations obtained by means of these adjustments are used to compare growth patterns of the analysed populations. Demography is analyzed over the age structure of each population. Mortality rates for the well represented fraction on the samples are also obtained. We approximate the present recruitment pattern by the relative abundance on samples of young and medium aged individuals.

Maximum longevity observed on the area is 10 years. Growth rates (K parameter from VBGM) range from 0.39 to 0.55 $year^{-1}$. There are significant differences between populations of the maximum length, which is around 160 mm. The demographic structure of these populations are quite homogeneous, both on recruitment and mortality rates of adults. Recruitment is apparently low, but this situation is compensated by a low mortality rate typical of these environments so stable. This allows densities to remain stable or on a slow decline.

These results emerge from in the context of several conservation projects funded by two public institutions: "Diputació de Girona" and "Generalitat de Catalunya".

Expansion of the exotic unionid *Sinanodonta woodiana* in low Ter River flood plain (Catalonia)

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In the northeast Catalonia, 4 native unionid species have been cited: *Potomida littoralis* (Cuvier, 1798), *Unio mancus* Lamarck, 1819, *Unio ravoisieri* Deshayes, 1847, and *Anodonta anatina* (L, 1758). Recently the exotic *Sinanodonta woodiana* (Lea, 1834), has been cited on Ter and Fluvià rivers. Between 1995 and 2014, several specific surveys were carried out, always below the Pasteral dam, both in the Ter river and in several of its tributaries, including Lake Banyoles. Moreover, from 2010 to 2014 a thorough freshwater bivalves prospection campaign was performed in the alluvial plain of lower Ter, including the river and secondary water masses, mainly irrigation channels associated with the traditional system of agricultural irrigation. The prospections were done by manually on the river bed bottom. All over since 2010 a total of 90 sampling stations has been surveyed in this alluvial plain.

Status of native species is precarious, with populations intensely fragmented, and densities often low and heavily aged for lack of recruitment. This situation is clearly attributable to the severe rarefaction of native fish species, since in some areas even completely inexistent.

In contrast, the exotic *S. woodiana* is expanding and occupies already the whole alluvial plain and the lower course of the Ter river, where it is very abundant. Moreover, *S. woodiana* presents a well-structured global population in the alluvial plain of Ter River and a regular recruitment can be observed thanks to the fact that several exotic fish species are abundant in the area, are potential hosts to them.

This exotic unionoid appeared in most of the surveyed localities (81%), and was present in all the types of water bodies surveyed. In 16 % of the localities it was the only unionoid found, and it was present in all of the localities were other unionoid species were present. Specimens of this exotic species found alive constituted the 72 % of the total of alive unionoids collected during the surveying campaigns. Besides this species were quantitatively dominant in the whole of the alluvial plain. Therefore, it is the most spread and abundant unionoid species at present, often with very high densities. It presented a continuous distribution in the lower Ter river basin, as well as in the main irrigation canals.

Effect over naiad populations of flow drastic reduction in traditional irrigation channels in the low Ter River flood plain (Catalonia)

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In north-eastern Catalonia four species of naiads are considered native: *Potomida littoralis, Unio mancus, Unio ravoisieri* and *Anodonta anatina*. All these species, except *U. ravoisieri*, are present in the low Ter River flood plain, where there's also present and expanding the exotic naiad *Sinanodonta woodiana*. Aquatic ecosystems in this area are currently in a process of gradual change affecting its hydrological and ecological status. The recent construction of modern water pipes is leading to the abandonment of traditional uses of ancient irrigation channels. Most of these channels come from middle ages, and some of them were actually ancient fluvial courses that cross the delta in the past. As a result of these changes, severe effects on the biota were expected in these channels, being naiads the most singular and endangered component of the fauna communities.

From 2010 to 2014 annual freshwater bivalves monitoring campaigns were performed in two of these cannels affected by flow change, called Rec del Molí de Pals (RMP) and Rec del Molí de l'Escala (RME). A maintenance flow ("environtmental flow") was established on both channels (RMP: 350 l/s, approx. 15% of the initial regular flow; RME: provisionally, approx. 900 l/s, approx. 50% of the initial regular flow). Eight sampling stations were visited annually on RMP, and fifteen on RME. Other fourteen control stations were positioned in other nearby channels not affected by the hydrological changes. Samplings were done by manually prospection along the channel bed bottom. A monitoring of the water quality and the aquatic vegetation was also conducted.

In the case of RMP, the degradation of plant and animal communities became increasingly evident. So far there has been a progressive decrease in the extent of submerged vegetation (hydrophytes communities), while helophytic vegetation (reed and bulrush, maily) has increased considerably, colonizing much of the bed of the channel. Regarding the naiads, a gradual decrease in the overall density has been observed in the upper stretches of the channel, most affected by these changes. *P. littoralis*, with a very low initial density, has apparently disappeared. *U. mancus* density has also declined sharply, and probably is doomed to his disappearance in the channel in a few years. *A. anatina* and *S. woodiana* show a gradual decline on the upper half of the channel, but a stable situation in the rest.

