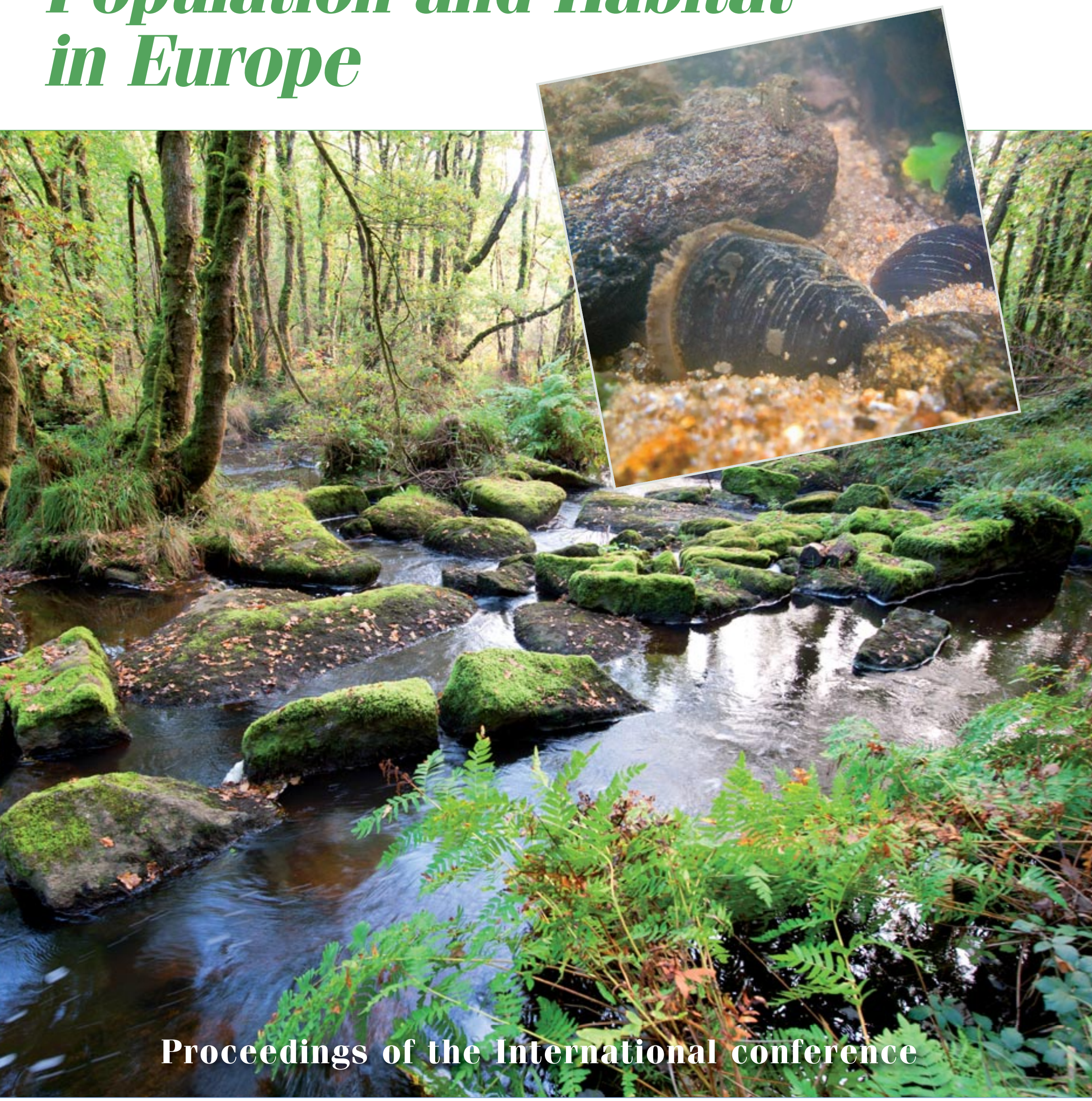
Conservation and Restoration of Freshwater Pearl Mussel Population and Habitat in Europe



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Une voix pour la nature

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European program LIFE 09NATFR000583

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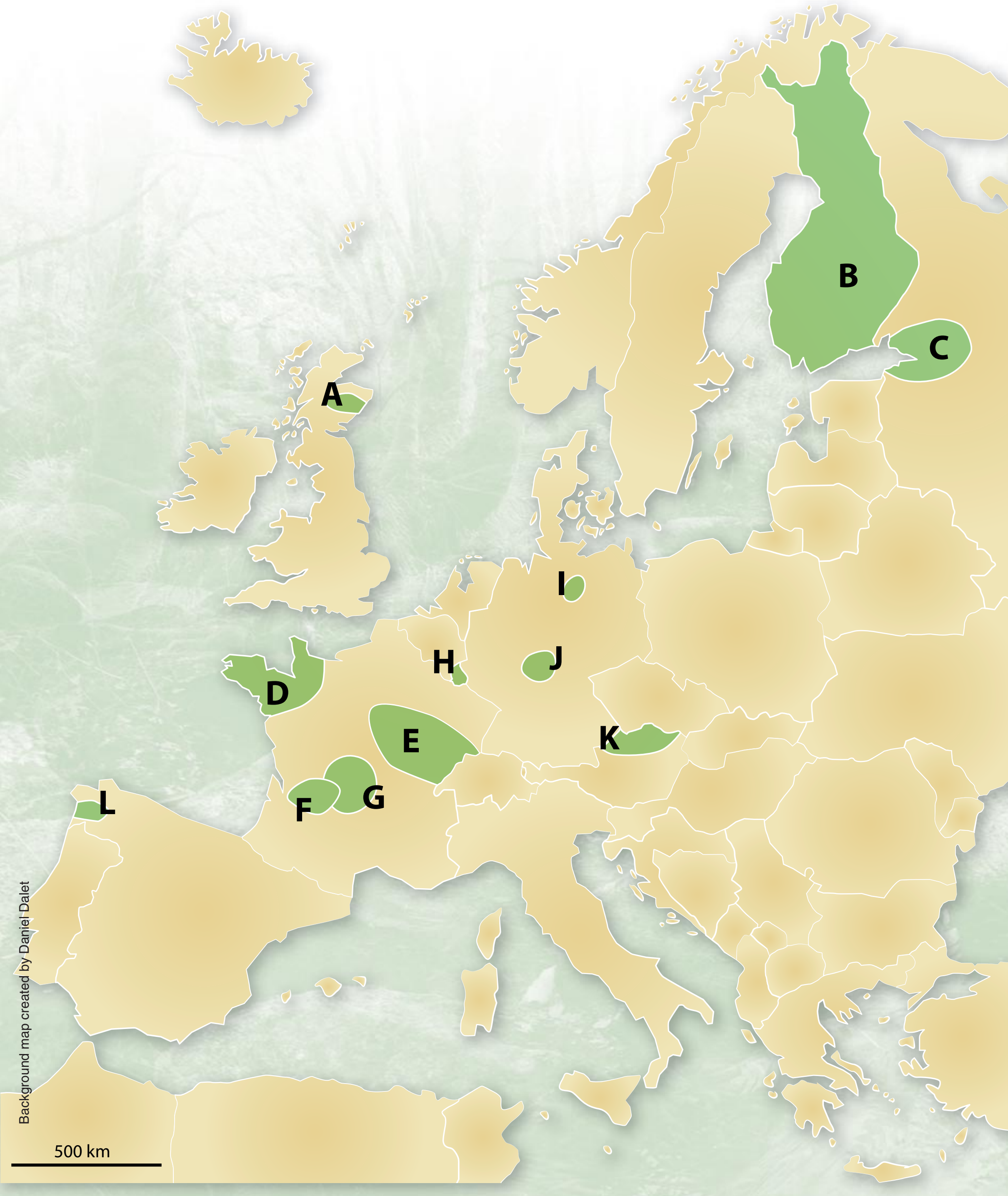
As members of scientific and organisation committees, we thank the Institute of Geoarchitecture and University of Western Brittany for hosting this conference. We thank the many conference participants for attending, the keynote speakers of each session and those of the roundtable, session chairpersons and those who gave the oral and poster presentations.

We would also like to say a big thank you to the University Restaurant for the catering, to the Translation Bureau for the interpreting during the conference and for translating the book of abstracts and conference proceedings, and to Julie Windebank (Urchin traduction) for revising the texts.



Location of study areas which were given during the oral presentations

A: river Dee, Scotland, United Kingdom; B: water system of Finland; C: rivers around Saint-Petersburg, Russia; D: rivers of Armorican Massif, France; E: regions of Bourgogne and Franche-Comté, France; F: river Dronne, France; G: river located in one of France's department called "la Haute-Vienne", France; H: river Our, Luxembourg; I: river Lutter, Germany; J: river Schondra, Germany; K: rivers Aist and Naarn, Austria; L: river Ulla, Spain.

Summary

International conference “Conservation and Restoration of Freshwater Pearl Mussel Population and Habitat in Europe”

Proceedings of the LIFE conference “Conservation of the Freshwater Pearl Mussel from the Armorican Massif”, September 2010 - August 2016, LIFE 09NAT/FR/000583
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H. Ronné

Foreword

Degemer mat d'an holl da re vras ha da re vunut e Skol veur Breizh izel.[1]

Dear colleagues and friends,

First, I would like to welcome you to Brittany and especially to Brest, which some of you are probably discovering for the first time.

Welcome also to the University of Western Brittany where we are pleased to be hosting this international symposium on the freshwater pearl mussel.

The Geoarchitecture research laboratory, *Conception, aménagement et gestion du cadre bâti de l'environnement. Doctrines et pratiques*. [Design, development and management of the urban environment. Doctrines and practices], which I lead, was formed following the creation of the Institute of Geoarchitecture, founded in 1976 within the Faculty of Sciences. Since its very beginning, the Institute of Geoarchitecture took an interest in environmental themes, from the point of view of teaching and research, especially the way in which the environment and biodiversity are taken into account in town and country planning. We should remember that the first law on the protection of nature in France also dates from 1976 and it is this same law which established the possibility of implementing lists of protected plant and animal species at national and regional levels, and the obligation to carry out environmental impact studies for any development project.

The Geoarchitecture laboratory has a multidisciplinary team including 22 faculty members, 15 co-researchers, 15 PhD students and 3 technicians, working in the fields of urban planning and development, ecology, geography, economics, sociology, law, etc.

It works in two main research directions: one dedicated to urban issues and urban planning, and one centred on environmental themes.

This second line of research concentrates on the following themes:
- Habitat and habitat complex assessment: typology, heritage assessment, conservation status assessment and mapping;
- Ecological restoration of degraded natural habitats: coastal zones, wetland areas;
- Management of natural areas: management plans, environmental diagnostics.

The history of the Institute of Geoarchitecture is partly linked with that of Bretagne Vivante – SEPNB. For many years, the *Société pour l'étude et la protection de la nature en Bretagne* [Society for the Study and Protection of Nature in Brittany] had its headquarters at the Faculty of Sciences in Brest. The association dates from 1953 and is recognized as a pioneering regional society for nature protection in France, including

1 - Welcome all of you, experts and amateurs, to University of Western Brittany.

the creation of a regional network of protected areas. All the naturalists of the Universities of Caen, Rennes, Nantes and Brest were members of the association's board of directors until the 1990s.

Some naturalists from Brest University have marked the history of the SEPNB, starting with one of its co-founders, Professor Albert Lucas, a marine biologist, but above all a passionate naturalist. We must also mention Jean-Yves Monnat, Maurice Le Démézet, Max Jonin, Mauritius L'Her and Michael Glémarec.

It is therefore natural that Bretagne Vivante – SEPNB and the Institute of Geoarchitecture would collaborate and work closely together: most of the naturalists in the university are actively involved in multidisciplinary teaching in the fields of ecology and environmental science, developing a field-based approach that allows students to make territorial evaluations. Some of these naturalists were part of the research team.

Since 1970, SEPNB has carried out a number of environmental studies: impact assessments, ecological diagnostics, proposals for development and restoration of sensitive natural sites, preliminary studies for the establishment of protected areas. These have sometimes been conducted in close collaboration with the Institute of Geoarchitecture.

Current collaborations continue in a more ad hoc manner:
- Participation in the development and evaluation of natural reserve management plans overseen by Bretagne Vivante – SEPNB;
- Long-term monitoring on the national nature reserves of Saint-Nicolas-des-Glénan and François le Bail, Groix Island;
- A thesis begun in 2014 on the conservation biology of *Eryngium viviparum*, a plant species threatened in Europe, with a single French population on a protected site managed by Bretagne Vivante – SEPNB.

It is a pleasure for us to be involved in the organization of this conference, allowing it to be held in the premises of the University of Western Brittany.

The themes of this conference dedicated to *Margaritifera margaritifera*, an emblematic species of the rivers of Brittany and Armorican Massif that is considered threatened at the European level, will enable us to take stock of the biology and ecology of this species, but also its protection and strategies to be implemented to maintain or restore populations. The issues to be discussed will be related to water quality and river management and restoration. The presentations will demonstrate that conservation management of a threatened species, including its lifecycle which depends on salmonid fish, goes well beyond the concerns of biologists and naturalists, but requires a comprehensive approach applied at the catchment scale, which works through the raising of awareness and involvement of politicians, managers and land users as well as closely collaborative work with naturalists and scientists. This is a vast endeavour, but we trust that your work and this conference will contribute to creating an impetus for its success.

I wish you fruitful discussions and an excellent conference.

Welcome speech by Frédéric Bioret

Director of Research Team EA 2219,
Institute of Geoarchitecture, Brest – France

H. Ronné

[1] The freshwater pearl mussel lives half-buried in the sediment of salmon rivers.

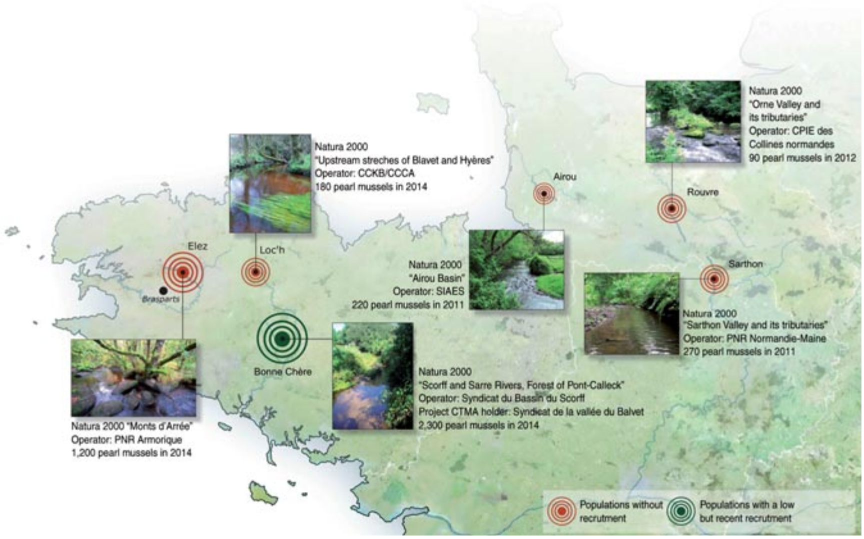
The association Bretagne Vivante – SEPNB has been working on nature protection in Brittany for 55 years. To this end, it unites the efforts of nearly 3,000 members, 19 local groups and 68 employees in the 5 departments of historical Brittany. The missions of Bretagne Vivante – SEPNB concern the improvement of naturalist knowledge, sharing of this knowledge through environmental education, protection of natural sites and militancy. Bretagne Vivante – SEPNB is a member of "France Nature Environnement" and a participant in the "Réserves Naturelles de France" network (it manages five State Natural Reserves and about 100 associative reserves). As part of its mission, the association is involved in setting up ecological restoration projects. As such, since 2010, it has led a programme to save the pearl mussel, a freshwater bivalve, on the Armorican Massif.

The freshwater pearl mussel (*Margaritifera margaritifera*), or pearl mussel, is a key indicator species of the quality of river ecosystems [1]. Its life cycle has a planktonic phase and a parasitic phase which takes place on the gills of a host fish (brown trout or Atlantic salmon). Its complex life cycle, ecological requirements and long lifespan (about a hundred years) make it an "umbrella" species: by protecting it, one protects an entire ecosystem.

The pearl mussel is a species of European Community interest listed in Annexes II and V of the "Habitat, Fauna, Flora" Directive, as well as Appendix III of the Bern Convention. It is protected by French law (Orders of December 16, 2004 and April 23, 2007).

The IUCN classifies the pearl mussel in Europe as "critically endangered". The next stage is "extinct in the wild". It is considered to be facing a very high risk of extinction in the wild in the near future, since a reduction of at least 50% of its population over 10 years has been observed by the scientists of this international organisation.

It is estimated that 90% of pearl mussel populations disappeared from Central Europe during the 20th century. A number of studies in Brittany and Lower Normandy have observed the same state of emergency for pearl mussel populations in western France, with gradual disappearance and aging. The strong heritage interest of the species, a living trace of the valley formation in the Armorican Massif, as well as its very demanding characteristics as a bio-indicator and its properties as an umbrella species, make the pearl mussel an important species to be preserved.



[2] The LIFE+ programme aims to conserve the six remaining major populations on the Armorican Massif.

In the face of this urgent need, a LIFE+ programme was granted to Bretagne Vivante – SEPNB in partnership with the Finistère Fishing Federation, the CPIE des Collines Normandes (an environmental education association), the Syndicat Intercommunal d'Aménagement et d'Entretien de la Sienne (interdistrict association for river preservation and development) and the Normandy-Maine regional natural park. The LIFE+ programme (2010-2016), completely in line with the national action plan, mainly involves rearing of the six remaining major strains of pearl mussels [2], monitoring of environmental quality, reinforcement of the populations and awareness raising to try to save the last populations in the Armorican Massif.

The LIFE+ programme, amounting to a total of about 2.5 million euros, is 50% funded by the European Commission. Other participants include the DREAL in Lower Normandy and Brittany, the Regional Councils of Lower Normandy and Brittany, the County Councils of Côtes-d'Armor, Finistère and Manche, as well as Agence de l'Eau Seine-Normandy.

As a part of this LIFE+ programme, the international conference "Conservation and Restoration of Freshwater Pearl Mussel Populations and Habitat in Europe" was held on 26, 27 and 28 November 2014, and its proceedings are transcribed here.

Marie CAPOULADE

LIFE+ programme coordinator
"Conservation of the freshwater pearl mussel from the Armorican Massif",
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Oral communications

Session 1

Freshwater pearl mussel in Europe: status and conservation issues

Freshwater pearl mussel in Europe: Status and conservation

Juergen GEIST

J. Geist

Freshwater pearl mussels (*Margaritifera margaritifera*) are target species in the conservation of oligotrophic stream ecosystems, fulfilling criteria of indicator, keystone, flagship and umbrella species (Geist, 2010). Most European populations of the species are in decline, with insufficient recruitment being the core problem in most areas. Conservation of the freshwater pearl mussel faces many challenges and needs to consider the diversity of pearl mussel habitats [1] as well as the species' complex life cycle.

Consequently, conservation efforts need to include management of water quality, functional stream substrates, host fish populations, and the genetic diversity and differentiation of freshwater pearl mussels.

Since gravidity of the mussels is typically not reduced, even in sparse and over-aged populations, this offers great potential for recovery, even in small and declining populations. The interaction of pearl mussels with their fish hosts is more complex, due to different host suitability among species (*Salmo salar* and *Salmo trutta*), as well as among populations within the same species (Taeubert *et al.*, 2010). Due to their adaptation to oligotrophic conditions, functional pearl mussel populations only require low densities of fish hosts if the other stages of the life cycle remain intact (Geist *et al.*, 2006).

Since high loads of glochidia can affect mortality and swimming performance of host fish (Taeubert & Geist, 2013), the determination of optimal infestation densities is essential in conservation measures such as the release of infested hosts as well as in captive breeding. The strong temperature dependence of larval development in freshwater mussels (Taeubert *et al.*, 2013, 2014) suggests that the host-parasite interaction may be affected by climate change. In the postparasitic phase, juvenile pearl mussels depend on a functional stream bed (Geist & Auerswald, 2007). High amounts of fine sediment can clog the stream bed, resulting in adverse effects on buried juvenile pearl mussels, as well as their salmonid hosts (Sternecker & Geist, 2010; Sternecker *et al.*, 2013). Provision of functional stream substrates is probably one of the greatest challenges in stream restoration (Geist, 2011, 2014). Comparisons of commonly applied stream bed restoration measures revealed that they all require incorporation of catchment erosion management to become successful (Pander & Geist, 2013; Mueller *et al.*, 2014; Pander *et al.*, 2015; Denic & Geist, 2015).

In addition to knowledge about the conservation and restoration of their habitat, information on the genetic diversity and differentiation of freshwater pearl mussel populations is also important (Geist, 2010). Genetic studies can reveal, for example, colonization history and population bottlenecks, and can help secure the genetic and evolutionary potential of the species by identification and protection of unique and diverse populations (Geist & Kuehn, 2005, 2008; Geist *et al.*, 2010).

J. Geist

[1] ***Despite the strong degree of adaptation of freshwater pearl mussels (Margaritifera margaritifera) to oligotrophic streams, their Holarctic distribution comprises streams that are very different in geomorphology, size, and flow. These four pictures show pearl mussel streams from France, Finland, Scotland and Russia.***

Cross-exposure experiments of juvenile pearl mussels originating from different genetic backgrounds (an exposure experiment of some pearl mussel stocks in their native streams but also in others streams) suggest that a certain degree of adaptation to specific stream conditions can occur (Denic *et al.*, 2015). In the context of restocking extinct populations, genetic analyses of shell material may help identify the most suitable source populations (Geist *et al.*, 2008). To prevent extinction of genetically unique populations, these valuable populations can be artificially cultured as an emergency measure (Gum *et al.*, 2011), but such efforts must necessarily be integrated into holistic concepts of habitat restoration.

Some of the conservation challenges, such as the definition of priority populations and areas of conservation, can only be achieved on a European level, whereas aspects of sediment and fish host management can only be addressed by local catchment management and stakeholder involvement. This contribution proposes a conservation strategy that integrates systemic and aggregated processes in a step-wise approach. Following the definition of conservation objectives based on ecological, socio-economic and genetic targets, an analysis of bottlenecks and constraints is carried out before setting achievable targets. Stakeholder involvement, communication and adaptive management are crucial to the process, which also needs to include evaluation and publication of results, irrespective of success or failure. As evident from several examples in this presentation, conservation of intact populations and their habitats should have highest priority and is easier to accomplish than any restoration action. ■

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State of the freshwater pearl mussel populations in Finland

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J. Syväranta

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H. Ronné

P.-L. Luhta

The freshwater pearl mussel (*Margaritifera margaritifera*) is protected in Finland by the Nature Conservation Act since 1955. The species is also listed in Annex II and V of the European Union Habitats Directive as a species whose habitat must be protected for its survival. Despite the protection, the freshwater pearl mussel populations have been declining almost everywhere in its original habitat. In Finland, the decline of the populations has estimated to be about 70% since the situation at the beginning of the 20th century (Valovirta, 2006).

Indeed, the 1955 Act protected *M. margaritifera* in Finland from pearl fishing but not from destruction of its habitat. Since the era of pearl fishing, the reasons for the declining populations have increased and included the cleaning of rivers for timber floating, the construction of hydropower plants, eutrophication and pollution of the rivers, the building of forest roads, and other forestry operations such as drainage of forest and peat lands, which have led to the silting of rivers.

Although there is a general awareness of negative development of the freshwater pearl mussel populations in Finland, the knowledge of the state of the populations is scattered and an overall picture from the whole country has been missing. Systematic population status assessments have been carried out only recently in 24 different populations (Oulasvirta *et al.*, 2014; Oulasvirta *et al.*, 2012; Oulasvirta & Syväranta, 2012). Older and more scattered data of the populations is found from the Finnish Museum of Natural History (Valovirta & Huttunen, 1997) and from Parks & Wildlife Lapland, which have their own databanks. The objective of this paper was to summarize all the new data as well as the older records of the distribution and state of the freshwater pearl mussel populations in Finland.

Methods

The state of the population was evaluated by applying Swedish criteria, where the population status is based on the population size and proportion of juvenile mussels in the population (Bergengren *et al.*, 2010; Söderberg *et al.*, 2009). The populations were ranked into six categories of viability, which were (1) "*viable*", (2) "*viable?*" (maybe viable), (3) "*non-viable/partly viable*", (4) "*dying-out*", (5) "*almost extinct*" and (6) "*extinct*" **[Table 1]**. In the Swedish method, the viability of the population is basically determined according to the proportion of < 20 mm (~10 years) and < 50 mm (~20 years) mussels in samples. The proportion of these size classes was calculated from samples taken from random transects. The viability status of the populations was studied in 24 rivers, out of which 3 are located in southern Finland and 21 in northern Finland. The state of these populations was evaluated in 2010-2013. In addition, there are 96 known freshwater pearl mussel rivers in Finland. Population status in these rivers was based on the older data or extrapolated from the results obtained in the 2010-2013 studied rivers.

Results

Only one out of the 24 investigated populations could be classified as "*viable*". Two other populations were classified as "*viable?*", 18 populations were classified as "*non-viable/partly viable*", 2 "*as dying-out*" and 1 population as "*almost extinct*". Sometimes recruitment of the young mussels could be detected from certain restricted areas in the river –usually in the upper course– when rest of the population consisted of only adult mussels. These populations were classified as "*partly viable*", although the population as a whole was classified as "*non-viable*".

When the results were extrapolated to the other freshwater pearl mussel populations in Finland and using the existing knowledge on these populations, the approximate number of populations in different classes in whole Finland was following: 5 as "*viable*", 10 as "*viable?*", 31 as "*non-viable/partly viable*", 54 as "*dying-out*" and 20 as "*almost extinct*" populations [Table 2]. In addition, more than 100 populations are already vanished in Finland.

Discussion

The results show that the freshwater pearl mussel is seriously threatened even in the remote wilderness areas of northern Finland. Especially alarming is the situation in the big main rivers, where the Atlantic salmon (*Salmo salar*) used to migrate before these rivers were harnessed to hydropower production. At the moment none of the freshwater populations in Finland is known to use the Atlantic salmon as a host during the reproduction. It is obvious that, without urgent restoration measures, the extinction of the freshwater pearl mussel in the main rivers is inevitable, and the distribution of the freshwater pearl mussel will be fragmented into a few isolated headwater populations, in which the recruitment takes place using the brown trout (*Salmo trutta*) as a host. These small and isolated populations are vulnerable to extinction even without human influence.

The historical reasons for the extinction or decline of the populations are pearl fishing, cleaning of rivers for timber floating [1] and harnessing rivers for hydropower

Class	Status of population	Criteria			Number of freshwater pearl mussels
		Size < 2 cm	Size < 5 cm	Size > 5 cm	
1	Viable	> 0%	> 20%		> 500
2	Viable?	> 0%	> 10%		> 500
		0%	> 20%		
3	Non-viable/ Partly viabe	0%	< 20%		> 500
		0%	> 20%		< 500
4	Dying-out	0%	0%	All, high-density	> 500
5	Almost extinct	0%	0%	All, low-density	< 500
6	Extinct	Earlier documented density but population already vanished			

[Table 1] *Criteria for determining the viability status of the freshwater pearl mussel populations (Bergengren* et al.*, 2010; Söderberg* et al.*, 2009)*

Status of populations	Number of populations	%
Viable	5	4%
Viable?	10	8%
Non-viable/Partly viable	31	26%
Dying-out	54	45%
Almost extinct (or probably already extinct)	20	17%
Total	120	100%
Extinct	>100	

[Table 2] ***The status of the freshwater pearl mussel populations in Finland. The estimate is based on the results obtained from the 24 populations investigated in 2010-2013 (Oulasvirta & Syväranta, 2012; Oulasvirta* et al.*, 2012, 2014) and on the older data from some of the populations (Valovirta & Huttunen, 1997; Oulasvirta, 2010; Oulasvirta* et al.*, 2006, 2008).***

production. More recent reasons are especially forestry activities such as clearing, ditching and plowing operations and making of forest roads [2][3]. In Finland, especially the ditching operations have been intense. Indeed, according to some estimates, almost 40% of the world's forest ditches are in Finland (Joosten & Clarke, 2002). The biggest damage was done already in the 1960-1970s, when most of the ditching operations were done. As a consequence, the rivers were silted up, which has made the bottom substrate unsuitable for the development of juvenile mussels. In the mussel populations, this can be seen in the termination of recruitment or as a dip in the age class of the mussels that are 40-50 years old [4].

The conservation of freshwater pearl mussel in Finland would require actions in different levels: (1) Searching for new populations, (2) Status assessment and monitoring of known populations, (3) Restoration of damaged catchment areas, (4) Construction of fish ways to the old salmon rivers and (5) Captive breeding in the most threatened populations. Moreover, an action plan for the freshwater pearl mussel in Finland is needed. ■

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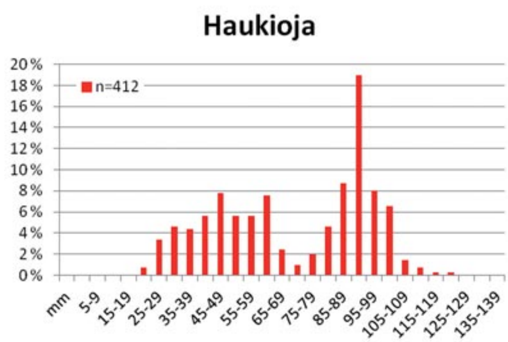
[1] ***Cleaning of freshwater pearl mussel river for timber floating in the 1950s***

[2] ***Clearing close to a freshwater pearl mussel river***

E. Moilanen

[3] ***A ditch in the woods which leads to a freshwater pearl mussel river***



[4] ***A recruitment gap was observed in Haukioja river in the 1960-70s. It coincides with intense ditching operations.***

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Status of pearl mussel populations in the Armorican Massif (France)

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H. Ronné

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C. Gouineau

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Pearl mussel populations are widely distributed in Europe but declining throughout their distribution area (Geist, 2010). The species is classified in the "Critically Endangered" category on the European Red List of the International Union for Conservation of Nature.

France is in the southwest of the mussel's European distribution with an estimated population of 100,000 individuals (Cochet, 2004). Freshwater pearl mussels are present in five major distinct regions: the Vosges, Morvan, Massif Central, western Pyrenees and Armorican Massif.

Study area

The Armorican Massif, covering an area of 58,000 km^2, is a natural region, both as a result of the geological unity of its subsoil, which is made up exclusively of Paleozoic rocks, and because of its isolation from other paleozoic massifs by Mesozoic or Tertiary plains (the Parisian and Aquitaine basins). In the Paleozoic era, granitic magmas or similar rocks made a sudden appearance in the middle of Paleozoic rocks and now form more or less larger marks on a part of this area. These old mountains were worn down over the course of time. The present day relief is generally low, varying between 100 and 400 m in the western part of Brittany (Montagnes Noires, Monts d'Arrée). In Lower Normandy, the hills of Normandy and Bas-Maine have about the same altitude, but this can exceed 400 m locally (including in the Monts des Avaloirs, 417 m, the highest point of the Armorican Massif).

Western Brittany therefore has a hilly relief on mainly granitic rocks and rainfall is above 900 mm per year, as on the hills of Normandy and Bas-Maine. In eastern Brittany and in the rest of the Armorican Massif, the relief is less rugged, the bedrock is schist and the rainfall is less than 800 mm per year.

The rivers of western Brittany and hills of Normandy and Bas-Maine are similar in many respects to mountain rivers, with steep slopes, sustained flow and cool temperatures in summer.

Distribution of the species before 2005

To establish the historic presence of the species in the rivers of the Armorican Massif, three types of sources were used: various written documents, collections and eyewitness accounts. Although imprecise, the oldest record dates from the 17th century: "Mr. de Ponchasteau told me that seven or eight leagues from Brest, at the foot of a mountain called Ménaré flows a stream in which pearls may be fished that are small but very white, which his lady wears on her collar, and as earrings and bracelets." (Dubuisson-Aubenay, 1636). In his "Histoire ancienne et naturelle de la Province de Bretagne", de Robien also evokes two rivers where there are mussels that produce pearls (de Robien, 1756). The "catalogues" published in the 19th century are much more accurate (Bourguignat, 1860; Taslé, 1867; Daniel, 1885; Locard, 1889; Leboucher & l'Abbé Letacq, 1903) but it is mostly the texts relating pearl fishing that provide the most information (Bonnemère, 1901; Ogès, 1953) [1].

The collections of the Museums of Natural History in Paris, Rennes and Nantes contain several samples of mussel pearl shells (mostly dating from the 19th century), which made it possible to confirm the presence of the species on 9 catchment areas.

The collection of eyewitness accounts provided information for 22 catchment areas.

The surveys carried out in the 1990s by Quéré and Cochet (Quéré, 1996, 1997; Cochet,1998), provided more up to date information on some populations but also new informations.

For the whole Armorican Massif, at least 52 rivers spread over 29 catchment areas have had a pearl mussel population [2].

Distribution of the species after 2005

Previous surveys enabled the LIFE+ program to update knowledge on several catchment areas (Hesnard, 2005, 2006; Holder, 2007; Mérot & Capoulade, 2009a, 2009b), but it is mainly those made since 2010 (Ribeiro *et al.*, 2012; Pasco & Capoulade, 2013; Pasco & Hesnard, 2013; Pasco, 2015a, 2015b) that allow us to have a slightly clearer view of the status of the populations at the scale of the whole Armorican Massif.

