Aquatic Conservation with Focus on *Margaritifera margaritifera*

Proceedings of the International Conference in Sundsvall, Sweden, 12-14 August, 2009

Lennart Henrikson, Björn Arvidsson and Martin Österling (eds.)
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Contents

Preface .................................................................................................................................................. 1

The EU LIFE project “The freshwater pearl mussel and its habitats in Sweden” ............................................ 1
Lennart Henrikson and Sofi Alexanderson

How much do we know about the population ecology of *Margaritifera margaritifera* in relation to its conservation? ................................................................................................................. 1
Mark Young

The freshwater pearl mussel – *Margaritifera margaritifera* (Linnaeus) – in Sweden during five centuries (1539-2009) – an outlook from the work on a Swedish bibliography on the large freshwater mussels ................................................................. 1
Ted von Proschwitz

The Freshwater Pearl Mussel *Margaritifera margaritifera* (L.) in Sweden - status, changes and threats ..................................................................................................................................................................................... 1
Håkan Söderberg, Lennart Henrikson, Andreas Karlberg, and Oskar Norrgrann

The freshwater pearl mussel in Austria – current status and prospects for the future ......................................................................................................................................................................................................................... 1
Daniela Csar, Christian Scheder, and Clemens Gumpinger

Conservation and restoration of a freshwater pearl mussel (*Margaritifera margaritifera*) population in Northern England .................................................................................................................................................. 1
Ian J. Killeen

Demographic structure, sampling and the inferred genetic structure of Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations .......... 1
Eef Cauwelier, Phil Boon, Lee C. Hastie, Ian Sime, Elizabeth C. Tarr C. Thompson, Eric Verspoor, and Mark Young
Conservation genetics of freshwater pearl mussel (*Margaritifera margaritifera*) populations in south Sweden; some preliminary results .......................... 1
Björn L. Arvidsson, Amra Hadzihalilovic-Numanovic, and E. Martin Österling

A summary of relationship of biotic and abiotic factors to recruitment patterns in *Margaritifera margaritifera* from two areas in Sweden .......................... 1
Martin Österling and Björn Arvidsson

A Catchment Management approach to the conservation and restoration of *Margaritifera margaritifera* SAC populations in the Republic of Ireland .......... 1
Evelyn A. Moorkens

Improving forest management plans to support implementation of the EU Water Framework Directive: the freshwater pearl mussel (*Margaritifera margaritifera*, L.) as a tool ........................................................................................................... 1
Johan Törnbloom, Per Angelstam, and Johannes Pålsson

The effects of liming on the freshwater pearl mussel (*Margaritifera margaritifera*) in a Norwegian river ................................................................. 1
Bjørn Mejdell Larsen

Do signal crayfish *Pacifastacus leniusculus* harm freshwater pearl mussels?
Some field observations .................................................................................. 1
Christine Schmidt and Robert Vandré
Proceedings of the International Conference on “Aquatic Conservation with Focus on the Freshwater Pearl Mussel *Margaritifera margaritifera*”, Sundsvall, Sweden, 12-14 August, 2009

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Preface

The freshwater pearl mussel *Margaritifera margaritifera* (L.) is one of the very few invertebrates that have attracted the positive interest of human all over its distribution range. The reason is that it is a fascinating species from biological, cultural, as well as environmental perspectives. It has a complicated reproduction system depending on host fish. It has great demands on its habitat (water quality, water flow, riparian zone, bottom substrate) and moreover it needs a viable host fish population. It is an environmental indicator and is used as an umbrella species. It is also a flagship species for aquatic conservation. Most interesting for people is, of course, the pearl fishing, which has been described from Sweden already in the 16th century and by the famous Carl Linnaeus in the 18th century.

The freshwater pearl mussel shows a dramatic decline – extinct populations, decrease of population numbers, and insufficient recruitment. Therefore it is red listed all over its European range. All over Europe there are conservation works on freshwater pearl mussel – legal protection, habitat restoration, infection of the host fish with glochidia, captive breeding. Actions to preserve the freshwater pearl mussel will also favour other aquatic species – freshwater pearl mussel conservation is aquatic biodiversity conservation!
WWF (World Wide Fund for Nature) Sweden implemented the project “The Freshwater Pearl Mussel and its habitats in Sweden” during 2004-2009 (LIFE04NAT/SE/000231). The overall objective was to improve the habitats for juvenile freshwater pearl mussels and the host fish brown trout Salmo trutta in 21 streams. The actions were improvements of the biotopes, re-introduction of mussels, information to the stakeholders, and development of planning methods. The practical experiences of the Swedish project, but also from other projects in Europe were summarized in a handbook “Restoration of Freshwater Pearl Mussel Streams”. An international conference “Aquatic Conservation with Focus on the Freshwater Pearl Mussel Margaritifera margaritifera” was held in Sundsvall 12–14 August, 2009. In these proceedings presentations from the conference are published. More information and project publications are found at www.wwf.se/fpm.

The printing of these proceedings was financed by WWF (World Wide Fund for Nature) Sweden.

Dr. Lennart Henrikson Dr. Björn Arvidsson Dr. Martin Österling
The EU LIFE project
“The freshwater pearl mussel and its habitats in Sweden”

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Abstract

WWF Sweden implemented the project “The Freshwater Pearl Mussel and its habitats in Sweden” during 2004-2009. The overall objective was to improve the habitats for juvenile Freshwater Pearl Mussel Margaritifera margaritifera (L.) and its host fish brown trout Salmo trutta in 21 streams. The actions were improvements of the biotopes, re-introduction of mussels, information to the stakeholders, and development of planning methods. The results of the actions were not possible to document during the project period due to the difficulty to detect the juveniles. However, monitoring programmes will give data in the coming years. One outcome of the project is a manual for restoration of Freshwater Pearl Mussel streams, based on experiences from the LIFE-project but also projects in other countries. The project was financed by the EU LIFE fund, Swedish Environmental Protection Agency, WWF Sweden and partners involved.

Introduction

Scandinavia is a core area for the endangered Freshwater Pearl Mussel (FPM) Margaritifera margaritifera (L.). In Sweden there are approx. 550 FPM streams corresponding to 1/3 of all FPM streams in the world (Söderberg et al. this volume). However, the species has gone extinct in 1/3 of the streams and there is no sufficient recruitment in 3/4 of the remaining streams. Therefore there is a great need of conservation and management work to save the FPM.
The Swedish FPM conservation is based on: (1) legal protection, i.e. setting aside whole or large parts of the catchment as nature reserve or biotope reserve, (2) general consideration rules according to the Swedish Environmental Code, The Swedish Forestry Act etc., (3) general rules for voluntary consideration for forestry and agriculture (in forestry often connected to environmental certification), and (4) habitat restoration.

Many Swedish FPM streams are Natura 2000 sites, the European Union (EU) network of protected areas – a legal instrument. Most these Natura 2000 sites do just include the stream, not the riparian zone. Even if there are rules saying that no Natura 2000 site may be damaged, it is not realistic to use Natura 2000 instrument for a whole catchment. Nature reserve may be an option but may just be used for small catchment or parts of a catchment. Today there are just around 10 FPM streams that have a sustainable protection as nature reserves. Legal protection is not enough to save the FPM in Sweden. Moreover there is a great need of habitat restoration and improvements to create biotopes especially for the juvenile mussels but also for the host fish brown trout *Salmo trutta*.

In 2004 WWF Sweden initiated a project “The Freshwater Pearl Mussel and its habitats in Sweden” by grants from the EU LIFE Nature Fund, the Swedish Environmental Protection Agency and the partners involved\(^1\). The partners were four county administrative boards, one city, Swedish Forest Agency, and Karlstad University. The main objective was to develop and test different restoration measures and to increase the knowledge of the pearl mussel among stakeholders.

**Project actions**

The actions were:
(1) Improvements of the biotopes: establishing new "mussel beds" consisting of gravel and stone material to counteract siltation and to get a clean substrate where the small mussel can survive, eliminating migration obstacles for host fish and restoring river bottoms that have been cleared to facilitate floating of mussels.

\(^1\) EU LIFE Nature project “The Freshwater Pearl Mussel and its habitats in Sweden” (LIFE04NAT/SE/000231)
timber, plugging ditches to eliminate input of fine particulate matter and hence prevent siltation.

(2) Re-introduction in one stream with just a few FPM specimens left.

(3) Information to the actors/stakeholders: in public and personal meetings with the actors (landowners, forestry operators, etc.) information and advice are given to achieve an improved consideration to water in e.g. forestry. Information material like brochures was produced.

(4) Development of planning methods: development of action plan at two levels – drainage area and biotope.

**Results and experiences**

The overall experience was that the actions were successful, but it was not possible to document any improvements of the recruitment of juvenile FPM in the projects streams during the project period. The reason is that it is very difficult to detect these small mussels. However, for each stream there is a monitoring programme, which will give answer if the actions were successful.

One purpose was to decrease the siltation, i.e. the clogging of the bottom substrate by fine particles. One action was to measure the degree of siltation. This was done by Martin Österling, Karlstad University, and he and his co-workers found a relation among the amount of fine particles and the occurrence of young mussels (Österling et al. 2010). This studied will be repeated and included in the monitoring programme.
Figure 1. Above left: Studies of freshwater pearl mussel. Photo: Per-Erik Jacobsen. Above right: The vision – a young mussel. Middle right: New (clean) bottom substrate. Below left: Restoration of the stream bed by adding stones which have been removed to facilitate timber floating. Below right: Fish way around a dam. Other photos: Lennart Henrikson.
The project is described in a “layman’ report” (Anonymus 2009). The practical experiences of the Swedish project, but also from other projects in Europe were summarized in a handbook “Restoration of Freshwater Pearl Mussel Streams” (Degerman et al. 2009). A travel report, describing other European FPM projects, was published (Henrikson 2009). Törnblom et al. (this volume) discuss how forestry management plans may be adapted to the FPM. An international conference “Aquatic Conservation with Focus on the Freshwater Pearl Mussel *Margaritifera margaritifera*” was held in Sundsvall 12–14 August, 2009 (this volume).

More information and project publications are found at www.wwf.se/fpm.

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The project was financed by EU LIFE fund, SEPA (Swedish Environmental Protection Agency), the Swedish Forest Agency, the County Administrative Boards of Kalmar, Västra Götaland, Örebro and Västmanland, the city of Göteborg and WWF Sweden.
References


How much do we know about the population ecology of *Margaritifera margaritifera* in relation to its conservation?

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Abstract

This paper reviews briefly what is known about the ecology and habitat preferences of the freshwater pearl mussel (*Margaritifera margaritifera*) in relation to its conservation, and then makes suggestions for the most pressing research needs and conservation activities. These suggestions, which focus on the needs of the juvenile mussels, are meant to be the opening of a debate, rather than a definitive statement.

Introduction

*Margaritifera margaritifera* is globally threatened (Young *et al.* 2001) but with sufficient viable populations surviving in Scandinavia, north-west Russia, Scotland and east Canada to provide a realistic hope that its status can be stabilised and then eventually improved. The challenge is to choose conservation activities that are truly effective, based on our knowledge of the ecology and habitat preferences of the mussel. Despite research now spanning several decades (e.g. Hendelberg 1960), there are still things to be discovered about the mussel’s requirements but nevertheless realistic conservation programmes are now showing signs of success (e.g. the River Lutter, Germany *vide* Altmüller and Dettmer 2006).

Can we answer the following two questions, at different scales? Firstly, we observe mussel populations ranging from north-west Russia to Spain and from Austria to Canada and these may be inhabiting large rocky rivers, or small sandy streams – given this huge range of habitats, can we identify the essential
common features that allow *M. margaritifera* to thrive? Secondly, at a small scale, we can observe the detailed distribution of mussels in a stream and find that groups of mussels occur here but not there – can we explain fully and predictably why mussels show this occurrence pattern in relation to stream variables? At present we struggle to answer either question successfully and so cannot yet claim to understand fully how to manage our rivers and streams to ensure mussel population viability.

As a contribution to the debate about these questions, this paper asks **what we know** and **what we do not know** about the ecology of *M. margaritifera* and then offers a series of personal ‘guesses’ about which topics are important and need urgent attention for wild populations and which are less important and so can be tackled at more leisure. It seems very likely that other mussel ecologists will disagree!

**Factors affecting adult mussels**

The organisation of the paper follows the life cycle of the mussel, beginning with the adults. References have been deliberately kept to a minimum.

**Adult food**

Adult mussels are general filter feeders, ingesting a variety of suspended planktonic and detrital particles (Fuller, 1974), immediately rejecting those of incorrect size and consistency as pseudofaeces and digesting those appropriate. Given that mussels survive and grow in rivers in Scotland of ultra-low nutrient status, it seems unlikely that adult food is ever limiting. However, there is speculation that mussels close to lake outlets may benefit from the outflow of lake plankton and so grow faster than expected and it is apparent that mussels do grow at different rates in different rivers, (although this may well reflect differences in many other factors, such as temperature or calcium concentration). In general it is likely that too much food, indicating high nutrient status or eutrophication, will be strongly disadvantageous but it can be predicted that in most cases there is no need to consider whether adult food is sufficient.
Predation on adults and other factors leading to their decline

It is usual to believe that predation on adult mussels is very low, except where pearl fishing has occurred. There is limited, anecdotal evidence that muskrats, beavers and gulls may occasionally prey upon adult mussels, but not so as to cause any significant effect. Adult decline does follow serious degradation of river quality. For example, in many English rivers and, as an example, in the River Ythan in Scotland, adults have declined to near extinction, following siltation and raised nutrient status associated with intensive agriculture.

If a population profile is made, this will immediately show where the population is deficient, including if there is an unexpected loss of adults. It is essential in all conservation schemes to produce a diagnostic population profile but this is not a subject requiring further research. Furthermore factors affecting adults will, at a much lower level, have already affected juveniles, so conservation should mostly focus on these. The exception is where monitoring indicates that adults are declining sharply in a river where immediate improvement is not realistic. In such a case it may be considered necessary to remove mussels into captivity, where they can be kept alive until conditions do improve.

Sperm transfer and fertilisation

This subject is almost completely unknown. It is presumed that males shed sperm in early/mid summer, which are then inhaled by females and that this leads to fertilisation, but this is supposition. However, almost all populations continue to show around 40% of gravid adults and Bauer (1983) claims that mussels can become hermaphrodite in very sparse populations. There is a deleterious genetic consequence if very few adult males are contributing to the output of young mussels in a population (Cauwelier et al. 2009) but otherwise it seems that there is no need for active research on the mode of fertilisation in *M. margaritifera*. 
Factors affecting glochidia

Glochidial release

Glochidial release can be amazingly highly synchronised and the reasons for this are not clear. Attempts to link this event to stream factors, such as changing temperature, oxygen concentration or flow rate, have so far failed (Hastie 1999). However, interesting though it may be to understand this striking phenomenon, it seems unimportant to study this further, in the context of conservation action.

The required numbers of host fish for glochidia

We know a great deal about the relations between glochidia and their host fish. In Europe host species include *Salmo salar* (the Atlantic Salmon) and *S. trutta* (the Brown Trout), in the latter including both the resident, sedentary and the migratory form. It is clear from many observations that each of these three hosts can support a viable population of mussels. It is also known that there is a loss of glochidia from the host fish, caused by an immune response (Bauer and Vogel 1987) and that there is some adaptive matching between local races of fish and mussels (Geist *et al.* 2006).

These fish populations are under threat throughout their range and so it is vital to know whether there is a relationship between juvenile fish densities and the output of juvenile mussels and, even more importantly, if there is a lower threshold of fish density, below which young mussel output is insufficient to sustain the population.

Various estimates have been suggested for the necessary number of juvenile fish. Bauer (1988) suggest >20 juvenile fish/100m$^2$; Zuiganov *et al.* (1994) quote >10 juvenile fish/100m$^2$ and Österling (this volume) reports a wide range of densities, at least including 5-20 juvenile fish/100m$^2$ (as well as stressing the importance of local adaptive matching). Surprisingly, apart from obvious local cases, where fish populations have become extinct or very sparse indeed, such as following the effects of acid rain events (Larsen, this conference), most workers appear to believe that 'normal' fish densities are adequate. Nevertheless, it is clear that it is vital to determine if there is a lower fish density threshold and to model if there is a clear relationship between the
density of juvenile fish hosts and the output of juvenile mussels. Österling’s recent work in Sweden, where fish sampling exactly matches the location of mussels, and samples are collected at times when glochidia will be on the fish, will be an important contribution to this topic. It is also clear that any culturing of young mussels should, as a precaution, match mussels with local fish stocks.

Factors affecting juvenile mussels

Initial juvenile mussel survival

Every study has concluded that the most important limiting factor for the maintenance of a viable mussel population is that the juvenile mussels are ultra-sensitive to habitat condition. They live buried in the hyporheic substrate, initially feeding on a bacterial film on the sand grains, and are crucially dependant on a free flow of water into this hyporheic zone. The precise reasons for this sensitivity are subject to continued research and, although there is agreement that anything that acts to reduce the free flow of water amongst the sediment is detrimental, there is currently little hard evidence of cause and effect and much reliance on observation and correlation.

Food for juvenile mussels

Buddensiek (1995) and others have shown that juvenile mussels use their feet to swipe bacteria off substrate particles and Hruska (pers. comm.) believes that grass roots in his culture lades somehow act to enhance the supply of this nutrient source. However, there are no published studies which identify exactly what sort of bacterial film is preferred and for how long young mussels feed in this way, before they change to filter feeding and need to have access to the free water column. Therefore there is much scope for original work in this area. However, it is likely that there is always sufficient bacterial film available and that food for juveniles is not limiting, so that work on this topic is not necessary for the success of conservation actions. However, too many nutrients may well be the cause of excessive algal growth and siltation, which is highly detrimental. Therefore too much, rather than too little, nutrient may be the usual problem. In culture, however, it is essential to consider this topic carefully and to develop effective feeding conditions.
The effects of predation and disease on juvenile mussels

We are used to assuming that predation is unimportant for adult mussels and mussel disease is more-or-less completely unstudied but thought to be unimportant. However, predation of juveniles is much more likely and Schmidt and Vandré (this volume) have shown that crayfish may prey on mussels and it is likely that fish such as eels (*Anguilla anguilla*) may also feed on them. Juvenile mussels die very readily in captivity, often from unknown causes, and it may be that fungal or other infections are involved. Compared to the attested problems of substrate siltation, these may be unimportant topics, but this is essentially unknown.

The impact of siltation on juvenile mussels

It is universally agreed that juvenile mussels need a substrate of clean, coarse sand in which to burrow and that in many circumstances this substrate is partially stabilised by the presence of large stones or boulders. It is also generally agreed that there needs to be a free exchange of water between the water column and the interstitial spaces, presumably primarily so that the water available to the mussels is fully oxygenated. There have been only limited measurements of interstitial chemistry, including oxygen saturation, partly because of the technical difficulties of taking spot measurements, without contamination from water column water, but some researchers, including Geist and Auerswald (2007) have measured the differences in the redox potential between the free water and the interstitial water, as a surrogate measurement that indicates the extent of the water interchange. They and others have observed free interchange (ie little difference in redox potential) in substrates where juveniles are found, compared with limited interchange (larger redox potential differences) in substrates where juveniles are absent. The factor which prevents this necessary free exchange is primarily silt. This is crucially important, although there are few studies which have attempted to quantify the levels of oxygen that are needed for juvenile survival and whether these change as the mussels develop. More research is urgently needed on this subject but we are already at the point where it is certain that controlling siltation is the primary conservation requirement.
Excess siltation can be caused by many activities, such as soil erosion during agricultural and/or forestry activities, run-off from contaminated hard surfaces, excess algal growth during unusually warm weather or caused by extra nutrients, insufficient turbulent flow and so on. Excellent examples, such as that of the River Lutter in Germany, or of rivers improved during the Swedish LIFE project, as reported in this volume, indicate that reduction in silt loading can rapidly lead to increased survival of young mussels.

*The relation between substrate conditions and flow patterns in a river*

Speculation, based on some important observational evidence, suggests that naturally variable river flows can act to produce optimal substrate conditions. High flows may re-suspend substrates, carrying away the finer silts and re-depositing cleaned sand beds. If this occurs at a time when young mussels are large enough to withstand the process, then the improved cleanliness of the substrate is very beneficial. However, if these high flows occur soon after the young mussels have dropped to the substrate, then they may be washed away or crushed (Hastie *et al.* 2001). It is likely that a year in which significant numbers of young mussels survive is a year in which the timing and magnitude of the high flows act to cleanse the substrate but not to damage any young mussels present. The acknowledged erratic year-to-year differences in recruitment may be partly due to variation in yearly river flows and the presence of lakes upstream of many viable mussel populations may be because they buffer heavy rainfall and damp down the height of floods.