In contrast, in the RME channel and in the control stations, no significant changes were observed in populations naiads located in the control stations.

So, it has been confirmed that to ensure the conservation of stocks naiads of traditional irrigation canals, the established maintenance flow is ineffective in the case of RMP. Unfortunately the prevision for the official final maintenance flow in RME is similar, i.e. approx 15% of the initial regular flow, so that even in this channel the future of the naiads is not ensured. It is necessary to note that these channels are the last refuge for native naiads of this flood plain, as they have practically disappeared from other bodies of water, including the main watercourse of Ter river.

River specific genetic diversity of *Margaritifera margaritifera* with respect to host fish and population size

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Effective conservation approaches for endangered species such as the freshwater pearl mussel (*Margaritifera margaritifera*) require integration of ecological and genetic information. Low genetic diversity is a matter of concern, as it may reduce the ability of the species to adapt to environmental changes. Therefore, maintaining genetic diversity has been identified as one of the key elements in successful conservation programs. We examined the genetic structure and diversity of 21 freshwater pearl mussel populations located in Finland, Sweden and Norway. We used mitochondrial DNA *COI* sequences and microsatellites to generate genetic information. From 18 observed *COI* haplotypes 7 were unique and restricted to certain populations. The number of observed haplotypes per population (haplotype richness, H) ranged from 1 in the River Hirvasjoki to 10 in the River Karpelva. An average of 5.2 alleles were observed for the 9 microsatellite loci used in this study. Hierarchical analysis of molecular variance (AMOVA) revealed that 0% of the genetic variation was among drainage systems, 31% among populations within drainages, and 69% within populations. Pair-wise F_{ST} values spanned a wide range and 88% of differences in all pairwise comparisons were highly significant ($P < 0.001$). The results of the Mantel test ($r = -0.041$ $P = 0.662$) confirmed that there was no isolation by distance between the populations.A regression model was fitted to the four response variables, mean observed and expected haplotype (H) and allelic (A) richness, using the host species and the mussel population size (N) as predictor variables, assuming asymptotic increase in the response variable with the increasing population size. *M. margaritifera* of Atlantic salmon rivers had a higher asymptotic haplotype and allelic richness than *M. margaritifera* of brown trout rivers (all $p < 0.05$). Allelic richness was at its asymptotic level already in the smallest observed N of < 10.000 while haplotype richness reached its asymptote with N around 50.000 mussels for both species. The results of this study show that current population differentiation does not match with current drainage systems. Most of the populations had relative high pairwise F_{ST} values, and in some populations there were also private alleles, indicating that there is a strong differentiation between those populations. Thus, no introductions should be carried out to increase genetic diversity or decrease inbreeding without the information of the genetic structure of the populations, because this could lead to outbreeding depression, which means a reduction in fitness by breakdown of the locally developed genetic adaptations.

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Rearing of *Unio crassus* in Northern Luxembourg

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The thick shelled river mussel *(Unio crassus)* was a very common species within its distribution area, often with high densities of 700 individuals/m^2. In the last decades there was a dramatic decline, for example in Germany approximately 90% of the populations were lost. For the freshwater pearl mussel (*Margaritifera margaritifera*) some rearing approaches were tested, but there is not much knowledge about how to rear *Unio crassus*. This work shows one method to rear juvenile thick shelled river mussels from ca. 0.2mm up to a size of 2.5 cm or more.

Juvenile mussels were reared in aquaria directly after excystment for one year until they they reached the size of approximately 1 cm: 60 juvenile mussels (age <24h) were put in aquaria (40x30x26 cm) with a water pump (to create water current) and a layer of sand (0.5 cm) at room temperature of 19-20°C. Water change occurred once a week. They were fed with Shellfishdiet 1800 and Nanno 3600 at different concentrations depending on the age. When mussels reached a size of approximately 1 cm they were reared inside the rearing facility in a flowthrough channel: 17-30 mussels were kept in 4 gravel baskets at the natural temperature regime of the Our river. No food was added. Increasing sediment deposition was removed regularly by gentle shaking. With the size of approximately 2 cm, the mussels were reared in an artificial rearing channel that runs over a wild meadow (until release to the wild). Mussels were kept in gravel boxes that were placed on the bottom of the channel.

Mussels grew up to approximately 1 cm in the aquaria and around 60% of the mussels survived in each aquarium during one year. In the flowthrough channel the growth was different depending on the season (temperature). Survival rates ranged between 85-100% during 9-12 month. The mussels reached a minimum of 2.5 cm in the gravel boxes after one year and the survival rate was 94%.

With this method it was possible to obtain mussels with an average size of 2.5 cm or more during four years. These results can help in mussel conservation projects to start directly with rearing *Unio crassus* if necessary. Especially the last two steps of this method (Flowthrough channel and channel in meadow) are very time-saving methods that do not afford much work.

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9 NOTES