Currently, 24 rivers spread over 11 catchment areas still have a pearl mussel population [3]. For 15 rivers, the population consists of less than 100 individuals, with

H. Ronné

[1] ***Pearls from pearl mussels harvested in the Horn River (Finistère)***

[2] ***Distribution of the pearl mussel in the Armorican Massif before 2005, in red color***

[3] ***Distribution of the pearl mussel in the Armorican Massif after 2005, in red color***

an absence of recent recruitment. Only 9 rivers thus have a population over 100 individuals **[Table 1]** with the presence of some young individuals, particularly in the sub-catchment of the Sarre. The total population is estimated between 5,000 and 6,000 individuals. The catchment areas of Aulne and Blavet are home to over half this population.

Conclusion

The presence of the pearl mussel has been documented on almost the majority of the catchment areas in western Brittany and ten water courses in Lower-Normandy. In less than a century, its decline is estimated at over 95%. The LIFE+ program "Conservation of freshwater pearl mussel from the Armorican Massif" aims to halt this decline and to safeguard the remaining populations.■

Region	Catchment area	Sub-catchment	Estimated population
Brittany	Aulne	Elez	1,000-1,500
		Fao	100-200
	Ellé	Aër	100-200
	Blavet	Loc'h	100-200
		Sarre	2,000-2,300
		Brandifrout	100-200
Lower Normandy	Sienne	Airou	200-300
	Orne	Rouvre	100-200
	Loire	Sarthon	200-300

[Table 1] ***Rivers with more than 100 individuals***

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R.-P. Bolan

Left: the Bonne Chère (tributary of the Sarre); right: the Elez

H. Ronné

Pearl mussels in the Bonne Chère

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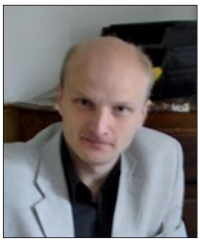
H. Ronné

The Sarthon

Conservation activities over the area around Saint-Petersburg, and their impact on pearl mussels

Igor POPOV



A. Kotova

I. Popov

Saint-Petersburg is located at the mouth of the Neva river flowing into the Gulf of Finland in the Baltic Sea. Seven populations of pearl mussels were recently described around it, within a radius of 250 kilometres (Popov & Ostrovsky, 2014) [1]. The total number of mussels is barely more than 50,000. However, their existence is still surprising, because Saint-Petersburg is a large source of negative influences on the environment. Its population numbers about 5,000,000 citizens.

Although no special programs on pearl mussel conservation have existed in the area, some nature protection activities conducted with other objectives have contributed to pearl mussel survival.

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[1] ***Location of the pearl mussel rivers around Saint-Petersburg: (1) Peipia, 2) Gladyshevka, 3) Roshinka, 4) Sestra, 5) Okhta, 6)Yanega and 7) Shotkusa***

Release of the young Atlantic salmons in pearl mussel habitats

In the Gladyshevka river young salmons were released for a long time to restore the decimated local population. The past condition of the pearl mussel population is unknown; just three samples from the area had been kept in the Russian Academy of Sciences. In addition to the Atlantic salmon, the brown trout was also present in the area (Khalturin, 1970).

Releases of young salmons had been conducted in the 1980s, but unsuccessfully, with no returns of spawners to the river being noted. Since 2000, the releases have started. Originally, these young salmons were bred for scientific purposes. During the breeding some surpluses of juveniles occurred and these fishes were released into the natural environment. These

Month and year	Number	Mean weight (in g)	Age
May 2000	8,500	8-10	1+
May 2001	1,500	100	2+
September 2002	10,000	8-10	0+
May 2003	1,500	15	1+
September 2003	10,000	8-10	0+
September 2004	10,000	8-10	0+

[Table 1] ***Releases of young Atlantic salmons in the Gladyshevka river***

juveniles were descended from the spawners of the Neva and Narova rivers flowing into the Russian section of Baltic Sea. The first release of the year 2000 was especially effective: 8,500 parrs weighing 8-10 g were released in two riffles of the river. Afterwards, in June and September of this same year, an electrofishing was conducted there. It showed a high parr density – about 1 parr per 1 m^2. This means that a large number of parrs occurred in the riffles during the season of pearl mussel reproduction (August). The following releases were less successful in this respect: either large juveniles were released in May, or small juveniles were released in September [Table 1], i.e. most migrated to the sea in spring, and parrs were therefore not numerous in the river in summer. In 2003, an attempt to estimate the results was undertaken: fishing to look for spawners was performed, and one of these spawners was caught (Popov, 2003).

In 2006, the first research for pearl mussels was conducted. A small number of mussels was found in the river and its tributary (Roshinka). In the riffle, where parrs had been released, two young pearl mussels had been found. During the search for pearl mussels, a redd with salmon fries was found with a young fish, *i.e.* the evidence of the Atlantic salmon restocking (Ostrovsky & Popov, 2008) [2][3]. Pearl mussels were observed in these waterways over 5 years [Tables 2 and 3]. It turned out that the situation did not change significantly: the pearl mussels were still not numerous, but some juveniles were found [4]. Most of these young specimens were found in the places where the reared parrs had been released. It is probable that the releases stimulated the recruitment of pearl mussel population. An untypical situation now exists in these rivers: the population of pearl mussel is very small (some tens of individuals), but reproduces. In Europe, some populations larger than this one have not reproduced for decades (Araujo & Ramos, 2001).

Protected areas around pearl mussel habitats

The rivers mentioned above were included in two sanctuaries. Gladyshevsky, one of these sanctuaries was created for the

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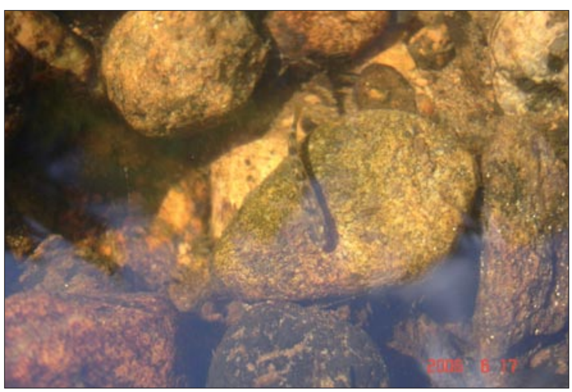
[2] ***Spawning site of salmon in Gladyshevka river***



[3] ***Salmon fry on the surface of the spawning site***

Year	Studied area on the bottom of the river (in m²)	Number of pearl mussels	Number of young pearl mussels (about 5 cm long)
2006	500	3	2
2007	500	5	0
2008	200	-	-
2009	400	2	1
2010	300	1	1

[Table 2] ***Results of pearl mussels' observation in the Gladyshevka river***

Year	Studied area on the bottom of the river (in m²)	Number of pearl mussels	Number of young pearl mussels (about 5 cm long)
2006	1,000	23	0
2007	200	4	0
2008	200	0	0
2009	200	2	3 empty shells
2010	200	3	0

[Table 3] ***Results of pearl mussels' observation in Roshinka river***

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[4] ***Young pearl mussel of the Gladyshevka river***

conservation of pearl mussel and salmonid. The aim of the other, Lindulovskaya rosha, was the conservation of rare trees – an old plantation of larch grove *Larix decidua*, which dates from the eighteenth century. Pearl mussel was discovered after the creation of this sanctuary and so by chance the conservation of the riparian vegetation contributed to the conservation of riverine environment.

"Rediscovery" of pearl mussel in these rivers stimulated the search for other populations. It turned out that pearl mussel had once again been protected "by chance" in another sanctuary (Kotelsky) which aims to protect the Kopanskoye lake and its surrounding forests. In the small Peipia river flowing from this lake into the Baltic Sea about 40,000 pearl mussels were found. The density of these individuals reached 1,000 per m² (Ostrovsky & Popov, 2011) [5]. The establishment of the sanctuary prevented some threats to pearl mussels. Although the lake attracted attention for aquaculture, it was forbidden in order to preserve this area from pollutions inherent in this kind of activities. The conservation of forests also contributed to the conservation of pearl mussel habitats. However, the lack of information about pearl mussel couldn't stop the creation of activities near the lake that affected pearl mussel negatively. On one of the river banks there was a sanatorium with a sewage outfall which was directly oriented into a concentration of pearl mussels' area. An insufficient pollution control led to the death of several thousands of pearl mussels here. Recently, several hundreds of mussels also perished because of works on the renovation of an electricity line. Now, due to recent studies, information on pearl mussels is available in all organizations that it might concern and such events are thus less likely.

Preservation of riparian vegetation

Four pearl mussel populations were found in the rivers flowing outside the protected areas. The conservation of pearl mussels had become possible thanks to the existence of a riparian vegetation on the river banks (Popov, 2015). In such a situation, banks subsiding, sand drift, acidification and other negative influences originating from surrounding territory only have a weak impact. Preservation of riparian vegetation had become possible because agriculture lands occupy small surfaces of the catchment area (Popov, 2015), and because of the particularities of land and river use. Waterways and their banks cannot be private property in Russia. Even if a private plot of land is located close to a waterway, free access to the bank must be kept. Some exceptions and violations often occur, but the main part of the banks is still "nobody's". Such a situation has resulted in "disordered" river banks: nobody clears the vegetation, whereas it turns out to be necessary [6]. At least small strip of natural vegetation usually exists there. Since the waterways are "nobody's" the state can introduce numerous bans on their use. According the "Water Code of Russia"

[5] *Pearl mussels of the Peipia river*

(03.02.2006. n° 74-FZ) "water protective zones" and "bank defensive zones" exist at the banks of each waterway. These notions indicate different methods of bans at distinct distances from the waterside. A "bank defensive zone" is usually 30-50 m, while a "water protective zone" is 30-200 m. In a water protective zone the following activities are not allowed: 1) use of sewage for fertilizing soil; 2) land use for cemeteries, burial places for animals or waste; 3) aerial pest control; 4) movement and parking of transport outside of roads and specially designated places. In "bank defensive zones", ploughing, addition of earth and pasturing are prohibited.

[6] *Pearl mussel habitat on the Yanega river*

Forestry management has also contributed to the conservation of the riverine environment. Forests also cannot be private property in Russia. They can only be rented. This resulted in numerous restrictions on their use and in the fact that they are used extensively rather than intensively. Tree branches, stumps, leaves and shrubs are usually not utilized [7]. A large organic mass remains in a forest after clearing (although European technologies of the intensive use of wood have been developing recently). Usually renewal of the forests happens naturally with no transformation of the forest into tree plantation. Under these conditions, natural drainage in the rivers persists.

"Red Book" in Russia

In the 1990s, Russian authorities established an organizational base for work on "Red Book of Russia" and local Red books for the members of Russian Federation. These books correspond to the early versions of the Red list of threatened species of the IUCN. Only rare and threatened species are included in them. The red books were taken into account in several Russian laws. Thus, according to the law "On protection of environment" (10.01.2002. n° 7-FZ) any action harmful to the species of Red Book is prohibited.

Pearl mussel was included in Red book of Russia (www.sevin.ru/redbook); meaning that, in a case of necessity, at least some actions which threaten a species can be reported by anyone (as in the case of the above mentioned Peipia river). However this system is not sufficiently effective because no database exists with the locations where species classified as "*dying-out*" occur. Cases of harm done to these species may therefore go unnoticed.

It should be mentioned here that some researchers tend to classify the information on pearl mussel habitats as secret and refer to rivers with letters in their articles (for example: River A, river B, etc.) (Buddensiek, 1995; Hastie *et al.*, 2000). Some Russian scientists decided to do the same (Ostrovsky & Popov, 2011). However, the case of Peipia river demonstrated a negative consequence of this practice: the lack of information caused a denial of river

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[7] ***Clearing at the pearl mussel habitat, near the Peipia river***

status. On the contrary, even very sparse information on pearl mussel occurrence in the Gladyshevka river prompted the creation of some conservation measures.

Brown trout populations of the Baltic Sea basin and the landlocked populations of the Atlantic salmon (including the salmon of Ladoga Lake) are also included in the Red book of Russia. Fishing of these species is prohibited, but the application of this ban is ineffective. Fisheries inspection usually concentrates the efforts on the most significant big waterways, while the fishing in small rivers is less monitored. The destruction of host fishes is now the most significant negative factor threatening the current status of pearl mussel populations in the investigated area.

Conclusion

Passive conservation measures aiming to protect the whole environment enabled survival of pearl mussel populations over the area around Saint-Petersburg, but do not guarantee species stability for the near future. The number of pearl mussels is decreasing. Active measures including artificial rearing of pearl mussels and their host fishes are required in most of the populations. ■

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Session 2

Recent advancements in the biology and ecology of freshwater bivalves in Europe, linked to their conservation

Culture of the freshwater pearl mussel and its contribution to species conservation

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B. Praun

Freshwater mussels are one of the most endangered taxonomic groups worldwide (Williams *et al.*, 1993), and many species are close to becoming extinct due to the pollution, degeneration and destruction of their habitats. The only possible means of saving these populations in the long term is habitat restoration. However, this process is time-consuming and will not be completed before many local populations are lost.

One possible action towards saving the genetic diversity of many of these indigenous populations is to facilitate the artificial reproduction of young mussels, thereby enhancing existing populations until environmental restoration is completed.

The freshwater pearl mussel *Margaritifera margaritifera* has shown a massive decline during the last few decades and many populations are no longer functional (Geist, 2010). Many local populations have already become extinct or are close to extinction. As the freshwater pearl mussel is protected by national and EU legislation (Directive 92/43/EEC), all countries hosting freshwater pearl mussel populations are obliged to protect them and, if possible, enhance remaining populations. The presence of healthy populations is a sign of a pristine river system with no overexploitation in the catchment. Ziuganov *et al.* (1994) suggest four strategies to protect freshwater pearl mussel populations. The first is to create refuge areas with sufficient protection for mussels to survive and reproduce. As a second method, Ziuganov proposes the transfer of adult mussels from healthy recruiting rivers to rivers with threatened populations. A third method is the release of infested host fish. The last method discussed is the culture of freshwater pearl mussels, attempts at which have become more and more numerous over recent years and will be presented in the following sections of this paper.

Culture of the freshwater pearl mussel

Short retrospection

The first attempts in Europe to culture *Margaritifera margaritifera* in the recent past were conducted by Hruška between 1980 and 1990 in the Czech Republic (Hruška, 1992, 1999). Buddensiek continued this work with a series of in-situ experiments using, for the first time, the concept of “hole cages”, or “Buddensiek cages” (Buddensiek, 1995). Between 1999 and 2001, the first culture attempts were made in Scotland by Hastie (Hastie & Young, 2003). Michael Lange from Saxony in Germany further improved the methods used by Hruška and produced very useful protocols on the rearing of juvenile mussels in Buddensiek cages and gravel boxes (Lange & Selheim, 2011). Meanwhile, rearing activities have begun or are ongoing in 14 European countries [1]. This paper will focus mainly on the freshwater pearl mussel (*Margaritifera margaritifera*), but some information will also be given for the giant river pearl mussel (*Margaritifera auricularia*) and two species of North American *Margaritifera* (*M. marrianae* and *M. falcata*).

How to culture freshwater pearl mussels

Life cycle

The life of a freshwater pearl mussel can essentially be described as a complex cycle in which parasitic larvae (glochidia) encyst on the gills of juvenile brown trout (*Salmo trutta fario*). The larvae remain on the fish for several months during the winter period before dropping off in spring and settling into riverbed sediments. Hidden in the sediment, the young mussels develop and become visible at the river bottom after three to five years. At the age of 10–15 years, they are considered to be mature. To begin culturing, it is essential to collect fully-developed larvae (Scheder *et al.*, 2011) from the adult mussels. This can be achieved through two different strategies, as described below.

Home stream strategy

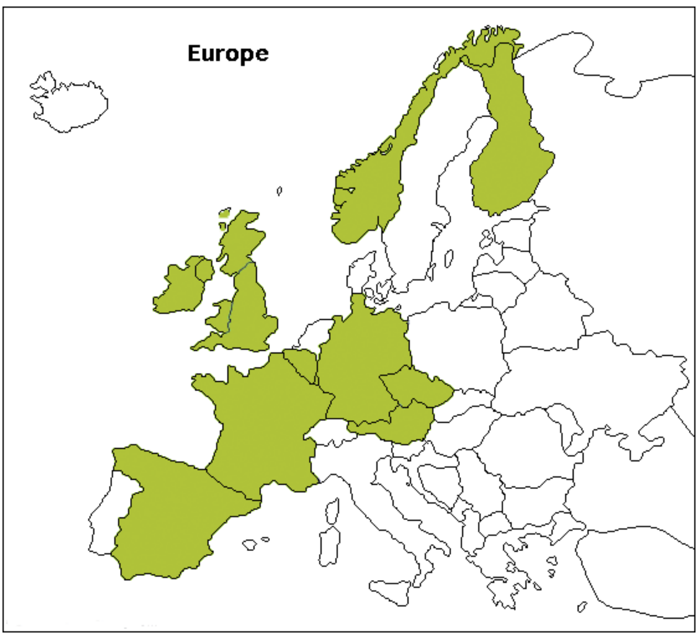
Adult mussels are sought in their home river and observed from late spring to late summer in order to track the development of larvae. Either larvae can be collected in the river at the right moment, or a few mussels carrying larvae can be brought to a hatchery or rearing facility and be kept in a tank until the fully-developed larvae are released. After releasing the larvae, the adult mussels are brought back to the home stream. The collected larvae are immediately used to infest the fish.

Ark strategy

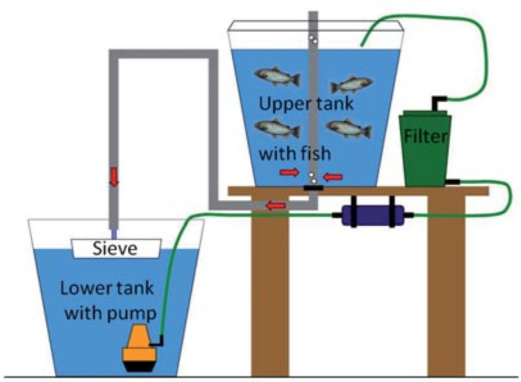
Adult mussels from one or more populations are taken from their respective home streams. Some individuals are brought to a hatchery or rearing facility. Here, the animals are kept in tanks for long periods lasting from a few months to several years. Flow-through tanks supplied with adequate water and food are necessary for the survival of the mussels. In late summer, released larvae are collected and used immediately for fish infestations.

The most commonly used culturing methods

Once the host fish are infested, they are kept in tanks over the winter. As early as January, a preterm juvenile mussel collection cycle can be started (Eybe *et al.*, 2014). A mussel-seed collection set-



[1] ***European countries with* Margaritifera *rearing activities (in green)***



[2] *Mussel-seed collection set-up*

up must be installed, and well-infested fish selected and transferred to the upper tank of the installation [2]. After having obtained enough degree-days (+/-2,500°C) (Hruška, 1992; Thomas *et al.*, 2010), the first juvenile mussels start to drop off the fish and can be collected from the sieve installed above the lower tank. Following the feeding protocol, the mussels reach a size of 1 mm after 100-120 days if cultured in boxes containing water rich in wetland detritus (Eybe *et al.*, 2013). Juveniles having reached the 1 mm stage are often transferred into Buddensiek cages (Buddensiek, 1995; Schmidt & Vandré, 2010) and returned to their home stream. Mussels reaching a larger size (>5mm) after 2-3 years are transferred into larger cages (e.g. gravel boxes, mussel silos). Having spent another few years (2-3) in these cages, the mussels can be released into their home stream.

Other rearing systems

During recent years, attempts to culture freshwater pearl mussels have become more and more numerous, as have the methods involved (Gum *et al.*, 2011). In Northern Ireland, semi-natural systems, such as artificial raceways, have been used successfully (Preston *et al.*, 2007), but more lab-intensive methods such as in vitro culture have also been tried. Another propagation method is the release of infested fish directly into the mussel stream.

Rearing activities throughout Europe and USA

Figures [1] and [3] show (highlighted in green) all the countries or federal states with freshwater mussel rearing activities. The type of rearing activity is shown according to the country or region in which it is practiced (Gum *et al.*, 2011) [Table 1].

Figure [4] summarises some aspects of rearing activities concerning project funding, runtime and the host fish, water, strategy and food used.

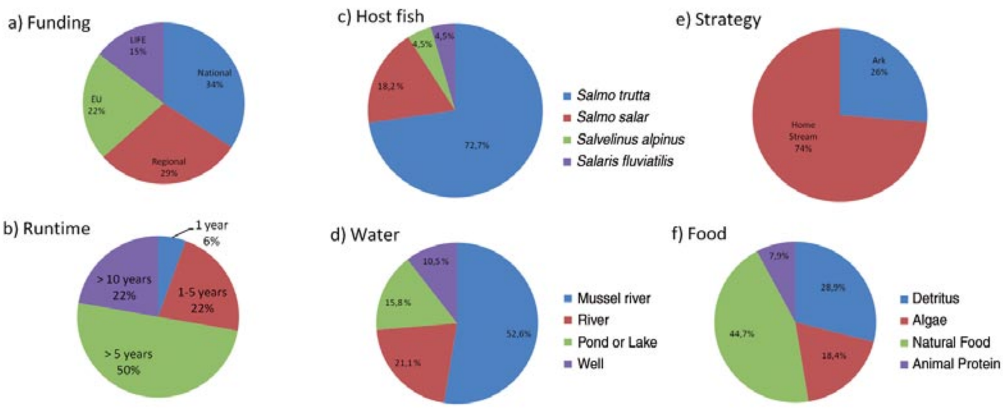
Taking into account all of the ongoing rearing projects throughout Europe, the following recommendations can be made:
- Start captive breeding before mussels are under stress or are already gone!
- Choose a hatchery suited to the needs of mussels (i.e. with regard to water quality), not to those of politicians!
- River restoration planning is time-consuming. Where will juvenile mussels be kept in the meantime?
- Caring for mussels requires passion and patience!

In conclusion (and in contribution to species conservation) it can be stated that:
- Almost all freshwater pearl mussel-hosting countries in Europe have begun rearing activities;
- Successful rearing of *M. margaritifera* is

[3] *US states with* Margaritifera *rearing activities (in green)*

Country	Years	Species/Number of populations	Host fish	Water used	Strategy	Method
Norway	2011-ongoing	M.m/19	*Salmo trutta fario/ Salmo salar*	Pond water	Ark/ Home Stream	Detritus boxes/ artificial stream
Finland	2005/2007/ 2012–ongoing	M.m/1	*Salmo trutta fario*	Lake water	Home stream	Release of juvenile mussels/ in vitro culture
Scotland	2001	M.m/1	*Salmo salar*	River water	Home stream	Gravel cages
England	2007–ongoing	M.m/9	*Salmo trutta fario/ Salmo salar/ Salvelinus alpinus*	Lake water	Ark	Gravel trays
Wales	2004–ongoing	M.m/7	*Salmo trutta/ Salmo salar*	River water	Ark	Gravel trays
Northern Ireland	1999–ongoing	M.m/1	*Salmo trutta fario*	River water	Ark	Semi-natural raceway/ release of infested fish
Republic of Ireland	2006–ongoing	M.m.d/1	*Salmo trutta fario*	River water	Home stream	Semi-natural rearing of juvenile mussels in long tanks with circular gravel covered bottoms.
Germany/ Vogtland	2000–2012	M.m/3	*Salmo trutta fario*	River water	Home stream	Detritus boxes/ Buddensiek cages/gravel cages
Germany/ Passau	2007–ongoing	M.m/4	*Salmo trutta fario*	River water	Home stream	Detritus boxes/ Buddensiek cages/ gravel cages/ release of infested fish
Germany/ Aachen	2006–ongoing	M.m/3	*Salmo trutta fario*	River water	Home stream	Detritus boxes/ Buddensiek cages/ gravel cages/ release of infested fish
Germany/ River Lutter	1973–2001	M.m/1	*Salmo trutta fario*	River water	Home stream	Infestation and release of (autochtonous) fish
Belgium	2005–2012	M.m/3	*Salmo trutta fario*	River water	Home stream	Juvenile mussels released in semi-natural raceway
Luxembourg	2008–ongoing	M.m/1	*Salmo trutta fario*	River water	Home stream	Detritus boxes/ Buddensiek cages/ gravel cages/sand aquaria/ release of infested fish, but also lab-intensive culture
Czech Republic	1990–ongoing	M.m/7	*Salmo trutta fario*	Well water and river water	Home stream	Detritus boxes/ Buddensiek cages/gravel cages
Austria	2010–ongoing	M.m/3	*Salmo trutta fario*	River water	Ark	Detritus boxes/ Buddensiek cages/ gravel cages/silos
France	2010–ongoing	M.m/6	*Salmo trutta fario*	River water	Home stream	Troughs with sand/ lab-intensive culture
Spain/ Galicia	2012–ongoing	M.m/2	*Salmo trutta fario*	River water	Ark	Detritus boxes/ Buddensiek cages/ lab-intensive culture/ release of infested fish
Spain/ River Ebro	2003–2014	M.a/3	*Salaria fluviatilis*	River water	Ark	Release of infested fish in the river/release of juvenile mussels collected in laboratory
USA/Alabama	2013–ongoing	M.mar/1	*Esox americanus*	Well water	Home stream	Detritus boxes
USA/ Washington State	2014–ongoing	M.f./1	*Oncorhynchus clarkii*	Well water	Home stream	Detritus boxes
USA/Missouri	2012–ongoing	M.f./1 from Washington state	*Oncorhynchus clarkii*	Well water	Home stream	Detritus boxes

[Table 1] ***Examples of artificial rearing of freshwater pearl mussels in Europe and in US States (M.m:* Margaritifera margaritifera*; M.m.d:* Margaritifera margaritifera durrovensis*; M.a:* Margaritifera auricularia*; M.mar:* Margaritifera marrianae*; M.f:* Margaritifera falcata*)***



[4] *Percentages of different aspects of Margaritifera culture: (a) funding, (b) runtime, (c) host fish, (d) water used, (e) strategy used, (f) food used.*

possible, but successful release projects are still rare;
- Rearing is and must remain an emergency solution;
- Reared mussels can be used as a bioindicator to find suitable rivers for their release;
- Follow-up monitoring of released mussels must be carried out;
- Knowledge-sharing is important; and
- By far the best solution is the restoration of natural habitat (watercourses). ■

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Establishment of a rearing method for freshwater pearl mussels of the Armorican Massif

Pierrick DURY


Bretagne Vivante – SEPNB

P. Dury

As part of the LIFE program "Conservation of the freshwater pearl mussels from the Armorican Massif" (or LIFE "Mussel"), six populations were put into rearing structures for their genetic conservation and population strengthening in different streams. Three rivers of Brittany (the Élez, the Loc'h and the Bonne Chère) and three watercourses of Lower Normandy (the Sarthon, the Airou and the Rouvre) are concerned.

A building designed especially for mussel rearing

The pearl mussel rearing station on the Favot fish farm in Brasparts, central Finistère, is now fully operational [1]. Each room is usable, making the building ideally-suited to the rearing requirements of the pearl mussel. The farm is spacious and well-designed, making work more effective.

The 300 m² building is equipped with a meeting room, a laboratory (for performing gill checks, mussel sorting, counting and observation under a binocular microscope, preparation of feed, etc.), two mussel rearing rooms, an algae production room and a hall for quarantining the Lower Normandy strains.

Production targets

As part of the freshwater pearl mussel LIFE program, some theoretical production objectives were put forward. Table 1 shows the number of mussels that should be obtained for each strain at the rearing station in 2016, the last year of the program. These objectives take into account production increases of up to 10% per year.

The theoretical objectives presented in the pearl mussel LIFE program application were very high. Meetings with partners in Europe conducting similar actions led to an understanding of the large manpower needs of such rearing activities, which had been underestimated in our case. With the resources available, we had to adapt and develop effective rearing systems that were efficient and had low maintenance requirements.

Cohort	Age	Theoretical expected umber
Cohort 0+	0-1 year	36,000-72,000
Cohort 1+	1-2 years	16,200-32,400
Cohort 2+	2-3 years	7,290-14,580
Cohort 3+	3-4 years	3,280-6,560
Cohort 4+	4-5 years	1,475-2,950

[Table 1] ***Expected production targets for each strain in 2016***

H. Ronné

[1] ***The mussel rearing station was built as part of the freshwater pearl mussel LIFE program***

Verifying maturity and larvae collection

In the field, the various adult populations are monitored by technicians from Bretagne Vivante – SEPNB during the spawning season. Using forceps to very slightly open the freshwater mussels (which requires authorisation), a check is made of genitors to determine which individuals are maturing. These are then monitored for several weeks until the glochidia reach their final stage of development. The larvae are then collected at the edge of the river before being transported to the rearing station. The adults are returned to the river after being marked.

Controlled host-glochidia contact at the rearing station

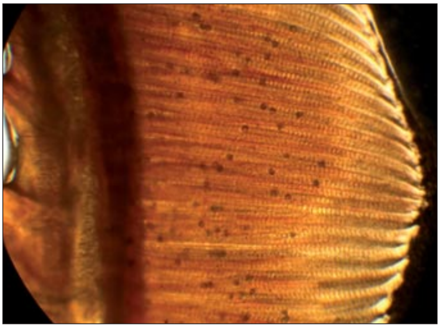
Once at the rearing station, each sample of glochidia is checked to verify its capacity for encystment. This is done by putting a grain of salt on a drop of water containing larvae under a microscope. If the larvae close instantly, this means that they are viable and that infestation is possible. The total number of glochidia is estimated by performing counts on various samples.

For controlled host-glochidia contact, one host fish is used for each 1,000 glochidia. Fish (brown trout) are first fasted, and then placed in a vessel adapted to their number, taking care not to provoke stress in them, which could cause mucus hypersecretion and disrupt infestation. Any external substance or element, such as drops of sweat, is excluded. Water bubbling and an oxygen supply are put in place to keep larvae in suspension and to maintain the dissolved oxygen level, as the water is not changed during the process. Controlled contact lasts between 30 min. and one hour. Some fish are then killed in order to monitor the success of the infestation, which is done by removing the gills [2].

When the controlled contact has been performed, the fish are returned to aquaculture basins where they are maintained in traditional rearing conditions for ten months.

The first production season, 2012

Following the collection of larvae in 2011, millions of young mussels were harvested,



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[2] *Checking the number of larvae encysted on brown trout gills*

including strains from the Bonne Chère River in 2012. For this first year of young mussel harvesting, we wanted to sort meticulously with the help of a large number of volunteers. Unfortunately, soon thereafter, the choice of rearing system proved catastrophic. The young mussels were placed in two Artemia sieves with a mesh of 150 µm. These systems were then placed in plastic incubation trays originally used for the hatching of young salmonids [3]. Almost all of the mussels died in the first few days because the mesh was too fine and became blocked, preventing the free flow of water in the rearing system.

Improvement of the rearing method

Later in 2012, after the failure of the first harvest, we decided to radically change the rearing method for the newly-collected young mussels. Following Frankie Thielen's advice (Luxembourg), mussels from the Elez, the Bonne Chère and the Loc'h were reared in aquariums. These tanks were connected in groups to a collecting trough in which a pump was installed to circulate water in a closed circuit between the different tanks [4].

Each aquarium had a capacity of 20 L and contained a 2cm-thick bed of calibrated sterilized sand. Approximately 1,000 mussels were placed on this bed and were fed daily with a mixture of "Shellfish diet 1800"[1] and "Nanno 3600"[2], which are commercially available algal pastes. The aim was to obtain a concentration of about 30,000 cells/mL, obtained by adding two drops of "Shellfish diet 1800" and 175 µL of "Nanno 3600" per 20 L.

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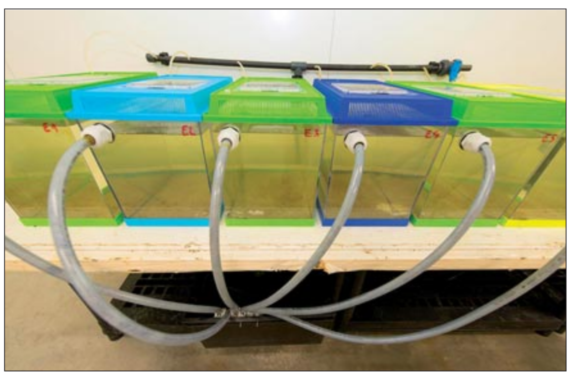
[3] *Artemia sieve in a drawer of a plastic incubation tray*

The rearing water was first filtered to 36 µm, then decanted and brought to the correct temperature. Each week, 80% of the rearing system water was renewed after mixing and siphoning the aquariums. The physicochemical parameters were monitored regularly, in particular the temperature, dissolved oxygen and nitrites.



H. Ronné

[4] *Aquariums installed for the rearing of young mussels*

1 - Shellfish diet 1800: microalgal solution with a concentration of 2 billion cells/mL (size 5-20 µm) composed of 40% *Isochrysis*, 15% *Pavlova*, 25% *Tetraselmis* and 20% *Thalassiosira weissflogii*.

2 - Nanno 3600: a microalgae solution composed of *Nannochloropsis* sp. at a concentration of 750 million cells/mL (size 1-2 µm).

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[5] ***Rearing troughs***

H. Ronné

[6] ***1-year-old mussels being placed in rearing troughs***

Although effective, this system was not optimal. Indeed, the production goals could not be achieved because an excessive number of aquariums would have been required and their maintenance would not have been possible due to lack of manpower. It should also be noted that these volumes are too low for physicochemical parameters to be buffered.