Climate change is already altering the frequency, timing and extent of floods in many streams and it seems vital to investigate and model the likely changes in stream flow regime that climate change will bring. It is possible, or perhaps likely, that changing flow regimes may make some streams less suitable for mussels, whilst perhaps making others more suitable.

*Priorities for research*

Based on the opinions expressed above, the author recommends the following priorities for research, which will underpin conservation action:

1. Further elucidation of genetic relationships between and within mussel populations and between them and their fish hosts.
2. Discovering whether there is a threshold of fish host numbers, below which mussel recruitment is insufficient, and whether above this there is a positive relationship between fish density and mussel recruitment.
3. Clarification of the mechanism by which increased silt harms young mussels.
4. An extension of the dataset relating to optimal values for various physico-chemical parameters in the hyporheic zone.
5. Modelling the impact of climate change on stream flow regimes and thereafter predicting the effects of any likely changes on mussel recruitment.

The following seem likely to be less important topics:
1. Identification of the necessary quantity and composition of food for adults (but note that this is necessary for success in mussel culture).
2. Clarification of the relationship between the release of glochidia and their success in attaching to their fish hosts.
3. Studies on the immune response of host fish to glochidia.
4. The identification of the exact nature of juvenile food (but note that this is necessary for success in mussel culture).
5. Studies on predation and disease in juvenile mussels.

Priorities for conservation action

The suggested priorities for conservation action are as follows:
1. Prioritising populations requiring conservation action, using population profiles, threat analysis and genetic studies.
2. Active catchment management and stream restoration, often including silt reduction.
3. Sustaining fish stocks.
4. If necessary, because of the inability to make sufficient and timely stream improvements, culture adults and rear juveniles for eventual release.

In the author’s opinion, less important action points are as follows:
1. Completion of surveys.
2. Re-introductions, because so few opportunities exist where streams have been restored adequately.
3. General pollution reduction (rather than targeted on streams being restored).
4. Learning from a comparison with the situation in Canada.
5. Ensuring that legal protection is enforced.
7. Making an alliance with those working on the conservation of salmonid fish.

Acknowledgements

I have benefitted from discussion with too many people to mention individually over the years and am most grateful to all of them, but the opinions expressed are wholly mine. A recent ‘thank you’ is owing to Lee Hastie, Liz Tarr, Jürgen Geist, Eef Cauwelier and Iain Sime.

References


The freshwater pearl mussel – *Margaritifera margaritifera* (LINNAEUS) – in Sweden during five centuries (1539-2009) – an outlook from the work on a Swedish bibliography on the large freshwater mussels

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Introduction

The work on an annotated bibliography of the large freshwater mussels (Margaritiferidae, Unionidae, Dreissenidae – totally 9 species) in Sweden started in the beginning of the 1990’s. The collecting of publications was initially a ‘by-product’ of a national mapping project of the species, a work which, beside all records from modern reports and material in museum collections, intends to trace and map also all literature records. By the time, the listing of papers did grow into the running bibliography project. A previous presentation of aims and results was given by von Proschwitz (2006). The final goal is to include all types of publications – books, scientific theses and papers, popular papers, conservation reports of all types (including ‘grey’ literature), pamphlets, newspaper articles – and to class them according to:

**Species:**

- A = Anodonta
  - Aa = Anodonta anatina
  - Ac = Anodonta cygnea
  - Dp = Dreissena polymorpha
  - Mm = Margaritifera margaritifera
  - Pc = Pseudanodonta complanata
  - Sw = Sinanodonta woodiana
  - U = Unio
  - Uc = Unio crassus
  - Up = Unio pictorum
  - Ut = Unio tumidus
- LMG = Large freshwater mussels general

21
Subjects:
Ar = Archaeology
Bi = Bibliography
Ch = Cultural history
Co = Conservation biology
Ec = Ecology
Et = Ethology
Fa = Faunistics
Fi = Fiction
Ge = Genetics
Hi = Histology
La = Law
Me = Methodology
Mo = Morphology
No = Nomenclature
Pe = Pearls
Ph = Physiology
Pf = Pearl fishery
Pg = Phylogeny
Re = Review
Sy = Systematics
Sc = Shell chemistry
Sf = Subfossil
Ta = Taxonomy
Zg = Zoogeography

Geographical area:
SG = Sweden, general
SSG = Southern Sweden, general
NSG = Northern Sweden, general
- = No geographical area indicated
[Administrative unit, län]
AB = Stockholms län
C = Uppsala län
D = Södermanlands län
E = Östergötlands län
F = Jönköpings län
G = Kronobergs län
H = Kalmar län
I = Gotlands län
K = Blekinge län
M = Skåne län
N = Hallands län
O = Västra Götalands län
S = Värmlands län
Example page from the bibliography 10


HJELLSTRÖM, M. & SAETRE, P. (1992): Utbredning och styrparametrar för vandrarmusslan *Dreissena polymorpha* i sjön Erken. – (D-arbete om 10...

Species: Dp [Subjects: Ec, Fa] [Geographical area: AB] [Type of publication: AT] [References: TvP].


Species: Mm [Subjects: Ec, Fa] [Geographical area: BD] [Type of publication: MR] [References: TvP].


Species: Mm [Subjects: Co, Ec] [Geographical area: SG] [Type of publication: PA] [References: TvP].


Species: Mm [Subjects: Co, Fa, Pf] [Geographical area: X] [Type of publication: CR] [References: TvP].


Species: A [Subjects: Sf] [Geographical area: AC] [Type of publication: SJ] [References: TvP].


Species: Mm [Subjects: Co, Ec, Fa] [Geographical area: O] [Type of publication: BO] [References: TvP].

1) The order of the references is strictly chronological and alphabetical. The English alphabet is followed, which means that the letters Ä and Å appear under A, Ö and Ø under O and Ü under U.

2) The remarks under ‘References’ mean: TvP = The title has been finally checked in original; ZR = The publication is listed in The Zoological Record.
The bibliography is meant to be a useful tool for all working on different aspects of the Swedish large freshwater mussels. Up to now (May 2009) it comprises 1545 titles. New titles are continuously traced from library catalogues, reference lists and the web – and added to the bibliography. All publications are checked in original before entered finally. At present, approx. 200 possible further titles are being traced. If necessary, special comments are given on different editions, year of publication, errors in titles, irregularities etc.

Not only do the publications make up a huge amount of knowledge on the species, they also constitute the historical development of the knowledge over the centuries. From this vast material I have chosen to present here some of the most important publications – to represent trends in the interest in the freshwater pearl mussel and the growth of knowledge.

The early works by Olaus Magnus

Olaus Magnus (1490-1557), was the last catholic archbishop of Sweden. He spent many years in exile at the papal court in Rome, and during this time he produced two works, which are of immense importance for the cultural-historical understanding of Northern Europe. The first is the ‘Carta Marina’ (1539), which is the first fairly accurate map of Northern Europe; and the second the massive ‘Historia de gentibvs septentrionalibvs’ [‘History of the Nordic Peoples’] (1555), a work of a classic scholar, which in its almost 900 sites (23 ‘books’, in four volumes), often in anecdotic form, gives information on a very varied range of topics, among them pearl fishery. In the Carta Marina drawings of mussels with pearls are inserted in Rivers Indalsälven and Ångermanälven in Central Sweden (Fig. 1). Chapter 21 of Book 22 of the History of the Nordic Peoples deals with pearls, pearl formation, pearl fishery and the use of pearls. It is, of course, to be read in its historical context and contains superstition and misinterpretations, often literally taken over from classic works on natural history. There is an interesting vignette picture to the chapter, which illustrates the text (Fig. 2). The similarity to the illustration in the Carta is striking and it probably shows the same rivers.
Figure 1. Part of Central Sweden from Carta Marina (1539) by Olaus Magnus, showing freshwater pearl mussels, containing pearls, in the Rivers Indalsälven and Ångermanälven.

Figure 2. Vignette picture of chapter 21, book 22 in History of the Nordic peoples by Olaus Magnus (1555), showing pearl fishery and use of pearls. The rivers are probably the same as in Carta Marina (cf. Fig. 1).
The monopolisation of the pearl fishery by the Swedish crown (The 'Regale')

During the years 1691-1723 and 1731-1861 the pearl fishery was monopolised by the Swedish Crown (a so called 'Regale'). The first regale was proclaimed during King Karl XI (Kong. Maj:t 1691) (Fig. 3) and gave the crown full control of the trade with pearls as all found pearls had to be sold to the state. Special pearl inspectors were to handle the trade in the different provinces. The crown found the exclusive privileges very important and had large expectations of financial gain from the monopoly. The first proclamation was followed by an explanation (Kong. Maj:t 1692) and an extension to Carelia and the Baltic states, which at that time were Swedish provinces (Kongl. Maj:t 1694). The first regale has been treated in detail by Awebro (1995), and his figures show clearly that both the number of delivered pearls and the paid compensation for the pearls, after initial high levels, decreased rapidly and at the end of the first regale dropped to a very low level and hence did not fulfil the expectations. It has been calculated that as many as 2.000.000 mussels / year were slaughtered in the end of the 17th century, when the fishery was intense (Awebro 1995), which lead to the collapse of the populations in several streams.

Only a few years after the abolition of the monopoly (Kongl. Maj:t. 1723), it was renewed in a somewhat altered form (Kongl. Maj:t. 1731). This 'second regale' comprised only pearls found on land belonging to the crown. Although it never became economically important, it was not abolished completely until 1861 Kongl. Maj:t. (1861). More information can be found in a rather overlooked paper by Sjöberg (1865).
PLACAT och Förordning/

Angående Wärse-Kistefjärne ut deß Norden.

Stockholm den 29 Maj År 1691.


STOCKHOLM/
Tryckt ut K. V. Sal. Nichol. Manhils Tryckeri,

28
Figure 3. Title page and first text page of the royal proclamation by King Karl XI of the crown monopoly (Regale) of the pearl fishery in 1691.

The age of Carl Linnaeus

One of the teachers of the famous Carl Linnaeus was Olof Rudbeck jun. (1660-1740), professor in Uppsala. In the account from his Lapland journey 1695, he gives extensive and important information on the freshwater pearl mussel, its biology and Swedish pearl fishery in the end of the 17th century. Although the manuscript of this work was destroyed in the great fire of Uppsala in 1702, a
copy survived (in the library of C. Linnaeus!), and a reconstruction was published 285 years later (Rudbeck 1987).

Carl Linnaeus (1707-1778) (knighted von Linné 1757), professor in botany and medicine in Uppsala, dominated the Swedish science during the 18th century. He dealt with freshwater pearl mussels, the formation of pearls and pearl fishery in several of his publications. His approach to the nature was the typical utilitarian of the 18th century scientists (Linnaeus 1740), but during his journeys he came in contact with pearl fishery in different parts of Sweden and expressed concern for the ‘uneconomic and thoughtless killing of thousands of mussels’. Especially in his Lapland journey 1732 (not published in full until 1889), during which he observed pearl fishing in River Pärälven, he gives extensive comments on pearl fishery – illustrated with own drawings (Fig. 4, 5). An important paper from the period, very much in the spirit of Linnaeus – based on observations in the nature – is the one by Gissler (1762) on pearl fishery in the North Swedish provinces of Medelpad, Ångermanland and Jämtland.

Figure 4. Drawing by C. Linnaeus of pearl fisher on a raft, collecting freshwater pearl mussels with a forceps (from Iter Laponicum [1732] 1889).
Linnaeus also tried to launch production of artificial pearls on a large scale in Sweden. As no freshwater pearl mussels were available in Stream Fyrisån in Uppsala, he experimented with duck mussels – *Anodonta anatina* (Linnaeus), into which he introduced objects, through drilled holes, to induce pearl formation. The first enthusiastic interest by the Swedish government was replaced by scepticism as they realised the long time aspect of pearl growth, and Linnaeus’ expectations were never to become realised. For details on Linnaeus and the project of artificial pearl production see Anonymus (1930), Drake (1930).

In this context should be mentioned a die-hard myth concerning the coat of arms of Linnaeus: It has been claimed that Linnaeus was knighted because he invented the artificial production of pearls and that the item in the middle of the arms is a pearl (!). This is of course pure nonsense. The method used by Linnaeus was the same, which for centuries had been known in China, and it had nothing to do with his knighting. Although several times demented in biographic literature on Linnaeus – it still pops up and was, embarrassingly enough, presented as a fact in one of the exhibitions in Uppsala during the Linnaeus jubilee in 2007! It can be added that the item in the coat of arms is not a pearl – it is an egg (the source of all life).

**Growing knowledge in the 19th century**

During the 19th century, papers on pearl fishery still dominated, but the growing knowledge of the biology of the freshwater mussels, and especially the reproduction, is reflected in the literature. As an example of a classical description of the freshwater pearl mussel in a larger zoological encyclopedia...
SVENSK ZOOLOGI,

FÖRFATTAD
AP
C. QUENSEL.
Med. och Med. Doktor, Prof. i Medicin och Farm., Lärare i Koninkl. Naturskolor vid K. Keijgwerck, på Carolberg. Lidare av K. Vet. och

MED ILLUMINERADE FIGURER,
TECKNAD
AP
J. W. PALMSTRUCH.

FÖRSTA BANDET.

Med Konungen Nådiga Privilég. avslut.

STOCKHOLM,
Tryckt hos CARL DELÉN, 1866.
can be cited: ‘Svensk Zoologi’ [Swedish Zoology] (1806-1825), edited by Palmstruch (Fig. 6). In the middle of the century, an interesting discussion of the nature of the glochidia, found in the gills of the mussels took place. Were they parasitic organisms or larvae of the mussels? An overview of the differing opinions among scientists is given by Lovén (1852).

**Pioneering publications from the 20th century**

In the 20th century, the growing thoughts on the need to preserve the freshwater pearl mussel and suggestions and recommendations of conservation measures can be seen in the literature. Early papers, dealing with the conservation aspect are those of Ekman (1905, 1908). Mentioned should also be Müller (1957). The old thinking – of the freshwater pearl mussel as a pearl
producing, economic resource – still lingered as late as 1950, when an exception from the species protection was granted to allow pearl fishery. This should finance measurements to improve the fishery in the same water course (Kellertz 1950).

Especially two papers, which both had important impact on later research and conservation work on *Margaritifera* should especially are worth attention: Hendelberg (1960), concerning age-determination and population structure, and Björk (1962) concerning ecological demands and habitats.

The break through of the modern conservation thoughts

The modern conservation thinking on the freshwater pearl mussel in Sweden had the final break through with a series of papers, dealing both with the ecology, real and possible threats and protection and improvement measurements both for the species and its habitats, in the beginning and middle of the 1980’s – especially should be mentioned: Eriksson *et al.* (1982, 1983) and Grundelius (1982, 1984, 1987). Important steps towards the present state of knowledge and conservation actions are the inter-disciplinary seminar in Jokkmokk 1992 (Åjte 1995), the compilation of status, trends and threats (Eriksson *et al.* 1998) and the latest conservation plan for the species (Schreiber *et al.* 2005).

Structural and chemical analysis of mussel shells

A special research field, analysing the structure of the yearly growth zones and the variation in their chemical composition was developed by H. Mutvei and E. Dunca at the Swedish Natural History Museum and T. Westermark at the KTH Royal Institute of Technology. These techniques have opened up a wide field of applied research, such as exact determination of age, growth patterns in different populations, water courses and geographical areas, use of the shells as chemical archives for studying of environmental changes, pollution etc. in past and present times – all applications, which are closely linked to the ongoing conservation work. As examples of the very large number of publications and reports on these linked subjects could be mentioned: Dunca (1999), Mutvei (1989), Mutvei & Dunca (1995) and Westemark *et al.* (1997).
Some conclusions from the present state of the bibliography

The growing interest in the species and its conservation has resulted in a still increasing stream of papers and reports during the latest decades – the dominance of the freshwater pearl mussel over the other species, as well as the increasing importance of conservation becomes evident if the classification of the titles is treated statistically:

- 76 % (1174) of the total number of papers (1545) deal entirely or partly with the freshwater pearl mussel.
- 47 % (727) of the total number of papers have conservation aspects.
- 73 % (1128) of the papers were published 1980-2009.
- 70 % (790) of these (1128) papers have conservation aspects.

In Fig. 7-9 the classification of the titles according to species, subjects and year of publication is showed in graphic form. Note that a publication may contain information on more than one species and one subject.

Figure 7. The bibliographed papers classed according to species.
Figure 8. The bibliographed papers classed according to subject.

Figure 9. The bibliographed papers classed according to year of publication. Note the different time intervals in the classes.
The bibliography is already today available for all working on large freshwater mussels (so far only in word format, but the preparation of an excel version is in progress), and it is the intention to – within a ten year period – publish it both in printed form and on the web.

Acknowledgements

I am very grateful to all colleagues, who during the years, have provided me with their own and others reprints and reports, as well as to the staff of many Swedish libraries, which also have done a great job in helping me with tracing and ordering old and rare publications. The staff of the Göteborg Natural History Museum has also offered me valuable assistance, especially should be mentioned T. Nordander, for help with the tracing of literature, J. Berg for help with the statistic treatment of the material and the construction of the excel version of the bibliography and E. Hagström for improving the language.

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MUTVEI, H. & DUNCA, E. (1995): Struktur och tillväxt av flodpärlmusselkall i relation till miljöförändringar. – (Flodpärlmusslan i tvärvetenskaplig


The Freshwater Pearl Mussel *Margaritifera margaritifera* (L.) in Sweden - status, changes and threats

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Abstract

The knowledge of the freshwater pearl mussel *Margaritifera margaritifera* (L.) in Sweden is rather good. It is widely spread all over Sweden with the most populations in the northern part. It was present in 551 streams in 2006 (around 600 pearl mussel streams are known in 2012). Sweden holds one third of all European freshwater pearl mussel populations. Half of the streams do not show any evidence of recruitment during the last 20 years, i.e. no mussels <50 mm are found. Just 13% of the streams have recent recruitment (mussels <20 mm, approximately 10 years old). Moreover at least 78 populations have gone extinct, mainly in southern Sweden. However, the conservation value assessments show that Sweden still has a number of healthy FPM populations – 98 populations have high or very high conservation values. During the last decade there are changes indicating lowered viability, increased length of smallest mussel found, and decrease of the streams with mussels <50 mm. However, in streams limed to counteract acidification there were no changes. Although Sweden has a number of viable populations the situation is similar to that in other countries: (1) extinct populations, (2) mostly non-recruiting populations, (3) negative trends. The main threats are considered to be forestry, migration obstacles, altered water flows, acidification, and land use causing sediment and nutrient load. The most serious problem is probably siltation of stream bottoms, due to forestry or agricultural activities in the catchment. This is in agreement with the opinion among European scientists. The needs of the
freshwater pearl mussels in Scandinavian streams are presented. Sweden and the other Scandinavian countries, Norway and Finland, is a core area for the freshwater pearl mussels with two-thirds of the populations. With this follows a great responsibility for conservation of the freshwater pearl mussel.

**Introduction**

The freshwater pearl mussel (FPM) *Margaritifera margaritifera* (L.) is one of the very few invertebrates that have attracted the positive interest of human all over its distribution range. The reason is that it is a fascinating species from biological, cultural, as well as environmental perspectives. Most interesting is, of course, the pearl fishing, which has been described from Sweden already in the 16th century and by the famous Carl Linnaeus in the 18th century (von Proschwitz this volume).

The FPM is red listed (EN, endangered) in Sweden. It is protected according to fishery legislation since 1994. The first Swedish national action plan for FPM was published in 1991 and updated in 2005 (Schreiber et al. 2005).

The first Swedish work on modern conservation aspects started in early 1980’s and Eriksson et al. (1982) found that several populations had gone extinct in southern Sweden, probably due to acidification of the water. During the following decades the mapping and other studies of the FPM included the whole country. In 1998 a standardized method for monitoring of FPM was developed (Söderberg 1998) and later updated several times (e.g. Bergengren et al. 2010). A lot of FPM populations have been investigated by this method. Henrikson et al. (1998) developed a method for assessment of the conservation value of FPM populations and Söderberg (2006) developed a method for simple classification of FPM population status.

Several studies since the 1980’s have increased the knowledge of the distribution, status and trends of the FPM. Eriksson et al. (1998) presented a synthesis of the studies until 1995. Degerman et al. (2009) presented a manual for restoration of FPM streams describing the biology, threats, conservation, and measures from a Scandinavian perspective with outlooks to other European countries. von Proschwitz (this volume) presented an outlook from
the work on a Swedish bibliography on the large freshwater mussels, where all Swedish publication between 1539 and 2009 may be found.

The time has elapsed and the objective of this paper is to describe the actual distribution, status, and recent changes of the FPM in Sweden until 2006. This paper is a summary and updated version of a report from the County Administrative Board of Västernorrland (Söderberg et al. 2008).

Methods

Data collection

A questionnaire was sent to all Swedish county administrative boards in Sweden where FPM is present to get data and information on former/actual occurrence, population characteristics, changes, and threats.

Definition of stream

We have chosen to use streams instead of populations when describing occurrence, distribution etc. Our definition of a stream is according to the Swedish Meteorological and Hydrological Institute (Fig.1).

Standardized method for monitoring

The Swedish standardized method for monitoring of FPM (latest update by Bergengren et al. 2010) is used to estimate population size and population density. A short description: The FPM distribution range within the stream is stratified into three parts. 15-18 stream sections (5-6 per stratum, length 3-20 m) are randomly chosen. At all sections the stream bottom is examined with a “bathyscope” (a plastic cone with glass at the bottom) and the number of mussels is counted (without digging the substrate). The length of the smallest mussel found is measured. Outside each of the counting sections the first 15 mussels observed (i.e. randomly chosen) are measured (length, height, width). This means that 225-270 mussels are measured. The same sections are used in follow-up-studies.
Figure 1. Illustration of how “streams” are defined by the Swedish Meteorological and Hydrological Institute. The map shows five freshwater pearl mussel populations in four streams (A-D).

Conservation value
The conservation value is assessed by six criteria and six score classes (Tab. 1). The scores are summarised and placed into three classes:
- Very high conservation value (18-36 scores)
- High conservation value (8-17 scores)
- Conservation value (0-16 scores)

This model can just be applied to populations investigated by the standardized method.

Table 1. Criteria and score classes for assessment of conservation value of freshwater pearl mussel populations (Henrikson et al. 1998).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Population size (thousand mussels)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Mean density (individuals m⁻³)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Distribution range (km)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Smallest mussel found (mm)</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Percentage mussels &lt;20 mm</td>
<td>1-2</td>
</tr>
<tr>
<td>Percentage mussels &lt;50 mm</td>
<td>1-5</td>
</tr>
</tbody>
</table>
Status class

The simple system for classification of population status is less time consuming. After a scanning of the FPM occurrence, the locality with best recruitment is chosen for length measurements of the first 100 mussels found. This simple classification may also be applied at populations investigated by the standardized method.

Table 2. Status classes and criteria for simple classification of status of freshwater pearl mussel populations (Söderberg 2006).

<table>
<thead>
<tr>
<th>Status class</th>
<th>Population status</th>
<th>Population structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Viable</td>
<td>&gt;20% &lt;5 cm and &gt;0 % &lt;2 cm (&gt;500 individuals)</td>
</tr>
<tr>
<td>2</td>
<td>Viable?</td>
<td>&gt;20% &lt;5 cm or &gt;10 % &lt;5 cm and &gt;0 % &lt;2 cm (&gt;500 individuals)</td>
</tr>
<tr>
<td>3</td>
<td>Non-viable</td>
<td>&lt;20% &lt;5 cm or &gt;20%&lt;5 cm and &lt;500 individuals</td>
</tr>
<tr>
<td>4</td>
<td>Dying out</td>
<td>All &gt;5 cm, many individuals (&gt;500 individuals)</td>
</tr>
<tr>
<td>5</td>
<td>Almost extinct</td>
<td>All &gt;5 cm, few individuals (&lt;500 individuals)</td>
</tr>
<tr>
<td>6</td>
<td>Extinct</td>
<td>Documented former presence but now disappeared</td>
</tr>
</tbody>
</table>

Results

Geographical distribution

According to different types of studies the FPM is found in 551 streams occurring from the southernmost to the northernmost part of Sweden, a distance of approx. 1,500 km (Fig. 2). All FPM streams were found below 600 m above sea level. Most of the FPM streams are situated in the northern two-thirds part of Sweden.
Figure 2. Distribution of freshwater pearl mussel populations in Sweden. Main catchments are shown.
**Distribution**

The FPM was present in 52% of the 118 main Swedish catchments (larger than 200 km\(^2\) and ending in the sea), and in 7% of 128 coastal catchments (smaller than 200 km\(^2\)) (Fig. 2).

In most (approx. 80%) of the streams the longitudinal distribution of the FPM populations was less than 6 km based on data from 123 streams. Ten streams (8%) held mussels more than 10 km with the longest stream being 29 km.

**Population sizes**

Based on 126 streams the populations size was less than 5,000 specimens in 29% of the streams, 5,000-10,000 11%, 10,000-50,000 27%, 50,000-100,000 13%, 100,000-200,000 11%, and >200,000 9 %. 20 streams had populations exceeding 100,000. The largest population is 1,400,000 specimens. All populations with >200,000 mussel, but one, were found in northern Sweden. There were significant positive correlations between the populations size, distribution range and density (p<0.05, Pearson's correlation coefficient).

The total number of mussels in the 127 streams (standard method) was 8,800,000 (mean 70,000, median 20,000).

**Population densities**

Based on 123 streams the mean population density is less than 5 specimens/m\(^2\) in 70% and higher than 10 specimens per m\(^2\) in 14% of the streams. The highest density found was 40 specimens per m\(^2\). Most of the streams with the highest densities (90%) were found in the northern part of Sweden.

**Size of the mussels**

In 60 out of 465 streams the smallest mussel found was <20 mm, in 174 streams 20-50 mm, and in 231 streams the smallest mussel was >50 mm. Figure 3 shows the distribution of the smallest mussel found in streams investigated by the standardized method. There was a tendency that more streams with small mussels were found in the northern part of Sweden. Mussels <10 mm were found in 10 streams, all but one in northern Sweden.
The conservation values among 118 streams were: very high conservation value – 27 streams (23 %), high conservation value – 61 streams (52 %), conservation value – 30 streams (25 %). Most of the streams have status class 3-5, i.e. lowest viability (Fig. 4).

**Conservation value and status class**

![Figure 3](image1.png)

**Figure 3.** The distribution of the smallest mussel found in 123 freshwater pearl mussel streams investigated with Swedish standardized method (Bergengren et al. 2010).

![Figure 4](image2.png)

**Figure 4.** The distribution of the freshwater pearl mussel status classes (based on viability) at 285 streams. Highest viability to the left and extinction to the right.
**Extinct populations**

The questionnaire sent to regional authorities (the counties) resulted in an estimate of 78 FPM populations that had gone extinct. Two-thirds of the streams with extinct populations were situated in the southern Sweden (Fig. 5).

![Extinct freshwater pearl mussel populations according to the questionnaire to the county administrative boards. Main catchments are shown.](image)

**Changes**

241 streams were investigated during the 1900’s and 2000’s (149 streams (200 localities) with simple status classification and 41 streams with the standardized...
method) allowing analysis of changes during approx. 10 years (Tab. 3). Several significant changes could be recognized: increased status class, increased mean length, increased size of the smallest mussel found, and decreased percentage of mussels <50 mm. All these changes indicate deterioration.

Table 3. Changes of FPM population characteristics during around 10 years in the 1990’s and 2000’s. Statistical significant differences are presented as + or – depending on increase or decrease. One +/- means p<0.05, ++ P<0.01, +++ p<0.001. 0 = means no significant difference. n = number of paired samples. Sign test has been used for status class and Wilcoxon matched-pairs sign test for the other variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple status classification or standardized method</th>
<th>Standardized method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total n=200</td>
<td>Not limed n=111</td>
</tr>
<tr>
<td>Status class</td>
<td>+++, (n=197)</td>
<td>0, (n=88)</td>
</tr>
<tr>
<td>Mean length</td>
<td>+++, (n=158)</td>
<td>0, (n=67)</td>
</tr>
<tr>
<td>Smallest mussel</td>
<td>++, (n=172)</td>
<td>0, (n=74)</td>
</tr>
<tr>
<td>Largest mussel</td>
<td>0, (n=160)</td>
<td>0, (n=68)</td>
</tr>
<tr>
<td>Share &lt;20 mm</td>
<td>0, (n=170)</td>
<td>0, (n=75)</td>
</tr>
<tr>
<td>Share &lt;50 mm</td>
<td>-, (n=172)</td>
<td>0, (n=76)</td>
</tr>
<tr>
<td>Density</td>
<td>0, (n=163)</td>
<td>0, (n=69)</td>
</tr>
<tr>
<td>Population size</td>
<td>0, (n=40)</td>
<td>0, (n=25)</td>
</tr>
<tr>
<td>Conservation value</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When data was divided into limed and not limed streams, respectively, there were no significant changes over time for the limed ones but deterioration for the not limed populations (Tab. 3).

Discussion

The knowledge of the spatial distribution and status of the FPM in Sweden is rather good. However, there is a lack of information from larger streams/rivers as the investigation methods are restricted to water depth where it is possibly to wade (approx. 1.3 m).
**Status**

The study shows that the FPM still is wide-spread in Sweden with the most of the 551 FPM streams in the northern part. After this study was finished (2006) additionally 50 FPM streams were found and even more are certainly to be found in the future, especially in the northern part of Sweden.

The population size is, as expected, related to the distribution range and density – long streams with high density have the largest population. The total number of mussels in Sweden is unknown. If the mean population size from 127 streams (investigated by the standardized method) is multiplied with all 551 streams the total number will be around 39,000,000 mussels. In a European perspective, Sweden holds around one-third of the FPM populations and probably circa 11% of the number of mussels (Tab. 4). Hence, Sweden and the other Scandinavian countries Norway and Finland is a core area of the FPM in the world with around two-thirds

**Table 4.** Estimation of the number of freshwater pearl mussel streams and individuals in Europe. Revised from Geist (2010) – figures in italics are updated.

<table>
<thead>
<tr>
<th>Country</th>
<th>Estimated number populations</th>
<th>Estimated number of individuals</th>
<th>Reference for revision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>29</td>
<td>50,000</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>5-6</td>
<td>2,500-3,000</td>
<td></td>
</tr>
<tr>
<td>Czech Republic</td>
<td>6</td>
<td>80,000</td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>Max,.1</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>1</td>
<td>35,000-40,000</td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>174</td>
<td>&gt;12,000,000</td>
<td>Oulasvirta (2010)</td>
</tr>
<tr>
<td>France</td>
<td>84</td>
<td>Max.,100,000</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>69</td>
<td>Max.,144,000</td>
<td></td>
</tr>
<tr>
<td>Great Britain</td>
<td>105</td>
<td>&gt;12,000,000</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>135</td>
<td>&gt;12,000,000</td>
<td></td>
</tr>
<tr>
<td>Latvia</td>
<td>8</td>
<td>25,000</td>
<td></td>
</tr>
<tr>
<td>Lithuania</td>
<td>1?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Luxembourg</td>
<td>1</td>
<td>150-200</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>360</td>
<td>143,000,000</td>
<td>Larsen (2010)</td>
</tr>
<tr>
<td>Poland</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>6</td>
<td>&gt;1,000,000</td>
<td></td>
</tr>
<tr>
<td>Russia</td>
<td>24</td>
<td>&gt;140,000,000</td>
<td>Makhrov (2010)</td>
</tr>
<tr>
<td>Spain</td>
<td>36</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>551</td>
<td>39,000,000?</td>
<td>this paper</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>Approx. 1,600</strong></td>
<td><strong>Approx. 360,000,000</strong></td>
<td></td>
</tr>
</tbody>
</table>
Half of the FPM streams do not show any evidence of recruitment during the last 20 years, i.e. no mussels <50 mm are found. Just 13% of the streams have recent recruitment (mussels <20 mm, approximately, 10 years). This means that most of the populations are not functional, which will, of course, in the long run lead to extinction. This is also shown by the status classes (Fig. 4). Just 36 out of 285 streams have population with status class 1 or 2 (viable and viable? respectively).

Moreover 78 known (12 %) populations have gone extinct according to the questionnaire but this is probably an underestimate due to lack data on former occurrence (Fig. 5). Eriksson et al. (1998) estimated that one-third of the FPM populations have disappeared since 1900-1950, most of these in the southern part.

However, the conservation value assessments show that Sweden still has 98 streams had high or very high conservation values The highest score was 27 (out of 36 possible). This may be compared to the River Varzuga at the Kola Peninsula, NW Russia, which had a score of 32 (Bergengren et al. 2004, Henrikson & Söderberg 2010).

A general pattern is that most FPM streams and healthy populations are found in the northern part of Sweden. This is probably due to lower human activity (less population, industries, agriculture, and acidification). Also the small catchments close to the coasts are more affected by humans, which may explain the greater loss of FPM in those areas.

**Changes**

According to local people several FPM populations have decreased in number of mussels or gone extinct. We have no scientific data describing the changes in FPM populations during the last century. However, studies in southern Sweden have shown a statistical correlation between the disappearance of FPM and acidification, which also is verified in field experiments (Henrikson 1996).

The present study shows that during the last decade there have been changes indicating lowered viability – increase of status class, length of smallest mussel, and the share of mussels <50 mm respectively (Fig. 4). This is especially the case for those streams that are not limed to counteract acidification.
**Threats**

There is no scientific method developed for identification of the specific threats towards a certain FPM stream. According to the questionnaire to the county administrative boards the most common threat towards the FPM were said to be: forestry, migration obstacles, un-natural water flows, acidification, and agriculture (Tab. 5). The most serious problem is probably siltation of stream bottoms, due to forestry or agricultural activities in the catchment. This seems to be a general opinion among European scientists (e.g. Buddensiek 1995, Geist & Auerswald 2007, Hastie 2011). Many dams and road culverts are migration obstacles affecting the FPM host fish populations. There is one obstacle per 2 kilometres according to inventories of around 15,000 km streams in southern Sweden (Swedish Forestry Agency, unpublished data). Probably also other habitat changes as clearing for timber floating are of importance. Large dams and regulation of the water flow may also be important. In large parts of Sweden acidification, i.e. deposition of air-borne acid pollutants still is a great problem for the salmonid host species as well as FPM (Henrikson 1996).

**Table 5. Overview of the threats towards freshwater pearl mussels in Sweden.**

<table>
<thead>
<tr>
<th>Human activity</th>
<th>Habitat effects</th>
<th>Biological effects</th>
<th>References (primarily Scandinavian ones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forestry</td>
<td>Siltation, changed Light climate</td>
<td>Oxygen deficit High temperature</td>
<td>Nyberg &amp; Eriksson 2001, Högberg 2009, Österling <em>et al.</em> 2010,</td>
</tr>
<tr>
<td>Road and dam constructions</td>
<td>Migration obstacles</td>
<td>Decreased host fish populations</td>
<td>Vaughn &amp; Taylor 1999, Jensen 2007</td>
</tr>
<tr>
<td>Electricity production</td>
<td>Water regulation – un-natural water dynamics Migration obstacles</td>
<td>Physiological stress on mussels and host fish</td>
<td>Watters 2000, Dunca &amp; Larsen this volume</td>
</tr>
<tr>
<td>Combustion of coal an oil</td>
<td>Acidification – lowered pH, increased aluminium Migration obstacles</td>
<td>Toxic impact on mussels and host fish</td>
<td>Henrikson 1996, Taskinen <em>et al.</em> 2011, Larsen this volume</td>
</tr>
<tr>
<td>Agriculture</td>
<td>Eutrophication – increase of nitrogen, phosphorus etc, leading to e.g. siltation</td>
<td>Oxygen deficit</td>
<td>Bauer 1988, Geist &amp; Auerswald 2007</td>
</tr>
<tr>
<td>Introduction of non-native species</td>
<td></td>
<td>Failed reproduction, predation</td>
<td>Henrikson &amp; von Proschwitz 2006, Schmidt &amp; Vandré this volume</td>
</tr>
</tbody>
</table>
No authority listed pearl fishing, which in former time was threat but today is not seen as a problem. There is some concern of the introduction/invasion of non-native species. The American brook char *Salvelinus fontinalis* is a competitor to the native brown trout *Salmo trutta* in small streams (Öhlund et al. 2008) and it is not an acceptable host for FPM. The suitable host are native brown trout or Atlantic salmon, and there is some concerns about the importance of introduced strains which may be poor hosts (e.g. Taubert et al. 2010). The introduced predators the American muskrat *Ondatra zibethicus* and the American signal crayfish *Pacifastacus leniusculus* may affect mussel populations and can be a potential problem (Henrikson & von Proschwitz 2006, Schmidt & Vandré this volume).

**Needs**

Generally spoken FPM needs flowing water of good water quality, a stable riverbed consisting of a suitable material (sand, gravel), good water flow in the hyporheic zone and good availability of host fish. Degerman et al. (2009, in press) tried to make this more precise from data of Scandinavian streams with healthy FPM populations (Tab. 6). As far as we know, brown trout is the only host fish species in Swedish streams, but in main rivers Atlantic salmon *Salmo salar* may be host.

**Table 6.** Summary of the habitat needs of the freshwater pearl mussel based on current knowledge for healthy populations in Scandinavian waters (Degerman et al. 2009, in press).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>( \geq 6.2 ) (minimum)</td>
</tr>
<tr>
<td>Inorganic aluminium</td>
<td>&lt;30 ( \mu g ) l(^{-2} ) (maximum)</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>5-15 ( \mu g ) l(^{-1} ) (average)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>&lt;125 ( \mu g ) l(^{-1} ) (median)</td>
</tr>
<tr>
<td>Turbidity</td>
<td>( &lt;1 ) FNU (average, spring flood)</td>
</tr>
<tr>
<td>Water colour</td>
<td>&lt;80 mg Pt l(^{-1} ) (average, spring flood)</td>
</tr>
<tr>
<td>Water temperature</td>
<td>&lt;25 (^\circ) C (maximum)</td>
</tr>
<tr>
<td>Fine grain (&lt;1 mm) substrate</td>
<td>&lt;25 % (share of particles, maximum)</td>
</tr>
<tr>
<td>Redox potential</td>
<td>&gt;300 mV (corrected value)</td>
</tr>
<tr>
<td>Number of juvenile salmonids</td>
<td>( \geq 5 ) per 100 m(^{-2} ) (minimum, summer)</td>
</tr>
</tbody>
</table>
Conservation

The Swedish FPM conservation is based on: (1) legal protection, i.e. setting aside whole or large parts of the catchment as nature reserve or biotope reserve, (2) general consideration rules according to the Swedish Environmental Code, The Swedish Forestry Act etc., (3) voluntary consideration from forestry and agriculture (in forestry often connected to environmental certification), (4) habitat restoration and (5) liming of acidified waters.

Today there are just around 10 FPM streams with sufficient legal protection in Sweden (nature reserves). Natura 2000 is it is not sufficient in its present form, and has to be complemented with other legal measures, especially regarding land use and riparian zones. It is also possible to protect streams and buffer zones by “biotope reserves” according to the Swedish Forestry Act, but this is seldom done. Most of the Swedish “protection” of FPM streams relies on voluntary measures by the land users.

The most large-scaled measures taken for the FPM is liming of acidified streams (e.g. Henrikson & Brodin 1995). Liming is also an important measure in Norway (Larsen this volume). Other examples are eliminating of fish migration obstacles and in-stream habitat restoration like in streams cleared for timber floating. An EU LIFE project was implemented during 2004-2009 testing different in-stream measures as well as management of the riparian zone (Henrikson & Alexanderson this volume).

Conclusions

Sweden, and the other Scandinavian countries, is a core area for the FPM in Europe with around two-thirds of all FPM streams, which means that Scandinavia has an international responsibility for the conservation of FPM. Although Sweden has a great number of FPM streams the situation is similar to that in other countries (e.g. Geist 2010): (1) extinct populations, (2) mostly non-recruiting populations, (3) negative trends. But still there are a number of viable populations.

If we are going to get an effective protection, the FPM as well as the host fish populations must be evaluated. This means that human activities in the streams
as well as in the catchment must be analysed and appropriate measures is taken. This is a great challenge for the future.

Acknowledgements

This study was financially supported by grants from the Swedish Environmental Protection Agency and WWF (World Wide Fund for Nature) Sweden. We thank Erik Degerman for valuable comments on an earfier draft of this article.