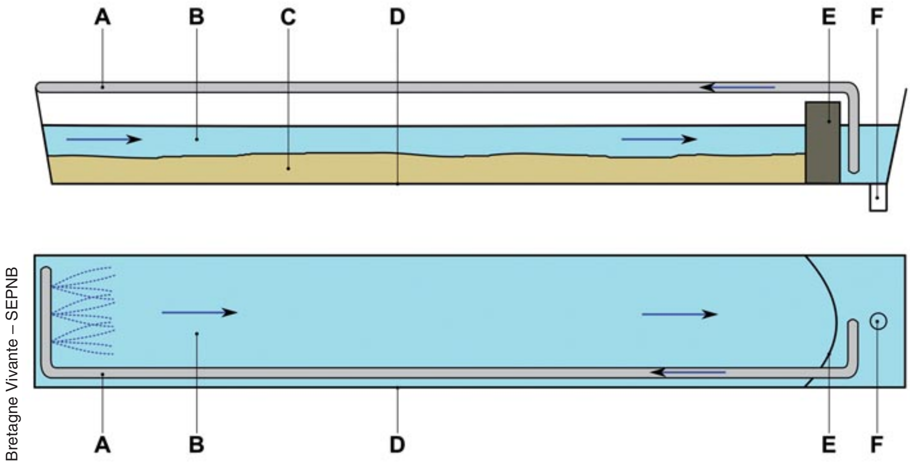
2013-2014: a new rearing system

Applying the experience gained with aquariums, a new system on the same principle was implemented on a large scale. Troughs typically used for rearing salmonids were recycled for the cultivation of pearl mussels [5][6][7].

These systems, with a capacity of 100 to 200 L, were modified to create closed systems that reproduce a miniature artificial watercourse. A fine grid was placed over the end of the trough in order to retain a bed of sand 2–3 cm thick. An aquarium pump was placed behind this grid so as to allow a continuous flow of water. A daily intake of food was supplied (1 mL of "Shellfish diet 1800" and 1 mL of "Nanno 3600"). After trials at different concentrations it seemed that a number ranging from 5,000 to 10,000 mussels is optimal for the first year of rearing. The system maintenance was identical to that of the aquariums used the previous year, but a much larger storage capacity was possible [8].

H. Ronné

[7] ***1-year-old mussels at the surface of the substrate in the rearing troughs***



[8] ***Diagram of the operation of rearing troughs, side view and top view. A: return pipe of the water supplied by a pump; B: water filtered at 36 µm; C: aquarium substrate; D: plastic trough; E: grid containing sand on one side; F: the trough purging system***

2014 Results

Tens of thousands of freshwater mussels have already been grown in the Brasparts rearing station. Brittany strains have been reared since 2012; in 2014 there were three cohorts at the rearing station. In 2011, the quarantine imposed by the veterinary services for Lower Normandy strains prevented the collection of glochidia from the streams involved (and therefore prevented the collection of young mussels in 2012). In 2012, the rise in water levels in the Lower Normandy region prevented the monitoring of maturing, hindering the collection of larvae (as well as the collection of young mussels in 2013). It was only in 2013 that we were able to collect larvae from the Airou and Sarthon rivers and in 2014 that we obtained larvae from all Lower Normandy populations. In 2015 all Lower Normandy strains were in rearing [Table 2].

At harvest, a maximum of 10,000 young mussels are kept for each population, with the hope of high survival rates that will enable us to reach the theoretical targets for older cohorts. We can therefore theoretically rear 60,000 young mussels each year, although this result will depend on the number of mussels collected.

Sixty days of quarantine

The waterways of Brittany are considered free of contagious diseases, including viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN). However, the rivers in Lower Normandy are not considered free of these diseases. For the needs of our program we transported mussel larvae and a small amount of river water from the Lower Normandy streams. In order not to jeopardize the health status of aquatic animals in the watershed, in accordance

Cohort	Elez	Bonne Chère	Loc'h	Airou	Rouvre	Sarthon
Cohort 0+ (0-1 year) - 2015	10,000	10,000	0	2,000	15,000	5,000
Cohort 1+ (1-2 years) - 2014	5,000	10,000	(2,000)	(3,000)	0	(7,000)
Cohort 2+ (2-3 years) - 2013	2,500	2,500	2,400	0	0	0
Cohort 3+ (3-4 years) - 2012	1,210	5	30	0	0	0

[Table 2] ***Young mussels being reared at the station in June 2015. This temporary data in brackets remains to be confirmed.***

H. Ronné

[9] ***The effluent treatment system (ozonizer and ultra-violet filter)***

with Directive 2006/88/EC, the *Direction départementale de la protection des populations* (DDPP) of Finistère asked that quarantine be set up [9]. This unplanned operation represented a considerable additional cost in the construction of the building.

Specifically, the establishment of such a quarantine involves compliance with strict guidelines. During the quarantine period, all effluents must be treated with ozone. To lift the quarantine, virological and serological analyses are conducted on sentinel trout (rainbow trout present in the basins) 15 days before the end of the quarantine period to certify the absence of these diseases.

With more than 58,000 freshwater mussels, the station completely fulfils its role as the main conservatory for the mussel strains of the Armorican Massif. Their long-term conservation now depends on the success of river-improvement operations. Our efforts must now be concentrated on the restoration of high-quality watercourses!

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Three year old pearl mussels

Influence of excystment time on the breeding success of juvenile freshwater pearl mussels

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F. Thielen

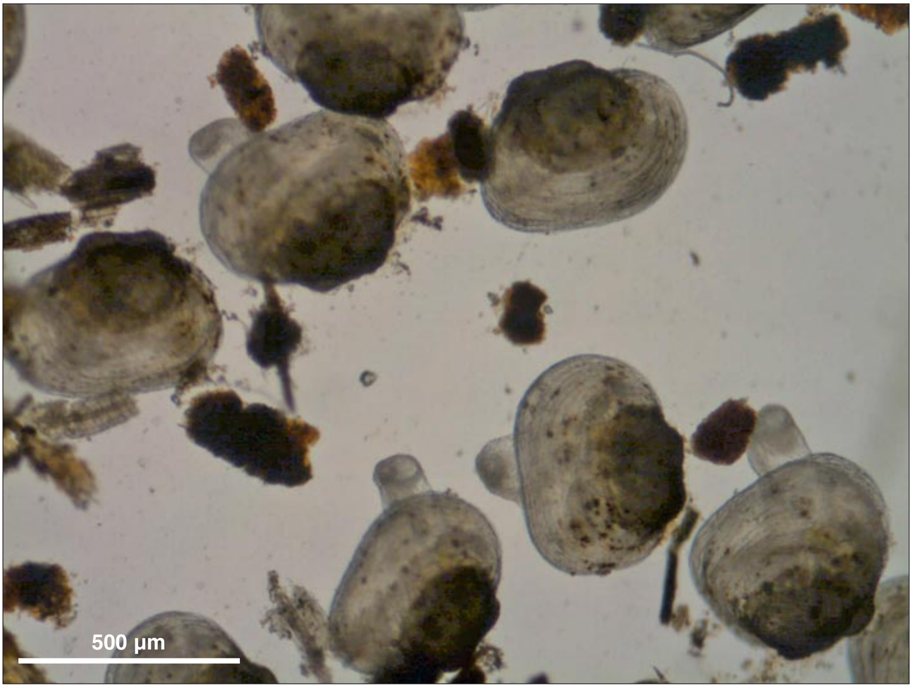
T. Eybe

Rearing in captivity and successive release of the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.) can help to increase the likelihood of its survival in various rivers. Because of time-consuming rearing methods, it is important to choose the strongest and healthiest mussels in order to obtain the highest possible number of fit and fast-growing individuals in the shortest period of time.

Another method used to rear a high number of freshwater pearl mussels is the collection of "mussel seed" early in the year by artificially increasing the temperature in tanks containing infected host fish carrying larvae. Using this method, it is possible to have multiple successive excystment periods in one year and to thereby increase the total number of young mussels collected. However, there is no data available regarding the rearing success of juvenile mussels collected in an artificial preterm excystment period.

In this study, two excystment methods (one artificial in January and one natural in May) were analysed. The growth and survival rate of juvenile mussels were compared in order to determine whether or not artificial excystment periods negatively influence the breeding success of the young mussels. Furthermore, for the two methods, the growth and survival rate of the mussels relative to the excystment period were observed.

An early excystment cycle (January) did not influence the growth or survival rate of the juvenile mussels, and individuals collected during the middle of the excystment period were among the best-suited to captive breeding. Growth of up to 1 mm or more over a period of 110 days and a survival rate of 62-98% was observed. The survival rate of the mussels from the natural excystment cycle was lower than that of the early excystment cycle (7-38%), presumably due to poorer water quality conditions in the river. Thus, an early excystment cycle can represent an advantage if river water conditions become worse in spring and summer (due, for example, to high nitrite or ammonium concentrations or pesticides), as the juvenile mussels which have just detached can grow and become more resistant before fertilisers or pesticides begin to be applied around the catchment area of the river.



T. Eybe

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EYBE T., THIELEN F., BOHN T. & SURES B. 2015 – Influence of the excystment time on the breeding success of juvenile freshwater pearl mussels (*Margaritifera margaritifera*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, pp. 21-30, DOI: 10.1002/aqc.2471.

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Conservation of freshwater pearl mussels in Austria: advances in a controlled rearing system

Daniela GSTÖTTENMAYR, Christian SCHEDER
& Clemens GUMPINGER

M. Huemer

D. Gstöttenmayr

Since 2011, the highly endangered freshwater pearl mussel (*Margaritifera margaritifera* L.) has been the focus of the Austrian species conservation project "Vision Flussperlmuschel". This project aims to establish healthy reproductive populations for two particular mussel strains originating in the Upper Austrian river systems Aist and Naarn. The initial strategy is based on a high annual reproductive success rate as well as on high survival rates of juvenile mussels under controlled conditions.

C. Scheder

C. Scheder

C. Gumpinger

In 2011, in the course of a preliminary project, the infection of the host fish was performed in a semi-natural water body – a millrace – that is inhabited by a freshwater pearl mussel population. In subsequent years the infection process was conducted in an artificial flow-through "mussel-channel" and fish basin system. In both cases, the infection process took place without human intervention, to avoid stress for both the mussels and the brown trout in this critical stage of reproduction. In the following summer, after the transformation of glochidia into juvenile mussels, the latter are collected upon release from their hosts and reared in vitro in climate chambers, where they are kept until they are big enough to be transferred into Buddensiek boxes and relocated to various streams. A major experimental innovation of the year 2014 was the installation of new flow-through channels for juvenile mussels, to be launched completely in the 2015 breeding cycle.

During 2011, the first year of breeding, 658 juvenile mussels were obtained, followed by 1,156 in 2012, 19,295 in 2013 and 40,239 individuals in 2014. In contrast to the high numbers of "harvested" juvenile individuals, survival rates during the first months were low, which strongly reduced the respective cohort.

Introduction

One of the most endangered unionid species in Europe is the freshwater pearl mussel (*Margaritifera margaritifera* L.), listed in Annexes II and V of the European Habitats Directive (Council of the European Union, 2006) and Appendix III of the Berne Convention. As in the rest of their natural distribution area, Austrian mussel populations have declined over the course of the last century as a consequence of intensified forestry activity and intensive agriculture. Other negative anthropogenic impacts, such as riverbed degradation, loss of natural floodplain (lateral connectivity), or problems relating to hydroelectric plants, represent a growing threat to remaining mussel populations (Hastie *et al.*, 2003).

In Austria, the freshwater pearl mussel continues to survive in remnant populations in Upper and Lower Austria. In 2012, the Department for Nature Conservation of the Government of Upper Austria initiated a long-term species conservation project to protect the remaining Austrian freshwater pearl mussel populations and to restore new habitats in watercourses already recognised as having suitable conditions. A captive rearing system was put into place with the aim of supporting indigenous mussel populations with a continuous external input of individuals. As a consequence, the rearing strategy emphasises a high annual reproductive success. The project is built upon two main strategies:
1. Captive breeding, budgeted at about one third of the time and cost of the whole project;
2. Catchment restoration, budgeted at two thirds of the project's resources.

The following text focuses on the captive breeding portion of the project.

Materials and Methods

Infection of host fish and mussel "harvesting"

1) The preliminary project

The preliminary study was conducted in the Gießenbach millrace, a semi-natural waterbody inhabited by 220 freshwater pearl mussels. The Gießenbach stream is situated in Upper Austria and discharges into the River Danube. The millrace divides into two different stretches, which are linked to each other: a 400 m-long semi-natural section that is occupied by the mussel population in question, and a directly adjoining 20 m-long concrete box section that channels the water through the former mill yard. These two sections are separated from each other by a 3.5 m-high dam, formally used as the mill weir. In August 2010, 255 brown trout (*Salmo trutta fario*) yearlings were held in the box section by means of transversal metal bars. When the mature mussels in the upstream millrace section released their glochidia, the host infection took place naturally without human intervention, passing through the respective section of the watercourse. The trout were kept in the concrete box stretch throughout the winter. During the following spring, the 25 fish showing the highest infection counts were transferred into a 2,000 L conical-bottomed water tank with a hole in its centre. The tank was connected through the hole to a 250 L water barrel and water was pumped in a circuit between these two containers. On its way from the fish-tank to the water barrel, the water was sieved through a mesh (100 µm pores), to collect the juvenile mussels. This mesh was checked daily and the contents were brought to appropriate laboratory facilities in Wels, Upper Austria. (Scheder *et al.*, 2014).

2) The Vision Flussperlmuschel project

One of the first steps of the project was the construction of a breeding facility in 2011. Two rearing systems in the form of rearing channels were installed in a container situated on the tributary of the Aist River. For each mussel strain, i.e. the Aist and the Naarn mussels, a separate flow-through channel system was created. Each channel (continually supplied with fresh water from the Aist river system) consists of one mussel basin directly connected with a fish basin. The mussel basins, made to resemble a natural watercourse bed, are about 3.5 m long and 0.54 m wide and provide an artificial habitat for 50 mussels per strain. The fish basins, where about 150 brown trouts are kept per infection cycle, are 1.45 m long and 0.63 m wide. Every summer, between July and August, yearling trouts are put into the fish basins and then undergo an undisturbed acclimatization period. Through the constant water flow, infection with ejected glochidia again takes place without any human intervention. After an infection check, the fish are held over the winter in special cages in an adjacent fishpond. The following May, the fish are retransferred into the container and held in cylindrical water basins with conical bottoms through which water is pumped in a closed circuit. As in the preliminary project, this water is routed through a fine-meshed sieve that enables the team to collect the released juvenile mussels (Gumpinger *et al.*, 2013).

Laboratory work: mussel rearing in climate chambers

The mesh contents are examined after each collection and checked for released mussels (Thomas *et al.*, 2010). The juveniles are transferred into plastic boxes filled with 250 mL water taken from the Aist river system and enriched with a mixture of algae food (Shellfish Diet 1800TM and Nanno 2600TM) and 12.5 mL of detritus [Editor's note: detritus is taken from wetlands close to the watercourse] (Eybe & Thielen, 2010). The water-detritus mixture is renewed weekly concurrently with a count of mussels and the removal of dead individuals. The plastic boxes are stored at 18°C in climate chambers until the mussels are large enough (> 1 mm) to be transferred into special cages in the field (Buddensiek, 1995; Scheder *et al.*, 2014).

In the first year of stage II of the project, new flow-through channels for juvenile mussels, based on the Dury system (Dury *et al.*, 2013), were installed and tested. These channels are 2.16 m long and 0.43 m wide and contain about 90 L of water and a 2 cm-thick sandy substrate layer. Once per week the water of these channels, which originates from rivers of the Aist river system and is pumped in a closed circuit, is changed. In the future, juvenile mussels will be transferred into these channels directly after they are harvested. Furthermore, new wooden cages, based on the system of F. Elender (F. Elender, pers. comm.), and mussel silos similar to Barnhart's structures (Barnhart *et al.*, 2007) have been created. In the following summer the recently released juveniles will be transferred into these new systems which will immediately be placed in the two river systems in question.

Fieldwork: mussel rearing in the rivers

Juvenile mussels that survive the critical period in the climate chambers are transferred into slightly modified Buddensiek boxes (Scheder *et al.*, 2014; Buddensiek, 1995) and then exposed in natural habitats. These streams, officially recognised as suitable mussel habitats, belong to the Aist and the Naarn river systems. The Buddensiek boxes are checked twice a year.

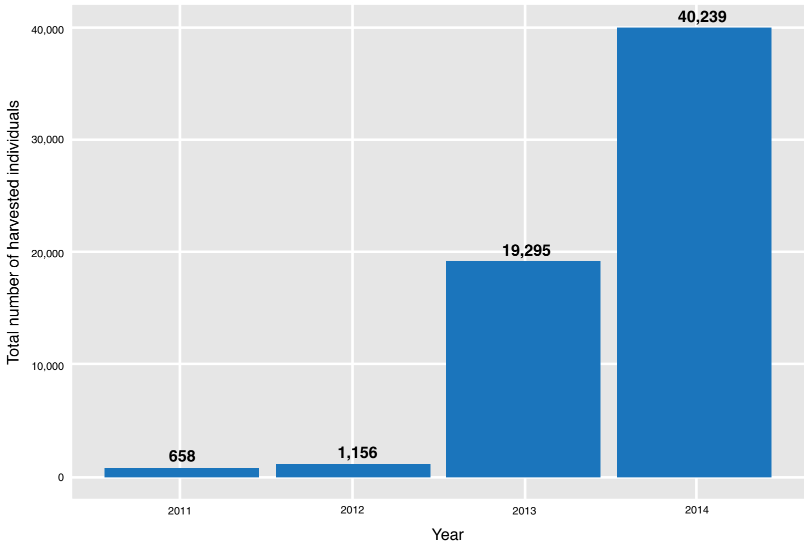
Results

In the preliminary study of June 2011, 658 juvenile mussels were obtained. From May to July 2012, in the first year of the project, 1,156 juvenile mussels were obtained, followed by 19,295 specimens in 2013 and 40,239 mussels in 2014 [1]. In 2012, all of the reared mussels originated from the Naarn mussel strain, while in contrast all juveniles of 2013 were descended from the Aist mussel strain. There was no reproductive success of the respective other strain in either year. However, in 2014 reproduction was successful for both strains, with no notable difference in the "harvest success" (18,982 juveniles of the Aist strain as compared to 21,257 juveniles of the Naarn strain).

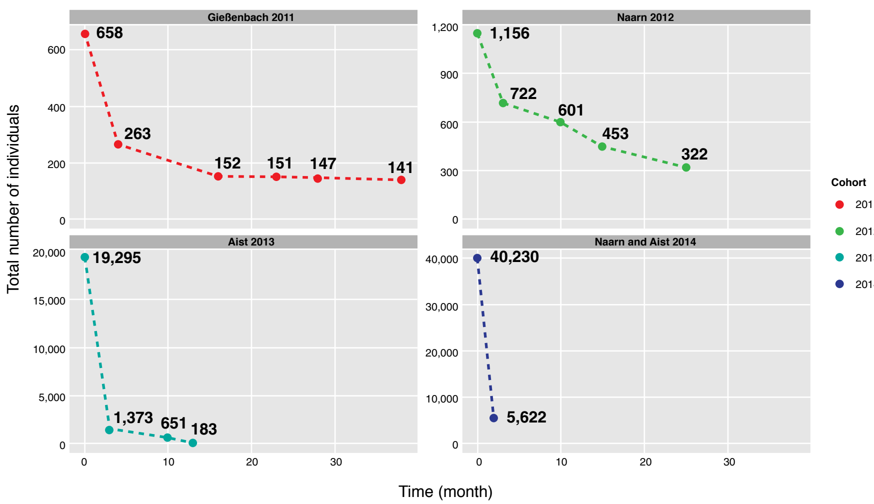
Survival rates during the first summer were identified as a crucial factor for annual breeding success. The survival rates of juveniles of the particular cohorts up until October were: 39.97% in 2011, 62.46% in 2012, and 7.12% in 2013. In 2014, 13.97% of the harvested mussels survived until September. After this bottleneck in the rearing system, the numbers of mussels in the particular cohorts tended to stabilise. Between October 2013 and August 2014, when survival was assessed, the survival rates of the cohorts 2011, 2012 and 2013 were 95.92%, 71.08% and 13.33% respectively [2].

Discussion

The comparison of the "harvesting success" in consecutive years indicates that the workflow concerning the first stages of the breeding process was constantly improved. Most notably, the 40,239 juveniles gathered in 2014 represent a major advance in the mussel rearing method since 2011. However, while the numbers of harvested mussels increased annually, the numbers of individuals that survived the first summer season did not. Indeed, in the last two years of the project, the mortality rates of juvenile mussels in their first month after metamorphosis were very high (92.88% in 2013 and 86.03% in 2014). Furthermore, the results indicate that mortality rates of mussels decrease after the first year. This phenomenon can particularly be observed in the 2011 cohort. The findings highlight the need for methodological improvements in the second stage of rearing. In order to



[1] *Total numbers of harvested freshwater pearl mussels per year*



[2] *Numbers of juvenile freshwater pearl mussels in time (per month) and by distinct cohorts*

achieve a higher rate of annual reproductive success, the mortality rate of juvenile mussels in their first months must be reduced. ■

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Session 3
Monitoring - Ecotoxicology

Initiation of a population dynamics study of the freshwater pearl mussel in the upper valley of the Vienne (France) using "N-Mixture" models of abundance

Cyril LABORDE, David NAUDON, Cloé MARCILLAUD & Aurélien BESNARD

Trace metal accumulation and bioavailability in the Ulla basin (NW Spain): evaluation of the potential effects on the freshwater pearl mussel

Juan ANTELO, Manuel SUÁREZ-ABELENDA, Cristina PASTORIZA, Jesús BARRAL, Paz ONDINA, Adolfo OUTEIRO, Sabela LOIS & Juan Manuel ANTELO

Ecotoxicological study of sensitivity to metal contaminants of the pearl mussel in the upstream part of the Dronne, Dordogne (France)

Magalie BAUDRIMONT, Patrice GONZALEZ, Alexia LEGEAY, Nathalie MESMER-DUDONS, Éric GOURSOLLE, Julie CHEVALIER, Bénédicte PÉCASSOU & Romain PAPIN-VINCENT

Climatic and environmental control of shell growth in the endangered freshwater pearl mussel (Brittany)

Julien THÉBAULT, Clémence ROYER, Aurélie JOLIVET, Pierre-Yves PASCO, Marie CAPOULADE, Philippe MASQUELIER & Laurent CHAUVAUD

Initiation of a population dynamics study of the freshwater pearl mussel in the upper valley of the Vienne (France) using "N-Mixture" models of abundance

Cyril LABORDE, David NAUDON, Cloé MARCILLAUD
& Aurélien BESNARD

C. Laborde

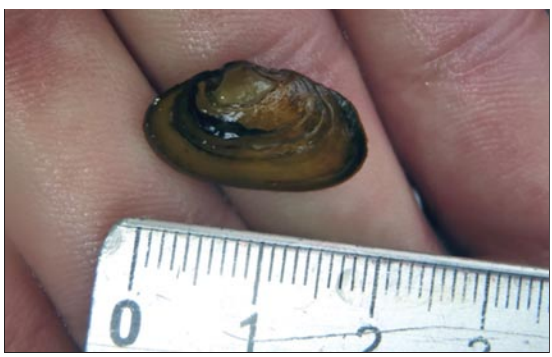
The Limousin region is situated in the western foothills of the Massif Central, a granite massif with a very dense river network. This is a favourable habitat for the freshwater pearl mussel (*Margaritifera margaritifera*), with at least 46 rivers occupied by the species. However, very little is known about the species in this area: less than 1% (Naudon, 2015) of the river stretches suitable for the species have been surveyed (ONEMA, 2009).

D. Naudon

C. Marcillaud

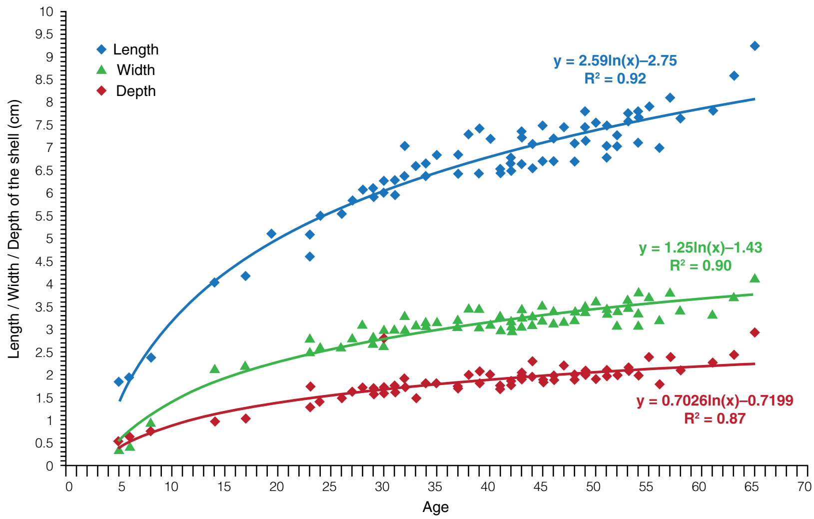
A. Besnard

A study conducted in 2011 (Laborde, 2011) identified a 15 km area (between the municipalities of Nedde and Tarnac) where 746 individuals were recorded over 2.44 km. The youngest mussel observed measured 1.8 cm [1], the proportion of juveniles (<6 cm) was 43%, and reproduction was observed (regular presence of glochidia on brown trout). This population appears to be a "hotspot" of the Vienne catchment and exhibits a relatively good state of conservation demographically [2].



[1] ***Photo of a juvenile pearl mussel of 1.8 cm found in Nedde***

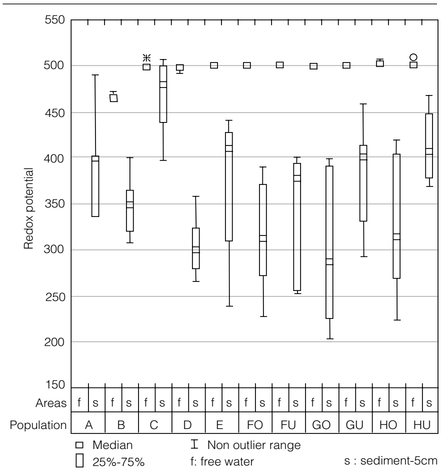
In 2013, a genetic study was conducted (Kuehn & Geist, 2014). According to Geist, the population of the Vienne presents



[2] ***Growth curve of the freshwater pearl mussel in the upper valley of the Vienne***

"a very high genetic variability and low influence of genetic drift [...]. Substrate quality [...] corresponds to the quality observed in functional populations, [...] this population deserves a high conservation priority" [3].

In order to monitor population dynamics, a survey was conducted in 2014 by repeated counts on a systematic random sample of river sections between Nedde and Tarnac, taking into account the detectability of the species [4]. This work was carried out with official authorisation from the prefecture.



[3] ***"Boxplot" of redox potential in open water and at a depth of 5 cm in the substrate in Margaritifera margaritifera populations in the Limousin region (France). Site "C" is the Vienne at Nedde (Kuehn & Geist, 2014).***

Materials and Methods

N-Mixture models

Mixed models of abundance or N-Mixture models (Royle, 2004) are based on random sampling and repeated counting on several sites. This type of model assumes that local abundances follow a Poisson distribution, and that the distribution and counting of individuals are independent of time. It provides an estimate of the average abundance per site, the site occupancy rate, and the probability of detecting individuals. The method makes it possible to observe the changing demographics of a population at each repeated survey (mortality, recruitment, colonisation, extinction, etc.).

The assumptions for using this method are that:
– the population is closed during each year of study;
– detections at each site are constant and independent between visits. Variations can be modelled using covariates; and
– the integration factor K must be defined (upper limit of abundance per site).

Method application

84 study sites were selected by systematic random sampling done by taking a section of 20 linear metres (by 20 m wide) every 200 m over a length of 15 km (the study area). A survey of the river bottom was made using a bathyscope to cover each site in 30 minutes; this was done 3 times [5].

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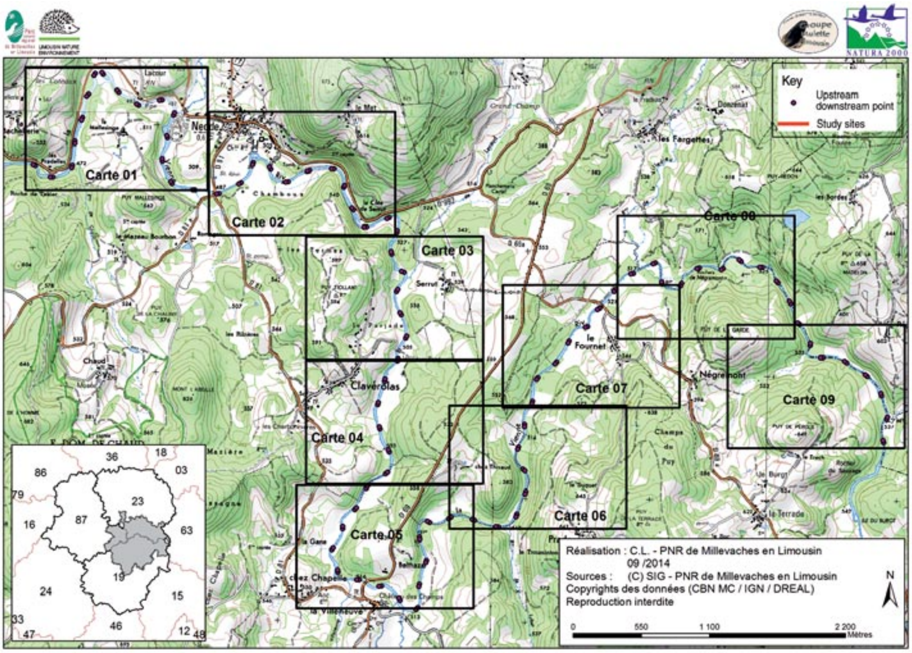

[4] ***Area of study and systematic random sampling***

Surveying consisted of making 4 return journeys in the river, covering the entire area and noting the abundance observed (living and dead mussels). Each river section was located with a GPS and marked with paint. The hydromorphology of the river and its surroundings was also noted.

Data analysis (N-Mixture)

N-Mixture models are robust statistical tools that make it possible to estimate all calculable probabilities for the river sections studied. Several models could be compared, notably for exploring the impact of covariates on the probability of detecting individuals or on local abundance.

The "Akaike Information Criterion" was used to compare models, making it possible to select the model that best described the data with the least possible parameters. It is provided, together with the estimations, in the PRESENCE© program (Hines, 2006).

We then tested the model's suitability to the data using a goodness-of-fit test. Following these tests, random effects on detection and abundance were introduced. To do this, the analyses were reproduced in the Bayesian framework to improve the reliability of results in a manner that took into account variability in abundance caused by burrowing and other phenomena. By this method, the probability of detection and the abundance observed are allowed to vary around a mean, following a normal distribution.

Results

Raw results of the N-Mixture model

The monitoring made it possible to pass 3 times over 51 river sections. The sections

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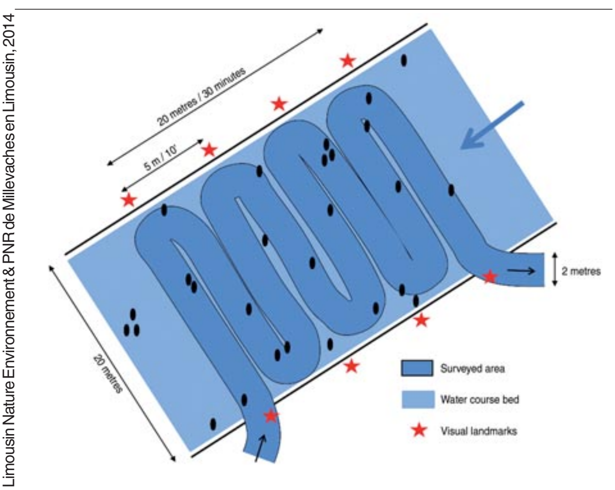


[5] ***Surveying protocol for a 400 m² study area***

that were not surveyed were excluded due to lack of time and/or accessibility. The raw data are presented in [Table 1].

With regard to the data analysis, factor K was set at 200. The covariate used was the section code. We used the "Royles biometric-Repeated Count Data" model in PRESENCE© (Hines, 2006). The analysis was performed 3 times: once for living mussels, once for empty shells and once for both [6]. Empty shells can "exit" or "enter" the study population, leading to a breach of the assumption that the population is closed between the survey passes. The high variability in shell observations during the 3 passes invalidates any analysis of this data. We therefore went no further with interpretations concerning these shells.

AIC = 1,291.6		
Visible occupancy rate: **0.8039**		
Total visible abundance: **321** (living mussels)		
Estimated occupancy rate (psi): 0.9999		
Estimated occupancy (psi)	std.err	95% Confidence interval
psi (site): **0.9999**	0.0000	0.9999-0.9999
Total estimated abundance (N): **2,974 individuals** (living mussels)		
Total estimated abundance		95% Confidence interval
N(site): 2,974		455-14,040

Limousin Nature Environnement & PNR de Millevaches en Limousin, 2014

[6] ***Results of the analysis by mixed model of abundance for living individuals performed in PRESENCE©***

As the goodness-of-fit test was relatively inconclusive on the data set, we added "Bayesian" random effects [Table 2], in order to better take into account burrowing phenomena.

Site occupancy and detectability

On each of the 3 passes, we found 32, 37 and then 27 sites where the species could be detected. The cumulative results made it possible to show that at least 80% of sites were occupied. In a single pass, the species was thus detected in 5 to 7 cases out of 10. The N-Mixture model selected indicates that 99.99% of the sites would be occupied, but with varying abundances. Hence, when no mussels were observed 3 times on a site, there were in fact very probably 3 to 4 individuals.

The probability of individual detection of mussels is between 0.00% and 19.32% depending on site and pass. By performing 3 passes of 30 minutes on 400 m², we "miss" on average 84 to 100 individuals per pass, with an overall detectability of 15.57% for the study as a whole.