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The freshwater pearl mussel in Austria – current status and prospects for the future

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Abstract

The freshwater pearl mussel (Margaritifera margaritifera Linné, 1758) is one of the most threatened species in Austria. Once having occurred in the northern parts of Upper and Lower Austria in enormous densities, there are only some isolated scattered beds left nowadays. Most populations lack juveniles, as the natural reproduction does not work anymore.

In order to conserve the few remaining populations, several protection projects have been carried out in the last decade, dealing mainly with the support of natural reproduction by releasing juvenile autochthonous brown trout (Salmo trutta Linné, 1758) infected with glochidia. In the past few years successful breeding was impeded and previous efforts were destroyed by unforeseen circumstances. These were mainly uncontrollable activities in the catchment area, e. g. the flushing of an impoundment or the release of toxic substances into the river.

The continuous decline of the freshwater pearl mussel populations in Austria during the past ten years requires a new conservation strategy which is currently being worked out. The new strategy is focused on two central measures: artificial breeding and the restoration of an appropriate river, including its catchment area.
Current status in Austria

The distribution of pearl mussels in Austria has always been restricted to the foothills of the Bohemian massif in Upper and Lower Austria (Gumpinger et al., 2002) (Fig. 1). In the 1920s more than 50 rivers in Austria were home to pearl mussels (Riedl, 1928). The populations have declined drastically since then; more than half of the stocks have totally vanished. Nowadays, there are only some isolated scattered populations left (Scheder & Gumpinger, 2008).

Fig. 1. Potential distribution area of the freshwater pearl mussel in Austria (dark grey area) and currently inhabited rivers (dark grey lines).

Especially in the past few years the situation of the freshwater pearl mussel has worsened catastrophically. In the year 2002, the total population in Austria was estimated to about 70,000 individuals (Gumpinger et al., 2002). Recent investigations have pointed out that the populations have declined by 50% on average since then. Additionally, in most of the inhabited rivers no successful recruitment has taken place over the past 15 years. When in the early 1990s the largest remaining mussel population in an Upper Austrian river called Waldkist...
counted some 20,000 individuals, occurring in a reach about 30 km long, there are not even 1,000 specimens left nowadays, all living in one single, isolated bed (Scheder & Gumpinger, 2008). The largest population in Lower Austria in the River Großer Kamp currently comprises approximately 10,000 specimens – compared to more than 20,000 in the year 2002 (Csar & Gumpinger, 2009) (Fig. 2).

![Graph comparing population sizes](image_url)

**Figure 2.** Comparison of the estimated population sizes in Austrian freshwater pearl mussel rivers based on several investigations made over the period from 1993 until 2009.

The decline is caused by various factors, including pollution, river regulation or adverse fish stocking strategies. But the crucial factor is the massive input of fine sediments that derive from the heavy agricultural use of the catchment areas. Those fine sediments inhibit the successful reproduction of the freshwater pearl mussel, as they clog the interstitial where the juveniles dwell and disable the supply with oxygen and nutrients.

**Activities in the past**

In order to conserve the few remaining populations, several protection projects have been carried out in the last decade. It was obvious that the heavy fine sediment loads and the adverse land use could not be dealt with promptly and easily. So the primary objective was the infection of a preferably large number of juvenile brown trout with glochidia that were gained from gravid mussels in
situ. The host fish were then immediately released in preferably clean brooks and streams with appropriate habitats. The protection scheme dealt with providing an amount of juvenile mussels as large as possible and with spreading the offspring in the maximum range as a bridging until the currently adverse environmental conditions should have been improved and natural reproduction made possible again.

During the past years significant fluctuations in gestation rates and larval development were detected, relating to a variety of abiotic factors, mainly water temperature. Thereby the gradual development of the glochidia turned out to pass through five morphologically discriminable stages (Scheder et al., in prep). Apart from the protection measures in the river Waldiaist several protection measures are taken in several other regions.

**Setbacks in the past**

In the years 2006 and 2007 the whole protection programme was put at risk due to constant careless dealings with the river and its catchment area. In 2006 a so far unknown toxic substance obliterated a whole mussel population in a brook called Harbe Aist, a tributary to the river Waldiaist. This was the second largest known population in the river system and one of the largest remaining ones in the whole of Upper Austria. In the regarding year, the mussels in the large bed in the river Waldiaist, located some 20 km downstream of the extinguished population in the small tributary, showed a significantly lower breeding effort, only very few mussels bearing larvae at all. Apart from that, the actually pregnant mussels expelled their larvae much too soon, as a result of which no material for the infection could be gained.

In the year 2007, the normal amount of female mussels was pregnant again; the assisted breeding programme could be carried out on schedule. One day before the planned infection a reservoir of an upstream hydropower plant was flushed and an estimated amount of 3,000 - 5,000 m$^3$ of fine sediments was mobilised (Fig. 3). The fine sediment load caused the pregnant mussels to expel their larvae at once in distress. Once more no larval material was available for the infection of the brown trout provided. Furthermore the fine sediments clogged the interstitial some 15 km downstream of the hydropower plant.

It must be assumed that the juvenile mussels that had arisen either from natural reproduction or from the assisted breeding within the past five years will not have survived the impact.
Prospects for the future

In spite of the current conservation efforts the numbers of populations as well as the numbers of individuals in the mussel beds are still declining. According to this, the simple continuation of the previous conservation measures cannot be expected to be sufficient. It becomes clear that only a major protection project can save the few remaining populations from becoming extinct.

As a basis for a future approach, a new action plan has been worked out, scheduled for the next 18 years. Basically the project will focus on two central strategies: First, the breeding will be transferred to a system free of interference, a so-called „vivarium“ (Roberts, 2006) (Fig. 4). There, the undisturbed development of the glochidia will be ensured.
After infection, the fish will remain in the fish farm all winter long. In spring, juvenile mussels that drop from their hosts are collected and transferred into refrigerated breeding boxes. There they are fed with detritus until their gills are fully developed and they are able to start filter-feeding. At that stage they will be put into „Buddensiek-cages“ (Buddensiek, 1995) and brought out into preselected brooks within the original river system, but in smaller catchment areas. The juvenile mussels are then used as indicators: the growth-rate and the rate of survival will help to find out about the most suitable small catchment area which shall then be restored in the long term. Juvenile mussels with a total length of more than 1 cm will be transferred into iron cages in order to monitor their survival and growth-rate. Based on these results, a decision which of the preselected catchment areas should be restored will finally be made possible. Beforehand several investigations in the preselected brooks are made, e.g. the examination of the redox potential (Geist & Auerswald, 2007). Over the course of the years, the system of breeding will gradually progress from the described artificial method to a fully natural method with as little human impact as possible (Fig. 5).

**Figure 5. Development of the breeding-method.**

Meanwhile, somewhat time-displaced, the most suitable brook and its catchment area within the River Waldaist-system will be restored. Firstly, the siltation must be restrained in order to restore an appropriate habitat for juvenile mussels. The restoration of riparian areas as for instance the elimination of spruce trees, the generation of buffer zones and the rewetting of drained areas are imperative necessities. Along with this, sediment traps will be
installed in order to reduce unnaturally high sediment loads of tributaries and drainage ditches referring to the successful pilot project in the River Lutter in Lower Saxony (Altmüller & Dettmer, 2006). Modifying land use schemes and implementing the measures is only possible if a sufficiently large spread of land is purchased and managed from an ecological point of view.

The success of both strategies – breeding and habitat restoration – will be evaluated after the completion of each partial project. The measures will mainly concentrate on the system of the River Aist. As soon as the new action plan works properly, we hope to be able to expand the project to other catchment areas in order to ensure the genetic variability of the Austrian freshwater pearl mussel populations.

Furthermore the measures will be accompanied by various strategies in public relations. On the one hand the information, clarification and sensitization of the public, especially protagonists around the rivers (landowners, farmers, forest workers, power plant or mill operators,…) who could put the program at risk is of particular importance for the success of the protection program. On the other hand statutory consulters and administration authorities often have problems to deal with the specific requirements of the freshwater pearl mussel in (potential) pearl mussel rivers when examining (negative) impacts of projects. For that reason a manual will be worked out which will help with the implementation and verification of projects.

Complementary to this is the establishment of a national database where administrative bodies and local authorities can find details on rivers which are home to freshwater pearl mussels.

Acknowledgements

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Conservation and restoration of a freshwater pearl mussel (*Margaritifera margaritifera*) population in Northern England

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Abstract

There is only one remaining large population of *Margaritifera margaritifera* surviving in England. A major survey in 2006 (10 years after the previous survey) showed that whilst the river still had a population of over 350,000 individuals, the river was not in favourable condition and there was virtually no recruitment. There had been a shift of the entire population to a higher size/age class since the 1996 survey, with the adults now 10 years older. Mussels under 65 mm comprised less than 1.5% of the overall population and juveniles <10mm (i.e. 4 years old) were rare and found only in the most highly oxygenated riffles. Redox potential measurements showed high levels of siltation in the gravel substrate and young mussels were present only at sites with high redox potential.

Given the small size of the upper catchment and relatively few environmental pressures it was considered that the river and its mussel population had the potential to be recoverable to favourable condition. Since the 2006 survey, a restoration programme has been instigated in the upper 4km of the river. Most of the environmental threats to the mussels have been identified and addressed. In the last 2 years non-native conifer forestry has been sympathetically removed, sections of river have been fenced to prevent stock access, fallen trees have been removed, turbidity meters have been installed, and field drains are being blocked to prevent silt input. Early indications show that all the measures are having a positive effect and that the river and mussels are showing signs of recovery.
The River Ehen

The River Ehen lies in west Cumbria in north-west England. The river drains from Ennerdale Water, a moderate-sized (3km$^2$ surface area), relatively deep, oligotrophic lake, and flows for a distance of 27km before entering the Irish Sea. The lake drains an area of moorland, forestry and rough grazing of the central western fells in Ennerdale, an area of approximately 44km$^2$. The SAC comprises the first 13km of the river from the outlet of the lake with a total catchment area of <100km$^2$. The lake and river catchment is very sparsely populated with farming, tourism and forestry being the predominant land-uses, and there are very few population centres within or adjacent to the SAC. The River Ehen has a regulated flow regime with a compensation flow set at 31.8ML/day.

Mussel population

No scientific studies of the pearl mussel in the River Ehen were carried out until 1995, and prior to this date, what little information there was with respect to distribution and abundance came from historical accounts of pearl fishing (see Killeen & Oliver 1998). In 1995 English Nature commissioned a survey of six rivers in England where substantial populations of the freshwater Pearl Mussel once existed – this included the River Ehen. The findings of this survey (Oliver & Killeen 1996) indicated that the Ehen, supported a large population with a structure that suggested recent recruitment. On the basis of that survey, the R. Ehen was identified as supporting one of the most significant populations of the freshwater pearl mussel in the United Kingdom. In 1996, a second survey was commissioned by English Nature. This survey was designed to provide a complete picture of the distribution, population size and structure of mussels in the R. Ehen and its major tributaries (Killeen & Oliver 1997a, b). In the third report from this study, the survey data was complemented by a review of relevant environmental parameters (Oliver & Killeen 1997). This comprised a management plan which included a summary of the life history of the mussel and a review of the data that was available at that time status, threats and conservation. The results from this study showed that the river supported a mussel population in excess of 300,000 individuals, with some evidence of juvenile recruitment. On this basis, the River Ehen was by far the most...
important river in England for the freshwater pearl mussel and was subsequently selected for SAC designation.

No further pearl mussel survey work was carried out until 2003/2004 when a study (funded by the Environment Agency and English Nature) was carried by the University of Aberdeen (Gibbins et al. 2005) principally to undertake a fluvial audit to examine the river's hydrology and sedimentology, but with some work to determine the age profile, mortality rates and long term viability of the river's mussel population.

In 2006, ten years after the previous major study of the River Ehen mussel population, a new study was commissioned by the Environment Agency (EA) to assess the current status of the pearl mussel population in the River Ehen in a number of ways (Killeen 2006).

The distribution and abundance of the mussels were assessed by repeating counts of adult mussels in sections previously surveyed in 1996. Previous baseline transects were repeated and new ones set up for monitoring purposes. The results indicated that adult mussels were still present in the river in high numbers. The population estimate for the Ehen River for 2006 was within the range of 350,000 to 500,000 individuals.

The size and therefore age profile of the mussels in the river was assessed by measuring a total of 1540 mussels from 33 quadrats taken from all areas of the mussel population. These results showed that there had been a shift of the entire population to a higher size/age class since the 1996 survey, with the adults now 10 years older (Figure 1). Mussels under 65 mm were found to comprise less than 1.5% of the overall population. Juveniles less than 10mm long or approximately 4 years old were rare and found only in the most highly oxygenated riffles. The results show that the river is not in favourable condition as there was insufficient recruitment, resulting in an ageing population which would inevitably lead to extinction unless the trend was reversed.
**Restoration Programme**

Given the small catchment size and relatively few environmental pressures it was considered that:

- The River Ehen should be given the highest possible priority in terms of conservation effort and resources
- The greatest effort should be directed towards river restoration
- The river was recoverable if immediate action was taken
- Removal of risk was the only way to safeguard this population of pearl mussels into the future
- While there would be considerable expenses required for effective risk removal, the Ehen has by far the most important population in the UK outside of Scotland and the costs required to restore it to Favourable Condition will be a lot less than in any other river in England or Wales

Since the 2006 survey, a restoration programme has been instigated. As most (~90%) of the mussels are living in the first 4km of the river, downstream of the lake, and the catchment is relatively small, most conservation efforts have focused on this section. The following sections outline the measures that have been implemented and give some preliminary results.
Forestry

The first major restoration efforts were undertaken by The Forestry Commission (FC) in 2007. Much of the land in the Ehen catchment, both upstream and downstream of the lake is forested by conifer plantations. As part of its Forest Design Plan for Ennerdale, the FC wished to clearfell c. 44ha of conifers on the slopes above and adjacent to the Ehen, downstream of the lake. Following felling, the intention was to allow the area to regenerate as a mix of open habitats and native woodland. Some native woodland planting and seeding has been carried out in areas where seed sources are thought to be limited to aid the development of native woodland (as part of the Wild Ennerdale Initiative). The long term future management is aimed at conservation management and low intervention systems with possibly fuel wood produced for local use. An Appropriate Assessment was carried out prior to felling with particular attention paid to mitigation for the pearl mussels.

Skylining operations were used to minimise vehicular movement on the steep slopes close to the river. Two particular risks were identified: from the breakdown of brash during timber removal (where rotting brash and timber debris can leach nutrients and tannins into the river directly or through surface run off/percolation), and the threat of pollution and silt transportation from soil erosion or stream crossing points.

The threat from brash was mitigated by moving the brash piles created at the bottom of the slope to a suitable short term storage areas where there were no direct pathways to the river, so that they could dry, lose their needles and then be supplied into the fuel market. A long list of measures was implemented to ensure that the risk of silt and pollutants entering the river were avoided, including silt-trapping of all drains and channels. Automatic turbidity loggers (see below) were installed to monitor the effectiveness of the silt traps. All of these measures were far more stringent than those applied for normal felling operations and incurred considerable additional cost. However, the operation appears to have been highly successful and there do not appear to have been any short-term, nor are there likely to be long-term deleterious effects.
Turbidity Loggers

Measurements of redox potential throughout the SAC clearly indicated that much of the substrate had levels of siltation that was too high for successful juvenile mussel recruitment. Coniferous forestry, overgrazing of land, agricultural drains, direct access of grazing animals to the river leading to bank erosion and poaching, and fallen trees leading to bank erosion had all been identified as potential sources of silt to the river. However, there was no information on actual levels of siltation or the most serious sources.

Automatic turbidity loggers (sondes) from the EA were first installed in the upstream part of the river as part of the mitigation for the forestry felling operations (see above) and subsequently monitored by the FC. Two loggers were installed, one at a location immediately upstream of the forestry drains and the other immediately downstream. The loggers were set to take turbidity (NTU) and temperature readings at 15 minute intervals. The data gathered showed that there was no significant increase in turbidity between the sondes and that the forestry mitigation measures were successful. The data also revealed that actual levels were very low (mostly under 1NTU, Figure 2) and higher levels correlated with high rainfall (Figure 3).

![Figure 2. Example of results from turbidity logger (daily means of NTU over a 6 month period) (EA data).](image-url)
These turbidity loggers showed that field drains are open to the river and were one of the major sources of silt. They have also been used to identify which field drains (mainly agricultural in this case) are having the greatest silt input.

In-river Tree Removal

Survey work on the upper River Ehen (Killeen 2006, 2007) identified several locations where large trees had fallen into the river. These were considered to be having a significant impact on the mussel population by changing the hydrodynamics of the river, particularly upstream ponding, which was leading to silting of the substrate, and scouring where the water was forced through narrow channels. A programme of tree removal was instigated in autumn 2007.

At the 6 locations initially selected for tree removal, the work was preceded by a brief survey to map out the location and numbers of mussels in the area of the proposed works. This was carried out a) to inform the contractors of areas of safe and unsafe working (i.e. where no in-stream trampling could be permitted), and b) to provide a baseline from which the after effects of the removal could be monitored. Pre-surveys were carried out in October 2007 and the post works surveys in June and September 2008.

The results of the surveys to monitor the numbers and locations of mussels post-tree removal works were very encouraging. All the initial signs are positives with ameliorated flows across the channel, layers of accumulated silt and detritus have been dispersed, and the beds appear to have stabilised. At
several of the locations the number of mussels had increased from the pre-
survey numbers, almost certainly as a result of mussels that had previously been
obscured or covered with silt or detritus becoming exposed. There did not
appear to have been any significant negative impacts. The exposure of the
mussels that had previously been obscured has allowed them to return to a
healthy habitat where they were filtering open water and contributing to the
reproductive effort of the population. On the basis of those results, further
tree removal was carried out in 2008/2009.

### Water Quality & Auto Samplers

Water quality in the River Ehen is generally very high. There is a long dataset
extending back over 15 years of monthly samples collected by the EA at a
location within the core area of the mussel population.

The level of orthophosphate since 1995 has, on most sampling dates, been less than
0.005mg/l with a mean of 0.00195mg/l. However, there have been a few occasions
when the orthophosphate level has exceeded 0.02mg/l. The total oxidised nitrogen
levels since 1995 have been relatively stable with the majority of determinations
being less than 0.5mg/l and with a mean of 0.338mg/l. There have been levels
of over 2mg/l recorded but most high values were prior to mid-1998. Similarly
levels of BOD and ammonia are all very low. All of the median levels of the
critical determinants are in line with those given by Moorkens (2006, 2007) as
being required for effectively recruiting populations. In spite of this apparent
very high water quality, the river persistently experiences blooms of filamentous
algae, particularly in the upper 2km reaches.

During high temperatures and sunlight, with continuous nitrate in the water,
any introduction of available phosphorus to the system will be taken up
immediately by organic growth. This was leading to continuous growth and
decay and chronic filling of interstitial spaces within the gravel substrate. The
water quality monitoring was not showing up phosphate elevations during times
of filamentous algal growth and in spite of several lines of investigation,
attempts to locate the source of the problem proved to very difficult.

In winter 2008/2009, auto-samplers were installed by the EA at 2 locations in
an effort to pinpoint the source of the phosphorous to the river and
demonstrate phosphorous levels in the river were elevated on a rising flood.
Sampling were taken on the rising flood rather than at or after the peak flood as both silt and phosphorus concentrations tend to peak at the start of a flood event. The auto-samplers were triggered by mobile phone when the hydrographs from a nearby gauging station indicated a forthcoming rising flood. Eight samples were then taken at hourly intervals. Results from these trials of the auto-samplers on 3 flood events showed a similar pattern. For example (Figure 4), at an upstream location the orthophosphate level rose gradually over the 8 hour period from 0.003 to 0.006mg/l, whereas the level at a downstream location rose rapidly to 0.035mg/l within 2 hours, and then gradually fell back to 0.005mg/l over the ensuing 6 hours. This demonstrated that elevated levels of phosphorous enter the river on a rising flood then rapidly fall to low levels. Run-off from farmland between the 2 samplers will be the next focus of attention.

**Riparian Management**

Although the upper catchment of the Ehen is small, there are very few landowners, and the agriculture is low intensity, farming practice is one of the major issues to be addressed in the conservation and restoration plan for the River Ehen and its mussel population. The principal issues are lack of buffer zones, stock access to the river, and nutrients and silt entering the river from field drains. To date, considerable progress has been made with fencing the adjacent land immediately downstream of the lake. Cattle drinking troughs with mains water have also been installed and measures are underway to solve the problems with the field drains which are the most serious sources of silt.

![Figure 4. Example of phosphorous results from auto-sampler (EA data).](image)
The future

Although the restoration process is in its infancy, and there is still a great deal to be done, early indications show that all the measures implemented thus far, are having a positive effect and that the river and mussels are showing signs of recovery. It is very early days for the conservation and restoration efforts to be showing a significant change in juvenile pearl mussel recruitment, but there are places where 1-2 year old individuals can be found. Also, EA electro-fishing surveys in 2007 and 2008 have recovered encysted salmon throughout the length of the SAC. The possibility of using bankside techniques as used by Altmueller & Dettmer (2006) in Germany to encyst young salmonids to ‘kick-start’ juvenile mussel recruitment is being investigated. In-stream tree works, management of field drains, provision of stock watering and stock exclusion fencing will all continue. It is hoped that farming practice in the valley will continue to change with pearl mussel conservation becoming a higher priority in landowner’s agri-environmental agreements.

Two highly significant and potentially highly beneficial changes will come into effect over the next 5-10 years based upon 2 major reviews by the EA. Although there is only limited published information on water levels and flow velocity requirements of pearl mussels, it was concluded that the amount of water flowing down the Ehen was inadequate and may be one of the main interacting reasons for increased siltation (through inadequate flushing). The EA could not conclude that there was no adverse effect of the current abstraction licences for public water supply from Ennerdale Lake on the River Ehen SAC. The EA sought advice from specialists and reviewed the data on velocities from the River Kerry in Scotland (Hastie et al. 2000) and developed hydrological models to back up their decisions. As a result, the water abstraction license will be changed so that there is a staged compensation flow and a reduced take from the lake. The increased compensation flow arrangement will be provided by a state of the art fish pass (funded by United Utilities) that will move in response to lake levels.

A review of the geomorphological regime in the river concluded that there was an inadequate supply of coarse sediment to the river. Therefore, it is proposed to re-divert a mountain stream, which prior to the 19870s flowed into the upper part of the river but currently flows into the lake, back into the main river.
Acknowledgements

Implementation of the restoration plan has been a collaborative effort between the conservation agencies, stakeholders and landowners. The Environment Agency (NW Region) has provided the impetus and much of the funding towards this restoration project, and the funding towards the research and monitoring carried out by the present author. In particular, Gail Butterill is thanked for her unstinting commitment and interest in the River Ehen and its mussels, and without whom, saving the mussels would be more a more distant and less optimistic prospect. The author also acknowledges and thanks Gareth Browning of the Forestry Commission, and United Utilities, Natural England, the National Trust and the National Park Authority for their practical and financial contributions. Lastly I thank Evelyn Moorkens for her contributions to the field research and her invaluable input into development of the management & restoration plans.

References


Demographic structure, sampling and the inferred genetic structure of Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations

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Abstract

Inferences drawn from the analysis of genetic data are increasingly used to inform conservation management and require samples that are representative of the populations under investigation. Furthermore, a range of assumptions variously underlie the theoretical framework underpinning such analyses, such as infinite population size, non-overlapping generations and random breeding. Problems can therefore arise when investigating population genetic structure in long-lived species, such as the endangered freshwater pearl mussel (*Margaritifera margaritifera*), with its complex life cycle, encompassing iteroparity, overlapping generations, selfing, and where field collections could encompass unrepresentative samples. To test for the representativeness of genetic samples and the robustness of the results obtained, a comparison was made between the demographic structure (based on shell lengths) of 11 pearl mussel populations and genetic findings (based on the analysis of nine microsatellites) from a random sample from each location. Furthermore, size-stratification was carried out to divide the samples with significant deviations from Hardy Weinberg equilibrium into a number of size classes and the genetic analysis was performed on those. A single sample was unrepresentative of the demographic population, with an overrepresentation of older mussels and
characterised by a significant heterozygote excess. Four other samples showed significant heterozygote deficiency. Overall, genotypes of the samples that were size-stratified were not in Hardy-Weinberg (HW) equilibrium. However, HW equilibrium was found within each size class, suggesting potential within-population structuring. This study highlights the need for larger-scale size-stratified samples to elucidate possible within-population structuring in freshwater pearl mussels.

Introduction

Population genetic analyses are increasingly used in support of species conservation, from helping to define breeding populations, to monitoring levels of genetic diversity, estimating effective population sizes and identifying conservation units (Frankham et al., 2002). Such analyses, however, are based on drawing inferences from a sample of individuals that, for analytical robustness, need to be representative of the populations under investigation. Furthermore, much of the theoretical framework that underpins many aspects of such analyses are based on various assumptions, such as infinite population size, non-overlapping generations, random breeding, equal contribution of all breeders to the next generation, absence of mutation and selection and independence of loci (Hartl & Clark, 1989; Frankham et al., 2002). It is therefore easier to be confident of results in the case of semelparous organisms with an annual or seasonal life cycle, e.g. the isopod Asellus aquaticus (Murphy & Learner, 1982; Verovnik et al., 2004). In species with a more complex life cycle, e.g. salmonids, which includes some overlapping of generations, the results using the basic population genetic model can still be interpreted with considerable confidence (Allendorf & Waples, 1996; Waples, 2002). Furthermore, new theoretical frameworks have been developed for such situations (Waples, 1990a,b, 2002). However, it remains unclear whether this is still the case with regard to the interpretation of results from samples taken from long-lived (> 100 years), iteroparous species, such as the endangered freshwater pearl mussel, Margaritifera margaritifera (Bauer, 1992), which potentially have many overlapping generations.

*M. margaritifera* has a complex life cycle, though detailed knowledge of its reproductive behaviour, such as whether individuals reproduce every year or consistently with age, is lacking, as is even a basic understanding of its
reproductive biology with regards to glochidia production and survival (Bauer, 1987). Although fecundity is generally high, the actual size of breeding populations is unknown, as is whether reproductive success is skewed among breeders across age cohorts. What is known is that, at low mussel densities, female mussels can become hermaphrodites and self-fertilise (Bauer, 1987), adding a further complexity to the life cycle that may, potentially, confound the interpretation of patterns of distribution of genetic variation within and among samples. Thus, it may be difficult to draw robust inferences from samples using standard population genetic models that do not take this complexity into account. Whether or not this might be the case has not been examined in *M. margaritifera*.

Recently, a number of genetic studies on *M. margaritifera* have been carried out, which focused on providing insights in support of their conservation (Geist & Kuehn, 2005; Machordom et al., 2003; Bouza et al. 2007, unpubl. data), as numbers have declined dramatically in the last few decades (Young & Williams, 1983a; Bauer, 1986, 1988; Cosgrove et al., 2000; Young et al., 2001). Thus, there is an urgent need to develop effective conservation plans that address genetic issues, such as the loss of intraspecific biodiversity, the potential for outbreeding depression when conducting population supplementation, and the best sources of material for (re-)introductions. In studies to date, non-destructive sampling of randomly picked, visible mussels at a particular location has been the method to obtain individuals for analysis. Potentially at least, this can lead to an overrepresentation of larger/older mussels and an underrepresentation of small/young mussels (< 40 mm) in samples, as juvenile mussels are mostly buried in the substrate (Hastie & Cosgrove, 2000) and smaller individuals are harder to find. Furthermore, even where they are found, smaller mussels may not be sampled because obtaining sufficient tissue from them is more problematic and potentially more harmful. If so, a drawback of such samples is that the genetic sample may not be representative of the overall population, where genetic diversity is not randomly distributed among cohorts, and will be particularly unrepresentative where populations are dominated by small mussels.

The unrepresentativeness of samples may extend to collections of larger individuals. *M. margaritifera* larger than 60 mm can be easily detected in the river bed and they can grow up to 150 mm in length (Bauer, 1992; Hastie et al., 2000), encompassing a large number of age cohorts and overlapping
generations (Bauer, 1992; Hastie et al., 2000). Taking into account the complexities of the mussel’s life cycle, it is potentially possible that genetic diversity varies across age cohorts, in cases where all individuals do not reproduce every year, numbers of breeders vary, and there are varying levels of self-fertilisation across years. As such, it may be more difficult to interpret departures of the distribution of genetic variation from randomness across a sample without taking variation across cohorts into account.

We report here the results of a preliminary study that examines how representative samples of *M. margaritifera* taken for genetic analysis were of the overall demographic structure of the mussels at the collecting sites. The extent of within-population structuring among cohorts, and the effect which sampling across a large number of generations can have on the inferences drawn from standard genetic analyses was investigated. A comparison was made of the distribution of mussel lengths between the sample taken for genetic analysis and a larger, independent demographic sample from 11 Scottish pearl mussel populations. Based on the analysis of microsatellite loci, genetic diversity parameters and Hardy-Weinberg (HW) and linkage disequilibrium (LD) tests were compared between representative and unrepresentative samples. Finally, genetic analysis was performed on a single size-stratified sample, together with those samples that were unrepresentative of the demographic population of origin or showed significant deviation from HW equilibrium. These samples were divided into a number of size classes to reduce the number of overlapping generations in each size class.

**Methods**

**Demographic analysis**

Between 1995 and 2007, the lengths of 1898 pearl mussels were recorded at 11 locations in different Scottish rivers. The mussels were obtained along a 1 m x 50 m transect, according to the method described by Hastie *et al.* (2000). This method ensured that small mussels were included and that the size distributions were complete. For purposes of confidentiality, because of the persistent threat of illegal pearl fishing in Scotland (Young *et al.*, 2001), the locations are referred to as sites A – K.
In 2008, 30 mussel samples were collected at random from the same locations, together with a further, size-stratified sample \((n = 39)\), which was collected from an additional, large, healthy, reproducing Scottish population (site L). Mussel lengths were recorded and used to divide the sample into a number of size classes. A non-destructive sampling method was used to collect haemolymph (Geist & Kuehn, 2005). Mussels were taken from the riverbed; a 19G x 1.5” sterile needle, attached to a 1 ml syringe, was inserted into the base of the mussel and approximately 150 - 300 μl of haemolymph was collected and stored in 2 ml vials filled with absolute ethanol. The mussels were then carefully returned to the riverbed.

**Genetic analysis**

Haemolymph was spun at 17,500 x g for 10 min. Ethanol was discarded and the samples were left to dry at 37°C for 1 h. Total DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer’s protocol for the purification of total DNA from animal tissues. Nine microsatellite loci \((\text{Marma2671, Marma4277, Marma5280, Marma3116, Marma3621, Marma5167, Marma4143, Marma4315 and Marma4726})\) were amplified using the primers sequences described in Geist et al. (2003). PCR reactions were carried out in a 15 μl reaction volume, containing 5-100 ng DNA, 75 mM Tris-HCl (pH 8.8), 20 mM \((\text{NH}_4\text{})_2\text{SO}_4\), 200 μM of each dNTP, 0.5 μM of each primer, 0.25 units of ABgene Taq DNA polymerase (ABgene, England) and 1.5-3.0 mM MgCl₂, depending upon the marker (Geist et al., 2003). PCR cycling conditions and annealing temperatures were according to Geist et al., (2003). Electrophoresis was carried out on a MegaBace 500 capillary sequencer (GE Healthcare), and allele sizes were determined using MegaBace Fragment Profiler version 1.2.

**Statistical analyses**

Kolmogorov-Smirnov tests were carried out to test for differences in length distributions between the genetic and demographic sample in each location. Allele frequencies, expected \((H_e)\) and observed \((H_o)\) heterozygosities were calculated using GENEPOP 3.1 (Raymond & Rousset, 1995). This program was also used to test if the genotype proportions for each sample were according to Hardy-Weinberg \((\text{HW})\) expectations and to test for linkage disequilibrium \((\text{LD})\).
Size-stratification was carried out for the samples that showed significant differences in length distribution between the samples and those where genotype proportions were significantly different from HW equilibrium. The sequential Bonferroni method was used to correct for multiple tests with regards to the mussel length distributions, and the probabilities associated with deviation from HW and LD across locations. Fisher’s test for combining probabilities (Sokal & Rohlf, 1995) was used to test for HW equilibrium and LD across size classes within each location.

Results

Representativeness of genetic samples

A significant difference (p = 0.022), which remained after correcting for multiple tests, was found between the length distribution of demographic and genetic sample for one location, river E (Figure 1). In this case, there was both an over-representation of larger mussels and an under-representation of smaller mussels in the genetic sample compared with the demographic sample. For the other 10 locations, the length distributions between the demographic and genetic sample did not differ significantly, even though the smallest mussels were absent from the genetic sample in five locations - sites B, C, D, F and H (Figure 1). At three locations, sites G, J and K, both the smallest and the largest mussels were not sampled, resulting in a cohort distribution of the genetic sample that was more restricted, though not significantly so, than the larger demographic sample.

Significant deviations from HW proportions were observed, after sequential Bonferroni correction, in genetic samples from five of 11 locations - sites A, B, C, D and E. Of these, tests for heterozygote excess and deficiency were significant in four and one (site E) cases, respectively. No evidence for significant LD was found for any of the 11 samples, suggesting that the loci, in all cases, were segregating independently. Average \( H_r \) ranged from 0.12 for site A to 0.74 for site F, with an average of 0.58 ± 0.17 across all samples. \( H_0 \) varied between 0.09 for site A and 0.72 for site I, with an overall mean of 0.56 ± 0.18.
Figure 1. Distribution of freshwater pearl mussel, Margaritifera margaritifera, lengths from a demographic (black bars) and genetic sample (white bars) for each of the 11 locations (A – K) in Scotland.
Table 1. Genetic diversity parameters for each *M. margaritifera* sample at 11 locations (A – K). Number of individuals (n), p-values associated with tests for HW-equilibrium (pHW) and linkage disequilibrium (pLD), and estimates for observed (*H*<sub>o</sub>) and expected (*H*<sub>e</sub>) heterozygosity are given. P-values in bold are significant after correction for multiple tests.

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>pHW</th>
<th>pLD</th>
<th><em>H</em>&lt;sub&gt;o&lt;/sub&gt;</th>
<th><em>H</em>&lt;sub&gt;e&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>0.010</td>
<td>1.000</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>B</td>
<td>29</td>
<td>&lt; 0.0001</td>
<td>0.873</td>
<td>0.62</td>
<td>0.57</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>&lt; 0.0001</td>
<td>0.742</td>
<td>0.66</td>
<td>0.57</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>&lt; 0.0001</td>
<td>0.082</td>
<td>0.52</td>
<td>0.43</td>
</tr>
<tr>
<td>E</td>
<td>30</td>
<td>&lt; 0.0001</td>
<td>0.746</td>
<td>0.64</td>
<td>0.65</td>
</tr>
<tr>
<td>F</td>
<td>30</td>
<td>0.446</td>
<td>0.888</td>
<td>0.74</td>
<td>0.70</td>
</tr>
<tr>
<td>G</td>
<td>30</td>
<td>0.788</td>
<td>0.905</td>
<td>0.61</td>
<td>0.65</td>
</tr>
<tr>
<td>H</td>
<td>29</td>
<td>0.511</td>
<td>0.511</td>
<td>0.52</td>
<td>0.51</td>
</tr>
<tr>
<td>I</td>
<td>29</td>
<td>0.471</td>
<td>0.902</td>
<td>0.70</td>
<td>0.72</td>
</tr>
<tr>
<td>J</td>
<td>25</td>
<td>0.644</td>
<td>0.998</td>
<td>0.68</td>
<td>0.65</td>
</tr>
<tr>
<td>K</td>
<td>27</td>
<td>0.520</td>
<td>0.143</td>
<td>0.60</td>
<td>0.59</td>
</tr>
</tbody>
</table>

**Differences between size classes**

The mussel length distributions of the genetic samples of sites A - E and L were used to divide the sample into a number of size classes, three classes for sites A and E (Table 1) and four size classes for the sites B, C, D and L (Figure 2). As mentioned above, in all locations genotype proportions deviated from HW equilibrium when the sample as a whole was considered, and there was no evidence of significant LD. In contrast, after correction for multiple tests, genotype proportions were in HW equilibrium when each size class was analysed separately for all locations, apart from site A (Fisher’s: p = 0.009). There was no evidence of significant LD in any of the size classes. Furthermore, there appeared to be some heterogeneity among size classes in estimates of heterozygosity (Table 2).
Figure 2. Separation of samples where genotypes were not according to Hardy-Weinberg proportions (Sites A – E) and a size-stratified sample (Site L) of pearl mussels into a number of size classes. Each datapoint represents an individual’s length (mm) and the vertical lines between points denote separation from one size class to the other. Details on the range of mussel lengths in each size class for each location can be found in Table 2.

Discussion

In 10 out of 11 comparisons, genetic samples appeared to be representative of the size ranges present at the collecting sites, based on the comparison of shell lengths with the larger, independent demographic samples. The single unrepresentative sample, from site E, showed a significant heterozygote excess, while four of the other samples showed a significant heterozygote deficiency. In the samples that were size-stratified, significant HW departures observed in the overall samples were not found within the size classes, where the genotypes in each size class were in HW equilibrium, with the exception of site A. At the same time, there was heterogeneity among size classes in the other diversity estimates.

For the genetic samples analysed, mussels were taken at random, mostly on a first-see first-pick basis. The most obvious way to take an unrepresentative sample is by sampling a disproportionate number of larger mussels, given they
Table 2. Genetic diversity parameters for six size-stratified pearl mussel samples. The analysis was performed on both the whole sample (Site A - E and Site L) and on each size class (mussel lengths in mm) separately. Number of individuals (n), p-values associated with tests for HW-equilibrium (pHW) and linkage disequilibrium (pLD), and estimates for observed (H_o) and expected (H_e) heterozygosity are given. P-values in bold are significant after correction for multiple tests.

<table>
<thead>
<tr>
<th>Size class (mm)</th>
<th>n</th>
<th>pHW</th>
<th>pLD</th>
<th>H_e</th>
<th>H_o</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A</td>
<td>21</td>
<td>0.011</td>
<td>1</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>76-79</td>
<td>6</td>
<td>0.045</td>
<td>0.990</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>80-83</td>
<td>8</td>
<td>0.060</td>
<td>0.994</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>90-93</td>
<td>7</td>
<td>0.076</td>
<td>0.986</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Site B</td>
<td>22</td>
<td>&lt; 0.0001</td>
<td>0.836</td>
<td>0.69</td>
<td>0.64</td>
</tr>
<tr>
<td>66-69</td>
<td>5</td>
<td>0.592</td>
<td>1</td>
<td>0.68</td>
<td>0.65</td>
</tr>
<tr>
<td>71-76</td>
<td>5</td>
<td>0.511</td>
<td>0.992</td>
<td>0.67</td>
<td>0.65</td>
</tr>
<tr>
<td>78-80</td>
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<td>0.354</td>
<td>1</td>
<td>0.69</td>
<td>0.68</td>
</tr>
<tr>
<td>88-96</td>
<td>7</td>
<td>0.931</td>
<td>1</td>
<td>0.61</td>
<td>0.67</td>
</tr>
<tr>
<td>Site C</td>
<td>24</td>
<td>&lt; 0.0001</td>
<td>0.641</td>
<td>0.73</td>
<td>0.76</td>
</tr>
<tr>
<td>76-83</td>
<td>6</td>
<td>0.747</td>
<td>0.998</td>
<td>0.70</td>
<td>0.88</td>
</tr>
<tr>
<td>90-93</td>
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<td>0.121</td>
<td>1</td>
<td>0.70</td>
<td>0.82</td>
</tr>
<tr>
<td>94-97</td>
<td>6</td>
<td>0.742</td>
<td>0.999</td>
<td>0.65</td>
<td>0.69</td>
</tr>
<tr>
<td>99-100</td>
<td>5</td>
<td>0.804</td>
<td>0.998</td>
<td>0.63</td>
<td>0.69</td>
</tr>
<tr>
<td>Site D</td>
<td>23</td>
<td>&lt; 0.0001</td>
<td>0.050</td>
<td>0.52</td>
<td>0.43</td>
</tr>
<tr>
<td>54-58</td>
<td>8</td>
<td>0.176</td>
<td>0.765</td>
<td>0.52</td>
<td>0.47</td>
</tr>
<tr>
<td>92-95</td>
<td>5</td>
<td>0.446</td>
<td>0.463</td>
<td>0.57</td>
<td>0.54</td>
</tr>
<tr>
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<td>0.64</td>
<td>0.65</td>
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</tr>
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<td>0.987</td>
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<tr>
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<tr>
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<tr>
<td>93-96</td>
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<td>1</td>
<td>1</td>
<td>0.65</td>
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<tr>
<td>97-100</td>
<td>8</td>
<td>0.878</td>
<td>1</td>
<td>0.58</td>
<td>0.62</td>
</tr>
</tbody>
</table>

are both more visible than smaller mussels and easier to sample for haemolymph. This appeared to be the case at site E, and probably accounts for the significant difference in mussel length distributions between the genetic and
demographic sample. This bias towards larger mussels at this particular location may have been promoted by the fact that the location is generally characterised by more large mussels and a shortage of small mussels when compared with locations elsewhere in Scotland (Tarr, Hastie and Young, pers. comm.). However, in most cases, it would appear that the genetic samples were demographically representative of the mussels at most sampling locations and sampling biases can be minimised by assessing a larger sample at a location for size distribution and then ensuring that those sampled for genetic analysis are broadly representative.