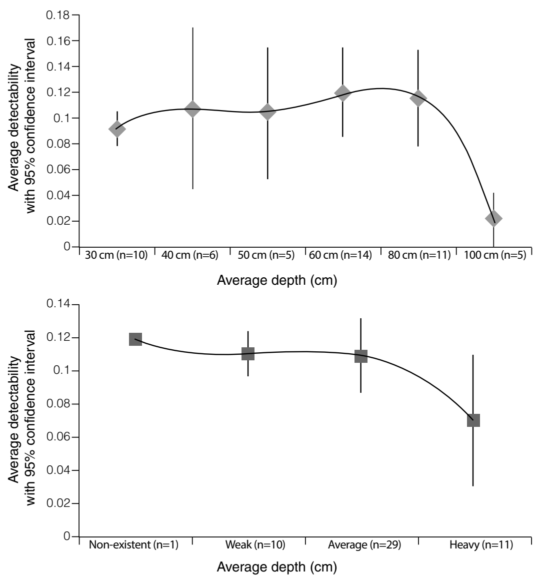
We searched for correlations between detectability and the hydromorphological parameters noted. The Figure [7] illustrates the decrease in detectability when the depth of the water exceeds 80 cm. At this depth, visibility is very poor. To a lesser extent, heavy shade decreases detectability.

Abundance and density

There is a more than 95% chance of finding between 177 and 255 individuals during a pass of the study sections, while the cumulative observations suggested that there were at least 321 distinct individuals observed over the 3 passes.

On the passes, we counted successively 188, 254, and 205 living individuals. The model indicates that the true abundance is about 2,974 living individuals (with a broad 95% confidence interval between 455 and 14,040) on these 51 sites.

The densities per site were between 0.009 and 1.82 living individuals per m², with an average of 0.146. The 10 sites for which no observations were made had densities below 1 individual per 100 m². The figure [8] shows that detectability is optimal for densities that are neither too weak nor too strong.



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[7] ***Effect of depth (at the top) and shading (in the bottom) on detectability***

Limousin Nature Environnement & PNR de Millevaches en Limousin, 2014

Abundance				Hydrology				River bank, riparian vegetation & riparian plots		
Id site	Abundance 1st passage june 2014	Abundance 2nd passage june 2014	Abundance 3rd passage june 2014	Average depth (cm)	Shading	River facies	Warping class (Archambaud *et al.*, 2005)	Main substrate couple (1 and 2)	Qualification of main substrate couple	Land use on riparian plots
10	0	0	0	100	>75%	Rapids	4	Stone/Stone	Bad	Leafy forest/Leafy forest
20	0	0	1	80	>75%	Rapids	2	Stone/Stone	Bad	Leafy forest/Leafy forest
30	1	0	0	80	<25%	Rapids	1	Stone/Stone	Bad	Leafy forest/Coniferous forest
40	1	2	2	60	25<X<50%	Slow flat facies	1	Stone/Sand	Average	Leafy forest/Meadow
50	2	2	2	60	25<X<50%	Rapids	1	Stone/Sand	Average	Leafy forest/Meadow
…	…	…	…	…	…	…	…	…	…	…
540	1	3	2	60	25<X<50%	Flat rapids	1	Gravel/Stone	Good	Leafy forest/Leafy forest
550	0	4	0	50	25<X<50%	Flat rapids	1	Sand/Pebble	Average	Leafy forest/Leafy forest
640	0	1	0	80	25<X<50%	Slow flat facies	1	Gravel/Pebble	Good	Leafy forest/Culture
650	0	0	0	50	25<X<50%	Rapid flat facies	1	Pebble/Stone	Bad	Leafy forest/Culture
660	0	0	0	40	25<X<50%	Waterfall	2	Gravel/Pebble	Good	Meadow/Meadow
670	0	0	0	100	<25%	Rapid flat facies	3	Sand/Stone	Average	Urban area/Meadow

[Table 1] ***Raw data from the 3 surveys of the 51 study sites***

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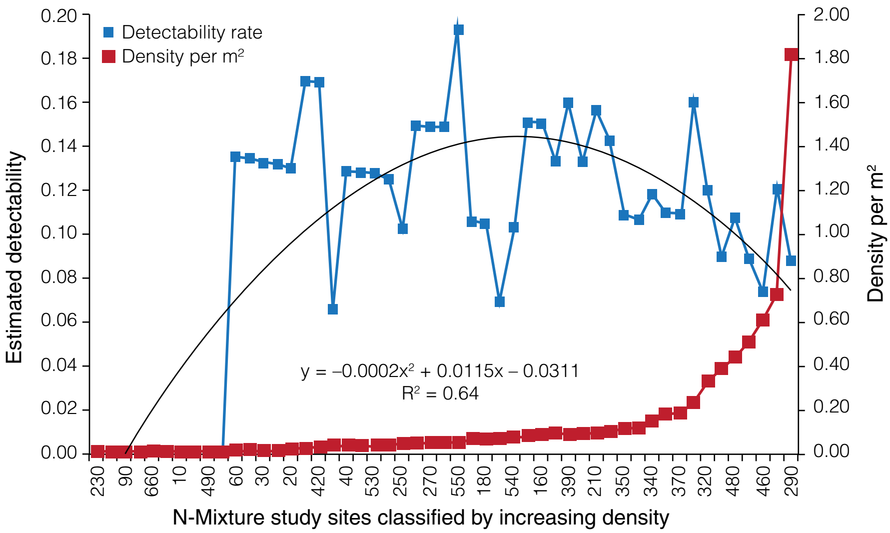
ID	Abundance 1st passage (06/2014)	Abundance 2nd passage (07/2014)	Abundance 3rd passage (08/2014)	Id	Detectability	Mean (estimated abundance)	Mean (estimated density au m²)	Standard dev.	2.5%	25.0%	50.0%	75.0%	97.5%	Rhat
10	0	0	0	N[9]	0.00%	3	0.009	7.99	0	0	1	4	22	1.12
20	0	0	1	N[10]	13.05%	8	0.019	14.33	1	2	4	8	40	1.01
30	1	0	0	N[8]	13.27%	8	0.019	12.42	1	2	4	8	39	1.01
40	1	2	2	N[19]	12.87%	16	0.039	22.33	2	4	8	18	75	1.01
50	2	2	2	N[20]	6.91%	29	0.072	43.69	2	7	15	33	139	1.01
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
550	0	4	0	N[11]	19.32%	21	0.052	26.52	4	7	12	23	94	1.02
640	0	1	0	N[4]	13.22%	8	0.019	12.74	1	2	4	8	40	1.01
650	0	0	0	N[30]	0.00%	4	0.009	8.05	0	0	1	4	22	1.13
660	0	0	0	N[43]	0.00%	3	0.009	7.38	0	0	1	4	22	1.09
670	0	0	0	N[51]	0.00%	3	0.009	7.61	0	0	1	4	22	1.11
				total N		2,974	0.146	3,366.55	455	950	1,792	3,575	14,040	1.02
				alpha.lam		2.5367		0.86	1.195	1.859	2.436	3.112	4.44	1.01
				beta		-2.1404		1.00	-4.229	-2.839	-2.1	-1.382	-0.4038	1.02
				mean.N		58.3093		66.01	8.922	18.63	35.14	70.1	275.3	1.02
				mean.det	15.57%	0.1557		0.12	0.0159	0.06188	0.1231	0.2277	0.4256	1.02
				sd.lam		1.5814		0.24	1.165	1.413	1.562	1.729	2.11	1.00
				sd.p		0.7146		0.32	0.3262	0.5024	0.6312	0.8342	1.578	1.00
				fit		86.5031		20.86	44.6	72.49	87.63	100.9	125.6	1.00
				fit.new		80.5761		18.05	44.65	68.52	81.07	92.76	115.5	1.00
				deviance		425.2300		36.55	333.1	407.7	432.5	450.2	479	1.00

[Table 2] ***Results of the estimates following Bayesian N-Mixture Modelling***

We searched for correlations between detectability and the hydromorphological parameters measured. Surprisingly, no effect of the substrate, clogging, or facies could be observed on abundance, which can be explained by the fact that the habitat is generally favourable. The figure **[9]** shows the only relationship observed, in which the pearl mussels are more abundant in deciduous forest sectors.

Discussion

This survey has limitations: observer bias, the fact that the mussels burrow into the sediments, etc., which are illustrated by the wide range of estimates. To overcome this, we made detectability and abundance variables in our model, allowing the latter



Limousin Nature Environnement & PNR de Millevaches en Limousin, 2014

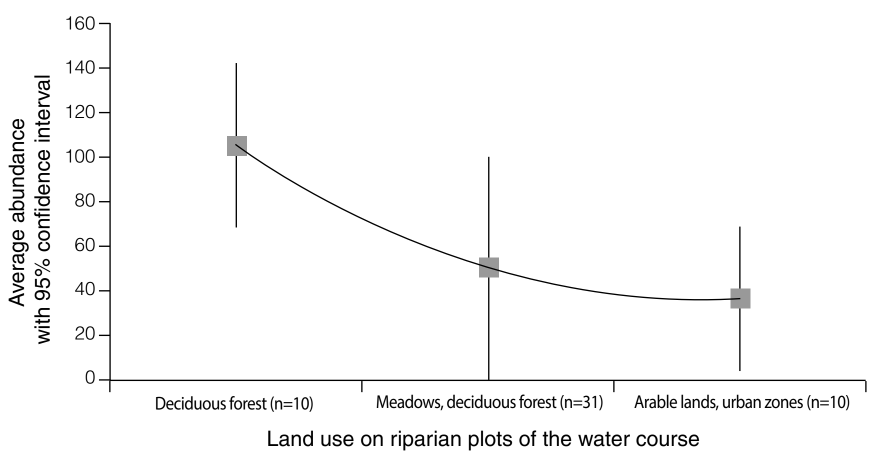
[8] *Correlation between detectability and density of pearl mussels (n = 51)*

to take into account individuals that spent part of the monitoring survey buried. However, we also need to ask what should be done about individuals that remain buried throughout the 3 passes.

A proportion of buried individuals between 24 and 61% was observed in Normandy (Beaufils, 2012) in 3 study sites over a period stretching from May 2012 to July 2012.

We could study burrowing by a Capture Marking Recapture (CMR) method (Otis *et al.*, 1978), or perform the 3 passes on a site in the same day. However, this would add a new bias to the detectability related to memorisation of observations from one pass to the next.

These results were compared with a similar survey made in 2014 based on CMR (Naudon, 2015), which only took into account non-buried individuals. These studies are consistent with one another and support the use of the statistical models developed here.



Limousin Nature Environnement & PNR de Millevaches en Limousin, 2014

[9] *Effect of land use on the abundance of pearl mussels*

Conclusion

Our study estimated the abundance of pearl mussels in our sampling area at 2,974, or an average of 0.146 individuals per m² (0.009 and 1.82 depending on the site). The species is present at 100% of the sites. We can conclude that it is fairly easy to detect the species on a site of 400 m² with the densities present.

With regard to abundance, on a wide river and with a single operator making 3 repeated passes, the detectability of the species was very low, and the operator observed only between 0 and 19% of individuals. This led to very wide confidence intervals for the abundance estimates. A more accurate modelling of detectability and variations in abundance between sites could help to refine these estimates.

This study illustrates the importance of taking into account detectability in pearl mussel population monitoring. Because the observer sees very few individuals, only robust statistical models can estimate and monitor the population dynamics of a species such as the pearl mussel over time. ■

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Trace metal accumulation and bioavailability in the Ulla basin (NW Spain): evaluation of the potential effects on the freshwater pearl mussel

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D. Arán

Like many threatened species, unionoid bivalves are facing severe extinction rates. Because of their sensitivity to ecosystem stress, many of this group are considered bioindicator species. The highly threatened *Margaritifera margaritifera* is a clear example of this. A benthic filter feeder, sedentary and long-lived, the freshwater pearl mussel is highly susceptible to anthropogenic contamination and to the presence of trace metals in surface waters and sediments.


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In order to assess the accumulation and mobility of trace metals (Cu, Zn, Ni, Cr, Cd and As) in the Ulla River basin, measurement of metal content was conducted and enrichment factors were calculated to evaluate anthropogenic influence on the presence of trace metals in the riverine sediments. The observed results show an accumulation of trace metals in the lower reaches of the basin, with the largest concentrations of Cu, Zn and As present in sites affected by acid mine drainage[1]. Statistical results showed a negative relationship between the

1 - Acid mine drainage is the steady runoff of an acidic mineral solution resulting from certain types of mine or from the storage of mine waste. It locally contributes to the global acidification of fresh water, a phenomenon that has been noted for several decades on a large scale.

distribution of trace metals in the river basin and the abundance of *M. margaritifera*. Additionally, the freshwater pearl mussel was found to be absent or minimally present when the sediments had the highest contents of Cu, Zn, Ni and As, whose synergistic relationship would affect species survival.

Introduction

M. margaritifera populations have diminished or even disappeared over recent decades, with many populations incapable of reproducing successfully (Young *et al.*, 2001). In Europe, the species is currently listed as “critically endangered” (Cuttelod *et al.*, 2011) or “endangered” by the International Union for Conservation of Nature. Various types of impact, such as climate change, introduction of invasive species, eutrophication, habitat alteration, or decline of salmonid host populations have been considered as triggers of this population decrease (Österling *et al.*, 2010). Freshwater mussels are considered highly susceptible to increases in the basal content of trace metals in sediments as a consequence of their long and sedentary life in direct contact with the substrate (Farris & Van Hassel, 2007).

Riverbed sediments are major sinks and carriers of metals in aquatic systems, with 99% of pollutants being stored in sediments throughout the hydrological cycle (Filgueiras *et al.*, 2004). The concentration of accumulated trace metals depends on their inflow from catchment areas, mineralogy, grain size, and physicochemical properties. However, trace metal content in sediments determines not only the survival, but also the biogeochemical mobility and availability of these metals.

The present study, designed as a long-term project, has as its goal a description of the likely relationship between the trace element content of river sediment and the abundance of freshwater pearl mussel populations. The total content of trace metals and arsenic (considered *a priori* as a determinant of pearl mussel decline) was measured to assess its accumulation in the sediments. Sequential chemical extraction was carried out to analyse its mobility and potential availability. Multivariate statistical analysis was used in the interpretation of the metal/metalloid dataset, with regard to distribution throughout the river basin and the effects on the *M. margaritifera* population.

Materials and methods

The Ulla River is the second largest basin in Galicia (NW Spain), comprising a total surface area of 2,764 km^2. Several areas of the river basin are included in the network of Special Areas of Conservation (SACs) as per European Union Directive 92/43/EEC, for the purpose of ensuring the long-term survival of threatened species and habitats. Thirty-nine surface sediment samples (0-15 cm) were collected from the Ulla basin between May and September 2012. These samples were air-dried for 8-10 days and sieved to collect the fraction < 63 µm, which was used for the chemical analysis and sequential extraction. The total concentration of trace metals was determined by ICP-OES following microwave-assisted digestion. BCR sequential extraction (Ure *et al.*, 1993) was carried out to obtain information on metal speciation in the sediments. This extraction selectively determines those metals associated with carbonates or exchangeable metals (BCR-1), metals bound to amorphous iron and manganese oxides (BCR-2), metals associated with sulphides or organic matter (BCR-3), and metals linked to geological materials (BCR-4).

The *M. margaritifera* populations and their densities were estimated in a previous study in which the Ulla River and its tributaries were divided into sections of 1 km in length (Lois *et al.*, 2014). For each section, several stretches of 50 m were sampled using aquascopes or by free diving. Visible individuals were counted and superficial digging (15 cm depth) was conducted to find the buried young mussels. The transect density was calculated as described by Krebs (Krebs, 1999).

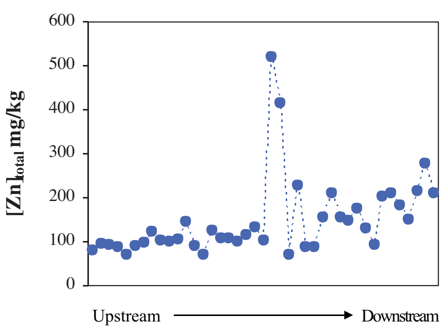
Results and discussion

Distribution of pearl mussel populations in the Ulla basin

The results obtained in the transect studies show an irregular distribution of *M. margaritifera*, allowing a differentiation among zones. In the upper basin, including the upper Ulla and Arnego rivers, the *M. margaritifera* patches extend along several kilometres in a more or less continuous distribution, with a notable absence near the Portodemouros reservoir, a dam located in the centre of the river catchment.

In the Arnego River this species reaches its top abundance with densities of up to 8.90 individuals/m², and an estimated population of 14,085 individuals. Nevertheless, the low representativeness of young mussel specimens in its age structure indicates a critical state in the conservation of the population.

Downstream, certain *M. margaritifera* populations exhibited a notable decrease in their range of distribution and the number of individuals. In both the Deza River and the lower Ulla River, the species distribution consisted of isolated specimens and/or low-density patches at a wide distance from one another. 75 specimens are estimated in the Deza River, and 161 in the middle and lower reaches of the Ulla (Lois *et al.*, 2014). The Deza River also suffers from population ageing, with 65% of the specimens >50 years old whilst individuals <5 years old were not observed.

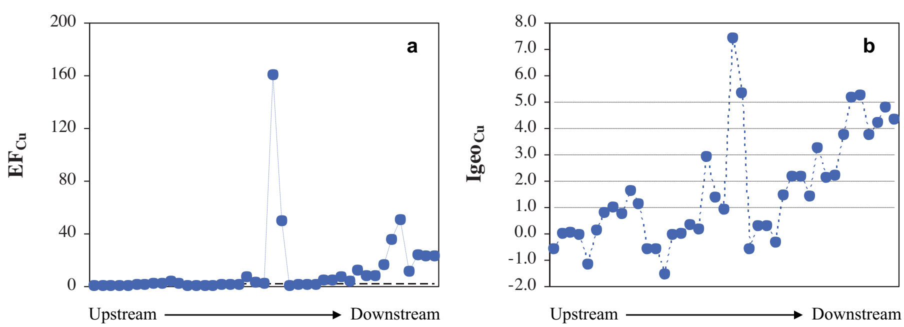


[1] *Spatial variation of Zn in the riverine bed of the Ulla River basin*

Spatial variation of metals and geochemical indexes

Throughout the riverine basin, the total concentration of major elements (Fe, Al) showed less variation than the distribution of trace elements (Cu, Ni, Zn, Cr, Cd, and As), which tended to accumulate downstream [1]. The highest levels of trace metals (excluding Cr) were found in the samples collected at those sites affected by mining activities (Cu, Sn and W). The results obtained suggest that the accumulation of trace metals in sediments might be caused by the discharge of acid mine drainage.

Accumulation of the trace elements found along the Ulla basin was assessed using the enrichment factor *(EF)* and the geo-accumulation index *(I_{geo})*. Both indexes provide a normalisation of the metal/metalloid concentration (using Al as a normalising element) and are widely used to separate natural and anthropogenic contributions. As an example, Figure 2 shows the calculated *EF* and I_{geo} values for Cu along the riverine basin [2]. Generally, *EF* and I_{geo} values indicated a moderate to strong accumulation of Cr and Ni in the upper reaches (attributed to the presence of Cr- and Ni-bearing minerals). As for Cu, Ni, Zn and Cd, accumulation was accentuated (from moderate to moderately strong) in the watercourses close to the copper mine and at the confluence with the Ulla River. Moreover, the I_{geo} index indicated there was no As or a very low accumulation of this element in the sediments, while *EF* indicated moderate accumulation at the confluence of the Sn/W mine.



[2] *Values of the enrichment factor (a) and geo-accumulation index (b) for Cu along the riverine basin*

Trace element speciation

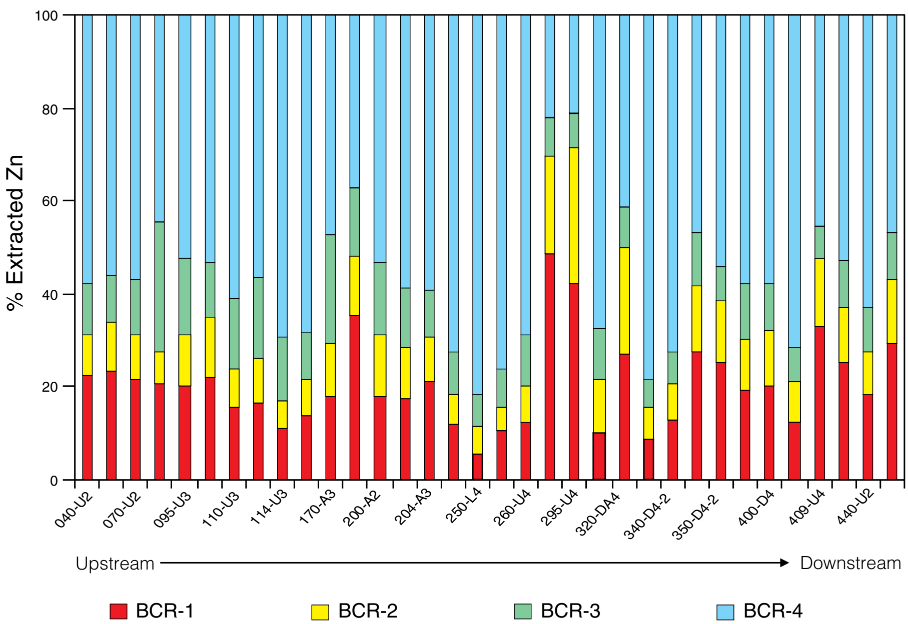
The total content in and of itself does not completely explain the likely metal interaction on the studied pearl mussel population. The mobility, bioavailability and toxicity of trace metals in natural systems depend strongly on their specific chemical form and on their associations with mineral and organic fractions.

The residual fraction (BCR-4), assigned to lithological materials, predominates for Fe, Al and Cr in most of the samples, comprising >80% of the total concentration. Ni, Zn, Cu and Mn show larger contributions in the most mobile fractions (BCR-1 and BCR-2, assigned to the presence of carbonates and amorphous oxides, respectively), highlighting the relatively high mobility and potential bioavailability of these elements. Changes in the physicochemical conditions of the substrate, such as acidification or changes in the redox potential, might release significant amounts of these trace elements into the aquatic system. The fraction associated with organic matter (BCR-3) was found to be important for Ni, Zn and especially Cu [3]. This conforms to the affinity that trace metals have for organic matter and indicates a lower mobility.

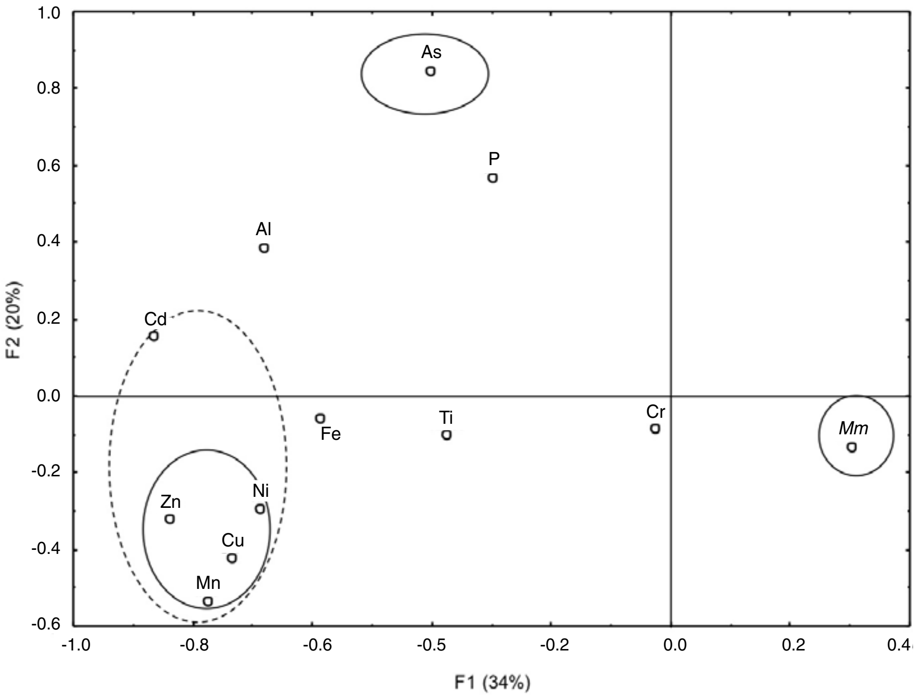
Statistical analysis

One-way ANOVA was used to discriminate differences in the grain-size, TOC and metal content (including total and BCR concentrations) between the defined regions of the river basin. Factorial analysis provides the most detailed appraisal of the distribution of trace elements in the river basin and of the behaviour of *M. margaritifera* populations in response to the accumulation of such elements.

Accordingly, factor loadings indicate a clear contraposition between the abundance of *M. margaritifera* and high concentrations of trace elements (excluding Cr), which coincides with the results from the ANOVA analysis, where a negative correlation is found between the population of bivalves and metals associated to the amorphous oxides and organic matter [4]. The combined information between factor loadings and factor scores indicates that sampling sites in the upper reaches, for both the Arnego and Ulla rivers, have the largest abundance of *M. margaritifera* and the lowest amounts of trace metals (excluding Cr, which is mostly present in the BCR-4 fraction). Moreover, sites from the lower reaches of the basin showed a lower density of bivalves and a higher accumulation of trace elements in the sediments



[3] *Distribution of Zn in different geochemical phases of sediments from the Ulla River*



[4] *Projection of factor loadings in the F1 F2 space*

Conclusions

The present study offers some indications of a probable way that *M. margaritifera* abundance is affected by the presence of the metal concentration in river sediments above their critical levels. The survival of *M. margaritifera* populations is not directly affected by trace metal content in sediments, but by the degree of mobility and potential availability of these metals. *M. margaritifera* population decline should not be exclusively ascribed to the accumulations of trace elements, since there must be other underlying causes. *M. margaritifera* is a good indicator of environmental quality as well as that of the riverbed and surface waters, pointing out the better preservation of the higher reaches of the studied basin. ■

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Ecotoxicological study of sensitivity to metal contaminants of the pearl mussel in the upstream part of the Dronne, Dordogne (France)

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The pearl mussel *Margaritifera margaritifera* is rare in French rivers today, although a relatively large population of 15,000 individuals was recorded in the River Dronne (Dordogne). Although the water quality of the Dronne is generally considered good, a source of sporadic pollution has recently been identified.


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This is an unauthorised dumping site used for many years to leave all kinds of waste, including batteries. Runoff is discharged into the river close to a mussel bed of 1,000-1,500 individuals. Among the metal contaminants that may be present, cadmium (Cd) is well-known to cause varying degrees of cellular or physiological dysfunction in different species (Percival *et al.*, 2004; Gonzalez *et al.*, 2006; Marie *et al.*, 2006). However, until now, no ecotoxicological study has been made on the effect of metal pollution on the pearl mussel.

The objectives of this study were therefore to add to knowledge about the potential impacts of metal pollutants on *M. margaritifera* through paired field and laboratory studies, using (i) an in situ study of the potential harmful effects of leaching from uncontrolled dumped waste on pearl mussels in the Dronne; and (ii) a laboratory study of the impact of a well-known toxic metal, cadmium.

Materials and Methods

Field Study

Adult individuals of *M. margaritifera* (n = 113 individuals) were sampled in April and July 2009 and again in March 2010, upstream and downstream of the uncontrolled waste dump, following an authorization to collect obtained from the Ministry of the Environment in 2009. The analyses concerned 10 individuals from each site for each of the 3 periods considered.

1) Experimental study with Cd

Thirty *M. margaritifera* individuals collected in July 2009 were used to perform a Cd exposure trial under controlled laboratory conditions. Five experimental treatments were set up, with 6 individuals per experimental unit: an "upstream control" treatment corresponding to individuals collected upstream of the waste dump; a "downstream control" treatment corresponding to individuals collected downstream from it; 2 Cd exposure treatments made by direct supply of the metal at 2 and 5 µg/L; and finally an exposure to estradiol at 100 µg/L as a positive control for endocrine disruption, given that Cd is a metal that can alter the endocrine regulation of individuals, as well as reproductive hormones (Pierron *et al.*, 2008). After 14 days of acclimatisation, individuals were exposed to each of these treatments for 7 days at 12°C in water from the Dronne on a 7 cm-thick layer of ultra-pure sand substrate.

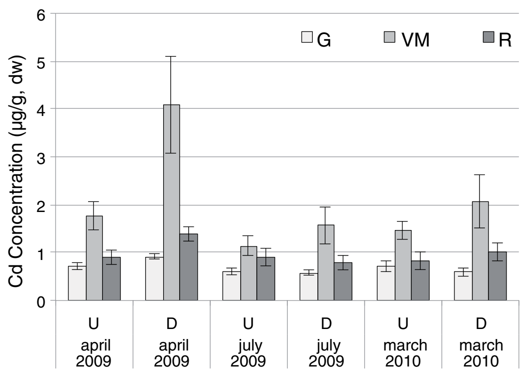
2) Parameters analyzed

Morphometric measurements of shell size were made. Condition indices were calculated from the weight of the flesh relative to the shell weight, and 3 tissue types were then separated: the gills, the visceral mass and the remaining tissues (mantle and muscles). The metal concentrations were determined by ICP-MS or atomic absorption spectrophotometry. Detoxification mechanisms were assessed by measuring concentrations of metallothioneins (MTs), which are cytosolic proteins capable of sequestering metal ions (Baudrimont *et al.*, 2003; Marie *et al.*, 2006; Paul-Pont *et al.*, 2010). In parallel, expression measurements were made on some genes involved in different cellular functions: countering oxidative stress (cytoplasmic and mitochondrial superoxide dismutase: *sod* and *sodMn*, respectively), mitochondrial metabolism (cytochrome c oxidase subunit: *cox1*; and 12S ribosomal gene) and metal detoxification (metallothionein: *mt*). Gene expression was measured by qPCR compared to a reference gene encoding ß-actin, after the sequencing and characterisation of genes previously unstudied in this species. Finally, histological analysis of the gonad was performed on pearl mussels exposed to Cd in the laboratory to determine the sex of the individuals.

Results and Discussion

Field Study

Cd bioaccumulation analyses and measurements of other metals in the pearl mussel tissues sampled upstream and downstream of the waste dump showed that there were significantly higher accumulations downstream relative to upstream, particularly for Cd [1] but also for Pb, Cr, As and Co. This pattern was strongest in April 2009, but also appeared in July.



[1] *Cd concentrations measured in mussel tissues (G: gill, VM: visceral mass, R: rest of the body) taken from the Dronne, upstream (U) or downstream (D) of the waste dump in April and July 2009 and March 2010 (mean ± SEM, n = 10). Different letters indicate significant differences at the level p = 0.05.*

The highest Cd concentrations reached up to 10 µg/g (dry weight) in the visceral mass. This value exceeds the threshold of 5 µg/g (dw) for shellfish judged fit for human consumption. Although the pearl mussel is not consumed, this Cd accumulation is occasionally high. If we compare these values with measurements in pearl mussels from Germany or Finland, which were found to have Cd concentrations of 50 and 7 µg/g (dw), respectively, in their visceral mass (Frank & Gerstmann, 2007), we can say that the levels found in the present study are among the lowest measured in Europe.

The July 2009 gene expression analysis indicated that the expression of the main gene involved in mitochondrial metabolism *(cox1)* is higher in individuals from the downstream sampling point than those from upstream, regardless of the tissue

Function	Organ	Gills	Visceral mass	Kidney
Countering oxidative stress	*sod*	1.09	1.91	0.72
	sodMn	0.65	1.65	0.79
Mitochondrial metabolism	12S	0.75	0.75	0.51
	cox1	1.53	2.61	2.15
Detoxification	*mt*	0.31	1.81	0.58

[Table 1] ***Downstream/upstream regulation factors for different genes studied in* M. margaritifera *after sampling upstream and downstream of the waste dump at Saint-Saud-Lacoussière in July 2009 (n = 10). Factors > 2 show a significant up-regulation and those < 0.5 a down-regulation.***

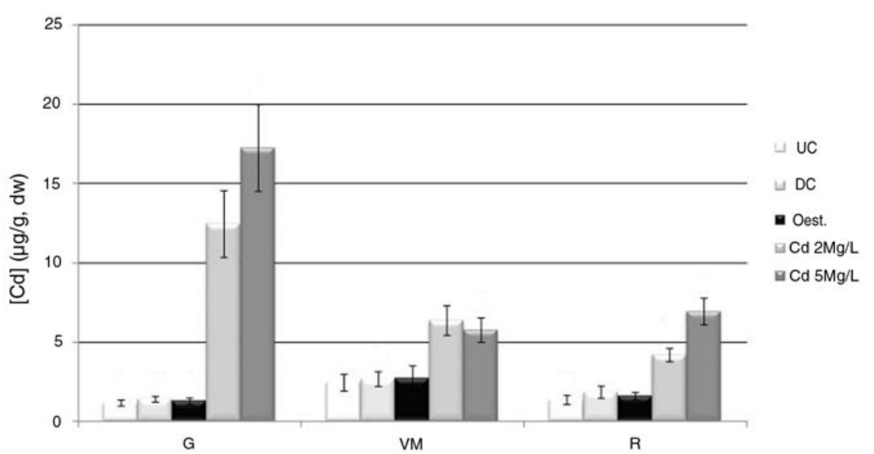
type analysed [Table 1], indicating a disruption of the mitochondrial respiratory chain in mussels. Expressions of the genes *sod*, *sodMn* and *mt* in the visceral mass also indicate that oxidative stress was generated in this tissue. The pearl mussels thus seem to be impacted by the previously-observed metal contamination, particularly in the visceral mass and kidney, whereas for this period of sampling, metal accumulations were not the highest observed.