The departures in 5 out of 11 samples from HW equilibrium cannot be accounted for by mistyping caused by factors such as allelic drop-out during amplification or to null alleles. These can be ruled out based on double blind scoring and retyping of samples. This means that, where HW deficits were detected, they could be from samples derived from multiple, independent genetic populations. However, this seems unlikely, given the more or less random dispersal of sperm. Furthermore, there is no other biological evidence suggesting the existence of multiple reproductively isolated populations within one mussel bed. A more likely explanation is that mating among mussels within a single demographic/breeding unit is not random. Different groups of potential breeders within a population may actually contribute to the production of different cohorts. This might occur if only a small number of breeders contribute to the next generation in a given year or, if they do not, that, for other reasons, there is a high variance in reproductive success among breeders that varies from year to year. Genotype proportions are expected to be in HW equilibrium in samples when they derive from a breeding population that is large, has non-overlapping generations, random mating, equal contributions of all breeders to the next generation, and no selection favouring particular genotypes over others; it also assumes no mutation or migration. The freshwater pearl mussel is long lived (100+ years) (Bauer, 1992), has many overlapping generations, and the breeding population size in a given year may be small. In addition, while individuals can be assumed to reproduce many times in their life, whether they do so every year or consistently with age is unknown. If resources for growth and reproduction are limited and reproduction energetically costly, it may not be physically possible for mussels to breed every year, resulting in an increased scope for the non-random mating and the non-random association of genetic variation across cohorts. Also, given the high individual fecundity, reproductive success could potentially be highly
skewed among breeders across cohorts, either by chance or because certain individuals are genetically superior and suffer lower mortality under the particular conditions to which a given year class are exposed as they develop. Finally, mussels are facultative hermaphroditic and potentially capable of selfing (Bauer, 1987). All of these various aspects of the complexity of the freshwater pearl mussel life cycle could potentially give rise to heterozygote deficiencies and, without a better understanding of species biology at each location, will be difficult to disentangle.

The potential causes of significant heterozygote excess are fewer. The most likely explanation is dissortive mating or outbreeding between different genetic groups. However, it seems unlikely that dissortive mating strategies have developed in pearl mussels, especially as the females can self-fertilise (Bauer, 1987). Furthermore, due to the limited dispersal capacity of M. margaritifera (Young & Williams, 1983b; Bauer, 1987) and the absence of significant population differentiation within rivers (unpubl. data), it is unlikely that outbreeding occurs, unless it is due to directed artificial hybridization of mussels from different populations and their re-introduction into the sampling location. What seems more likely is that it is a chance effect that reflects the non-random contribution of small numbers of breeders to a given cohort. This could occur if the offspring of only a few individuals dominate a year class if, by chance, they have a particularly different set of allelic variants at the loci sampled, something that may be marked if the individuals involved are from different cohorts.

The existence, at least in some locations, of within-population structuring is demonstrated by the fact that in some of the samples, when size classes were analysed separately, the significant deviation from HW disappeared. Again this strongly suggests that each cohort may, in at least some locations, be produced by a small number of individuals, with the genotypes in each new generation in HW equilibrium (Christiansen, 1988), unless the new generation is solely produced through selfing. This could be the case for the small population at site A, which was characterised by a high selfing rate both across and within size classes (unpubl. data). In any case, this suggests that, though mussels at a given location are likely to be part of a single genetic population, when considered over evolutionary time scales it is likely that there is significant structuring among cohorts associated with chance non-randomness in breeding each year caused by small numbers of breeders producing each new cohort. If so, then it
may be difficult to obtain a representative sample of the overall population at a location that is not in HW equilibrium, particularly where numbers of breeding mussels are small. This could be addressed by age or size-stratified sampling and ensuring that the full demographic range of the population is sampled in an unbiased way so that observed genotype proportions give an accurate estimate of allele frequencies for the population in a given location.

One interesting observation in the case of site E is that heterozygosity seemed to increase with age, as it was higher in the larger size classes. This could be conceived as supporting the general view that fitness in heterozygotes is higher (Turelli & Ginzburg, 1983; Danzmann et al., 1988). However, the number of individuals in each size class was low, and the result may just be an artefact or chance difference. Despite this, it is interesting and more in-depth research, using larger sample sizes, would be useful to examine these possibilities in more detail, as well as to elucidate the true nature and extent of within-population structuring in this species. This could be most effectively achieved either by taking large size-stratified samples or by sampling glochidia on infected fish over a number of generations (e.g. at 5 year intervals) to ensure that individual cohorts are being analysed, rather than a restricted bin of cohorts, as is the case when a restricted size range of individuals is considered. Examining potential within-population structure in more detail would provide some insight into the basic reproductive biology of this species, which is currently not well understood, though this knowledge is vitally important for pearl mussel conservation.

This preliminary study strongly suggests that care needs be taken when mussels are sampled for genetic analysis, though it also suggests that samples collected, in most cases, will be representative of the overall demographic population. It also points to the likely existence of some within-population structuring which might to some extent be predicted from the fact that the species has many overlapping generations, iteroparity and high potential fecundity of individuals leading to highly unequal reproductive success among individuals. Furthermore, it seems likely that not all individuals of breeding age will breed each year and that, in a given year, only a relatively small number of individuals may breed successfully or even at all. To investigate these issues in more detail, it will be necessary to analyse the genetic variation within and among individual generations, something that is most easily achieved by the sampling of glochidia from infected host fish in different years. Alternatively, size-stratified samples
could be taken from large populations, so that a sufficient number of individuals can be included in each size class. However, with this approach, it will be difficult to be sure that all individuals are from one year class. Combined with this, there is also a need to develop theoretical genetic analysis models that take complex life histories, such as those of *M. margaritifera*, into account, particularly with regard to the effects of overlapping generations and small numbers of breeders per generation, and distinguishing the effects of these factors from those of selfing and the admixture of distinct genetic populations.

**Acknowledgements**

We would like to thank P. Cosgrove and many others who helped with the sampling of mussels. Furthermore, we would like to extend our thanks to the landowners and managers for allowing access to the sites.

**References**


Conservation genetics of freshwater pearl mussel (Margaritifera margaritifera) populations in south Sweden; some preliminary results

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Abstract

Few populations of freshwater pearl mussels in south Sweden have the 20% mussels smaller than 50 mm suggested for viable populations. Therefore it may be necessary to reinforce some populations with mussels relocated from other functional populations. For this to be successful it would be preferable to relocate mussel with a similar genetic constitution. For this reason we investigated the genetic diversity and differentiation of 17 populations in south Sweden. We collected haemolymph from live mussels in Blekinge (4 populations), Småland (2), Västergötland (1), Dalsland (1), Värmland (5) och Närke (4), and used this for microsatellite analyses. An average of 13 alleles was found for the six microsatellites used. Allelic richness and genetic diversity was generally large but varied considerably between populations. Of the total genetic variation, 31% were found among populations. Genetic distance revealed that populations differentiated in three main groups Blekinge, Småland and populations around Lake Vänern. This probably reflects immigration after the glacial period since the genetic differentiation is too high to be the result of selection and/or genetic drift during the relatively short time of population being isolated.

Introduction

The freshwater pearl mussel (FPM) is decreasing in Sweden and the rest of Europe (Young et al. 2001; Söderberg et al. 2008). In Sweden, less than 50% of all populations are viable, meaning that they have less than 20 % mussels smaller than 50 mm (approx. < 20 years of age). In south Sweden this figure is
even lower; approximately 25 % is regarded as viable. This decrease may lead to reduced genetic variation and ultimately reduced fitness.

Although many populations have 10 000 individuals or more, there is reason to believe that the effective population size is much smaller, often less than 10 % of the observed population size (c.f. Paetkau et al. 1998). Therefore studies of genetic variation within streams and drainage areas are important for several reasons.

In the case that it is impossible to protect and restore all populations, genetic studies may be used to determine different conservation units. In Sweden where there are at least 551 streams (Söderberg et al. 2008) it may prove impossible to protect all streams and the economic cost of restoring these streams may prove too large. This implies that it will be necessary to have a conservation strategy based on a selection of streams, and in such a selection process the aim should be to identify different evolutionary significant units (ESU; Fraser & Bernatchez 2001) for future protection.

After protection and restoration, some populations may still fail to produce juveniles, and it may therefore be necessary to enhance these populations with mussels translocated from other genetically similar populations. The reason for this is that it would be favourable to collect genetically close mussels in order to save locally adapted gene complexes and avoid outbreeding depression.

In this paper we report some preliminary results from a study of conservation genetics in 17 populations in south Sweden.

**Material and Methods**

**Study sites**

Samples as collected from 17 populations in the counties of Värmland, Västra Götaland, Örebro, Kalmar and Blekinge, and from 7 main drainage areas (Figure 1, Table 1).
Methods

Samples were taken as biopsies (only from Värmland) or as haemolymph. The number of individuals sampled from each population varied between 33 and 50 except in 2 streams where the number of mussels were lower. In these streams all individuals found were sampled. Estimated population size varies from 10 to 69 200 (Söderberg et al. 2008, own calculations).

DNA was extracted according to standard protocol for cells and tissue (Macherey-Nagel). The amount of DNA was quantified by standard methods and stored at -20°C until used for analyses. Thirteen microsatellite loci were tested on randomly chosen mussels, and six with different levels of polymorphic bands were chosen for this study: MarMa 5167, 4277, 4143, 4315, 3621, 3116 (Geist et al. 2003).

PCRs were performed with 2 µl DNA, 2,5 µl mMdNTP, 1 µl 10XB, 0.075 µl Taq (with MgCl2 in buffer) and 0.1 – 0.4 µl primer. To different PCR reactions were used; TD 55-45 for MarMa 5167, 4277, 4143, 4315 and TD60-50 for MarMa 3621, 3116. PCR products were analysed using the CEQ 8000 Genetic Analysis System (Beckman Coulter).

Figure 1. The location of sample streams in south Sweden.
Table 1. Drainage area and population size of FPM in streams in different counties.

<table>
<thead>
<tr>
<th>Stream Code</th>
<th>Drainage Code</th>
<th>Population size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Billan</td>
<td>Bil</td>
<td>32600</td>
</tr>
<tr>
<td>Dalsälven</td>
<td>Dal</td>
<td>4800</td>
</tr>
<tr>
<td>Öjenäsbacken</td>
<td>Oje</td>
<td>8100</td>
</tr>
<tr>
<td>Torgilsrudsälven</td>
<td>Tor</td>
<td>Göta älv</td>
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<td>Älgån</td>
<td>Alg</td>
<td>66000</td>
</tr>
<tr>
<td>Teåkersälven</td>
<td>Tea</td>
<td>54700</td>
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<tr>
<td>Kolarebäcken</td>
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<td>40700</td>
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<td>Trösläven</td>
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<td>Lerkesån</td>
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</tr>
<tr>
<td>Mieån</td>
<td>Mie</td>
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</table>

Data analyses

Population genetic analyses were performed using GenAlEx software (Peakall and Smouse 2005). Genetic divergence between populations and the hierarchical portioning was estimated using the AMOVA (GenAlEx 6.2). The unbiased genetic distance (Nei et al. 1983) were calculated for all pair-wise comparison of populations (Popgene 3.2) and used to construct phenogram using Neighbour-Joining option in MEGA (Kumar et al. 2003, version 4.1).
Results

The allelic richness varied from 2.17 in Mieån (remember the small sample size) to 5.33 in Pauliström, and the number of effective alleles 1.516 in Ojenåsbäcken to 2.989 in Nättrabyån (Figure 2). Genetic diversity seems to be lower in Göta älv and Norrström drainage areas. Private alleles were found in 8 populations. Most in Nötån with 10 private alleles but the highest frequency was in Nättrabyån with close to 10 percent.

Observed genetic diversity ($H_o$) varied between 0.077 in Torgilsrudsälven and 0.554 in Bräkneån, and the expected heterozygosity ($H_e$) varied between 0.182 in Torgilsrudsälven to 0.643 in Nättrabyån (Figure 3). The observed values are slightly lower than the expected indicating a disequilibrium in some loci. A statistic test of all loci and populations revealed some significant deviations from Hardy-Weinberg equilibrium indicating non-random mating but the pattern was not consistent over different loci for all populations.

Figure 2. Observed allelic richness ($Na$) and expected richness ($Ne$) of FPM in streams in south Sweden.
There was no significant relation between estimated genetic diversity and population sizes (Figure 4). Theory predicts decreasing genetic diversity in decreasing populations but in this case it is unclear if these populations have declined and if so how much. In 11 of these populations no small mussels have been found, and in the rest of the streams the proportion of young mussels are less than 5%, except in Lerkesån with close to 15%.
Of the total genetic variation, 31% were found among populations. Analyses of genetic differentiation and of genetic distances indicate the populations may be divided in three groups (Figure 5). One consists of populations in Kalmar (Emån drainage), one of populations in Blekinge (different drainage areas) and a third consists of populations in the counties Värmland, Västra Götaland and Örebro, and in two different main drainage areas.

**Discussion**

This study indicates, together with an earlier study using RAPD (Hadzihalilovic-Numanovic 2005) that populations of freshwater mussels have a large genetic variation (Geist & Kuehn 2005). The only species with comparable genetic variation in freshwater seems to be the brown trout (Salmo trutta), the host of freshwater mussel larvae. This may indicate that populations originated from different refuges when they settled after the glacial period (Bernatchez 2001) since it seems unlikely that such a large genetic variation is the result of mutations during the short time populations have been present in south Swedish streams. However, part of the genetic variation may naturally be explained by
genetic drift and local adaptations during the time from the postglacial settlement (approx. 6 500 years BC).

Genetic differentiation also indicates different origin of south Swedish populations. After the glacial period a large freshwater lake (Baltic Ice Lake) was created east of Sweden with no contact with the Atlantic Ocean (Tikkanen & Oksanen 2002). At this time there was a connection of the Baltic to the White Sea in the east, and it is possible that populations of freshwater pearl mussels and its host the brown trout in the south-east have an origin in refuges in the east. For about 8 300 years ago a connection was opened with the Atlantic and much of the Baltic ice lake was emptied. After a period (couple of hundred years?) salt water entered the Baltic Sea. At this time Sweden was divided in two parts by the Yoldia Sea, the south part connected to Denmark. This study indicates that population in the counties of Värmland, Västra Götaland and Örebro have the same origin despite having main drainage areas both to the west (Götä Älv to Atlantic Sea) and the east (Norrström to the Baltic Sea) today. It seems possible that all these populations have an origin from refuges in the Atlantic region.

The conservation implications of this study would be that the investigated populations belong to three different evolutionary lineages. Conservation strategies should thus focus on the conservation of a number of populations within each lineage in order to maintain maximum genetic variation if not all population can be maintained for economical reasons. It is also important that genetic studies are conducted in populations in other parts of Sweden in order to maintain all evolutionary lineages present in Sweden. Since the fate of the mussel is linked to populations of its host, it is also of great importance to determine the number of evolutionary lineages of brown trout in Sweden. This may however be a difficult task since trout have been translocated between streams in the past without any consent from different authorities. Unless all populations can be saved, the concept of evolutionary significant unit should be used when conservation strategies of both mussel and trout are developed, i.e. authorities should use a strategy based on conservation units.
References


A summary of relationship of biotic and abiotic factors to recruitment patterns in *Margaritifera margaritifera* from two areas in Sweden

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**Abstract**

The freshwater pearl mussel has declined dramatically all over Europe. Here, we summarize work with the mussel from twenty-four pearl mussel streams in the Vänern area, and twenty-five pearl mussel streams in the Ljungan area. The aim was to investigate the age structure and growth of mussel populations, to identify at what life stage recruitment is failing, to see if recruitment is related to trout distributions and glochidia infection, and to investigate the influence of turbidity and sedimentation on recruitment.

Streams with and without recent recruitment could be separated when using the age structure of each population. Mean annual growth of adult mussels was higher in streams with than in streams without recruitment. There was no difference in the maximum proportion of gravid mussels between streams with and without recent recruitment. Trout densities were similar in streams with and without recent recruitment in the Vänern area. However, trout density of 0+, but not of older trout, was higher in streams with than in streams without recent recruitment in both autumn and the following spring in the Ljungan area. Glochidia infection did not differ between streams with and without recent recruitment in the Vänern area. However, glochidia infection of both 0+ trout and older trout was higher in streams with than in streams without recent recruitment in the Ljungan area.

Mean turbidity was lower in streams with than in streams without recent recruitment in both areas. There were also positive relationships between age of the youngest mussel and turbidity in both areas. Sedimentation was lower in the
streams with recent recruitment than in the streams without recent recruitment in the Vänern area. Generally, there were positive relationships between sedimentation and age of the youngest mussel in the Vänern area.

Turbidity and sedimentation mainly seem to negatively affect the benthic, post-parasitic juvenile stage of the mussel, but may also negatively affect 0+ trout. Sedimentation may hinder water flow in the gravel, resulting in low oxygen and pH conditions and high ammonia concentrations. Sedimentation has several possible sources and often affects whole stream systems. A holistic approach at the catchment scale is needed to reduce the input of fine materials to streams.

**Introduction**

Mollusks are highly threatened worldwide, comprising 42% of all species extinctions (Lydeard et al. 2004). One mollusk group, the freshwater mussels of the order Unionoida, are highly threatened throughout their distribution (Bogan 1993). The threats responsible for the decline are difficult to identify, as many anthropogenic activities probably contribute. Among the major threats, habitat alterations and destructions are among the most common. In freshwaters, such anthropogenic activities lead to an increased input of material to the water, which contributes to large scale habitat degradation (Box & Mossa 1999).

The cause of the decline of a species may be difficult to identify if this species has distinctly different life stages. Thus, it is not simply a question of which factor is threatening the species’ existence, but also which life stage is being affected. This may be particularly problematic for the long-lived unionoid freshwater mussels, which life cycle includes a parasitic stage on a host fish. Thus, any decline in the species with a complex life history may not only be related to direct threats to its own population, but also to threats to the host population (Hastie & Young 2001; Malcolm et al. 2003). Therefore, one may also need to examine the ecology of the host fish population (Bogan 1993; Haag & Warren 2003; Vaughn & Taylor 2000).

Habitat degradation by increased fine particulate material may act on all the stages in the complex life cycle of unionoid mussels. As the mussels are filter feeders, the cost of filtering may increase with decreasing quality of suspended material and result in reduced clearance rates and growth rates (Aldridge et al. 1987; Alexander et al. 1994; Baldwin et al. 2002). Clearance rates also decrease
during brooding, as the mussels brood the larvae on their gills (Tankersley & Dimock 1993a). Factors such as increased turbidity may therefore potentially restrict larval production. If larval production is low, infection on the host fish may also be low. Furthermore, survival of host fish may be reduced by fine particulate matter (Malcolm et al. 2003; Wood & Armitage 1997; Chapman 1990), and reduced numbers of host fish can result in lower numbers of larval infections and consequently reduced recruitment of mussels to the benthic population.

Increased sediment load into streams may also reduce survival of the unionoid mussels’ juvenile life stage, as the juveniles often lives within the sediment. Sedimentation can clog the interstitial spaces within the sediment, which can result in reduced water circulation in the sediment and thereby too low oxygen levels for juvenile mussel survival (Box & Mossa 1999; Wood & Armitage 1997; Buddensiek et al. 1993).