With regard to metallothionein (MT) protein synthesis, a significant increase was observed in the downstream site relative to the upstream one in July 2009, consistent with the metal accumulations observed. These defence proteins' detoxification mechanism was therefore activated.

Experimental study with Cd

Cd bioaccumulation results following direct exposure showed marked accumulations of Cd in the gills in the 2 and 5 µg/L treatments [2]. Such concentrations are dependent on the exposure dose, particularly in the gills and remaining tissues. In contrast, the concentrations measured in the "downstream control" and "estradiol" treatments show no accumulation of this metal. The pearl mussel *M. margaritifera* therefore has a relatively large Cd accumulation capacity since, in 7 days, it multiplied its concentration in the gills by a factor of around 15.

Concentrations of MTs measured in parallel show only a weak response to this metal compared to the "upstream control" treatment [3]. This means that, unlike most species, the pearl mussel seems to react very little in terms of detoxification by these proteins in the presence of Cd. This result is interesting because it may suggest a particular sensitivity of the pearl mussel to this metal. In contrast, a very strong induction of these proteins was measured in the visceral mass of the mussels from the



[2] ***Cd concentrations measured in 3 mussel tissues (G: gill, VM: visceral mass, R: rest of the body) in relation to exposure conditions (mean ± SEM, n = 6). UC: Upstream Control, DC: Downstream Control; Oest: Estradiol***

downstream site. These values are similar to those measured in situ in July 2009, suggesting the presence of other contaminants able to induce this response.

Comparative gene expression between the upstream and downstream sampling points indicates the generation of oxidative stress in the gills by up-regulation of the gene *sodMn*. The expression of the main gene involved in mitochondrial metabolism (*cox1*) is also higher, indicating a disruption of the mitochondrial metabolism. Finally, for the mt gene, the strongest up-regulation was noted in the kidney, followed by the gills and visceral mass. These results are consistent with the protein concentration measurements. For exposure to Cd, a dose-dependent response was found in all three organs. In the gills, Cd causes up-regulation of the genes *cox1* and *mt* but also down-regulation of cytoplasmic sod. The same trends are found in the kidney and visceral mass. These results are consistent with previous studies in exposure to Cd in model organisms such as zebrafish *Danio rerio* (Gonzalez *et al.*, 2006) or the bivalves *Corbicula fluminea* and *Crassostrea gigas* (Legeay *et al.*, 2005; Marie *et al.*, 2006).

Finally, histological analysis of the gonads revealed a predominance of hermaphrodite individuals among the controls. It is largely accepted that pearl mussels have separate sexes; however, in adverse conditions, females may develop hermaphrodism in order to maintain their reproduction (Bauer, 1987). It seems that the mussels of the Dronne are in this situation, which is positive in the sense that these individuals are able to reproduce (mature gonads with oocytes and sperm). However, these results suggest a reaction or adaptation of populations to their current conditions in the river, which can be considered as rather unfavourable. ■

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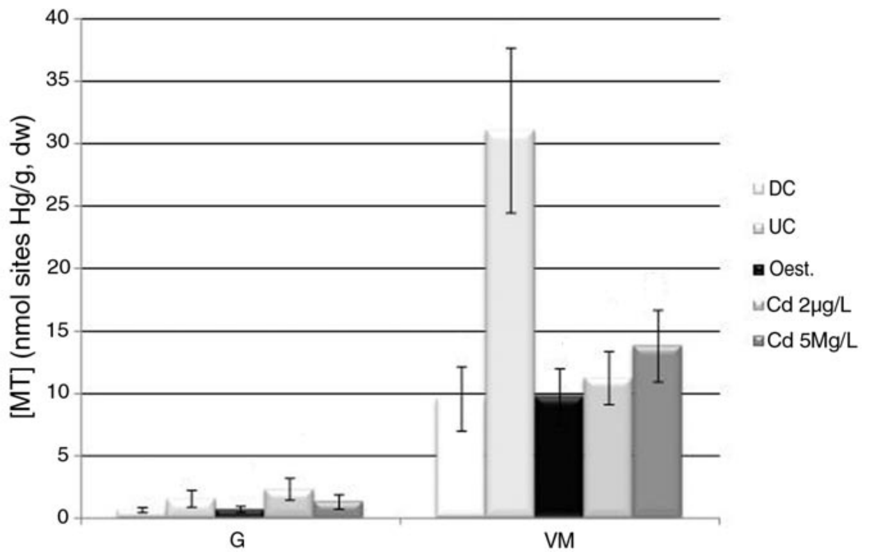

[3] *Metallothionein concentrations (MTs) measured in the two main tissues of* Margaritifera margaritifera *(G: gills and VM: visceral mass), according to exposure conditions (means ± SEM, n = 6).*

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Climatic and environmental control of shell growth in the endangered freshwater pearl mussel (Brittany)

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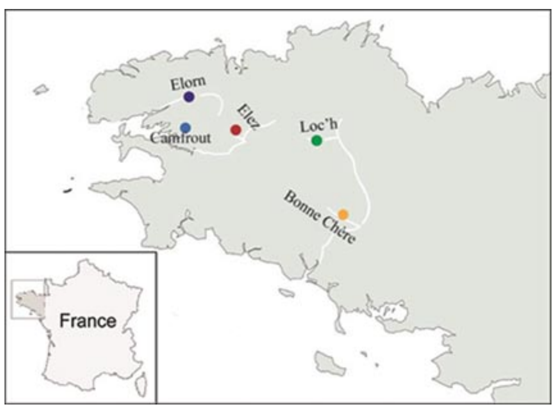
The freshwater pearl mussel (*Margaritifera margaritifera*) is a long-lived bivalve with a broad biogeographic distribution spanning European rivers and eastern North America (Geist, 2010). Since 1996 it has been classified as an endangered species on the IUCN Red List. Over the last century, there has been an estimated decline of 90% in freshwater pearl mussel populations living in European rivers (Geist, 2010) and of 99% in Brittany, one of the two main areas in France still hosting healthy freshwater pearl mussel populations (Cochet, 1998).

The preservation of *M. margaritifera* is based on sound knowledge of its biology, and especially its growth, which can be inferred from growth lines that appear as concentric striations on the shell surface and within the valves (internal striations visible in shell cross-sections). Rypel *et al.* showed that the deposition rate of growth lines is annual, with a winter slowdown in shell growth (Rypel *et al.*, 2008).

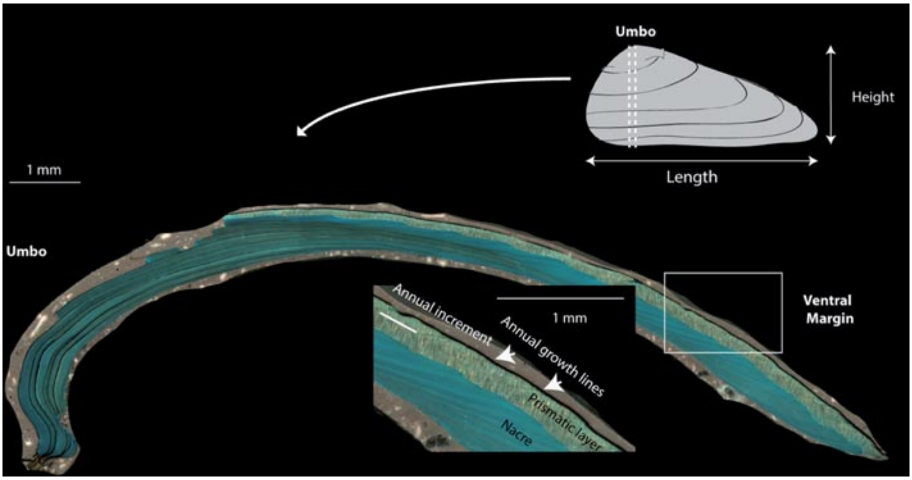
The present study sought to analyse the shell growth of pearl mussel populations in Brittany over the second half of the 20th century through the analysis of internal growth lines, and to make comparisons with other European populations. A further objective was to study the influence of climate and environment on pearl mussel shell growth by comparing Standardised Growth Indices (SGI) with several environmental and climatic variables.

Materials and Methods

Sixty-eight *M. margaritifera* shells were collected from 5 rivers in Brittany between 1996 and 2011 [1]. Only the shells of well-preserved dead mussels were kept and measured. The left valves were prepared according to sclerochronological methods as described by Thébault *et al.* in order to obtain one 1 mm-thick cross section per



[1] ***Map of harvesting locations: (1) Elorn River, (2) Camfrout River, (3) Elez River, (4) Loc'h River and (5) Bonne Chère River***



[2] ***Shell morphometric measurement axes (top right) and cross-section of a shell after immersion in Mutvei's solution (below)***

specimen (Thébault *et al.*, 2009). Annual growth rates were estimated by measuring the width of each growth increment visible in the outer shell layer on cross sections of all the specimens. Absolute dating of each annual increment was performed by backdating from the ontogenetically oldest increment **[2]**.

To enable comparisons with other European populations, shell growth in each population was modelled using the Generalized von Bertalanffy Growth Function. The model's mean asymptotic shell length (L_∞) and growth coefficient (k) were then used to calculate the overall growth performance index φ' (Pauly & Munro, 1984). This index was calculated (1) for each population in Brittany, (2) for Brittany as a whole by the pooling of data from all populations, and (3) for previously-studied European populations.

Shell width increment naturally decreases throughout the organism's lifespan. Annual growth increment time-series therefore require a correction for this ontogenetic trend in order to (1) compare the annual shell growth anomaly between individuals of different ages, and (2) to isolate environmental signals. To this end, we calculated Standardised Growth Indices (SGIs), a method that is based on the ratio between measured and predicted increment width (derived from the Generalized von Bertalanffy Growth Function). This ratio was calculated for each annual increment and for each specimen (Schöne, 2003).

Physicochemical data on the rivers were obtained from Osurweb[1], with the exception of the Camfrout River (Réseau rade de Brest[2]). Air temperature measurements were obtained from the European Climate Assessment & Dataset[3] website and the inter-annual variations of AMO[4] index were obtained from the NOAA[5] website as a time series of annual values. The AMO is known to affect the European summer climate, air temperature, frequency of extreme events, and ecology of organisms.

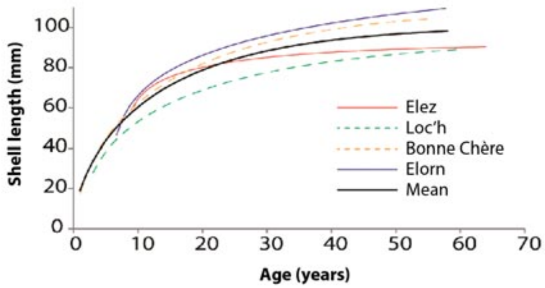
Results

The oldest shell (66 years old) was found in the Elez River and the youngest (10 years old) in the Bonne Chère River; the mean age of specimens was 42 years at the time of death. Populations from the Elez and Loc'h rivers were characterised by low L_∞ and high k values, in contrast with populations from the Bonne Chère and Elorn rivers **[Table 1][3]**. Growth parameters of the overall mean growth curve for Brittany were 105.8 mm and 0.069 y^{-1} for L_∞ and k, respectively, resulting in a φ' value of 2.89 **[Table 1]**. At the European level, φ' ranged from 2.51 (Northern Finland) to 3.02 (Ireland). There was a statistically significant inverse linear

1 - Osurweb: Agence de l'eau Loire-Bretagne website: www.eau-loire-bretagne.fr
2 - Réseau rade de Brest: The goal of Rade de Brest network is the acquisition and analysis of water and environmental data: www.rade-brest.fr
3 - European Climate Assessment and Dataset: http://eca.knmi.nl
4 - AMO: Atlantic Multidecadal Oscillation
5 - NOAA: National Oceanic and Atmospheric: www.esrl.noaa.gov

River	L_∞ (mm)	k (y^{-1})	φ'
Bonne-Chère	104	0.068	2.87
Elez	88	0.11	2.92
Elorn	110	0.047	2.76
Loc'h	79	0.12	2.87
Brittany	105.8	0.069	2.89

[Tableau 1] **Von Bertalanffy** ***growth parameters fitted from growth data from Brittany pearl mussel populations***

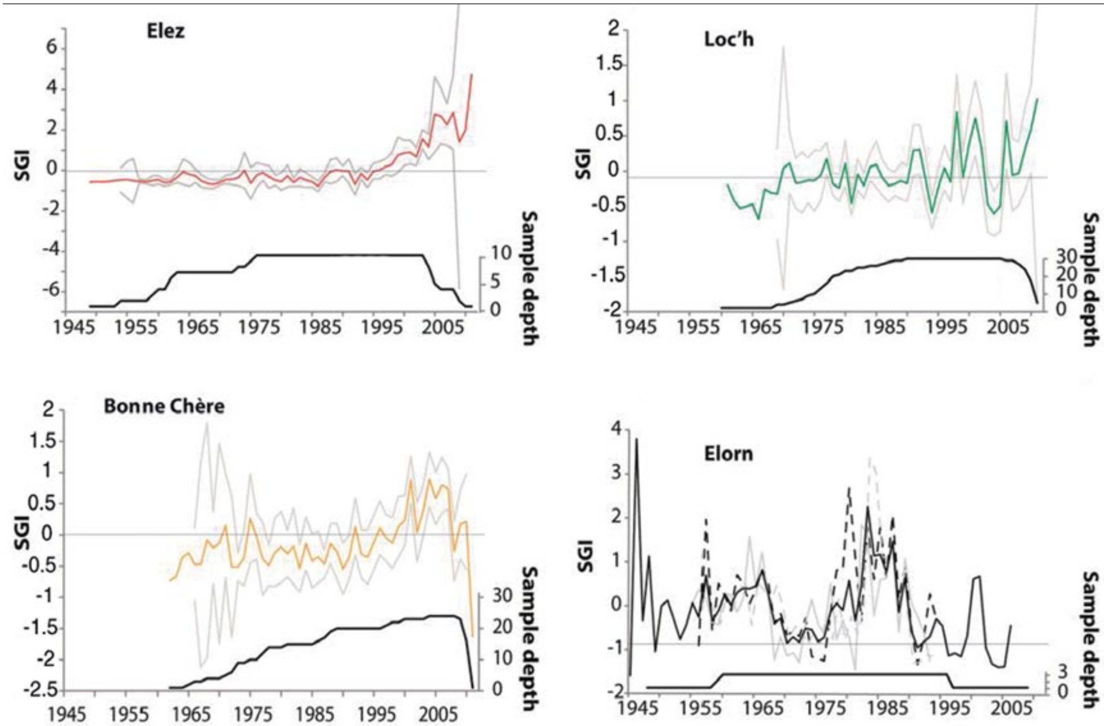


[3] **Von Bertalanffy** ***growth models for 4 populations investigated in the present study and for all of Brittany after pooling all our data (mean)***

relationship between latitude and φ' ($r^2 = 0.67$, $p < 0.05$) and a positive linear relationship between average air temperature and φ' ($r^2 = 0.8$, $p < 0.01$).

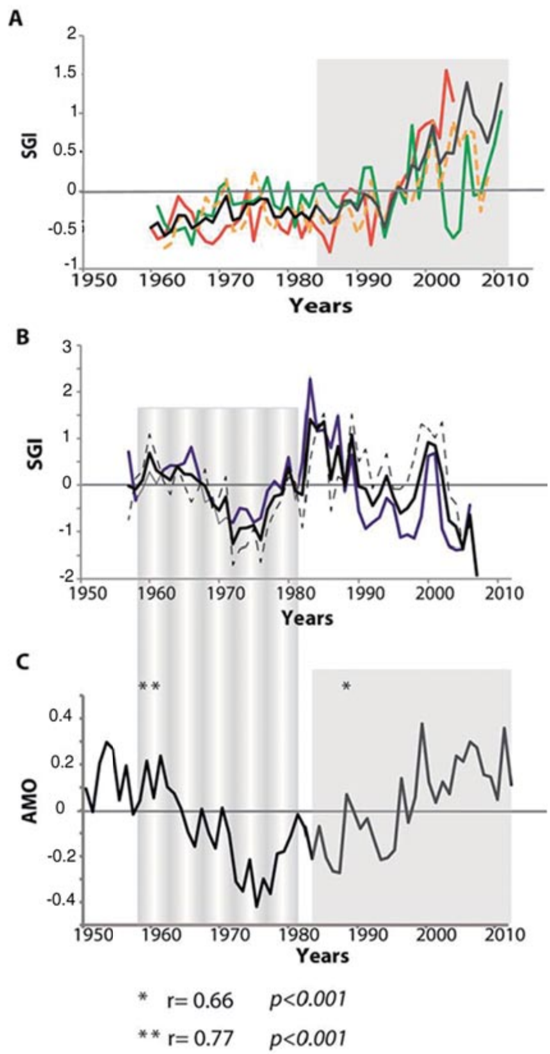
The SGI mean chronology from the Elez, Loc'h, and Bonne Chère populations presented a negative phase until 1995-1996, followed by a positive phase. The Elorn population displayed a negative phase between 1968 and 1979 and two positive phases over the periods 1946-1967 and 1980-1989 périodes 1946-1967 et 1980-1989 [4]. The Elez, Elorn and Loc'h SGI mean chronologies were positively correlated with temperature ($0.52 > r > 0.89$, $p < 0.05$). In addition, positive correlations with minimum conductivity (Elez: $r = 0.88$, $p < 0.05$) and with mean flow (Elorn: $r = 0.74$, $p < 0.001$) were observed.

The five populations were then divided into two groups according to the shape of their SGI time-series, each being correlated with a different part of the AMO: (1) the Elez, Bonne Chère, and Loc'h populations [5a],



[4] ***Mean SGI chronologies with 95% confidence intervals. "Sample depth" represents the number of shells used to build the chronology, for each year.***

and (2) the Elorn and Camfrout populations [5b]. Until the early 1980s, a strong (r = 0.77) and statistically significant ($p < 0.001$) relationship existed between the AMO and the SGI chronologies of the Elorn and Camfrout shells [5c]. After that period, AMO and SGI patterns from these two populations moved apart, and the AMO was better described by the SGI chronologies of shells from the Elez, Loc'h, and Bonne Chère (r = 0.66; $p < 0.001$).



[5] *Mean SGI chronologies split into two groups:*
A: Elez (red line), Loc'h (green line), Bonne Chère (yellow line), and mean (black line);
B: Elorn (purple line), Camfrout (dotted line), and mean (black line);
C: Time-series of AMO index variations between 1950 and 2010, with correlations between this index and the SGI of the two groups of populations.

Discussion

Previous analyses of shell growth patterns along a latitudinal gradient throughout Europe highlighted two growth strategies in *M. margaritifera*: rapid growth in early ontogeny that quickly leads to a maximum length (high k, low L_∞) and slower but longer growth with larger maximum sizes (low k, high L_∞) and a longer lifespan (Bauer, 1992). These two strategies may be linked to temperature variations along this gradient, with stronger shell growth at juvenile stages occurring at lower latitudes (with a high average temperature), and higher asymptotic length occurring at higher latitudes (with a lower average temperature). It is assumed that higher temperatures result in increased metabolic activity and hence faster shell production. Our SGI chronologies also showed that temperature was the common environmental factor correlated with population growth in the Elorn, Elez, and Loc'h rivers. However, our populations presented quite different SGI chronologies, suggesting that local conditions such as river quality can also influence shell growth.

The Elez population is located between a decommissioned nuclear power plant and a dam built in 1936. Although this river's nitrate concentration is one order of magnitude below those in other Breton rivers, the maximum chlorophyll *a* concentration can reach relatively high values (6.25 $\mu g.l^{-1}$). Thus, there is enough food to sustain high metabolisms and a low enough nitrate concentration to prevent negative impacts on survival, which may explain the high k value for this population. Dam management drastically changed in the late 1990s/early 2000s, resulting in a less variable/more regulated river flow. This regulation could explain the increase in SGI of the Elez shells at the end of the 20th century and the absence of any relationship with climatic oscillations, like the AMO, before this date.

SGI time-series for the Loc'h and Bonne Chère populations tended to present the same trend as that of the Elez shells, with an increase in SGI values in the early 1990s, in line with an increase in AMO. However, the SGI increase for the Loc'h and Bonne Chère populations is smaller than for the Elez population. Moreover, the sample depth was low before the early 1970s, which means that the AMO decrease observed between 1950 and the mid-1970s cannot be captured by SGI variations before 1970. If we restrain the SGI chronology for these populations to the period 1970-2010, a relatively strong and positive correlation with the AMO index appears. It is assumed that local environmental conditions in these rivers are relatively positive and stable for the growth of *M. margaritifera*, and that it is primarily the climatic conditions that control shell growth. This assumption is supported by the occurrence of recruitment events in these rivers

The profiles of the Elorn and Camfrout SGI chronologies were very different from those of the other populations, with a strong SGI-AMO correlation until the mid-1980s. Then, an almost linear decrease in SGI values occurred until the end of the 2000s. This shift could be related to a change in nitrate concentration in the Elorn River over the past 40 years (from 5 $mg.l^{-1}$ in the late 1960s to 50 $mg.l^{-1}$ in the mid-1990s). We assume that SGI chronologies built from Elorn and Camfrout shells tracked the AMO index variations until around 1980, when the river quality was still relatively good, but that local environmental conditions had a bigger impact than climate on shell growth after this date.

To conclude, SGI chronologies built from *M. margaritifera* shells are good proxies for the reconstruction of past variations in climatic oscillations like the AMO, as long as local environmental conditions in rivers are stable (e.g. river flow) and within the ecological tolerance limits of the species (e.g. nitrate concentration). The local environmental conditions will have a bigger control over shell growth than climate if those ones are not fulfilled. ■

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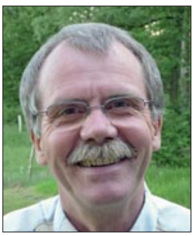
Session 4

From population to catchment area management

Restoring freshwater pearl mussel habitat in Lower Saxony (Germany): an overview of 40 years of protective measures

Reinhard ALTMÜLLER



A. Steinmann

R. Altmüller

The freshwater pearl mussel (*Margaritifera margaritifera*) once inhabited five catchments in the Lüneburger Heide in Lower Saxony (Taube, 1766). The first freshwater pearl mussel inventory in this region was carried out in the 1930s and its results showed that only around 50,000 specimens had survived in one catchment of the River Lachte and its tributary, the River Lutter (Wellmann, 1938).

In the 1960s, the medical practitioner W.D. Bischoff took an interest in the freshwater pearl mussel and became a pioneering figure in the field of its conservation (Altmüller *et al.*, 2001). His lifelong passion for these interesting and important molluscs led him to play a central role in what would turn out to be a long and successful plan of action to implement and improve species protection measures for the freshwater pearl mussel in Lower Saxony.

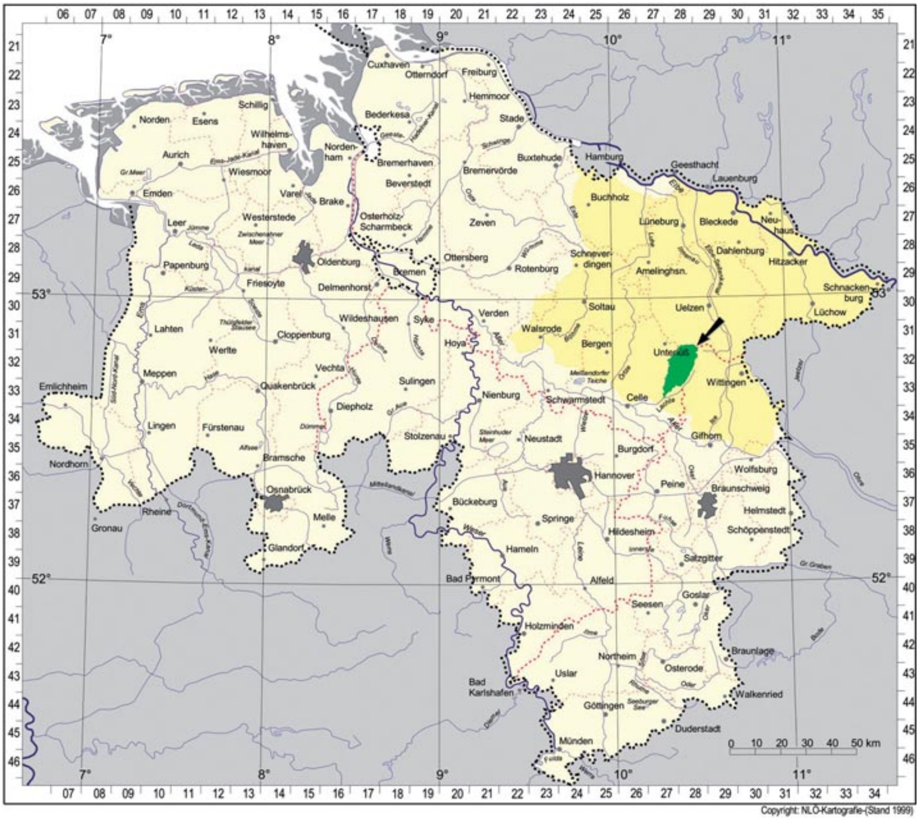
The River Lutter, habitat of the last remaining freshwater pearl mussels in Lower Saxony

The River Lutter is situated in the southern part of the Lüneburger Heide in northwest Lower Saxony [1]. Its catchment area of about 150 km², of which 75% is forested, giving a low population density of around 13 inhabitants per km². The river's total length is around 26 km with a downstream semi-natural section of about 7 km containing the pearl mussel population.

A study of freshwater pearl mussel reproduction biology with the aim of increasing pearl production

After discovering that only about 3,000 mussels remained, Bischoff decided to breed more mussels to produce pearls. However, before this goal could be achieved, he had to study the species' reproductive biology, as very little information existed about it at that time.

For this purpose, an experimental canal was built in 1968 that constituted a small



[1] ***Lower Saxony and its capital Hanover. In the north-east, the "Lüneburger Heide" area (in yellow) and River Lutter catchment (in green) are shown.***

diversion at a bend in the River Lutter about 1 km upstream from the mouth of the River Lachte (Bischoff, 1971). The researchers Bischoff and Utermark (the latter a student at the time) showed that the brown trout is the only suitable host fish for freshwater pearl mussel reproduction in the River Lutter catchment (Utermark, 1973; Bischoff & Utermark, 1976). They also showed that the parasitic phase of pearl mussel glochidia lasts for around ten months and not one month as previously believed (Bischoff & Utermark, 1976).

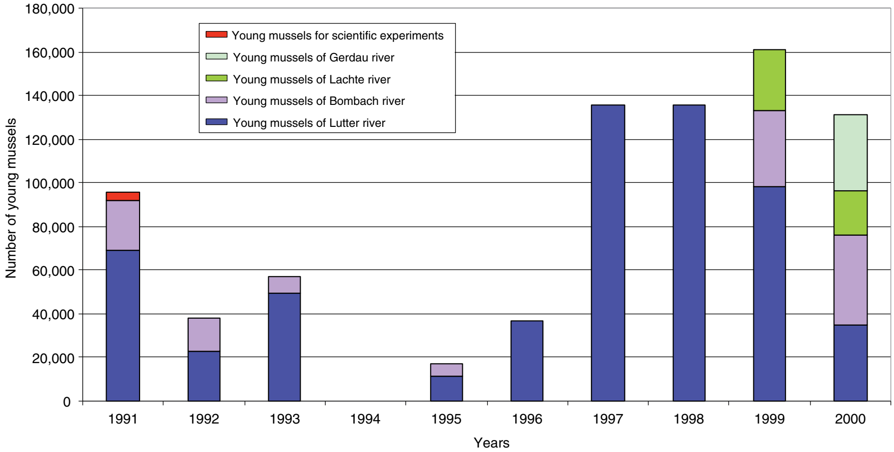
Artificial infestation of brown trout to produce young pearl mussels

After Bischoff and Utermark had discovered the secrets of the reproductive biology of this species, they sought to increase the production of young mussels. For this purpose, hatchery-raised brown trouts (3–4 years old) were artificially infested with freshwater pearl mussel glochidia from 1972 to 2000. After spending a period of about nine months in a hatchery in a pond, most of the fish were released into the River Lutter. From 1986 to 2000, some of this brown trout population was kept in the laboratory to collect the emerging young mussels that were initially used for scientific research (Buddensiek, 1991). From 1991 to 2000, the harvested young mussels were released into various rivers **[2]**.

From 1985 to 2001, electrofishing was used to catch wild brown trout in the lower 7 kilometres of the River Lutter. Specimens from 10 cm to 30 cm in length were infested with pearl mussel glochidia. Altogether, around 9,500 brown trouts were infested with around 25 million glochidia **[3]**. As young mussels had been found in this stretch of water, electrofishing was stopped from 2001 onwards to avoid any potential damage to these animals from fishermen walking in the watercourse.

The 1980s: a period lacking in successful artificial species protection measures but which saw the emergence of new knowledge about river ecology

The very few young mussels still extant in the 1980s (R. Dettmer, pers. comm.) were



[2] ***Number of young freshwater pearl mussels that were collected in a laboratory after they had left the host fish. The young mussels had been introduced into the River Lutter to sustain the existing population and in three other rivers for reintroduction experiments.***

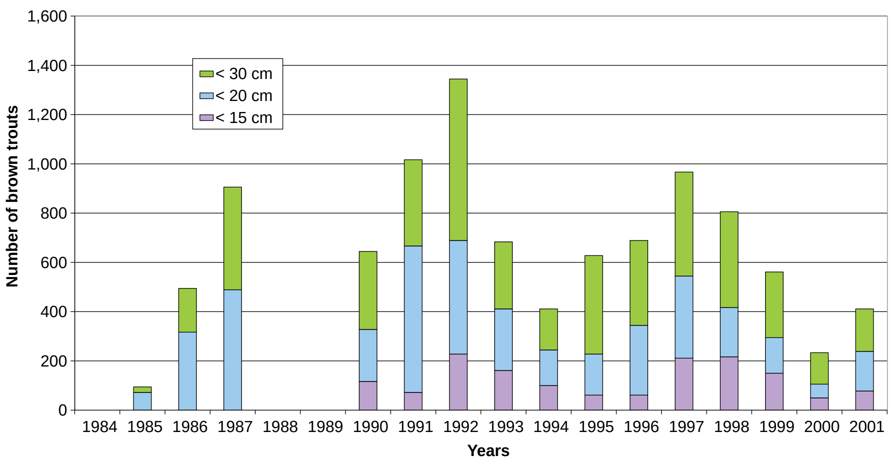
ultimately killed off by large amounts of sand sedimented upstream from a fallen tree.

This accident was a strong indication that drifting sand posed a major problem for river-bottom species. Since 1985, field operations such as electrofishing have led to deeper understanding of this subject through observations made while river-wading. The position of sand dunes was seen to change yearly; stretches that were gravelly one year would be covered by sand the next year and vice versa.

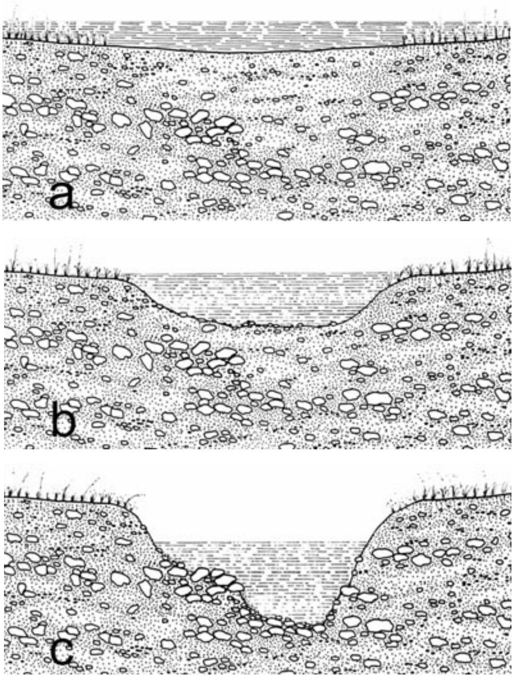
Subsequently, Buddensiek showed in his thesis that sand which covered gravelly stretches of the river bottom prevented interstitial water exchange, thus generating lethal conditions for typical interstitial fauna (Buddensiek, 1991; Buddensiek *et al.*, 1993).

The large quantities of sand are human-induced. River development – or "genesis" – is an erosion process (Altmüller & Dettmer, 1996). A "mini river genesis" can be observed almost every year at the end of winter in the Lachte River and Lutter River catchments on arable land following erosion caused by surface runoff.

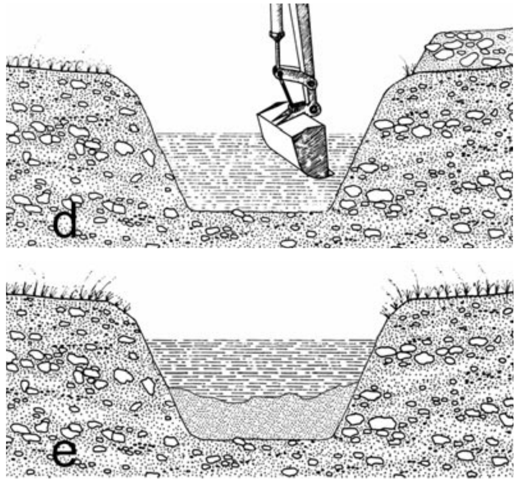
The natural development of watercourses in the Lüneburger Heide is comparable with



[3] ***Number of wild brown trouts caught in the semi-natural lower 7 km of the River Lutter and infested with freshwater pearl mussels' glochidia***



[4a, b and c] ***Development – or genesis – of a creek in the Lüneburger Heide as an erosion process with the formation of a relatively stable river bottom, consisting primarily of gravel and rocks (the hollow spaces between the gravel and rocks – the interstitial zone – is the most important habitat for river fauna).***



Altmüller & Dettmer, 1996

[4d] ***This indispensable part of a river is irreversibly destroyed by river maintenance, especially by machines.***
[4e] ***Elimination of the protective gravelly river bottom initiates sand erosion within the watercourse.***

these erosion channels **[4a, b and c]**. The landscape is formed by ground moraines. Variably-sized particles of this moraine material have been washed downstream; the heavier particles have remained and form a relatively stable river bottom. This stable river bottom, with spaces between gravel and rocks – the interstitial zone – is the habitat of the greatest part of the river biocenosis, and especially of young freshwater pearl mussels. River works and maintenance **[4d]** cause irreversible destruction of the river bottom and increase the amount of sand in the watercourse **[4e]**. Drifting sand clogs and covers downstream stretches as well as near-natural sections and consequently damages the river ecosystem downstream to the sea.

This growing knowledge about river development and the harmful consequences of river works and maintenance operations formed the basis for the following river protection measures, which ought to save the freshwater pearl mussel from extinction.

Reduction of river maintenance and sediment drift is the key to successful species protection measures, most notably for the freshwater pearl mussel through the *Lutterproject*.

Reducing sediment drift was clearly the most important measure to help young mussels to survive in the interstitial zone. However, such a measure is difficult to implement in Lower Saxony, where watercourse maintenance is a legal obligation for Water Maintenance Associations and landowners can demand a defined water level for their land.

Buying up the floodplains and wetlands was the only efficient way to stop the excavation of the natural and semi-natural watercourses within the River Lutter catchment and to save freshwater pearl mussel populations. Therefore, in 1989, the Celle and Gifhorn districts applied for funding for a nature conservation project (the Lutterproject) to protect the Lutter and to save the pearl mussels (Abendroth, 1993). The application was filed in the context of the German government's Riparian Land programme (Scherfose, 2002).

The grant application was approved and funding of €16.5 million was secured. The Lutterproject was conducted between 1989 and 2006 by the administrations of both districts and was supported by experts from different fields and administrations. Buying up the floodplains and the wetlands was the most important action that had yet been taken.

The fine sediment load which had been observed in the lower reaches of the River Lutter diminished significantly after the Lutterproject management purchased the rights to an old mill in the village of Eldingen in 1992. Since this time, no sand has been washed downstream from the mill and a stretch of about 7 km downstream of the mill has been washed free from overlaying sand (Altmüller & Dettmer, 2006). The stony and gravelly substrate re-emerged, ready for colonisation by flora and fauna. The species typical of a natural stream began immediately to return to the river bottom. One example of this phenomenon was the high reproductive rate of minnows (*Phoxinus phoxinus*) recorded in 1994 (Altmüller & Dettmer, 2006).

Freshwater pearl mussels reacted much later than the minnows to the improved conditions of the interstitial zone. The first young mussels were observed in 1997 (Altmüller & Dettmer, 2000). In 2000, the monitoring programme "Development of the freshwater pearl mussel population in the River Lutter by snorkelling" was initiated by the author. Alternating halves of the 7 km stretch downstream of the mill have been painstakingly searched annually for mussels [5].

High pearl mussel population growth was recorded in the first years; however, since 2008, there has been almost no growth. The reason for this phenomenon is not clear. However, it appears that the current in some stretches has changed, with other parts of the river bottom having become subject to faster water flow than before. Some habitats have become clogged by mud and the mussels are dead. Still, other parts of the river bottom seem to have developed into suitable habitat for young mussels. Altogether, it could be said that freshwater pearl mussels have a good chance of survival in the River Lutter. Furthermore, the moment has come to use this growing population to reintroduce the species into former pearl mussel streams through an innovative project launched in 2009. ■

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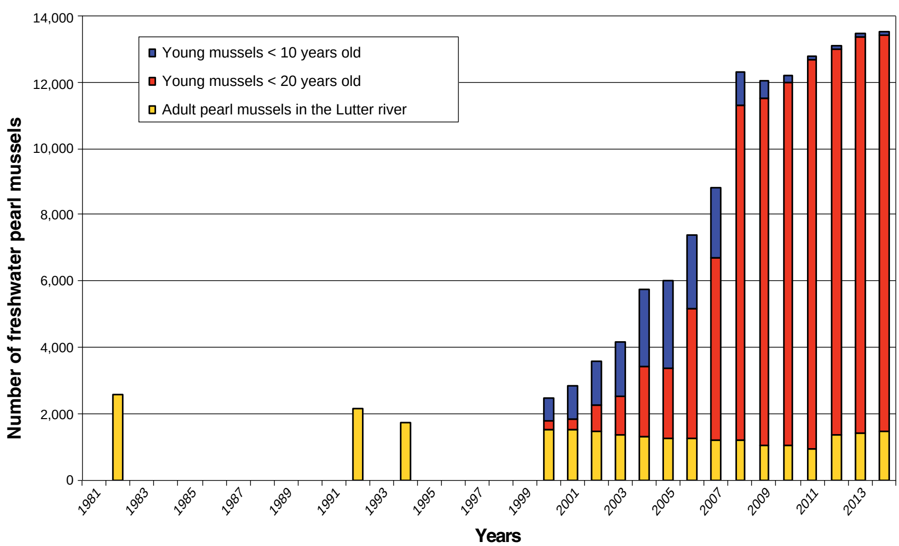

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Conservation measures for the freshwater pearl mussel in the River Dee in North East Scotland

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D. Riley

S. Cooksley

The freshwater pearl mussel *Margaritifera margaritifera* was once widespread in the UK (Cosgrove *et al.*, 2000). However, these populations have undergone a serious decline: in England, Northern Ireland and Wales the species is now restricted to a few sites and the majority of the UK's viable individuals are found in 72 Scottish rivers. In the UK, 26 SACs[1] have been designated for the species.

Pearls in Peril

Pearls in Peril (*PIP*) is an EU LIFE+ Nature project which aims to safeguard Great Britain's important pearl mussel populations (pearlsinperil.org.uk). Twenty-two organisations are working together to deliver 48 actions across 21 SACs. *PIP* aims to: (1) restore the habitat of pearl mussels and salmonids, (2) secure the long term survival of existing pearl mussel populations and (3) communicate with local, national and international audiences to raise awareness of pearl mussel conservation issues. *PIP* began in September 2012 and will continue to September 2016. One of the *PIP* project's largest initiatives is taking place on the River Dee in North East Scotland.

The River Dee

Geography

The River Dee is one of the UK's largest rivers. From its source at 1,220 m above sea level on the Cairngorm plateau, the Dee flows 130 km east from mountain and moorland, through farmland, to enter the North Sea in Aberdeen harbour [1]. The catchment area of 2,000 km^2, drained by 17 main tributaries, is relatively unusual in the UK in that it is characterised by predominantly upland, semi-natural ecosystems. The Dee catchment is of exceptional conservation value, supporting flora and fauna typical of an uncontaminated highland system. The Dee and its tributaries are designated as an SAC due to internationally important populations of Atlantic salmon, pearl mussel and European otter. The catchment consists of two geographically distinct regions: an upland western area dominated by mountain and moorland and a lowland eastern area of arable and improved grassland.

Land management – upland areas

Semi-natural land characterises the upper (western) part of the catchment. The land cover is predominantly moorland, consisting of a mosaic of blanket bog and heather moorland on the upper and middle slopes, with montane and alpine heath

1 - SAC or Special Area of Conservation: http://jncc.defra.gov.uk/

Dee Catchment Partnership

[1] ***The River Dee catchment and the main ecosystem services it provides***

vegetation on the highest summits. The soils, climate and topography are not suitable for intensive agriculture, and extensive sheep farming, deer and grouse shooting predominate. Their management, which involves "muirburn" (burning heather to promote new growth) and maintaining high deer densities, favours open moorland and suppresses tree regeneration which results in a largely treeless landscape.

Without tree cover, watercourses are subject to high summer temperatures and in recent years water temperatures of over 26°C have been recorded in the Dee catchment. In northern climates, the freshwater pearl mussel requires temperatures below 25°C (Hastie *et al.*, 2003) and perhaps even less than 20°C (unpublished information), the lethal limit for young salmon and trout being 28°C. Therefore the temperatures recorded are a serious cause for concern, and the situation is likely to become worse as government climate change scenarios project an increase of 4°C in mean summer temperatures by 2080 (UK Climate Projections UKCP09). The lack of trees also promotes erosion, resulting in watercourses becoming wider and shallower, and these effects are increased by deer and sheep trampling the riverbanks.

The upper catchment is not entirely devoid of trees. A high proportion of the few remaining areas of semi-natural Caledonian pine woods in Scotland are within the catchment and managed coniferous and deciduous forests have been established on many of the lower slopes.

Land management – lowland areas

The eastern lower half of the catchment is an agricultural mosaic managed for beef cattle, fodder crops and cereals. The river system in this area has been affected by widespread land drainage and changes to morphology associated with agricultural improvements. Most steams have been realigned and many are incised. The development of light industry in this area in the 1800s (e.g. mills, small-scale hydropower) involved the construction of weirs and dams which have formed long-term barriers to fish migration.

The river enters the sea at Aberdeen harbour, a world-class port handling around five million tonnes of cargo annually for a wide range of industries. The harbour, one of the busiest ports in Britain, is the centre of activity for the offshore oil and gas industry's marine support operations in northwest Europe.

Human population

The majority of the catchment's population resides in the City of Aberdeen (220,000 people) surrounded by commuter settlements and light industrial estates. Beyond the city, towns are small, and are concentrated around the river and in the lowlands. The 1970s oil boom led to the expansion of these settlements and the catchment is faced with continued pressures as local populations grow rapidly, bringing an increased need for domestic water supply and waste water disposal along with an expansion of associated infrastructure.

Water supply and waste water disposal

The River Dee and its tributaries are an essential water resource. Two large water catchments provide domestic water to the whole of Aberdeen City and over half of Aberdeenshire, supplying 300,000 people daily. The Dee's waters are also affected by effluent discharges. Although the focus has previously been on upgrades to waste water treatment plants, recent work has shown that inputs from private septic tanks are, collectively, a significant source of nutrients (Withers *et al.*, 2014).

Recreation

The Dee is one of Britain's top four salmon rivers and is internationally famous, especially for its multi-sea winter spring salmon[2], and also provides excellent summer fishing for salmon, grilse[3] and sea trout. Annually, over 100,000 anglers visit Deeside, which is worth £15 million to the local economy and supports 500 jobs. Past management in support of the fishery has led to widespread bank reinforcement on the river's main stem and the creation of instream structures such as current deflectors.

The catchment is an attractive centre for a wide range of outdoor pursuits such as canoeing, walking, cycling, camping, climbing, mountain-biking and skiing. There are associated pressures from littering, fires and erosion in some hotspots.

Condition of the river

Overall, the lack of heavy industry or intensive agriculture means that the catchment is in relatively good condition, with 22 of the catchment's 56 Water Framework Directive waterbodies at "Good" and a further two at "High" ecological status, according to SEPA[4]. The two major causes of downgrade are morphological problems and diffuse source pollution and consequently the Dee is the subject of two targeted government programmes to address these pressures. Point source pollution and abstraction are significant in some waterbodies, and invasive non-native plant species are also an important issue. Since the freshwater pearl mussel requires the upper end of the "High" status to be achieved, there is a need for considerable improvement throughout the catchment if the population is to be sustainable.

Dee Catchment Partnership

All of the organisations with an interest in water management in the Dee are members of the *Dee Catchment Partnership*[5], which has been tackling the river's complex issues for over 10 years. The twenty partner organisations have published a management plan (Cooksley, 2007) that provides an agreed strategic framework for action. The Partnership promotes widespread awareness and discussion of the main problems, coordinates activities, develops projects to tackle key issues, and provides a central source of information and advice.

Dee Pearls in Peril project

Although the Dee pearl mussel population numbers around 1.3 million, the population is sparse and reproduction is not sufficiently successful to maintain numbers. Declines

2 - Multi-sea winter spring salmon: when they return to the rivers, adult Atlantic salmon populations include two age groups: grilse which have spent one winter at sea, and spring salmon, which have spent two winters at sea.
3 - Grilse: grilse are migrating salmon or trout which return to the river after having reached maturity at sea at a size (and often at an age) much lower than that of a normal adult individual.
4 - SEPA or Scottish Environment Protection Agency: http://sepa.org.uk
5 - Dee Catchment Partnership: http://theriverdee.org

have been linked to diffuse and point source pollution, degraded habitat and pearl fishing, and it is likely that a combination of these factors is collectively responsible for the population's "Unfavourable" status. To tackle these problems, *PIP* is undertaking five areas of work in the Dee, taking a catchment-based, long-term approach to improving habitat conditions.

Riparian woodland

An ambitious tree-planting scheme aims to facilitate the establishment of native woodland over 70 km of riverbank in the upper Dee. The intention is to plant 40-50% of the riparian zone in this area. The trees will provide a range of benefits, including reduced water temperatures in salmon habitat, stabilised riverbanks and reduced erosion, improved retention of rainwater and reduced flooding, creation of habitat for wildlife, and generation of instream woody material and leaf litter.

Agreeing plans for planting has involved working with a wide range of bodies. A bottom-up approach has been essential with every step in the work involving close liaison with the landowners and estate staff. All of these stakeholders have different requirements, e.g. to avoid certain areas for planting, a need to build appropriate infrastructure or to plant appropriately for the landscape. Different methods, tailored to meet the needs of the land managers, are being used to protect the trees, and range from small enclosures **[2]** to fences set back some distance from the riverbank. 40 km of planting is currently underway.

Riparian protection

The *PIP* project is tackling agricultural diffuse pollution in the middle catchment. The project aims to reduce the amount of soil, fertiliser and pesticide entering watercourses, and to allow natural bankside vegetation to develop by facilitating the construction of 45 km of riparian fencing. Ten km of 10-12 m-wide buffer strips have been completed, towards a total target of 45 km by 2016.

Habitat restoration

The *PIP* project is making morphological improvements at 8 sites on the main stem of the river. River engineering features have been linked to absences in the pearl mussel population (Cooksley *et al.*, 2012) and the removal of features such as current deflectors and bank protection will help to restore natural processes, benefiting pearl mussels by increasing habitat availability. To date, three current deflectors (constructed from boulders arranged in a line across the river) have been broken up and re-distributed in the river **[3]**.

Monitoring

The restoration work is underpinned by a long-term monitoring programme to determine whether the project delivers its expected results. This covers the effects of the project on water quality and temperature, levels of shading, salmonid and pearl mussel populations and habitat, and levels of uptake and implementation of habitat restoration measures.

Pearls in the Classroom

PIP is providing an education programme to raise awareness of the freshwater pearl mussel amongst children and in local communities. During classroom visits, primary school children are being taught about the species' unusual lifecycle, its habitat requirements, threats to its survival, and its important cultural history in Britain.

Conclusion

The *PIP* project is tackling the issues affecting the River Dee's pearl mussel population at a catchment scale and is establishing measures and knowledge that will continue to have benefits for generations to come. This ambitious programme of work has been possible due to the opportunity to work within the established framework of the *Dee Catchment Partnership* and to use the delivery experience of the *River Dee Trust* and *Dee DSFB.* Partners are confident that monitoring will demonstrate that the project has, in time, contributed significantly to the recovery of the River Dee freshwater pearl mussel population. ■

Pearls in Peril LIFE+ programme is led by *Scottish Natural Heritage.* In the Dee catchment the project is delivered by the *River and Fisheries Trusts of Scotland* with support from Cairngorms National Park Authority, *Dee Catchment Partnership*, *Dee DSFB, Forestry Commission Scotland, Scottish Environment Protection Agency*, and *Scottish Natural Heritage*.
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River Dee Trust

[3] ***Breaking up current deflectors using a hand-winch***

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Experimental work on soft techniques for the restoration of freshwater pearl mussel habitat in Morvan (France)

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A. Boutin

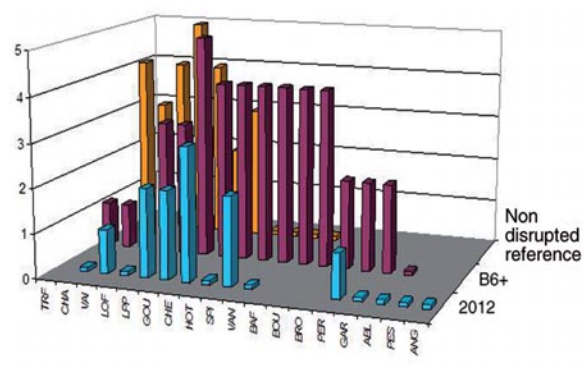
N. Galmiche

The rivers of the Morvan area are still home to 5 populations of freshwater pearl mussels, but these rivers suffer disturbances that have caused this species to become scarce. To halt its decline, the Morvan Regional Natural Park (PNRM) conducted a European Life Nature programme, "Ruisseaux" [Streams] from 2004 to 2009. Besides testing techniques for the management, preservation and restoration of the water quality of these environments, the programme made it possible for an initial reflection on ideas about biological continuity.

Wishing to further pursue this reflection, the PNRM has been coordinating a European LIFE+Nature programme, "Ecological continuity, management of catchment area and associated heritage fauna", since 01/09/2011. Some of the actions are demonstrations and/or employ innovative techniques.

Restoration of ecological continuity on a site of natural and historical heritage importance

In the 19th century, most settlements in the Morvan area had at least 3 or 4 mills. In the Cousin Valley between Avallon and Magny, there were 24. Poor sill management resulted in permanent partitioning of the Cousin River, and the absence of current has limited self-purification. The proportion of facies with slow flow, under the influence of the sills, is 35% (BIOTEC, 2013a). Sediment accumulates in the forebays, which store sand and small pebbles more or less temporarily, but retain cobbles of over 5 to 10 cm and boulders of all sizes more or less definitively (SIALIS, 2013). Fish can no longer access their spawning grounds and trout disappear gradually from such rivers. Of the structures on the river, 66.7% are to be considered impassable or very difficult to cross (BIOTEC, 2013a). Compared to what the Cousin River would be like without the problems caused by structures in this area, the current situation shows natural populations degraded in terms of both species richness (notably the absence of trout) [1] and abundance (Bouchard, 2012). The water temperature increases considerably in summer. The greatest change is +0.8°C (mean monthly temperatures) and +2.4°C for the Tmax [2]. This thermal "anomaly" is clearly caused by the succession of sills (SIALIS, 2013). The implications are considerable because the Cousin Valley has a pearl mussel population upstream of a Natura 2000 site.

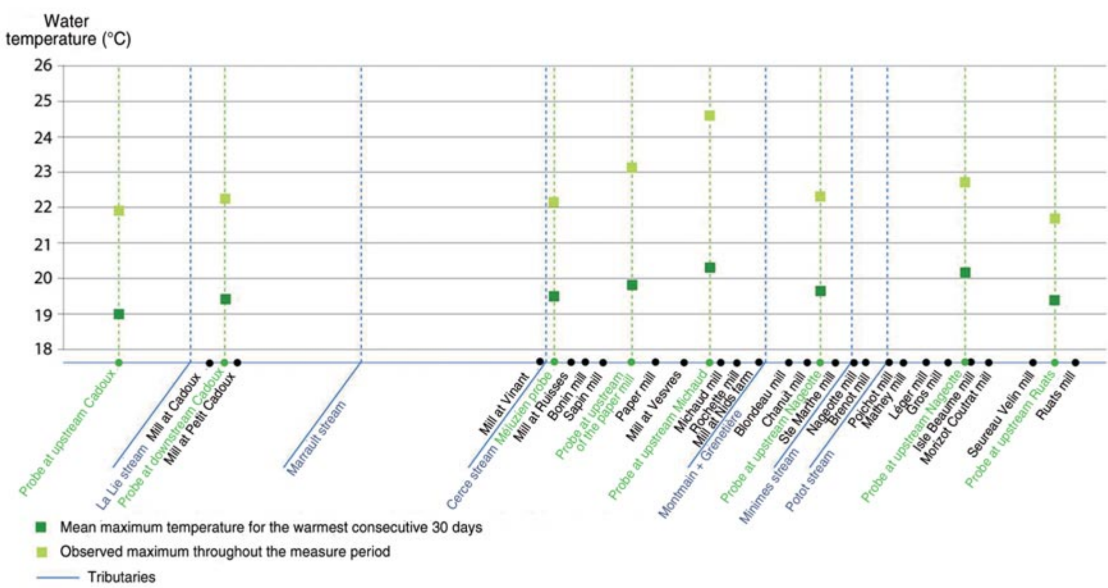


ONEMA

[1] ***State of fish stock at the station upstream of Nids farm on the River cousin in 2012. Comparison with observed stock with theoretical stock calculated based on environmental conditions at the station***

Restoration of ecological continuity can be accomplished in various ways, depending on the context of each structure. Demolition is a solution that restores full functionality to a stream, which is why the first step was to begin work on the old mill at "Petit Cadoux". The building was a hydraulic complex of which only ruins remained, consisting of a 26 m transversal paved section which maintained a 179 m stretch of stagnant water and marked a discontinuity in the pearl mussel population. The restoration of full hydraulic and sediment transparency could only be successful by widening an existing gap. However, this work was made difficult due to the established pearl mussel population upstream and downstream of the structure. Great care was taken to limit the impact of the restoration work on these mussels. First, a comprehensive assessment was made of the initial state of the population (Fouillé, 2013), notably through the use of protocols such as the "Mark and Recapture" method on the pearl mussel population; evaluation of the habitat with an oxidation/reduction potential probe; and habitat mapping by the Morphodynamic Attractiveness Index (IAM). The trees on the embankment were cut down in stages starting from the top so as to avoid dragging them through the streams, which could potentially dislodge freshwater pearl mussels. Then, the existing gap was widened to the desired size with a mini excavator. Foundation blocks were removed by hand and crowbar to lower the sill level and finish levellin [3]. A mini excavator was sometimes needed to extract the larger blocks and load them onto a raft [4]. Indeed, a raft was built to move the blocks to the old forebay to diversify the flow.

The raft was employed mainly to avoid using machines, which could have crushed individual pearl mussels. Today, the sill is gone and has been replaced by a large riffle. The site will also be strictly monitored



SIALIS

[2] ***Upstream-downstream evolution of thermal maxima at eight sites on the Cousin in 2012***

N. Galmiche

[3] ***Manual demolition of the Côte Cadoux sill***

N. Galmiche

[4] ***Raft transport of granite blocks making up the Côte Cadoux sill***

in order to observe the potential impact on the pearl mussel population, or an eventual recolonisation of new facies favourable to the species. With regard to the other projects, which are in the consultation phase, the most serious obstacle is the disappearance of the water body caused by the sill. In certain cases, the construction of fish ladders could favour the continuity of fish migration. However, this solution has many drawbacks, including the lack of sediment transport and the inability of some species to use these structures. In an effort to compensate for these problems, the design of the fish ladders was quite ambitious. Rockfill ramps were designed at a size that would allow trout and accompanying species such as chabot, brook lamprey, and white water cyprinids to pass. They will consist of two spillways at different altitudes and will be systematically accompanied by levelling. The slope will be 6% and the bottom very rough. Seven projects are being considered for implementation in the summer of 2015: three rockfill ramps, one bypass river and three partial levellings. In addition, as part of a future extension to the Life+ project, seven new projects will be considered in 2015.

Restoration of the Upper Cousin catchment

The Upper Cousin is one of the last five rivers in Burgundy where the freshwater pearl mussel is still present. However, its ecological situation is still a concern, particularly with respect to large imbalances in the fish population. Young and adult brown trouts are in troublingly low abundance on the main stream (Bouchard, 2012). One of the conservation issues of the pearl mussel population on this river is the restoration of the population of brown trout, its preferred host. The main limiting factors listed on this site are thermal issues associated with the presence of multiple ponds, the disconnection of many tributaries, cattle trampling and habitat modification on certain stretches of the river (hydraulic work and coniferisation). To date, the restoration work carried out on this site has involved the development of disconnected areas of the Cousin River's tributaries, restoring 12.4 km of brown trout spawning grounds; the installation of 8,574 m of fences; the construction of animal drinking areas and the restoration of riparian woodland; removal of 1,121 m^3 of spruce from 6.5 acres of bog, without creating ruts; the restoration of 750 m of stream banks with conifer growth; the placement of five drain boxes (*moines*) in ponds; the redevelopment of the Champeau pond bypass; and the hydromorphological restoration of brown trout habitat on an 850 m stretch of the Cousin River.

Among the more recent actions, some have seen the use of innovative techniques. The Champeau, Chailloux and Morin ponds are all fully upstream of the pearl mussel sites, and do not possess a suitable drainage system. The downstream pearl mussel population is therefore very vulnerable. A second conservation challenge for the species on this river is to restore and maintain populations of trout in the main watercourse. It is therefore very important to supply cold water downstream of these ponds to maintain the populations of brown trout. A drain box is a system that extracts water at depth and controls its drainage by the successive removal of boards. The three ponds mentioned above have been equipped with drain boxes.

Managing bottom deposits is a recurrent problem in the placing of drain boxes, and it is often necessary to dredge using an excavator. Sometimes the sludge depth is such that the machines cannot reach the bottom with their shovel. One technical solution is to de-sludge by underwater vacuuming [5]. This method produces immediate results. It is done using an amphibious barge equipped with a suction pump. The pump speed is from 10 to 40 m^3/h, depending on the type of material to be extracted. Via pipes at the pump exit, the sludge is collected on the shore or in settling ponds [6]. During the construction phase, it is also important to provide effective sludge filtration equipment downstream of the pond. For example, doubled grid curtain systems can be used with straw between the layers. This system is much more effective than using a simple bale of straw. Straw trapped in the settling ponds can be removed by a motor-driven pump and a vacuum dump truck or a mini excavator.

Finally, it is not always easy to ensure that minimum instream flow is maintained while the work is being done and a pond is being emptied. A temporary bypass is very effective but can be difficult to set up. One of the solutions devised by the company Grossetête is to use a geotextile on an open trench. This approach has proven quick to implement and very effective. A final difficulty encountered is when the bottom cannot be reached with an excavator. The lowest level of the excavator is often higher than the pond bottom. It is then necessary to lay foundations for the drain box in the water. In the Champeau pond, it was decided to drive a concrete sheet piling. This new surface will be sealed and then sunk, providing a new solid base allowing for the safe installation of the drain box.

The Morvan Regional Natural Park (PNRM) has been working for several years to solve problems of "indirect" disturbances, but wishes to offer restoration solutions more intrinsically linked to the aquatic habitat

N. Galmiche

[5] *Pumping bottom deposits from the Chailloux pond with an amphibious barge equipped with a suction pump*

N. Galmiche

[6] *Discharge of bottom deposits from Chailloux pond into settling ponds*

(e.g. restoration of fish hides and riparian woodland, diversification of facies). To refine and implement its choice of projects, the PNRM has enlisted the expertise of Biotec (BIOTEC, 2013b) to draw up a draft project offering a range of technical solutions for the physical restoration of a stretch of the Cousin River, with the aim of improving living conditions for salmonids and pearl mussels. Because it is difficult to sustainably favour the formation of shelter areas, fish hides and other fish habitats by implementing isolated single actions, any technical alternation must be integrated as part of a larger project restoring a stretch of about 5 to 6 times the width of the bed when the river is full. This approach was applied along a 700 m stretch of the Cousin River between two pearl mussel zones. Pearl mussels were no longer present in the most damaged stretch. On the steep-sloped stretches (generally greater than a 0.3% grade), the stream appears to have natural self-adjusting capabilities. It is then a case of using the river's own capacity to balance itself against instabilities caused by the introduction of external elements. The establishment of structures (deflectors, stumps, etc.) diversifying flow and aquatic habitat will result in erosion and sediment transport, but will also produce an area with sand and gravel deposits. For steep-banked winding stretches, it is recommended to use the natural capacity of the stream to work laterally, while allowing it to meet obstacles on the banks (bank reconstruction) to create stable undercuts. On stretches with shallow slopes (generally not exceeding a 0.2-0.3% grade), the river does not seem to benefit from its full natural capacity for self-adjustment, and therefore requires more intensive development aimed at narrowing the low-water channel and boosting flow diversification in the high-water channel.■

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River dynamics, bank erosion and fine sediment load in freshwater pearl mussel rivers

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A detailed survey of bank erosion in a pearl mussel river was carried out. We conclude that measures for the reduction of the fine sediment load should be taken mainly at the headwaters. Further downstream, in the main mussel areas of the stream, river dynamics should be tolerated to support the formation of diverse physical habitat structures.

A survey of bank erosion was carried out as part of the catchment study of a pearl mussel river in Northern Bavaria, Germany. In the lower ranges of the river we recorded active and ongoing bank erosion at 2.8% of the bank lines. The headwaters showed a much higher portion of bank erosion (>25%).

The survey shows that bank erosion contributes to the loading of the river with fine sediments. On the other hand, river dynamics and bank erosion enrich physical river structures and contribute to the formation of new favourable habitats for mussels and fish. An extrapolation of the possible amount of annual sediment loading by bank erosion reveals that it is probably minor in comparison to the sediment load resulting from soil erosion on arable land.

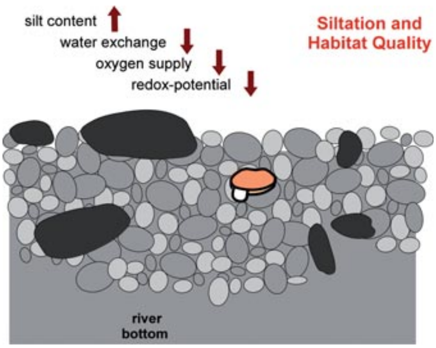
Thus, measures for the reduction of fine sediment load should be taken mainly at the headwaters: abatement of soil erosion, disconnection of the flow paths between arable land and the main river, restoration of the wet and shallow vales of small tributaries. Further downstream, in the main mussel areas of the stream, river dynamics should be tolerated to support the formation of diverse physical habitat structures.

Introduction

Loading of the naturally oligotrophic habitat of the freshwater pearl mussel with nutriments is believed to be the main reason for its decline throughout Europe (Bauer, 1988; Hruška & Bauer, 1995). Besides eutrophication, the clogging of the substrate of pearl mussel rivers with fine textured sediments is another factor of habitat degradation (Buddensiek, 1995; Geist & Auerswald, 2007). Elevated contents of sand and silt in the substrate inhibit water exchange and lead to oxygen depletion and unfavourable habitat conditions, especially for the young mussels living in the interstitial zone [1].

The current loading of a river with fine sediments is indicated by turbidity [2]. The detrimental effect of even low, but continuous, levels of turbidity on pearl mussel populations was demonstrated (Österling *et al.*, 2010). The concentration of suspended particles shows specific linear correlations for different materials with turbidity, measured as nephelometric turbidity units (NTU), and can be used to estimate the amount of fine sediments transported [3].

The main source of fine textured sediments is soil erosion. In many pearl mussel rivers, arable land is connected to the water channels via, for example, wayside ditches.



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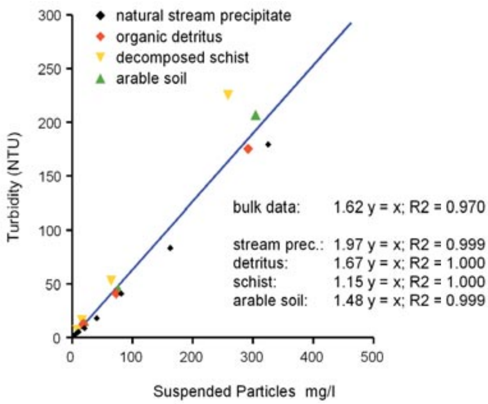
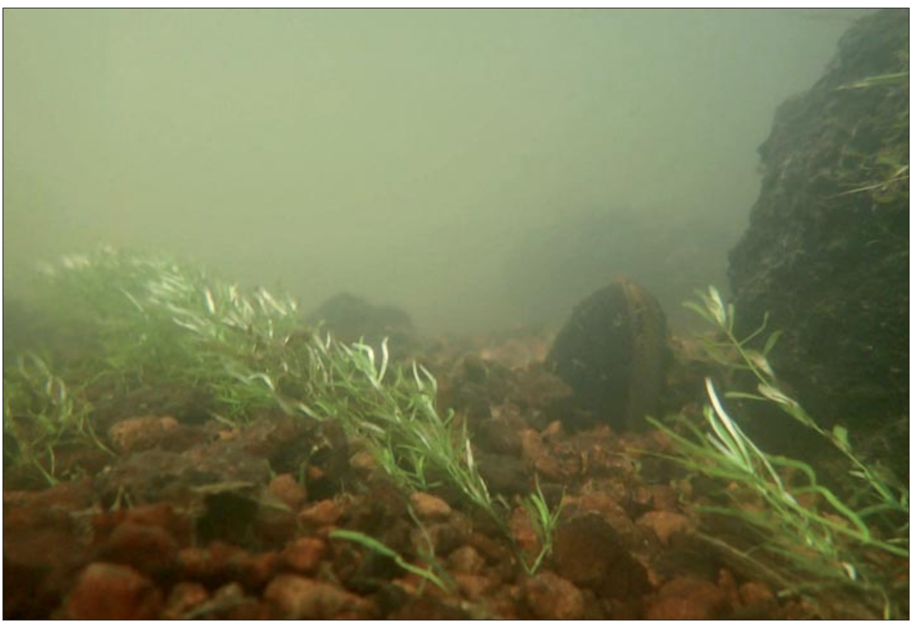
[1] *Degradation of the habitat of young pearl mussels by siltation of the river bottom*



Schmidt & Partner GbR, Non published data

[3] *Specific relations between the concentration of suspended particles and turbidity, measured as Nephelometric Turbidity Units (NTU). Results from serial dilutions with fine textured fractions of different materials that might typically be the cause of turbidity in streams*



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[2] *Visible turbidity in a freshwater pearl mussel river*


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[4] ***Wayside ditch connecting arable land to the Schondra river***

Thus, the main sources of siltation and turbidity often are situated at a considerable distance from the actual pearl mussel rivers [4].