The freshwater pearl mussel, *Margaritifera margaritifera*, is an endangered unionoid species with low levels of recruitment in many European streams (Bauer 1983; Bauer 1986; Cosgrove et al. 2000; Hastie et al. 2000; Morales et al. 2004). The larvae parasitize salmonid fish for 10 – 12 months. Life expectancy of *M. margaritifera* is one of the longest known for invertebrates, with individuals over 200 years (Mutvei & Westermark 2001).

*M. margaritifera* is considered as an umbrella and indicator species, and it is important to investigate the status and threats to the mussel. The aim of this summary was to investigate reasons for recruitment failure in the Vänern area, southwestern Sweden, and Ljungan area, in the middle of Sweden. Specifically, the aims were to investigate the age structure and growth of mussel populations, to identify at what life stage recruitment is failing, to see if recruitment is related to trout distributions and glochidia infection, and to investigate the influence of turbidity and sedimentation on recruitment.

**Materials and Methods**

Twenty-four streams were included in the investigation in the Vänern area in 2005, and twenty-five streams were included in the investigation in the Ljungan area in 2007-2008.
Different mussel surveys were performed, and included mussel counts in transects and squares, and the standardized survey of *M. margaritifera* in Sweden. To determine age and growth of the mussels, growth lines in the ligament and in the mussel shells were counted.

Gravid mussels were investigated every 10-14 days from June until the mussels were no longer gravid. Mussels were placed in buckets with water, whereupon they were carefully opened appr. one cm with tongs. Mussels were considered gravid when the gills had glochidia in their marsupium, which is seen as a “swollen”, creamy colored mass on the gills.

Electro-fishing was performed with a petrol-driven electroshocker (LUGAB), just after the last gravid mussel had been observed in autumn, and just before the mussels were released in spring. The density of fish was estimated from three successive removals of fish using the Zippin method (Bohlin et al. 1989). Captured fish were anaesthetized with tricain methane-sulphonate (MS222), whereupon length and weight were measured. Some fish were preserved in 4% formalin for later investigation in a microscope of encysted glochidia on the gills. In the Ljungan area, a photo was taken on one of the gills on the anaesthetized fish. The numbers of glochidia on the photo were counted and related to the total number of glochidia from the preserved fish. In some occasions, only 0+ fish were sampled, as local authorities wanted the older fish to remain in the stream for future reproduction of both the trout and *M. margaritifera*.

Turbidity (nephelometric turbidity units, NTU) was measured in the field with a portable TN-100 turbidimeter from June to September. On each occasion, water was collected at mean water depth at four random places within each site.

Sedimentation was measured from June to September using modified Whitlock-Vibert boxes. The boxes were filled with washed, small pebbles (16 – 32 mm) before they were placed in the sediment so that the top of the box was even with the sediment surface. Eight Whitlock-Vibert boxes were placed at randomly selected locations in each stream (at the uppermost 5 m length at each site), where the boxes remained until they were collected.
Results

Streams with and without recent recruitment could be separated in the Vänern area when using the age structure of each population. Mussels ten years or younger were always found in streams with recent recruitment, but not in streams without recent recruitment. The density of mussels in the 11-20 year class was higher in streams with than in streams without recent recruitment. There were also growth differences between the stream types, as mean annual growth of adult mussels was higher in streams with than in streams without recruitment (Table 1).

Gravid mussels were found between July and September in all streams in both areas. During this period, the proportion of gravid mussels increased up to a maximum, and then decreased until no gravid mussels were found. There was no difference in the maximum proportion of gravid mussels between streams with and without recent recruitment (Table 1).

Trout densities were similar in streams with and without recent recruitment in the Vänern area. However, trout density of 0+, but not older trout, was higher in streams with than in streams without recent recruitment in both autumn and the following spring in the Ljungan area (Table 1).

Analyses of glochidia infection revealed an effect of mussel density in the Vänern area, and on fish older than 0+ in the Ljungan area. Analyses of glochidia infection on 0+ trout revealed an effect of trout density in the Ljungan area. Furthermore, glochidia infection did not differ between streams with and without recent recruitment in the Vänern area. However, glochidia infection of both 0+ trout and older trout was higher in streams with than in streams without recent recruitment in the Ljungan area (Table 1).

Mean turbidity was lower in streams with than in streams without recent recruitment in both areas. There were also positive relationships between age of the youngest mussel and turbidity in both the Vänern and Ljungan area (Table 1).

Sedimentation of all size classes of inorganic material was lower in the streams with recent recruitment than in the streams without recent recruitment in the Vänern area. In contrast, only the smallest size class of organic material was
lower in streams with than in streams without recent recruitment (Table 1). Generally, there were positive relationships between sedimentation and age of the youngest mussel in the Vänern area.

Table 1. Adult mussel growth, proportion of gravid mussels, trout density of 0+ and older trout, glochidia infection rates, turbidity and sedimentation in the Vänern and Västernorrland areas. --- not measured.

<table>
<thead>
<tr>
<th>Biotic and abiotic property</th>
<th>Area</th>
<th>Streams with recruitment</th>
<th>Streams without recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult mussel growth</td>
<td>Vänern</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Proportion of gravid mussels</td>
<td>Vänern</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>Trout density (0+)</td>
<td>Vänern</td>
<td>No difference</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Trout density (older than 0+)</td>
<td>Vänern</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>Glochidia infection rate</td>
<td>Vänern</td>
<td>No difference</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Vänern</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>Vänern</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Västernorrland</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Discussion

Age structure

The results showed that many Swedish *M. margaritifera* populations have low proportions of juvenile mussels. Viable mussel populations have relatively high proportions of the 11 – 20 year class and at least some mussels in the < 10 year class, which shows that some populations are still viable (Young *et al.* 2001). Age structures also estimate status more exactly compared to length structure since growth may vary between populations. Another advantage of measuring age is the estimate of maximum age, which seemed to differ by 60 years between populations (Österling 2006). A high maximum age may result in a longer time to re-establish recruitment compared to a low maximum age. Furthermore, mussel density is supposed to decrease in the populations where recruitment has ceased, which may have negative effects on future
reproduction. In fact, the density has declined in many streams in Sweden from the 1990s to the 2000s. It is therefore urgent to identify the threats to this mussel species to be able to change the conditions and/or restore the streams.

**Gravid mussels**

The reproduction of *M. margaritifera* generally seemed to function, as the mussels were gravid in every stream and that there was no relationship between proportions of gravid mussels and mussel recruitment. Furthermore, we did not find that the proportions of gravid mussels were related to turbidity and sedimentation, although previous studies have shown that turbidity negatively affect growth of mussels (Aldridge *et al.* 1987; Baldwin *et al.* 2002; Osterling *et al.* 2007). As gravid mussels have lower clearance rates than non-gravid mussels, the effects of turbidity may thus be expected to be greater for gravid than for non-gravid mussels (Tankersley & Dimock 1993b). However, even if there seem to be no effects of turbidity on the gravid mussel life stage, declining densities of adult mussels may result in lower numbers of gravid mussels. This may in turn result in lower glochidial infection rates on trout, with the result of decreased number of juvenile mussels entering the benthic phase. Lastly, low mussel density may potentially result in low proportions of gravid mussels, for example if sperm transfer is reduced in sparse populations compared to more dense populations.

**Trout density**

The density of 0+ trout was lower in streams without than in streams with recent recruitment in the Ljungan area. A low trout density may result in lower total glochidia infections on trout than a high trout density, and thus result in a lower number of juvenile mussels entering the benthic stage (Arvidsson *et al.* 2006). Therefore, low trout density may contribute to a low recruitment of mussels. One explanation of this pattern may be a consequence of high turbidity and sedimentation, resulting in reduced survival of trout eggs buried in the stream sediment. A reduced survival of trout eggs may result in low density of 0+ trout, as was noticed in the streams without mussel recruitment.
**Turbidity and sedimentation**

Turbidity and sedimentation mainly seem to negatively affect the benthic, post-parasitic juvenile stage of the mussel, as this pattern was seen in both areas. Potential mechanisms that may be involved include sedimentation which may hinder water flow in the gravel, resulting in low oxygen and pH conditions and high ammonia concentrations (Wood & Armitage 1997; Buddensiek *et al.* 1993). Sedimentation and turbidity may also be positively correlated with nutrient levels, which can be associated with increased oxygen consumption and/or have toxic effects on the mussels, resulting in reduced survival (Bauer 1988; Dodds & Whiles 2004).

**Summary - restoration**

In summary, relatively clear streams with low sedimentation harbor juvenile mussels, whereas recruitment may fail in turbid streams with high sedimentation. Mussel populations without recruitment are thus facing the risk of extinction. Sedimentation has several possible sources and often impacts whole stream systems (Wood & Armitage 1997). Thus, a holistic approach at the catchment scale is needed to reduce the input of fine materials to streams (Wood & Armitage 1997; Harper *et al.* 1999). This will involve reducing erosion and sedimentation associated with human activities such as forestry and agriculture (Dodds & Whiles 2004; Kreutzweiser & Capell 2001; Nisbet 2001). Furthermore, flushing flows are needed if fine sediments are to be washed out (Ward & Wiens 2001), which is often a problem in regulated streams. Finally, restoring a stream often takes a long time (Wood & Armitage 1997), which means that restoration must start soon if recruitment of mussels is to be re-established.

**References**


A Catchment Management approach to the conservation and restoration of *Margaritifera margaritifera* SAC populations in the Republic of Ireland

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Abstract

The Republic of Ireland is currently compiling 27 sub-basin management plans in order to protect and restore the Natura 2000 populations of *Margaritifera* for which it has responsibility under the EU Habitats and Species Directive. These are for 27 populations of pearl mussels within 19 Special Areas of Conservation (SACs) for *Margaritifera margaritifera*, and one SAC also includes the population of *M. durrovensis*, the Nore pearl mussel. The statuses of the 27 populations vary greatly and are approximately equally divided into 5 different categories. It is demonstrated that the status of each population is strongly correlated with the intensity of landuse within its catchment. It is concluded that, as a first priority, large populations that are close to sustainable should be strongly protected, with a secondary priority being the maintenance of range where loss of that range would be at its most significant.

Introduction

The Republic of Ireland currently has records of live pearl mussels from 139 rivers. Some populations extend into a number of tributaries of the same river system, giving a total of 93 populations. The estimated number of live adult individuals in the country is greater than 12 million. Juvenile numbers vary greatly in different rivers, but from size profile survey work carried out between 1990 and 2009; their numbers are considered to be currently much lower than in previous years. The lack of adequate survival of juvenile mussels during the first 5 years of life when they are fully buried within the river bed sediment due
to unsuitable levels of fine sediment infiltration is considered to be the key problem in most if not all populations. While juvenile mussels can still be found in many rivers, their numbers are such that if these levels of recruitment continue, they will be unsustainable in the long term and the pearl mussel will become extinct in the country.

There are two protected taxa of *Margaritifera* in the country, *Margaritifera margaritifera* and *M. durrovensis*. The latter is a single population in the River Nore that lives in very hard water (Fig. 1). While genetic and other studies to date have had mixed results regarding the level of difference between the Nore population and its neighbouring mussels (Chesney et al. 1993, Moorkens 1996, Holmes 2001, Machordom et al. 2003), it remains protected in its own right by the EU Habitat’s Directive, and it is perilously close to extinction. The imminent extinction of the taxon has been documented for the last 15 years (Moorkens & Costello 1994, Moorkens 2004a, 2005, 2009), and the 2009 estimate of 300 living individuals, a decline of 85% since standard monitoring for the species was implemented 18 years ago (Fig. 2).

![Figure 1. The Nore pearl mussel Margaritifera durrovensis.](image-url)
The Catchment approach to *Margaritifera* conservation

There are 19 Special Areas of Conservation comprising 26 populations of *M. margaritifera* and the only population of *M. durrovensis* that form part of the Natura 2000 complement of designated sites under the European Council Directive on the Conservation of Habitats, Flora and Fauna (92/43/EEC). The lack of adequate recruitment of young in all Irish pearl mussel populations has led to the two Irish *Margaritifera* taxa being reported to the EU as being in unfavourable-bad conservation status (National Parks and Wildlife Service 2008). The Habitats Directive states in Article 1 that conservative status of a natural habitat will be taken as "favourable" when:

- its natural range and areas it covers within that range are stable or increasing, and
- the specific structure and functions which are necessary for its long-term maintenance exist and are likely to continue to exist for the foreseeable future.

There is a requirement, therefore, to improve the status of *Margaritifera* in Ireland. As the problems for the species are arising from catchment pressures, either as direct sediment passing into the river channel, or indirectly as decaying organic sediment following algal blooms and other decaying vegetation, the catchment management approach was considered to be essential for the

As the main mechanism for managing catchment protection in Ireland is the River Basin District level under the Water Framework Directive, 27 sub-basin plans are being prepared using the resources and approach of the larger River Basin Catchment Plans. The main approach is to assess the pressures within the catchments that are negatively affecting the pearl mussel populations, or would negatively affect their return to favourable conservation status, and to outline measures that need to be taken in order to remove these pressures. In order for the measures to be implemented, they need to be clear in nature, specific in their locations, and responsibility for their implementation identified. The location of each measure should ideally be shown on a map.

As the rivers are not in favourable condition for *Margaritifera*, it therefore follows that they should not be listed as in good condition under the Water Framework Directive. For that reason, rivers that are within SACs designated for *Margaritifera* have not been given “good” status. In addition to preparing sub-basin catchment plans, the Republic of Ireland has recently introduced stronger legislation to protect the freshwater pearl mussel (Statutory Instrument No. 296 of 2009) and so aid the implementation of the management plans.

**Information used to prepare sub-basin management plans**

Using information available for the sub-basins of the *Margaritifera* SACs from the Water Framework Directive Geographical Information Systems (GIS) datasets has meant that a wealth of information was readily available in digitised form. Important datasets include soil types, CORINE land cover maps, livestock densities, forestry coverage and age of planting of commercial plantations, damaged areas within commonage grazing areas, point source discharges, locations and numbers of on-site waste water sources, phosphorus risk areas, percolation risk areas, areas of peat cutting and river segments that have been arterially drained.

In addition to remote data, a series of surveys were undertaken in all 27 catchments, including a walk over survey to identify pressures along the river and hydro-morphological problems, a survey of salmonid fish and glochidial
loading, a macro-invertebrate and a phytobenthos survey to indicate where water quality pressures may be arising, a redox potential survey to assess river bed sedimentation and population surveys of the pearl mussels themselves. Surveys for *Margaritifera* included a) an assessment of the distribution of mussels within the designated river systems, b) an estimate of the numbers of adult mussels and their densities within each population, c) an estimate of recruitment success from size profiles measured from 0.5m x 0.5m quadrats at various locations of potential juvenile habitat within the population and d) from permanent repeatable transects used in the ongoing monitoring of these populations.

**Results of surveys and catchment information and their implications**

From the results of survey work, *Margaritifera* populations can be ranked into 5 different groups according to the size of the population, density of mussels within appropriate habitat, and presence of juveniles (Table 1).

**Table 1.** Ranking system used to divide *Margaritifera* SAC Catchments into status groups from 1 (best) to 5 (worst).

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Definition</th>
<th>Number of catchments (% of catchments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very large populations of adults (500,000+), all ages of juveniles, some juveniles in more than one area</td>
<td>5 (18.5%)</td>
</tr>
<tr>
<td>2</td>
<td>Large widespread populations of adults, or smaller numbers in good but restricted habitat, some juveniles in more than one area</td>
<td>5 (18.5%)</td>
</tr>
<tr>
<td>3</td>
<td>Large numbers of adults, some decline from larger numbers evident, few juveniles</td>
<td>6 (22.2%)</td>
</tr>
<tr>
<td>4</td>
<td>Small numbers adults from historical evidence (&lt;20,000), very few juveniles</td>
<td>5 (18.5%)</td>
</tr>
<tr>
<td>5</td>
<td>Very poor population of adults (&lt;10,000), few or no juveniles</td>
<td>6 (22.2%)</td>
</tr>
</tbody>
</table>

As the key pressures on the *Margaritifera* catchments in Ireland are those leading to siltation and nutrient enrichment, it is no surprise to see that the more intensively managed a catchment is managed, the greater the negative impact has been on the *Margaritifera* population. Indeed, the rivers that are closest to good status are within catchments that have close to natural habitats with very low intensity usage. Some summary statistics are presented in Table 2, based on
chi² calculation on deviations of the various status groups from the average of all 27 catchments. The average sizes of the poorest status catchments are on average 8 times larger than those of the best status catchments. Low intensity land use cover was estimated from the CORINE database, and in Ireland this includes peat bogs, natural grassland, moors and heathland, broad-leaved forests, transitional woodland scrub, inland marshes, bare rock and sparsely vegetated areas. The average percentage of these combined categories within a catchment for the highest status populations was 91.85%, compared with only 33.85% total for these combined categories in SACs in the worst status category. The corollary values of 8.15% (for the best status catchments) and 66.15% (worst status) were found for intensive land use categories, which included urban land uses, arable and intensive pastures, and coniferous forestry plantations. The top 14 best status catchments all have lakes upstream of Margaritifera habitat.

The correlations between the status of the Margaritifera populations and the land use of the catchment sub-basins within which they occur are unequivocal. However, the timeframe of the population declines needs also to be addressed in order to fully understand the implications into the future. From a simple look at the figures, it can appear as if it is possible to retain smaller populations in rivers, even with some intensive land use. However, this is misleading. The lifespan of adult Margaritifera individuals is longer than the timeframe of the intensification of land use in Ireland, thus the age/size profile and the success of the juvenile stage of the populations is of more use in predicting the long term fate of population survival into the future than the presence or numbers of adults alone. The lower the numbers of juveniles within these populations, the less chance there is for the populations to survive if present catchment management continues. Thus catchment issues need to be addressed in even the largest populations with the highest numbers of juveniles, because in the absence of remediation, the populations will eventually decline.

An example of a change in size profile in a large Irish population is shown in Figure 3. This shows the range of sizes measured in quadrat analyses in 1990 from work for a student thesis (Moorkens 1991), and from data collected during work carried out for the National Parks and Wildlife Service (Moorkens 2001, 2004b, 2006b).
Table 2. Status of *Margaritifera* population compared with size of catchment (km²), land use in catchment and presence or absence of a lake upstream (* = significant at 0.05; ** = significant at 0.01).

<table>
<thead>
<tr>
<th>Rank of <em>Margaritifera</em> population status</th>
<th>Number of catchments</th>
<th>Average size of catchment (km²)</th>
<th>Average percentage extensive land cover (CORINE)</th>
<th>Number (Percentage) of catchments with lakes upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>72</td>
<td>91.85%</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>117</td>
<td>80.85%</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>86</td>
<td>77.47%</td>
<td>5 (83%)</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>158</td>
<td>33.6%</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>613</td>
<td>33.85%</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Total (mean)</td>
<td>27</td>
<td>(218)</td>
<td>(48.31%)</td>
<td>(37%)</td>
</tr>
</tbody>
</table>

Significance

![Figure 3a. Example of a declining population 1990 - 2006. Size distribution profile 1990.](image-url)
Figure 3b. Example of a declining population 1990 - 2006. Size distribution profile 2001.

Figure 3c. Example of a declining population 1990 - 2006. Size distribution profile 2004.
The results of the survey work and the information on the catchments have helped to identify both the specific measures and the locations in which they need to be carried out, both in reduction of pressures at source, and along pathways towards the river.

In the largest populations, the pressures should preferentially be removed at source, i.e.:

- revert to lower grazing regimes
- final felling of coniferous plantation where it would remain a risk to the population if replanted
- installation of effective buffer zones
- fencing off of grazing animal access to the river
- provision of alternative drinking water troughs
- upgrading of on-site waste water treatment
- protection against further intensification of land use without evidence that it will not negatively effect the Margaritifera status of the population.

The greatest concentration of effort should be at the Margaritifera habitat areas and at important sources of silt and nutrient upstream.

In more intensively used catchments, more effort may be needed in removing negative influences along pathways, such as with the installation of sediment traps that are large enough to relieve pressure from the sub-catchment of origin.
of the sediment (e.g. a large drain or small stream). Direct connectivity of drains into rivers is a serious ongoing problem and considerable effort will be needed to restore a more natural situation, such as by diverting direct drains to alternative routes with sufficient silt and nutrient trapping areas.