Another potential source of the fine sediment load of pearl mussel rivers is bank erosion. In a recent river catchment survey we tried to map and estimate bank erosion and to evaluate its contribution to the loading of the river with fine sediments. In this paper we focus on this aspect, despite the fact that it is not the main factor of habitat impairment on this river. We present some results, observations and considerations of this survey, attempting to answer the question of whether measures to abate bank erosion should be considered to protect mussel habitats under the observed circumstances.

Materials and Methods

The catchment study was carried out on the Schondra River, in the Lower Triassic red sandstone region of Northern Bavaria. This river holds a pearl mussel population considered to be genetically distinct from other populations in Central Europe (Geist & Kühn, 2005), but that is now almost extinct. A catchment study was done to determine and estimate the sources of habitat degradation. As part of this study, a detailed survey of bank erosion was carried out. The catchment covers 160 km^2, 63% of which are forested. The lower parts of the river system, which are also the former mussel habitat, constitute flat floodplains covered by grassland and surrounded by forested slopes. In these parts, current bank erosion was surveyed. Length and height of breaking banks were mapped [5]. The headwater parts of the river system in the upper parts of the catchment are either small tributaries in forests or constitute small streams and water channels in meadows and arable


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[5] ***Mapping of river bank erosion: the length and height of freshly broken banks are recorded***

land. These channels were also surveyed, but the extent of channel and bank erosion could only be estimated. Historic maps were compared with recent aerial photographs to investigate whether stream and river channels have moved sideways through bank erosion. Rough extrapolations of the possible amount of sediments mobilised by bank erosion were compared to an estimate of eroded material from arable soils and its transport to the river, with "sediment dislocation ratio" (Auerswald, 1997) to assess the significance of bank erosion to the overall fine sediment load of the river.

Results

As in many of the declining pearl mussel populations in Central Europe, eutrophication from agriculture and, to a lesser extent, sewage, was found to be the most important factor of habitat degradation. Turbidity of the water and siltation of potential young mussel habitats is a second factor of habitat decline.

Floodplain

Before the clearance of forests in the headwater region in the medieval period, the floodplain zone of the river system was presumably constituted by gravel, cobbles and boulders, with the river flowing in changing and often multiple channels (braided zone) (Thorp *et al.*, 2008). Today, most floodplains in the lower mountain ranges of Central Europe are covered with alluvial loam. This loam was gradually built up during centuries from eroded soil material, mobilised during thunderstorms and deposited, layer by layer, in the floodplain. Usually the river still flows on the gravel bed, surrounded by steep banks composed of alluvial loam [6]. Concave banks are gradually undercut and break down. This process is typical of the meandering river zone (Thorp *et al.*, 2008). Geologically, the erosion of alluvial river banks can be considered to be a remobilisation of formerly eroded soil. From a short-term perspective, this is a form of soil erosion contributing to the pollution of the river with fine sediments. On the Schondra river, 1.4 to 7% of the bank lines (mean: 2.8%) show current erosion.

To protect the meadows from gradual erosion, farmers have paved the banks with boulders in most stretches of the Schondra floodplain, as on most rivers in Central Europe [7]. Pavement of banks creates a confined river structure, which can still be observed at many stretches of the Schondra: the river channel here is narrow. At floods, shear stress is high at the river bottom. Generally, the bottom is covered with a layer of cobbles serving as armouring, typical of a confined river zone (Thorp *et al.*, 2008). As the substrate underneath is protected from mobilisation, it ages and the spaces are clogged with fine sediments and encrusted with Fe and Mn oxides. Thus, bank erosion may contribute to the fine sediment load, but the continuous prevention of bank erosion creates unfavourable conditions for organisms living in the river substrate as well.

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[6] ***Floodplain of the Schondra, covered by a layer of alluvial loam***

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[7] ***Paved river banks result in the processes of a confined river zone.***

A large part of the floodplain of the Schondra was made a nature reserve in 1983. This might be the reason for the observation that the pavement of river banks is no longer maintained and that bank erosion has again intensified. On many stretches the old pavement is now found in the middle of the channel. The comparison of old maps and recent aerial photographs shows substantial bank erosion and movement of the river channel in certain stretches [8]. Where the river dynamic has removed the alluvial loam from a larger area, the braided river structure is again approached [9].

Headwaters

In the small water channels of the headwater region, extensive erosion as well as connections to erosion from fields was observed [10].We estimated that more than 25% of the channels are subject to ongoing bank or gully erosion. Erosion is facilitated by intensive agriculture which is carried out right up to the edge of the channels. Acute gully erosion, indicated by deep incision of small channels, tufts of grass breaking from the banks and tree roots protruding into the air in the channel, can be attributed to the disposal of rainwater from

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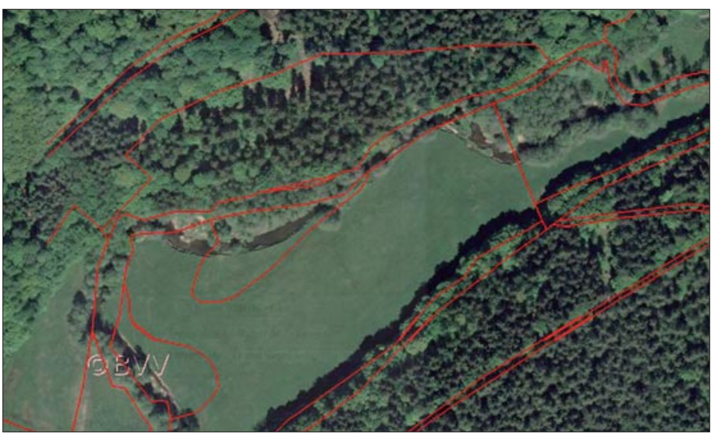

[8] ***Aerial photograph of a part of the Schondra floodplain. Red lines indicate the features shown by a map from the 1860s. In some places, the river channel has moved considerably since then.***

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[9] ***Where the alluvial loam has been removed by erosion, the river shows multiple channels on gravel and cobbles, indicating that a braided river structure is approached.***

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[10] ***Small streams in the headwater region show bank erosion as well as connection to erosion from fields.***

paved surfaces, such as run-off from roads and settlements.

Contrary to the observed extensive erosion, the comparison of old maps with recent aerial photographs shows that the watercourses in the headwater region have mostly remained stable. This is probably due to regular maintenance of channels and ditches.

Comparison of bank erosion and soil erosion from fields

As the actual rate of undercutting of banks is not known, the extrapolation of the sediment load is quite hypothetical. However, assuming that annual horizontal bank erosion is 5 cm in the floodplain and 2.5 cm at the headwaters, about 100 m^2 of fine sediments would be mobilised by bank erosion from the floodplain and about the same amount from the headwaters. An assumed moderate annual soil erosion of 5 m^3 (3.5 t) per ha from the 2,000 ha of fields in the catchment and a dislocation ratio of 12% (Auerswald, 1997) would result in a sediment load of 1,200 m^3 from agricultural soil erosion.

Thus, bank erosion can be expected to contribute significantly to the loading of the river with fine sediments, but the contribution might be minor compared with the sediment load resulting from soil erosion on arable land. On the other hand, river dynamics and bank erosion in the floodplain enrich physical river structures and contribute to the creation of new favourable habitats for mussels and fish.

Conclusions

The effect of bank erosion needs to be evaluated differently in various river zones throughout the catchment. Measures for the reduction of the fine sediment load are most effective at the sources of erosion, situated mainly at the headwaters: abatement of soil erosion from arable land (catch crops, etc.); disconnection of the flow paths between arable land and the main river; and restoration of the vales of small tributaries aiming to re-establish the shallow, wet channels of the natural discontinuous zone (Thorp *et al.*, 2008). Further downstream, in the main mussel areas of the stream, river dynamics should be tolerated despite the fine sediment load from bank erosion to support the formation of diverse physical habitat structures. ■

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The removal of bank protection to restore hydromorphology and salmonid habitat for freshwater pearl mussel conservation in a Scottish upland gravel-bed river

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F. MacDougall

K. MacDougall

Riverbank protection using boulder revetments to reduce loss of land and the input of sediment is a common morphological pressure in high energy, gravel bed-rivers. This pressure can limit the freedom of channels to naturally adjust their morphology to imposed watershed conditions and can create unnatural channel morphologies.

In turn, these physical changes can alter habitat conditions, for example by simplifying channel complexity, coarsening substrate and reducing sediment transport processes key to sustaining diverse habitats. However, case studies combining assessment of the morphological impacts of bank protection and river restoration options to remedy these impacts are rare. Here, we present findings from a river restoration project commissioned by Scottish Natural Heritage through the "*Pearls in Peril*" (PIP) LIFE + Nature project to restore habitat for salmonids in three reaches of the upper River South Esk catchment in Angus, Scotland, that illustrate bank protection pressures.

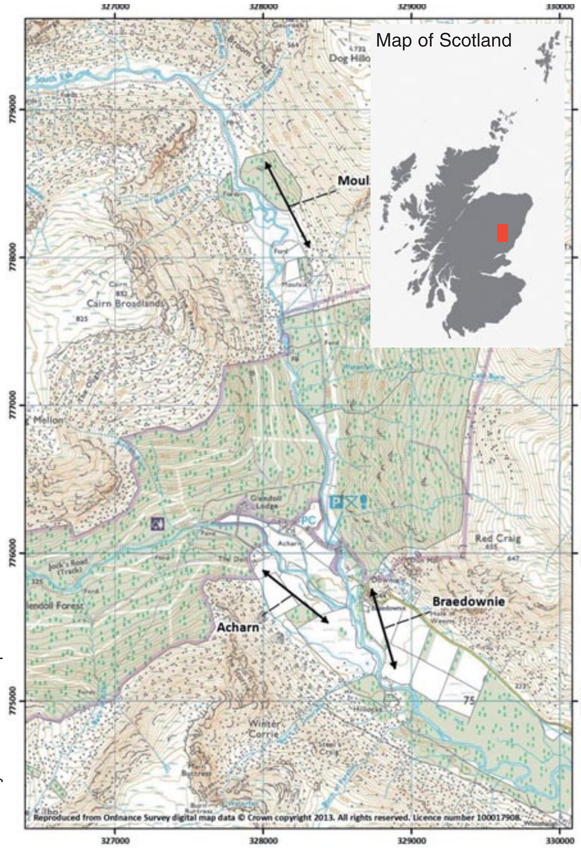
The River South Esk supports a freshwater pearl mussel population that is currently in an unfavourable condition. This project aims to benefit the freshwater pearl mussel population in the long term by improving salmonid habitat conditions and restoring key sediment transport processes. A combination of field-based mapping, topographical surveys and sediment characterisation combined with 1D hydraulic modelling were used to assess the baseline hydromorphology and habitat conditions. Using hydraulic modelling and expert-based judgment gained from the baseline assessment, predictions of hydromorphological changes created by removing structures were made. These predictions in turn informed the prioritised selection of bank protection sites for restoration action by using a multi-criteria assessment that considered constraints and likely benefits for morphology and habitat. It is hoped that the knowledge gained from the morphological impact of bank protection and the river restoration assessment approaches used in this study will be applicable in other similar settings.

South Esk river

The River South Esk is designated as a Special Area of Conservation (SAC) under the Habitats Directive due to its internationally important populations of freshwater pearl mussel and Atlantic salmon. It is a fairly typical high-energy Scottish upland gravel bed river, with a catchment area of 564 km^2 to the North Sea and mean flow of 13 m^3/s. The study area is in the upper reaches of the river at 250-280 m above sea level where the annual average rainfall is typically 1,500 mm. The catchment areas draining to the three study reaches varied between 20-56 km^2 with mean flows of 1.1-2.3 m^3/s and median annual maximum flood flows of 14-39 m^3/s [1].

Freshwater pearl mussels are most abundant in the middle reaches of the river where they attain densities of more than 20 individuals per m^2. The lower part of the river has a high proportion of juveniles (Langan *et al.*, 2007). However, the condition of the population is currently "unfavourable" as based on Site Condition Monitoring of the river due to habitat degradation. No change in the population status since 1997 and 2002 was observed at 14 out of a total of 18 sites, and juveniles were observed at 11 sites, compared to 10 sites during the previous surveys.

The furthest upstream pearl mussels are approximately 4.5 km downstream of the study reaches. Routine monitoring suggests there is a problem with recruitment in this part of the river although no reliable information exists on the earlier history of the population status in the upper catchment. Based on electrofishing of 7 sites throughout the river, juvenile salmon and trout densities are sufficient for freshwater pearl mussel recruitment. The number of sea trout and Atlantic salmon returning to the catchment has decreased in recent years and the SAC status is now "unfavourable"; however there have been some signs of recovery.



[1] ***Location of study areas***

Project Aims

The aim of the project is to prioritise restoration of sites on the basis of benefits to freshwater pearl mussels and restoration of natural processes through:
- assessing baseline hydromorphology (including impacts of bank protection) and habitat;
- identifying restoration measures and predicting impacts (channel, habitat and flood risk);
- prioritising restoration measures, by design and cost; and
- outlining effective monitoring methods to evaluate success of restoration work.

Establishing baseline conditions

The three study reaches contain bank protection that tends to occur where lateral channel migration would be expected, and comprise locally-sourced large boulders up to 2 m in diameter [2]. This protection was constructed in the 1980s with the aim of stabilising the river and reducing sediment input to improve salmonid habitat.

The study reaches were historically more dynamic and complex than they are at present, partly due to the bank protection work. The perceived impacts of bank protection according to the local fisheries trust include increased scour and coarsening substrate to the detriment of spawning fish.

The baseline conditions at the study reaches were established through:

• Field surveys:
- topographic survey of ~80 channel cross-sections;
- recording distribution of hydraulic habitats, sediment sources, sediment stores and bed sediment character;
- pebble counts in riffle and rapid channel units (23 in total);
- examination of structures and observations of potential options and implications.

• Hydrological assessment to determine design flows;

• Construction of 1D hydraulic models to derive geomorphic metrics (e.g. boundary shear stress, critical shear stress and Shield stress);

• Hydromorphological assessment:
- topographical, aerial photo and hydraulic information to derive descriptors of channel morphology (channel slope, depth, width and sinuosity);
- assessment of bed sediment size distributions;
- metrics of shear stress (τ) and specific stream power (ω) calculated to assess levels and distribution of energy available to do geomorphic work;
- critical shear stress (τ_{crit}) and the Shields parameter (τ^*) derived to give an indication of sediment transport and morphological sensitivity.

Hydromorphology and habitat conditions

The study reaches are set within the wide alluvial valley bottom in the glacial trough of a glen. The reaches are generally single thread, except an island at Braedownie, and review of historical maps typically


S. Addy

[2] ***Typical bank protection***

Characteristics	Moulzie	Acharn	Braedownie main branch	Braedownie left branch
Reach length (m)	1,202	809	712	199
Drainage area start (km^2)	18.0	25.7	56.1	56.1
Drainage area end (km^2)	22.2	26.1	57.7	56.2
Sinuosity (-)	1.24	1.23	1.19	1.02
Active channel width (m)*	13.5 (27-8)	13.8 (27-8.1)	23.4 (40-12.8)	9.8 (17.3-6.5)
Slope (m/m)*	0.009 (0.02-0.001)	0.013 (0.022-0.007)	0.010 (0.025-0.002)	0.013 (0.022-0.001)
Total length of bank protection (m)	738	487	190	30
Bank erosion length (m)	458	112	206	15
Max bank erosion length (m)	82	34	112	7
Bank material	Alluvium	Alluvium	Alluvium & fluvio-glacial	Alluvium
* Mean values with total range in brackets				

[Table 1] ***Physical characteristics of study reaches***

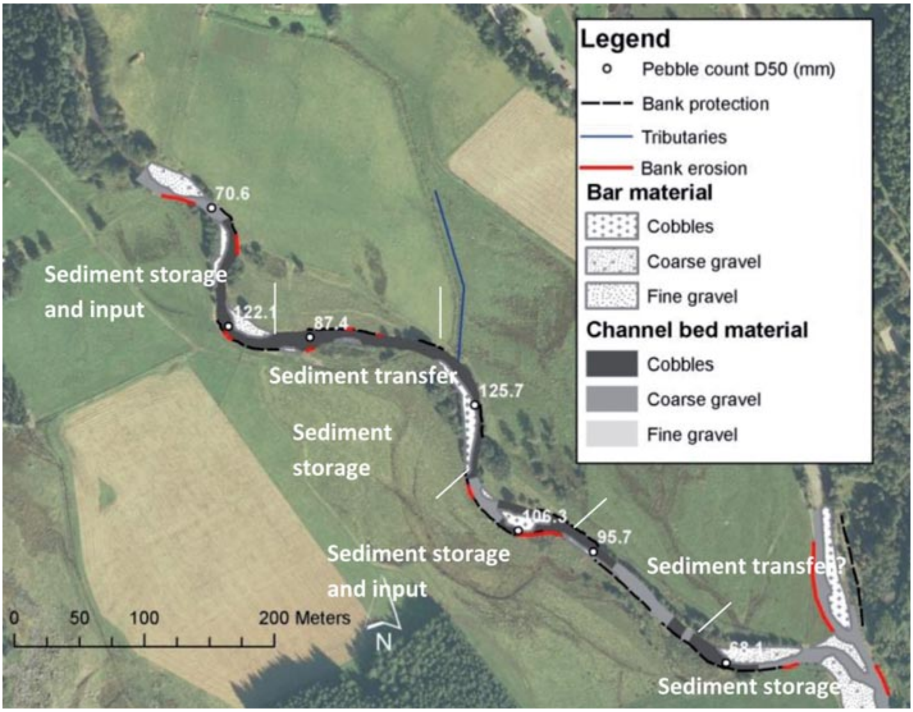
shows more dynamic processes with more channel branches. Despite the extent of bank protection within the reaches, active bank erosion processes are still occurring [Table 1][3][4].

Moulzie is the least energetic reach (lowest τ and ω) and the most stable (lowest τ^*), a condition that may have been enhanced by the bank protection increasing lateral resistance. Typical local bank erosion rates are observed to be <2 m since 2008. During median annual flood flow conditions (QMED), the reach is incompetent to transport median bed sediment particles but is competent to transport gravel sized material on average and potentially alter its morphology.

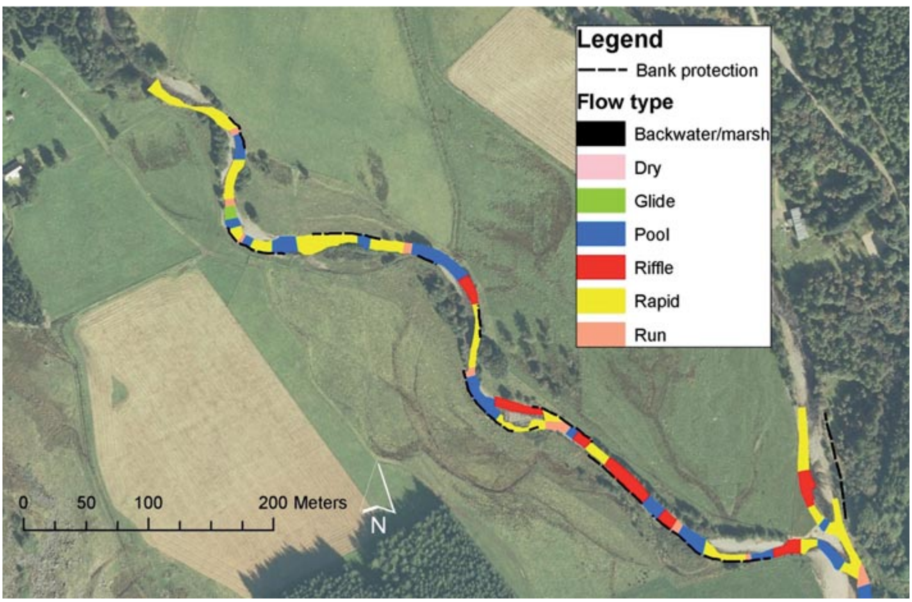
Acharn is the most energetic reach and locally can move median bed sediment sizes during QMED conditions and consequently adjust its bed morphology more readily than the other reaches, although its ability to adjust laterally is likely to have been reduced by bank protection and the establishment of mature riparian vegetation. Much of the reach is likely to be competent to flush sediment downstream delivered from bank erosion inputs and from above the upstream boundary. The total extent of bank erosion along the reach is limited (112 m) but is locally up to ~2.3 m since September 2008. Braedownie at the confluence of the White Water and the South Esk is split by a stable ~150 m-long island covered with mature deciduous woodland. This reach is incompetent to transport median grain size particles at QMED and the lower average value of τ^* indicates that the ability to move coarser framework grains and alter morphology through erosion is limited, particularly just downstream of the confluence, as indicated by the large extent of the bars. Bank erosion is locally severe, being up to 11 m since September 2011.

Predicted Restoration Benefits

The predicted hydromorphological effects of removing and restoring the bank



[3] ***Mapping of geomorphic features, sediment facies and interpretation of dominant coarse sediment regime styles***



[4] ***The distribution of flow types and bank protection***

protection works were assessed [Table 2]. Changes in flood risk were also examined and did not identify any significant changes apart from one location where channel migration towards an embankment could potentially lower the bank and increase the frequency of flooding.

The benefits to local habitats that could be achieved through restoration of the bank protection works were identified as:

- increased diversity of morphology in existing channel and through reconnection with palaeochannels;
- finer riffle substrate more suitable for spawning salmonids;
- bank undercutting and block input providing cover for fish;
- increased input of sediment for sustaining freshwater pearl mussel habitats downstream.

Restoration Strategy

The restoration strategy is focused on delivering benefits for freshwater pearl mussels by improving salmonid numbers through habitat improvement and increasing the delivering of sediment to downstream reaches needed to sustain freshwater pearl mussel habitat.

Restoration measures mainly comprised of removing bankside rock armouring, accompanied by enhancement measures including bank reprofiling and reconnection of palaeochannels. The impacts of these measures have been examined, and in the short term there is an increased likelihood of bank erosion and input of sediment, slight increases/decreases in bed mobility, while in the longer term increased lateral migration rates, more natural planforms, more natural distribution of bed sediments and increased morphological complexity are expected.

These restoration measures will promote a more natural distribution of bed sediments, thereby providing benefits to local habitats. The presence of finer substrates more suitable for spawning salmonids and improved habitat complexity will benefit all salmon life stages. These habitat benefits will also extend to freshwater pearl mussels and other biota.

A multi-criteria analysis (MCA) has been developed to prioritise sites on the basis of those bank protection structures which were having the greatest impact on natural processes, the potential benefit for habitat improvement and the risk posed to receptors (farmland and infrastructure). Using the MCA results, consideration of restoration work practicalities and discussion with local stakeholders, four zones were selected for design, which focus on seven of the prioritised sites. The restoration works are scheduled to commence in spring of 2015 along with a monitoring scheme.

Outcomes

Bank protection structures are a common morphological pressure but their impacts are rarely documented, and case studies of their removal in high-energy gravel bed river settings are rare.

This river restoration assessment is useful for gaining an initial insight into the impacts of bank protection which is rarely quantified and documented, and also demonstrates a simple, relatively low-cost approach to predicting the effects of restoration actions and prioritising sites.

It is recognised that there are uncertainties in terms of predicting the rates of hydromorphic and habitat changes. Robust monitoring will help to gain an insight into the rates of recovery from this type of morphological pressure to inform future restoration works. ■

Short term (< 1 year)	Longer term (1-10 years)
- Bank erosion and input of destabilised sediment - Bank erosion may be limited due to riparian vegetation and straight planform - Geomorphic predictions suggest slight increase in bed mobility	- Meander migration and extension - Further aggradation and channel widening - Future responses may be limited due to natural structure erosion and adjustment already occurred - Channel widening - Decrease in bed sediment size due to channel widening and greater local sediment input

[Table 2] ***Predicted hydromorphological effects***

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Posters

Theme 1

Freshwater bivalves in Europe: Status and conservation issue

Biological status and attempts to identify the causes of decline of the thick-shelled river mussel *Unio crassus* in the Allier catchment in Auvergne (France)

Sylvain VRIGNAUD

S. Botto

S. Vrignaud

The thick-shelled mussel, *Unio crassus*, benefits from national protection and appears in Annexes II and IV of the "Fauna-Flora-Habitat" directive. Despite this protected status, the little data available from different areas of its range show a major decline. However, studies on this species remain scarce.

Based on data from before 2012 and from collections, grey literature and personal observations, 10 rivers were selected for sampling. These rivers are small (less than 12 m wide and wadable). Variably-sized sections arranged irregularly along rivers were surveyed using an aquascope to verify the actual presence of these naiades. In addition, several variables were identified (electrofishing, mussel fauna, hydromorphology, etc.). Thus, more than 11 km of river was examined, representing an average of 4.23% of the length of the rivers concerned.

Detection of the species was particularly low, with only four rivers revealing the actual presence of this mussel. Furthermore, searches of the banks along the River Allier for shells left by floodwaters revealed nothing. The mussel was, however, reported as common in 1900. A drastic decline on this scale is equivalent to a status of "critically endangered" according to IUCN criteria.

Moreover, among the variables analysed so far, there do not appear to be significant differences in global ichthyofauna and associated mollusc fauna between streams with and without *Unio crassus*. However, there is a significant difference between these situations for the ratio between bankfull width and average water height. This hydromorphologic criterion reflects a worsening of river conditions for this mussel. ■

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S. Vrignaud

***Thick-shelled mussel,* Unio crassus**

The freshwater pearl mussel, a remarkable tool for our actions

Gilles BARTHÉLÉMY

G. Barthélémy

In the context of aquatic environment degradation, what levers for action do we have available to us? Some symbolic species, such as the freshwater pearl mussel, have an ability to mobilise action. The presence of the species suggests that "water quality is good"; however, our diagnosis indicates a deteriorated status. Populations are declining, and it is urgent to make the facts known. The production of valid indicators and technical guides can help in raising awareness.

An awareness of the environment makes it possible to set priorities and to bring parties together to work toward common goals around water and biodiversity. Actions are performed in the catchment: restoration of hedges and riverbeds, and elimination of fertilisation with the help of "Groupe Mulette Limousin / PRA". The species has led to the classification of watercourses as biological reservoirs for ecological continuity. Measures to protect the species have been imposed through administrative documents and police checks on the environment.

The freshwater pearl mussel represents a real opportunity to set ambitious overall objectives towards attaining a positive ecological status for waterbodies.

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ONEMA

Characterisation of the milieu for freshwater pearl mussel restoration in Limousin (France)

LIFE project: Giant river pearl mussel

Karl WANTZEN, Stéphane RIVIÈRE, Nina RICHARD, Philippe JUGÉ, Yann GUÉREZ, Élodie HUGUES, Guillaume MÉTAYER & Rafael ARAUJO

N. Richard

Since July 2014, the University of Tours and the Departmental Council of Charente Maritime, France, have been cooperating with partners in France, Spain and Germany to preserve the last populations of the "big sister" of the freshwater pearl mussel.

Margaritifera auricularia lives in the potamon of larger streams and rivers. Though it is less sensitive to water pollution than smaller species, it has nevertheless suffered dramatic population losses due to habitat deterioration and the disappearance of its host fishes, particularly European sturgeon species. Our project endeavours to overcome conservation bottlenecks, namely the high mortality of young mussels and anoxic mud accumulation at the site of the largest population in the Charente River, and to search for alternative host fish species. ■

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P. Jugé

Giant river pearl mussel, **Margaritifera auricularia**

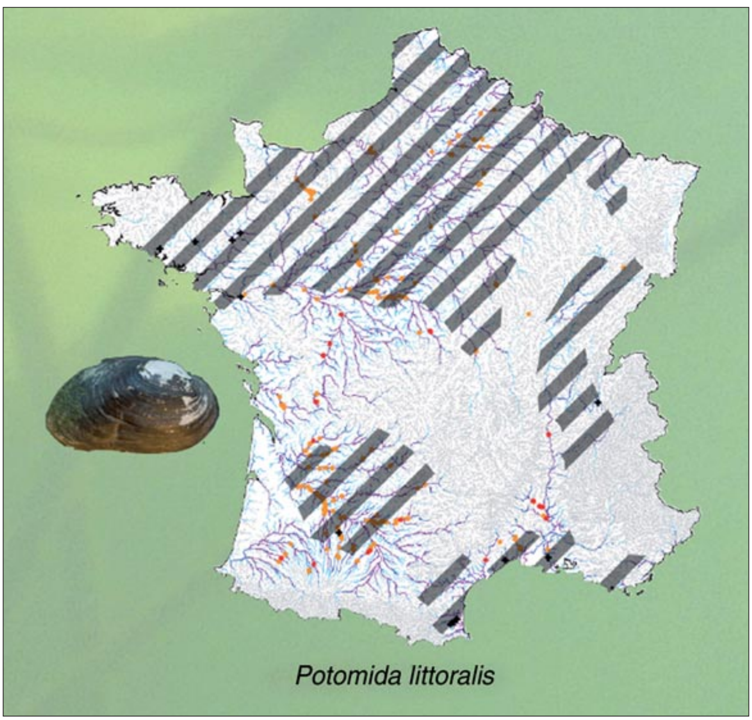
Out of sight, out of mind: the critical situation of the giant river pearl mussel and other naiads of France

Vincent PRIÉ

V. Prié

While the worrying situation of the freshwater pearl mussel *Margaritifera margaritifera* has mobilised nature conservation stakeholders throughout France and Europe, other species, less charismatic because of a lower profile and/or being less widespread, face a worse plight. The downstream areas of major watersheds are not well-known to naturalists, while anthropogenic disturbances are even greater there.

The natural ranges of various species of French naiads were modelled without taking into account limiting factors of anthropogenic origin such as pollution, barriers, etc. in order to infer an optimal distribution area in France. From this area of theoretical distribution we can draw convex polygons of presence or areas of occurrence ("extent of occurrence", *sensu* IUCN), which are used for example in the context of categorisation for red lists. Although it could be further improved, this method makes it possible to objectify and quantify the depletion of various species. Moreover, data from literature were compiled and field surveys were carried out to try and identify poorly-defined taxa.



V. Prié

Though it was widespread in major rivers from Denmark to Portugal, the giant river pearl mussel *Margaritifera auricularia* now remains in only a few population centres in France and Spain, all of which are threatened to varying degrees. The decline of its occurrence in France can be estimated at 90%, but its decline at the European level is even greater, and the population centres that remain are often on the verge of extinction.

Although it benefits from no conservation measures, the river mussel *Potomida littoralis* is the second most endangered species, with nearly 75% reduction in its area of occurrence in France. At the limits of its range in France, it appears to have completely disappeared from the Seine catchments and from coastal rivers in Normandy and Brittany.

The swollen river mussel *Unio tumidus* ranks third, with a 70% decrease of its area of occurrence in France.

The decrease in areas of occurrence of the freshwater pearl mussel *Margaritifera margaritifera* and thick-shelled river mussel *Unio crassus*, which receive over 90% of the funds allocated to bivalve conservation in Europe, is "only" 50% and 40% respectively.

The painter's mussel *Unio pictorum* and southern painter's mussel *Unio manus* seem less threatened, yet have seen 20% and 30% decreases in their respective areas of occurrence.

In addition, some taxa of poorly-known taxonomic status could not be found. This is particularly the case for *Unio pictorum deshayesii* and *Unio pictorum platyrhynchoideus*, the "bigoudaine" and "Landaise" painter's mussels, respectively, which seem to have completely disappeared before they could be studied. Degradation of the quality of Breton rivers may be to blame for the disappearance of the former of these species. It is more difficult to determine what may have changed in the lakes of the Landes since the late 19th century. The effects of mosquito control on freshwater bivalves have not been studied, but it is clear that areas treated in this way (Atlantic and Mediterranean coasts) are now very poor in naiads, whereas the literature shows that they were relatively abundant in the past.■

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Theme 2

Recent advancements in the biology and ecology of freshwater bivalves in Europe, in relation to their conservation

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

Pierre-Yves PASCO, Marie CAPOULADE, Pierrick DURY, Maria RIBEIRO, Benjamin BEAUFILS & Loïc ROSTAGNAT

H. Ronné

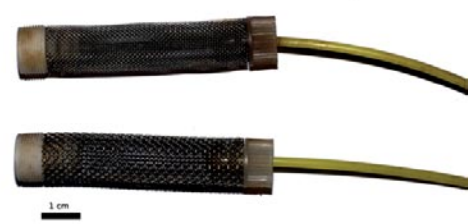
P.-Y. Pasco

The LIFE+ program "Conservation of the freshwater pearl mussel from the Armorican Massif" has as its objective the conservation of six major pearl mussel populations in the Armorican Massif. One of the problems identified is the lack of juvenile recruitment. A rearing station has been built and habitat restoration activities begun on rivers, with the first population reinforcements beginning in 2012. Cylindrical tubes of stainless steel mesh were used to test the survival and growth of some of the young mussels released.

In each stream, 12 mesh tubes (with a pore size of 0.42 mm or 0.8 mm), each containing five one-year-old mussels, were installed for 3 months (60 mussels per stream). In Brittany, these tubes were placed together at the heads of fast-flowing shallow areas where they were buried horizontally in a pile of gravel and/or stones. In Lower Normandy, the tubes were placed vertically at intervals of a few feet in fast-flowing shallow areas, driven into a hole previously made with a digging bar. In Brittany, the mussels used were from the same river as the one where they were released, while in Lower Normandy they came from a Breton strain.

The survival rate varied from 6.67% to 95%, being much higher in the Breton rivers than in the others. The size of the tubes mesh 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 were skewed because: (1) the tube installation method was not the same in Brittany and Normandy; and (2) the mussels used in rivers in Lower Normandy came from a Breton strain whereas, in Brittany, it was possible to use young mussels from the populations of the same rivers in which the releases were made. It is also possible that differences in the physicochemical parameters of the river water and/or sediments had an influence on these results.



P.-Y. Pasco

However, the use of this technique seems to be of interest in measuring the survival and growth of young mussels in the context of a population reinforcement program.

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Influence of stock origin and environmental conditions on the survival and growth of young freshwater pearl mussels in a cross-exposure experiment

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S. Beggel

M. Denic

The freshwater pearl mussel *(Margaritifera margaritifera)* is a highly specialised and sensitive freshwater bivalve, whose survival in the juvenile phase is indicative of high-quality habitats. This contribution investigates the use of young freshwater pearl mussels as bioindicators, considering the influence of various mussel stocks and study stream conditions on young mussel performance, as described by survival and growth rates.

A standardised cross-exposure experiment was carried out to investigate young mussel performance in four different pearl mussel stocks originating from the Rhine, Danube and Elbe drainages, representing distinct genetic conservation units. The young mussels were exposed in five study streams which were selected for their various water qualities and the recruitment status of their mussel populations. Per study stream, five standard mesh cages were installed, each containing an equal number of 20 (10 x 2) young pearl mussels per stock in separate chambers. Survival and growth rates of young mussels were checked after three months (i.e. before their first winter) and after nine months (i.e. after their first winter). Mussel stock and study stream conditions significantly influenced young mussel performance. Growth rates were determined by study stream conditions and increased with stream water temperature, organic carbon and C/N ratios. Survival rates varied stock-specifically, indicating different levels of local adaptation to their native streams. Due to the detection of stream-specific differences in young mussel performance, freshwater pearl mussels appear suitable as bioindicators. However, a careful of consideration of stock-specificity is necessary to avoid false interpretation of bioindication results. Cross-exposure of young mussels outside their native habitats could increase their survival rates and therefore serve as a complement to conservation programmes. ■

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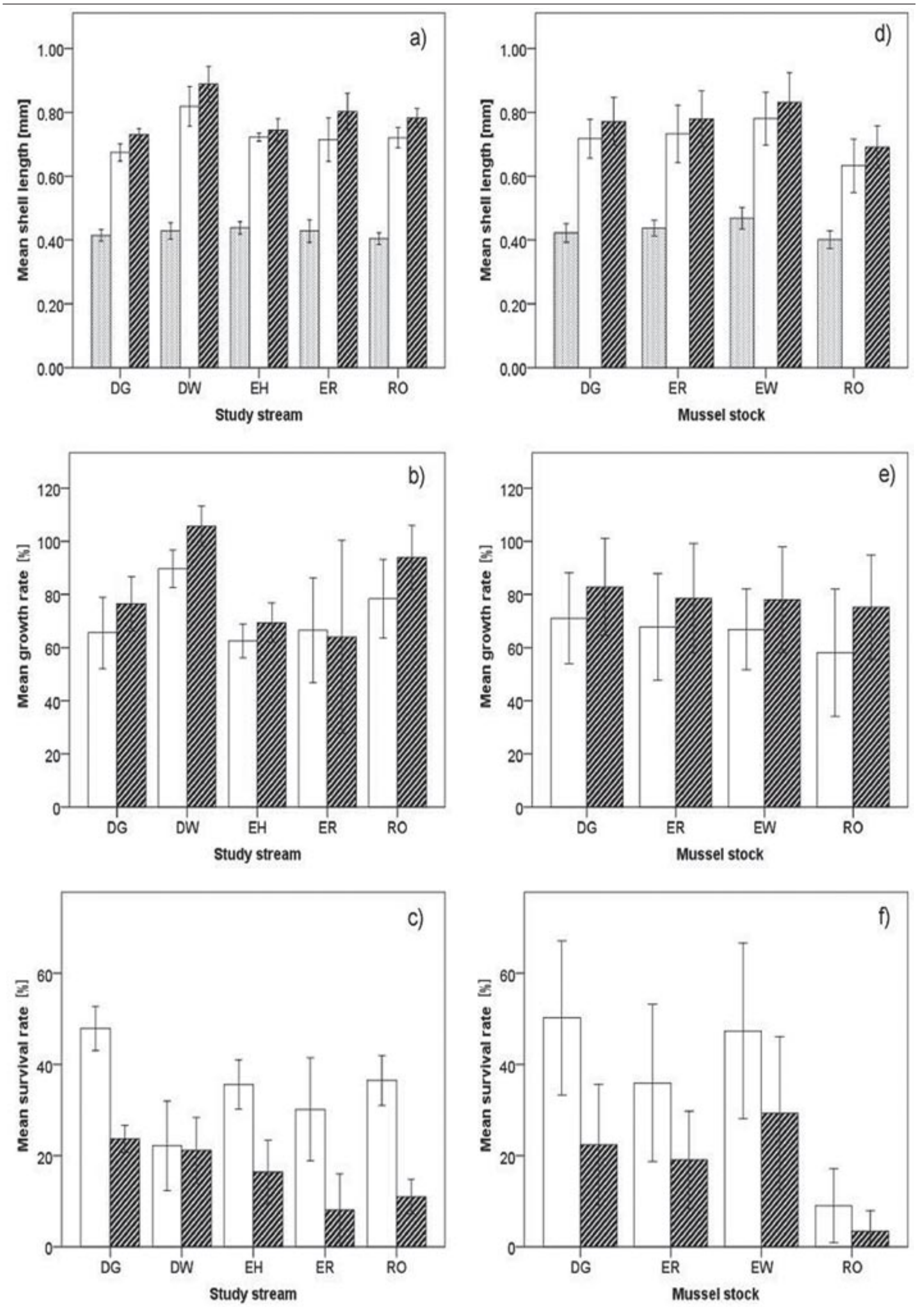

Mean shell length, growth and survival rates following studied streams (a, b and c) and mussel stock (d, e and f) at the initial state (in grey), before winter (in white) and in total (in dark). The abbreviations of abscissa mean principal catchment area (1st letter) and stream name (2nd letter):
DG=Danube, Giessenbach;
DW=Danube, Wolfsteiner Ohe;
EH=Elbe, Haarbach;
ER=Elbe, Rauner Bach;
RO=Rhine, Our.

Captive breeding of *Margaritifera margaritifera* (L., 1758) in Galicia (Spain): reporting preliminary results

Catarina VARELA, Sabela LOIS, Adolfo OUTEIRO, Ramón MASCATO, Rafaela AMARO, Eduardo SAN MIGUEL & Paz ONDINA

University of Santiago de Compostela

C. Varela

This work shows the first results of *Margaritifera margaritifera* captive breeding and juvenile culture during 2012 and 2013 in northwest Spain. Data on the infestation rates of several hosts, such as the Atlantic salmon (*Salmo salar*, L.), the brown trout (*Salmo trutta*, L.) and its ecotype sea trout are reported, as well as the growth rates of gill cysts. Moreover, we indicate the survival and growth rates of juvenile individuals in laboratory conditions over the course of a year. This work is part of one of the conservation actions for the recovery of this species, carried out in the framework of the "Margal Ulla" LIFE project (LIFE 09NAT/ES/000514).

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Mussels hide when you want to count them!

Xavier CUCHERAT, Damien FROMENT, Laurent PHILIPPE & Noélie TAPKO


V. Prié

X. Cucherat

Freshwater mussels are among the most threatened organisms in the world, and those in Europe have not been spared by the biodiversity crisis. Knowing their abundance is therefore of prime importance. They are mobile organisms that live wholly- or partially-buried in the soft sediments of rivers. When estimates of abundance in rivers or streams are made, it is highly likely that some of the individuals are fully buried and thus escape sampling, leading to a high risk of estimation bias. The abundance of mussels is therefore underestimated, which is particularly troublesome in population monitoring or in impact studies.

During three sampling campaigns carried out in three different types of rivers (large: the Vienne; medium: the Cure; and small: the Saulx), an unbiased estimate of the abundance of freshwater mussel species was made using double sampling involving excavations of the sediment. In the Vienne, Cure and Saulx, systematic sampling was the baseline survey technique. The mussels were searched for in quadrats of 50 x 50 cm. One quadrat out of three on the Vienne, one out of four on the Cure and all on the Saulx were excavated after a preliminary count of individuals visible on the surface. In all three studies, visibility was above a metre.

Out of 310 quadrats, 141 contained mussels, including 16 where there were only completely buried specimens, 103 with individuals only visible on the surface and 22 with individuals both visible and buried. There were thus 38 quadrats in which some individuals were buried. In the Saulx, 60 excavations were conducted. In these 60 excavations, 48 mussels were found, four of which were completely buried, making the percentage of undetected individuals 8.3%. In the Vienne, 282 mussels were found including 44 completely buried individuals found by our excavations, making the rate of buried individuals 15%. In the Cure, 28 excavations were performed in which four fully buried mussels were found, making the rate of buried individuals 100%.

In the Vienne, the thick-shelled river mussel, river mussel and painter's mussel show 25%, 13.2% and 50% of buried individuals respectively, in relation to the total number observed in the quadrats excavated. In the Cure, this percentage increases to 100% for the southern painter's mussel, while the percentage was 8% in the Saulx.

These results show the importance of taking into account buried individuals and thus making excavations in a river when pursuing the goal of obtaining an unbiased estimate of mussel abundance.

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N. Tapko

Theme 3

From population to catchment area management

Initiatives in support of freshwater pearl mussels in Lower Normandy (France)

Maria RIBEIRO, Benjamin BEAUFILS & Loïc ROSTAGNAT

Ecological restoration of rivers: how landscape analysis can help guide conservation of a target species

Marion DELISLE, Jérôme SAWTSCHUK, Isabelle MULLER & Ivan BERNEZ

Linking stream sediment deposition and aquatic habitat quality in pearl mussel streams: implications for conservation

Marco DENIC & Juergen GEIST

Establishment of a collaborative experimental network for wetland restoration in the department of Finistère (France)

Armel DAUSSE, Sébastien GALLET & Corinne THOMAS

Agricultural management of riverside land parcels in the Monts d'Arrée (France)

Jérémie BOURDOULOUS

Initiatives in support of freshwater pearl mussels in Lower Normandy (France)

Maria RIBEIRO, Benjamin BEAUFILS & Loïc ROSTAGNAT

H. Ronné

M. Ribeiro

The freshwater pearl mussel, which has now become an iconic species of our rivers, has for many years been exposed to degradation of the quality of its environment. Throughout its life stages, it encounters threats to its survival such as clogging of watercourse bottoms, inadequate quality of water flow, obstacles to salmonid movement, etc. All of these factors have consequences for the maintenance of the species in our region.

In order to restore river quality, local authorities and associations are making every effort and mobilising all possible means for action. Aid from the State, Europe or water agencies which assist operators all contribute to restoring the quality of the environment of the freshwater pearl mussel.

Actions that are being implemented include:

- protection of banks from trampling (fencing, restoration of riparian forest) and providing fields with systems for livestock watering (development of access to watercourses, nose pumps, gravity flow troughs);

- restoration of the ecological continuity of rivers (removal of dams, sills, conduits, etc.);

- restoration of stream hydromorphology through restoration work in the talweg;

- encouragement of vegetation maintenance along watercourses.

Thus, we can hope to, someday, see rivers regain their potential to host dynamic freshwater pearl mussel populations that evolve naturally with their host fish, such as salmonids, in the rivers of Lower Normandy.

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SIAES

Ecological restoration of rivers: how landscape analysis can help guide conservation of a target species

Marion DELISLE, Jérôme SAWTSCHUK, Isabelle MULLER & Ivan BERNEZ


H. Ronné

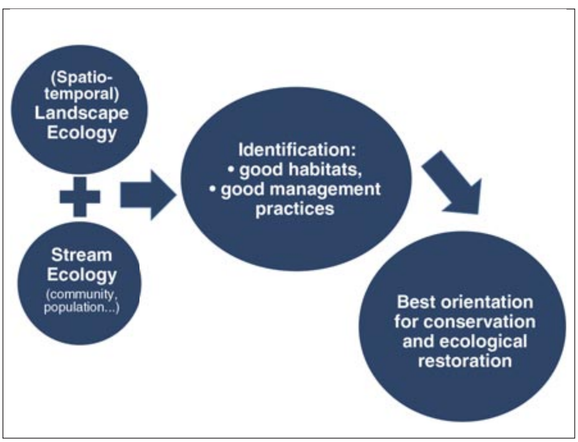
M. Delisle

By studying a large number of aerial photographs of the Oir catchment (Lower Normandy) it was possible to carry out a spatio-temporal analysis of the landscape near streams in this sector.

In addition, the Atlantic salmon (*Salmo salar*) and trout (*Salmo trutta*) are the target species of managers in this catchment and are the subjects of many scientific monitoring programmes as part of the *Observatoire de Recherche en Environnement sur les Petits Fleuves Côtiers* [Centre for Environmental Research on Small Coastal Rivers].

The linking of ecological data on streams and a landscape analysis allows landscape elements (e.g. density and age of riparian woodland) to be targeted which are correlated with the presence or absence of animal populations dependent on these streams. These results can be applied to guide management actions towards ecological restoration based on the large-scale ecological requirements of a given species.

This method could be adapted and applied to species other than salmonids. Particularly interesting sites for ecological restoration or reintroduction of species could be targeted. This approach is being tested in the Sélune catchment for white-clawed crayfish (*Austropotamobius pallipes*) and appears potentially interesting for the freshwater pearl mussel (*Margaritifera margaritifera*): it has a high protection status; its life history traits are related to other species of heritage interest; and ecological requirements both at the local scale and across the landscape as a whole are essential to its survival.



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Linking stream sediment deposition and aquatic habitat quality in pearl mussel streams: implications for conservation

Marco DENIC & Juergen GEIST


S. Beggel

M. Denic

The introduction of fine sediments into streams is considered to have a major effect on habitat quality, affecting the reproduction of sensitive species such as unionid mussels and salmonid fishes. To date, there is a lack of information on the magnitude and spatio-temporal resolution of sediment introduction.

This study aimed to quantify the spatio-temporal deposition of fine sediments in headwater streams in relation to the status of *Margaritifera margaritifera* and *Salmo trutta*. Fine sediment deposition was linked to the physicochemical conditions of the adjacent stream bed.

The mean observed deposition of fine sediments over the study period was 3.4 $kg.m^{-2}.month^{-1}$ with a high spatio-temporal variation ranging from < 0.01 upto 20.3 kg $m^{-2}.month^{-1}$. Discharge had the strongest influence on deposition rates.

Mean differences in redox potential between free-flowing water and the interstitial zone were 90 mV. The spatio-temporal variability of physicochemical parameters increased with the degree of degradation. High-quality reaches had more constant conditions.

Our results indicate that monitoring of sediment quality and deposition in streams has to include several time points and study reaches, or should at least be conducted during periods with the most adverse habitat conditions, to allow valid assessments of habitat quality. In streams with increased fine sediment deposition, instream restoration measures are insufficient for the enhancement of pearl mussel habitats due to the rapid clogging of interstitial pores. Only integrative catchment management based on detailed habitat analysis can ensure sufficient habitat quality for species sensitive to siltation. ■

DENIC M. & GEIST J. 2014 – *Linking stream sediment deposition and aquatic habitat quality in pearl mussels streams: implications for conservation. River Research and Application*, to appear.


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Establishment of a collaborative experimental network for wetland restoration in the department of Finistère (France)

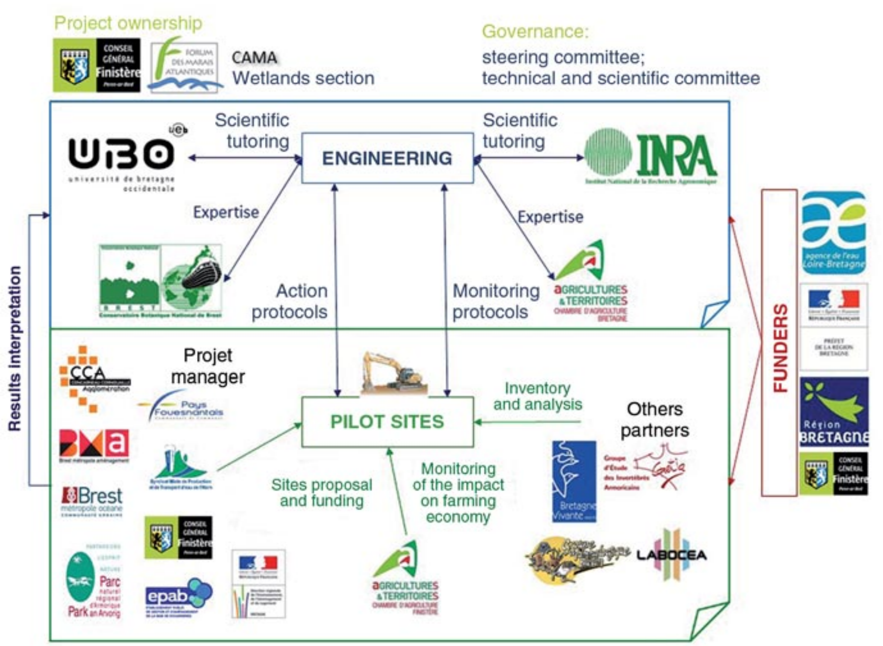
Armel DAUSSE, Sébastien GALLET & Corinne THOMAS

A. Dausse

For several years now, stakeholders in Finistère have raised important questions on how wetlands should be restored and how such operations should be monitored. These questions are particularly related to the toughening enforcement of ecological compensation plans or those on green algae. In response, a network of experimental sites dealing with the rehabilitation of wetlands in Finistère was established.

Their goals include:

- consolidation and validation of protocols for the rehabilitation of wetlands, including operations to remove drainage or filling and to clear conifers;
- quantification of environmental gains from rehabilitation, particularly in terms of the quantity and quality of water resources and biodiversity;
- quantification of the impact on farming;
- designation of simple monitoring indicators that can be proposed to project managers.



A. Dausse

The establishment of this network is coordinated by the CAMA (Cellule d'animation sur les milieux aquatiques) [Action group for aquatic environments – wetlands section], in which the Departmental Council of Finistère and the Forum des Marais Atlantiques [Atlantic swamp forum] are associated. There are a number of technical and scientific partners, including the University of Western Brittany, INRA Rennes, CBNB, Bretagne Vivante – SEPNB, GRETIA, the GMB and Chambers of Agriculture (at department and region levels). Its implementation is based on the monitoring of pilot sites selected following a call for proposals from the various actors likely to support work on wetland rehabilitation. Restoration and rehabilitation are carried out by local authorities who enter into agreements with farmers if necessary and also provide funding, the network providing technical and scientific support for the definition of rehabilitation protocols, monitoring and evaluation.

The unusual structure of this project allows synergy between the actions of various local stakeholders. Local authorities managing actions benefit from technical support in their implementation of the work and from monitoring and evaluation in connection with issues relevant to the local area. The network also permits the optimisation of resources. For scientists, the network makes "life size" experimental protocols possible, and enables knowledge dissemination as well as helpful feedback. For the promoters of the project, it highlights the actions of local stakeholders and facilitates the sharing of acquired knowledge.

The establishment of this network is a highly integrated example of collaboration at a regional scale for the rehabilitation of natural environments. ■

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Agricultural management of riverside land parcels in the Monts d'Arrée (France)

Jérémie BOURDOULOUS

GPO-PNRA

J. Bourdoulous

The freshwater pearl mussel population of the Elez, a tributary of the Aulne, is located in the heart of *the Armoric Regional Natural Park (PNRA)*. A remarkable river network runs across this area, including the sources of important coastal rivers of western Brittany. Local environmental factors are conducive to wetlands, which cover large areas of the catchment. These ensure the integrity of watercourses in this sector. Thus, since the 1990s, the Park has focused on guidance given to farming operations in direct interaction with riparian wetlands.

The wet meadows, moors and peat bogs of the Monts d'Arrée were a resource largely used by agriculture until the middle of the 20th century. Gradually, the evolution of farming systems lead to a loss of economic interest in these so-called low-value agricultural areas. The decrease in agricultural activity in these semi-natural habitats, resulting in a slow evolution of landscapes (natural afforestation dynamics), can temporarily lead to a homogenisation of the flora.

These wetlands nevertheless represent a unique biological heritage in Brittany. Thus, in the mid-2000s, the Monts d'Arrée were identified at the European level as a special area of conservation, or Natura 2000 site.

J. Bourdoulous - PNRA

Reaping of wet moors in Botmeur

Following the first successful experiments by the association Bretagne Vivante – SEPNB on the Cragou moors, the park started an experimental policy of contracts with local farmers, integrating the aspects of biodiversity, landscape and economic development. The land development group began operating in 1993. These 5-year contracts are applicable to the management of heathland and wetlands. Compensation to farmers is based on guidelines which imply the absence of fertilisation, soil turning and herbicide use. An initial diagnostic of land plots is carried out by the Park in concert with the farmers.

The Elez is considered a river of excellent quality. It is, however, vulnerable to anthropogenic pressures on its upstream catchment. Maintaining an ambitious agri-environmental policy in the context of the next Common Agricultural Policy will be a lever for the preservation of the pearl mussel population. ■

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Round table

What does the future hold for pearl mussel conservation in the Armorican Massif?

Summary of the round table,
chaired by Jean-Luc TOULLEC, Michel BACLE,
Marie CLÉMENT & Michel DY,
reported by Maëva AUFFRAY and Marie CAPOULADE

The LIFE "Mussel" programme officially ends on August 31st, 2016. After 6 years of work, monitoring, partnerships and raising awareness, the question now raised concerns the sustainability of the actions undertaken. Regional implementation of the national action plan seems to be the best framework available to us today.

Bretagne Vivante – SEPNB

The European Community and the French national context

At the Community level, the objective of the 1992 "Habitat, Fauna, Flora" directive is to preserve the natural habitats of wild flora and fauna, in complement to the "Birds" directive. It incorporates the general lines of the Berne Convention (1979), and strengthens and amplifies them on the territories of member States. The directive aims to establish a "coherent European ecological network of special areas of conservation, known as Natura 2000 sites", in which it is essential to ensure the maintenance of biological processes or elements necessary for the conservation of the habitat types or species for which they have been designated. In this context, the giant river pearl mussel and the freshwater pearl mussel were the subject of a European action plan in 2001 that outlined a set of broad objectives to save these species from extinction (Araujo & Ramos, 2001).

In France, the principal law for all actors in the field of nature protection is the 2009 Grenelle 1 law. Its main objective is to halt the loss of biodiversity. It mentions a large number of tools that have been discussed in presentations at this conference, such as the Natura 2000 objectives documents; the green and blue infrastructure; the strategy of protected area creation; and, more specifically, the development of plans for the conservation and restoration of critically endangered species.

With this 2009 Act, France gained a legitimate framework enabling various stakeholders to work towards nature protection, including state services, Natura 2000 actors, nature reserves, regional natural parks, associations, etc. Within this framework, a national action plan for the pearl mussel was developed (Prié *et al.*, 2012).

What are the benefits of pearl mussel conservation?

The well-known example of French-style gardens shows how our culture has involuntarily led us to control nature, which is not necessarily the case in other countries. Observing nature evolving independently, accompanying it and understanding it do not traditionally form part of our education. As a result, natural spaces and species are subject to very strong anthropogenic pressure, particularly in Brittany. The issues of agricultural practices, water quality, biodiversity, urbanisation, land take, etc. have blurred boundaries and are sometimes in opposition. In our society, it is therefore vital that nature is taken into account as an asset and a form of added value for a territory. Although efforts taken to preserve a tiny species such as the freshwater pearl mussel may seem amusing at first, they quickly lead to pressing questions about habitat, water quality, and activities in the catchment. Thus, pearl mussel conservation relates not only to issues of biodiversity but also to socio-economic aspects, quality of life, or even public health, notably through the example of drinking water. The pearl mussel is an exemplary species that highlights the management and life of our regions.

Working together

Rivers are environments with no borders. In the Armorican Massif, as elsewhere, these ecosystems run through various areas between their source and their arrival at the sea. In an analogous manner, the conservation of these ecosystems traverses different institutions such as communities, elected officials, associations, local populations, land users, farmers, etc. By bringing these actors together around the table, pearl mussel conservation rallies them around unifying projects and/or constructive partnerships. In addition to the involvement of volunteers (participation in inventories and development of conservation strategies), the "Conservation of the freshwater pearl mussel from the Armorican Massif" (or LIFE "Mussel") programme has made it possible for Bretagne Vivante – SEPNB to forge ties with actors with whom the association previously had little contact. The programme's territory, the Armorican Massif, meant that closer links were first established with partners in Lower Normandy. In both regions, aquatic environment protection stakeholders were then mobilised: federations of municipalities, regional natural parks, catchment authorities, associations, drinking water authorities, "aquatic environment" divisions of departmental councils, departmental services of the National Agency for Water and Aquatic Environments (ONEMA), water authorities, regional councils, Regional Directorates for the Environment, Development and Housing (DREAL), Departmental Directorates for Territories (and the sea) (DDT(M)), fishing federations, etc.

Example of the involvement of fishermen

On the stream of Loc'h pond in the Côtes d'Armor, the Federation for Fisheries and Aquatic Environment Protection, together with related authorised associations, are working to save the pearl mussel. After the initial surprise of finding that such a species existed in the river and the discovery of its distinctive features, some of the older members of the local population recalled that a large pearl mussel population was once present in this sector before the Kerné-Uhel Lake became a reservoir, and that some inhabitants consumed the mussels. This local re-discovery sparked a keen interest among the fishermen, or "protectors of aquatic environments", as they might better be called. They therefore naturally agreed to work with Bretagne Vivante – SEPNB on the LIFE "mussel" programme, participating in activities such as catching fish to monitor trout populations, normalising overall biological indices, and mapping and restoration of ecological continuity. On their initiative, the sector was even classed as a fishing reserve.

Example of the involvement of elected officials

Initially created about 50 years ago to encourage tourism, the Blavet Valley Syndicate turned its attention to the protection of aquatic environments and the issue of salmon migration in the 2000s. Although the left bank of the Blavet catchment is home to very intensive agriculture, altered watercourses and very high nitrate levels, the right bank is better preserved. Here, we find the Bonne Chère River, for example, home not only to the largest pearl mussel population in the whole of the Armorican Massif, but also to other small populations that were discovered thanks to surveys carried out by Bretagne Vivante – SEPNB. The Blavet Valley Syndicate wanted to improve its knowledge about the distribution of the species by funding inventory studies, which have been carried out annually by Bretagne Vivante – SEPNB since 2011. The presence of the species in the catchment demonstrates the value of all the work carried out by the Syndicate in the context of their Aquatic Environment Territorial Contract (CTMA): ecological continuity, bank restoration, installation of cattle-watering points, etc. The Syndicate's elected officials proved to be very receptive to this "mussel" introduction, particularly the explanations concerning the value of work aimed at attaining a status of excellence for these watercourses. So far, the work of the Syndicate has focused on aquatic environments, but now cooperation with farmers towards improving water quality will commence, with a particular focus on nutrients and pesticides.

Moves towards a more environmentally beneficial agriculture

Globally, the rural community is starting to realize that good practices can be a source of biodiversity. For example, at the level of the Finistère Chamber of Agriculture, actions to limit erosion and animal watering at streams are underway and contribute to limiting the transfer of fine particles into Breton waters. The problems linked to the excessive enrichment of streams, notably through nitrates, are beginning to be treated collectively. In this context, if agriculture encourages biodiversity, biodiversity offers just as much to agriculture. These actions must, therefore, be continued so that the farmers' efforts will eventually improve and sustain our quality of life. In Germany, solutions have existed for over 25 years to combat erosion or improve nutrient inputs into the watercourses. However, no solution has yet been found at the political level to initiate the necessary reforms. In France, we hope to do better in this area.

How will this be achieved in practice?

In general, the partners we have met so far wish to continue action for the pearl mussel through continued inventories, raising awareness, and cooperation. Currently, the most effective tool we have to continue the actions initiated by the "Mussel" LIFE programme is the National Action Plan 2012-2017 (Prié *et al.*, 2012). Its development in Brittany and then in Normandy would allow us to maintain a number of actions and to expand our vision of the mussel beyond the six sites on which Bretagne Vivante – SEPNB and its partners have been working since 2010. With a balance between practical restoration actions and knowledge improvement, we might find a future perspective that is manageable in terms of resource mobilization.

The benefits of shared toolboxes

In order to homogenise the monitoring done in France and in other countries, there have been plans afoot for some time now to establish norms for the measurements taken to characterize rivers and freshwater pearl mussel populations. Almost all of the countries with freshwater pearl mussel populations are involved, with contributions from scientists, private consultants, regulatory authorities and associations (such as Bretagne Vivante – SEPNB). Standardised protocols will soon be available, and unique criteria for the monitoring and survival of pearl mussel populations are being defined. Member countries will have to vote for the implementation of these measures, and fears of possible legal consequences

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could hold some of them back. The use of micro-power stations, dam outlets or other activities could, for example, be incompatible with the pearl mussel. Discussions must be continued at the national level to implement effective and operational toolboxes.

Yes to a regional action plan, but how to fund it?

The issues of biodiversity must be taken into account by Ministries at the national and regional levels. We are all aware of the present economic "crisis", but there is a striking and frustrating gap for stakeholders in the field between the funds granted for the development of slightly more intensive agriculture, for example, and those for the preservation of pearl mussel populations. Although the Economic Council for Sustainable Development recommends the use of compensatory measures to save heritage species, ensuring the consistency of the various policies and tools remains a priority. Bridges need to be built between the water authority CTMA tools, the regional action plan underway in Brittany, and that of Lower Normandy in order to make them consistent. In France, the number of pearl mussels has decreased by more than 99% since the beginning of the 20th century. We need to act now and allocate substantial resources. In Germany, for example, the project that was conducted on the Lutter River represents 16€ million, while in France, the most outstanding programmes have a budget of 2€ or 3€ million. If we want to save this species, the solution should also include the mutualisation of resources by the various catchment-scale stakeholders.

Protection of mussel sites

Within the framework of the LIFE "Mussel" programme, the DREAL in Brittany is committed to implementing measures for the regulatory protection of mussel sites. This commitment is reflected today by an extension of the Natura 2000 sites upstream of mussel sites and by the creation of biotope protection orders (APB). The provisions of these APB are currently under consideration. In addition, given recent discoveries of the presence of the species on sites other than those of the LIFE programme, the question of the introduction of these APB is currently being addressed in a broader manner.

Can rearing stations support populations in the long term?

On the Armorican Massif, as elsewhere, the establishment of pearl mussel rearing stations made it possible to revive aging populations. Unfortunately, not all populations will be able to benefit from this tool and priorities will have to be set. These priorities are based, amongst other things, on the capacity of local actors to restore the quality of watercourses and host-fish populations. Rearing stations will not be sufficient for the long-term health of pearl mussel populations, and the stakes are all the greater for the mussel populations that cannot benefit from this temporary support. It is therefore urgent to put questions of water quality and quality of life back into the public and political debate in coming years. ■

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H. Ronné

Field trips

Visit to the Brasparts rearing station on November 26th, 2014

[1] One hundred conference participants visited the rearing station at Brasparts, situated in the centre of Finistère [2]. This provided an opportunity for everyone to see the young mussels from six different rivers in Brittany and Lower Normandy that are being grown as part of the LIFE program "Conservation of the freshwater pearl mussel from the Armorican Massif" [3]. The visit continued with a trip to the nearby Elez River catchment, home to one of the pearl mussel populations [4].

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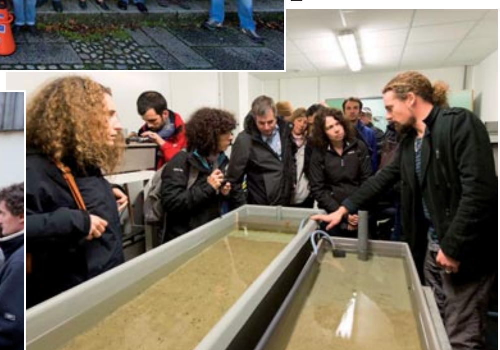
[1] The participants in front of the Faculty of Sciences of the University of Western Brittany before setting off for Brasparts.

[2] At the rearing station entrance, Pierrick Dury, technician at the Fédération du Finistère pour la pêche et la protection du milieu aquatique [Finistère federation for fishing and the protection of the aquatic environment], presenting a panel explaining the life cycle of the freshwater pearl mussel.



[3] The young mussels, although very small (the youngest are less than 1 mm and the oldest about 10 mm in length), posed in their troughs, ready to be photographed.

[4] It was a great day to admire the River Elez. But in late November, night falls quickly in the Monts d'Arrée and some of the party had to finish their tour with flashlights.

Visit to the Bonne Chère pearl mussel stream on November 28th, 2014

[5] After observing the mussels from the River Elez, a group of thirty participants strolled along the banks of another Brittany pearl mussel river, the Bonne Chère. This stream is home to the main known mussel population of the Armorican Massif, and includes some young individuals. [6] [7] [8].

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[5] An explanation of the localisation of pearl mussel rivers was given, intended particularly for the many international participants.

[6][7] Afterwards, the group looked at the many kinds of aquascope used to observe the mussels in their natural habitat.

[8] The last day of the conference took place in perfect weather conditions.

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Rouvre river

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Front cover photographies - Pearl mussel in Bonne-Chère (H. Ronné) and Elez (H. Ronné)

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LIFE + Program
« Conservation of the Freshwater Pearl Mussel from the Armorican Massif »