With the best of the *Margaritifera* populations living in small sized, remote catchments with adult mussel numbers of up to 3 million individuals, there should be no reason why numbers of juveniles should not be brought to sustainable levels into the future. However, in larger catchments that are very intensively managed, particularly with pearl mussel habitat restricted to lower reaches of the river, the task of restoring sustainable juvenile recruitment must be considered to be extremely difficult or in some cases impossible within the timeframe of the current population. In some cases the intrinsic habitat for the species is damaged (with silt) or lost (washed away). Many of the poorer catchments do not have lakes upstream, and in the past these upper catchment areas had natural peat habitats that absorbed large volumes of rainwater and slowly released them, thus buffering extreme rainfall events in the same way that an upstream lake does. Most of these upland peat habitats in large catchments have been very effectively drained for agriculture, forestry or peat exploitation. Damage in some of these catchments is irreversible within the mussels’ lifetime. In these situations, much of the *Margaritifera* gravel habitat in the upper river areas has been removed through scouring in high flood situations. In the absence of juvenile mussel habitat, the introduction of buffers and sediment traps would be of little use, thus measures to be implemented in the poorest mussel populations need to be considered very carefully in order to put limited resources to the best possible use.

Article 3.1 of the EU Habitats Directive specifies that Natura 2000 sites be designated so that the species' habitats concerned be maintained or, where appropriate, restored at a favourable conservation status in their natural range. This has resulted in a range of rivers in Ireland being designated across the country, both in remote westerly areas and the more intensively managed east. However, Article 4.1 states that for aquatic species which range over wide areas, such sites will be proposed only where there is a clearly identifiable area representing the physical and biological factors essential to their life and reproduction. This has normally been interpreted as the requirement to determine important areas for highly mobile species, but in the case of *Margaritifera*, it is clear that due to the longevity of the species, remnant
populations exist in rivers where factors essential to their life and reproduction have been destroyed. Much of this loss may have occurred prior to the implementation of the Habitats Directive.

The precautionary principle quite rightly demands that every effort should be made to allow for recovery of populations in rivers where decline has occurred if there is any chance that reversal of poor management practices could lead to improved and sustainable status for the mussels. This is particularly important for areas of the EU where remnant populations remain that are important range extensions that would otherwise be lost. However, it seems a particularly unsatisfactory interpretation of the Habitats Directive to place large efforts from limited resources into rivers that have very poor chance for restoration and to allow other rivers that have no designation, but have recoverable populations of *Margaritifera* to decline, because of choices based on minor range variation.

Of absolute importance in countries that still have a relatively large resource of pearl mussels is the need to focus efforts firstly on protecting and restoring populations that are close to sustainable. This can be achieved if effective catchment measures are put in place. In placing emphasis firstly on protecting the largest populations of the species and secondly on the maintenance of range within the EU, the future of the species would be most effectively protected from extinction.

**Acknowledgements**

The sub-basin plans have been compiled by RPS Consultants for the North South Share 2 project, and funded by the Republic of Ireland Department of the Environment, Heritage and Local Government. *Margaritifera* survey work was carried out by Ian Killeen and Eugene Ross as well as by the author, and thanks are due to them and to Sheila Downes and Bernie Ni Chathain for summary catchment information.
References


Improving forest management plans to support implementation of the EU Water Framework Directive: the freshwater pearl mussel (*Margaritifera margaritifera*, L.) as a tool

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Abstract

Implementing policies about ecological sustainability of riverine landscapes is a paramount challenge for planners and managers. Reflecting both instream and upstream catchment conditions and the fitness of local freshwater pearl mussel (*Margaritifera margaritifera* (L.)) populations is an indicator of the ecological integrity of forest streams. Land cover composition and land use intensity have been dramatically changed in the post-industrial forest landscape with increased proportions of coniferous forests, shorter regeneration times and increased drainage activities. This makes forest planners and managers key actors for introducing spatial planning at local to landscape scales using pearl mussel conservation as a tool. Along with viable trout (*Salmo trutta*) host populations, planning and management of riparian zones and catchment land cover is crucial for successful conservation of the freshwater pearl mussel. The planning process and design of forest management plans was studied by interviewing 15 forest planners in southern Sweden. We found that vital information associated to aquatic and riparian infrastructures were missing in the planning process. There were also gaps in the communication and information exchange among planners, managers and other actors involved with achieving good ecological status. Planning for conservation of viable freshwater pearl mussel populations as a focal species for ecological sustainability requires spatially explicit land cover data, and knowledge about host species at multiple scales. To put local in-stream conditions and land cover composition in a larger spatial context would increase the opportunity to understand consequences of cumulative effects on stream biota and to prevent decreased in-stream conditions, and thus ecological
sustainability. Landscape planning that considers both ecological and social systems is an approach to avoid this. To promote informed decisions associated to jurisdictional responsibility and precautionary activities in association to the EU Water Framework Directive there is a need for including additional spatial information into forest management plans. We discuss the need and importance of species like the freshwater pearl mussel for communication among actors, local participation and engagement of stakeholders.

Introduction

Water is an important component to consider for the development of ecologically sustainable landscapes. This is emphasized in the EU Water Framework Directive (Directive 2000/60/EC) and in many of Sweden’s 16 national environmental quality objectives (www.miljomal.se). Being dominated by forest landscapes, there is in Sweden an increased interest from forestry actors to consider water related issues, and to adapt forestry practices associated to aquatic and riparian environments (Ring et al. 2008). Streams and the environments surrounding water often are species-rich, and many plants and animals are dependent on their environment being regularly disrupted by flooding and cumulative up-stream structures and processes (Covich 1993, Törnblom 2008). High ecological integrity in headwater streams provides favourable conditions for ecosystems downstream (Törnblom 2008), and thus support the implementation of the EU and national water-related policies and ultimately ecological sustainability.

Streams and associated riparian habitats are species-rich ecosystems (Covich 1993, Décamps et al. 2009), and provide vital ecosystem services (Costanza 1991) and natural and cultural landscape values. Species richness and diversity is linked to the habitat structures and the functionality of processes in ecosystems (Törnblom & Angelstam 2008). Terrestrial animals like the otter (Lutra lutra), beaver (Castor fiber), pond bat (Myotis daubentoni), and a number of bird species are fully dependent on the catchments’ productions of insects, fish and aquatic plants and other food resources. Natural processes like fire, snow, wind and flooding affects the input of dead wood structures in streams, which is beneficial to many terrestrial specialists. Riparian zones offer not only food and protection, but also very important dispersal corridors at the landscape level (Törnblom 2008). Being dependent on factors at the scales of streams, riparian
zone and catchment land cover freshwater pearl mussel (*Margaritifera margaritifera*) is a focal species and potential indicator of ecological integrity as defined by the EU Water Framework Directive. Land cover composition and land use intensity have been dramatically changed in the post-industrial forest landscape with increased proportions of coniferous forests, shorter regeneration times and increased drainage activities (Ring *et al.* 2008). This makes forest planners and managers key actors for pearl mussel conservation as a proxy for planning in support of ecological sustainability of riverine landscapes.

Ring *et al.* (2008) reviewed how different forestry operations cause chemical and physical disturbances to aquatic habitats in Sweden. Forestry and forest management activities close to aquatic environments can influence stream morphology, water chemistry, substrate quality, insolation, water temperature, oxygen concentration, organic materials and nutrient supply. The risk for habitat deterioration is generally highest in conjunction with final felling, off-road forwarding of wood, site preparation, and soil drainage, since these operations can increase the amount of eroded material reaching the surface water and its rate of transport there (Ring *et al.* 2008). The effects of forestry on surface water are influenced by many factors as historical land and water uses, current environmental problems such as acidification and eutrophication, and natural disturbances like forest fires and wind throw (Ring *et al.* 2008). Also, a number of catchment associated attributes as land use, tree species composition and incorrectly or inappropriately sized road culverts constitute cumulative effects on stream biota at multiple scales within a catchment (Törnblom 2008).

In this context the forest management plan (FMP) and associated maps and data bases could be of vital importance as information documents showing today’s situation, and guide future land and forest management. This information is vital for building functional terrestrial and aquatic habitat networks that support ecosystem processes and species composition at multiple spatial and temporal scales of landscapes and catchments (Törnblom & Angelstam 2008).

Usually foresters develop and implement FMPs relying on tree inventories showing an area’s topographical features as well as its distribution of trees (by species and age class) and other plant cover (Maser 1994). FMPs may also include information about bearing capacity, roads, culverts, proximity to human
habitation, hydrological conditions, and soil information. The FMP includes the projected use of the land and a timetable for that use. Traditional FMPs focus on providing round-wood mainly for timber, pulp and paper, and bioenergy. Hence, considerations of product quality and quantity, employment, and economic profit have been of central, though not always exclusive, importance. Foresters frequently develop post-harvest site plans for reforestation, weed control, fertilization, or thinning. The objectives of landowners and leaseholder influence plans for harvest and subsequent site treatment. Foresters consider tree felling and environmental legislation when developing plans. Plans instruct the sustainable harvesting and replacement of trees. They indicate whether road building or other forest engineering operations are required.

In this study we investigated what forest planners knew about water issues and their opinions about how forestry affects the aquatic environments. Does this key group of planners consider water issues in their work with the development of the FMP? How is that knowledge or awareness implemented and manifested in the FMP?

We asked fifteen forest management planners in the South of Sweden four questions concerning their knowledge about: 1) the EU Water Framework Directive, 2) how they thought a riparian zone close to streams should be managed, 3) what type of advice they give concerning driving routes over or close to streams, 4) what improvements could be made in the forest management plan to develop considerations to aquatic environments within operational forestry practices. Finally, we discuss how the present FMPs can be developed to better consider water issues.

Material and methods

Study area and selection of forest planners

This study deals with forest planning in the Götaland region in the southern part of Sweden. Here, non-industrial privately owned forestry dominates. One key actor in forest planning is the forest owner organization Södra skogsägarna, which manages an associated member area just above 2.3 million hectare. On the market there are also several other companies and privately owned saw mills, which also buy raw timber. Common practice is that the buyer visits the
site in the field to establish a management description for afforestation after the contract on the right to cut has been signed. The respondents were associated to the Swedish National Board of Forestry (n=9) and “LRF Konsult” (n=6), which is a forest consultancy bureau of the national organization for agriculture (LRF).

The interview manual

The survey was conducted as telephone interviews with qualitative semi-structured questions (Trost 1991, Bryman 2001). A standard cordless desktop telephone was used. A recording apparatus was then connected to this, which in turn was connected to a computer. The interviews were recorded on the computer and then saved as digital files. Usually the interviews started immediately after telephone contact had been established. The interviewer then presented the case and conducted the interview as follows.

Hello my name is…and I’m doing a study dealing with water issues in forest management planning. I wonder if you’ve been involved with planning the last two years and if you then have time to answer four short questions?

Do you know about the EU Water Framework Directive and the Swedish water management ordinance (if so, could you explain how the EU Water Framework Directive will affect forestry and your own planning close to aquatic environments)?

How do you think a riparian zone along rivers and streams should be managed and is this something you apply in your own planning?

What kind of advice and instructions do you give concerning forwarding and off-road routes over or close to streams?

What improvements do you suggest could be done in the forest management plan to develop the considerations to aquatic environments within operational forestry practices?
Results

Understanding of policy

We used the EU Water Framework Directive as an entry and indicator of planners' knowledge concerning the need for a multi-scale catchment perspective to reach good ecological status. The first question had two parts. The first identified if the respondents had heard about the EU Water Framework Directive, and the second part was posed to validate the answer and see if they can relate to how the EU Water Framework Directive initiative could affect them selves in their own profession. A number of respondents answered “yes” very quickly at the first question, but hesitated and then admitted that they could probably only tell very little or anything about the EU Water Framework Directive, its ambitions and objectives or how it would affect their own planning activities of forestry. Only two respondents knew what the EU Water Framework Directive really is about (Figure 1). These respondents mentioned that the ambition was to reach a certain ecological status and that there are five Swedish water authorities that are now working with correlating their work with classification of surface waters. However, none of them could relate to how this EU Directive could affect forestry or the respondent own planning and management activities. In fact none of the fifteen respondents could describe or give an example of how the EU Water Framework Directive could affect forestry or have implications on their own work (Figure 2).

Figure 1. Two out of fifteen professional forest management planning respondents could say that they know what the EU Water Framework Directive is about.
Could you explain how the EU Water Framework Directive will affect forestry and your own planning close to aquatic environments?

![Bar chart showing responses](chart.png)

**Figure 2.** In this study none of the fifteen forest management planning respondents could describe or give an example of how the EU Water Framework Directive could affect forestry or have implications on their own forestry planning.

To be more concrete within the operational forestry planning, we asked the respondents how a riparian zone close to rivers and streams should be managed. The respondents came up with various suggestions. The three most frequently mentioned answers were that the riparian zone should be dominated by deciduous trees (n=10), or set-aside from normal production forestry (n=7). Suggestions like it should be a “varied riparian zone that follows the nature surrounding the stream” was followed by the respondent making a description of how the riparian zone should be designed and managed in the forest management plan (n=6). These suggestions were followed by several others not as frequently mentioned, but still a source of knowledge concerning forest management planners knowledge and attitudes concerning riparian zones (Table 1).
Table 1. How does a forest management planner think a riparian zone close to rivers and streams should be managed?

<table>
<thead>
<tr>
<th>Rank</th>
<th>Riparian management proposals</th>
<th>Respondents (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>It should be deciduous dominated</td>
<td>(n=10)</td>
</tr>
<tr>
<td>2.</td>
<td>I use the management classes NS and NO or PF</td>
<td>(n=7)</td>
</tr>
<tr>
<td>3.</td>
<td>Varied zone that follows the nature surrounding the stream</td>
<td>(n=6)</td>
</tr>
<tr>
<td>4.</td>
<td>I use to describe what I mean in the management plan</td>
<td>(n=6)</td>
</tr>
<tr>
<td>5.</td>
<td>It depends on the type of property and quality of streams</td>
<td>(n=5)</td>
</tr>
<tr>
<td>6.</td>
<td>It should at least be 10 meters wide</td>
<td>(n=4)</td>
</tr>
<tr>
<td>7.</td>
<td>I use general considerations (PG)</td>
<td>(n=2)</td>
</tr>
<tr>
<td>8.</td>
<td>It should be multilayered</td>
<td>(n=2)</td>
</tr>
<tr>
<td>9.</td>
<td>Dead wood is important</td>
<td>(n=2)</td>
</tr>
<tr>
<td>10.</td>
<td>I use to describe how to avoid driving damages</td>
<td>(n=2)</td>
</tr>
<tr>
<td>11.</td>
<td>I propose successive cutting</td>
<td>(n=1)</td>
</tr>
</tbody>
</table>

Swedish forestry is fully mechanized and harvesting is usually made by one processor and forwarder working as a team. When it comes to practical advice and instructions concerning terrain forwarding, off-road driving and the risks of rutting, soil compaction, erosion, oil, lubricants and oil spilling close to, or when passing streams and other aquatic environments, the three most frequently mentioned advices and instructions were to avoid even forwarding in humid areas (n=7), or if trespassing is urgently needed it should be done over a bridge or at least only at one site instead of over several different sites (n=7). These two were followed by suggestions concerning putting enough branches in the forwarding routes to decrease damages on the hydrology and prevent soil erosion (n=6). These advices and information were followed by several others not as frequently mentioned but still a source of knowledge concerning forest management planners ambitions to avoid damages on aquatic environments caused by driving and driving routes (Table 2).

Table 2. What kind of advice and instructions do forest management planners give concerning driving routes over or close to streams?

<table>
<thead>
<tr>
<th>Rank</th>
<th>Advice and instructions concerning driving routes over or close to streams</th>
<th>Respondents (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Avoid driving in humid areas</td>
<td>(n=7)</td>
</tr>
<tr>
<td>2.</td>
<td>If trespassing is needed do it over a bridge at one site</td>
<td>(n=7)</td>
</tr>
<tr>
<td>3.</td>
<td>Put enough branches in the driving routes to decrease damages and soil erosion</td>
<td>(n=6)</td>
</tr>
<tr>
<td>4.</td>
<td>Put in trees to avoid damages of streams</td>
<td>(n=4)</td>
</tr>
<tr>
<td>5.</td>
<td>Drive as little as possible close to aquatic environments</td>
<td>(n=3)</td>
</tr>
<tr>
<td>6.</td>
<td>Careful driving in general close to humid areas</td>
<td>(n=2)</td>
</tr>
<tr>
<td>7.</td>
<td>Build retention dams</td>
<td>(n=1)</td>
</tr>
<tr>
<td>8.</td>
<td>Written instructions for driving routes on map</td>
<td>(n=1)</td>
</tr>
</tbody>
</table>
Proposals to improve implementation?

The respondents were finally asked to come up with suggestions on improvements of how the forest management plan could be improved with consideration to rivers, streams and aquatic environments in general within the operational forestry practices. The three most frequently mentioned improvements were to develop more detailed aspects in the description codes that could be associated to aquatic environments (n=8). This was followed by the suggestion to write out more detailed forwarding routes on an actual maps (n=6), and also to develop and improve the verbal transmission of information between planners and operative forest management by concrete descriptions of codes associated to the size and composition of riparian zones (n=5). These suggestions were followed by several other interesting suggestions that could be seriously considered for future forest management plans (Table 3). Summarizing, respondents were aware of the need for more detailed information to improve forest management planning considering aquatic environments, both written on maps and orally transmitted.
<table>
<thead>
<tr>
<th>Rank</th>
<th>Improvements in the forest management plan to improve consideration to aquatic environments</th>
<th>Respondents (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Develop more detailed description codes associated to aquatic environments</td>
<td>(n=8)</td>
</tr>
<tr>
<td>2.</td>
<td>Write out more detailed driving routes on maps</td>
<td>(n=6)</td>
</tr>
<tr>
<td>3.</td>
<td>Develop verbal descriptions of codes associated to the size and composition of riparian zones</td>
<td>(n=5)</td>
</tr>
<tr>
<td>4.</td>
<td>Bring in more water related issues and information in the plan since they have been missed so far</td>
<td>(n=5)</td>
</tr>
<tr>
<td>5.</td>
<td>Use the map more so an understanding for the catchment perspective and downstream effects can be wider understood</td>
<td>(n=4)</td>
</tr>
<tr>
<td>6.</td>
<td>Add water related values as valuable species and red-listed species or other information concerning water quality in the descriptions</td>
<td>(n=4)</td>
</tr>
<tr>
<td>7.</td>
<td>Improve the oral dialogue and information transfer from planner to stakeholder concerning water related issues and other important values</td>
<td>(n=3)</td>
</tr>
<tr>
<td>8.</td>
<td>Since there are under entrepreneurs involved it doesn't matter what we say in reality</td>
<td>(n=2)</td>
</tr>
<tr>
<td>9.</td>
<td>Perhaps something should be mentioned concerning what is obliged to do close to water environments</td>
<td>(n=1)</td>
</tr>
<tr>
<td>10.</td>
<td>It is a balance between costs and richness in details</td>
<td>(n=1)</td>
</tr>
<tr>
<td>11.</td>
<td>You could work with more precautionary actions, but then you would probable get stuck with it doing nothing else</td>
<td>(n=1)</td>
</tr>
<tr>
<td>12.</td>
<td>Focus on a minimum deciduous proportion within the actual stand or unit from a landscape perspective</td>
<td>(n=1)</td>
</tr>
<tr>
<td>13.</td>
<td>Write in suggestions for improved ditching activities</td>
<td>(n=1)</td>
</tr>
<tr>
<td>14.</td>
<td>The forest management plan is already as good as it can be</td>
<td>(n=1)</td>
</tr>
</tbody>
</table>

Discussion

Several gaps

Swedish forestry and associated operations have been mentioned as serious threats to aquatic environments (Ring et al. 2008). The EU LIFE-project "Forest for Water" pointed out that the forest sector has no tradition of water management in their planning, and pointed to the importance of an adapted forest management plan (www.skogsstyrelsen.se/life). This survey of fifteen professional forestry management planners, in the most Southern region of Sweden, revealed that very few of the respondents knew about the EU Water
Framework Directive in spring 2009. This in spite of the fact that the EU Directive came into force in Sweden already by the year of 2000, and is expected to be the main tool for reaching good ecological status or potential in all Sweden’s surface waters in 2015. Our results shows clearly that the respondents could neither relate to how the EU Water Framework Directive, with its ambitions to manage water from a catchment perspective, encourage local participation and implement the polluter pay principle and finally reach good ecological status in all Swedish surface waters. Neither could the respondents relate the EU Water Framework Directive to their own planning activities of forestry practices. These results are far from an ideal situation for the implementation of the EU Water Framework Directive or for those endangered species that can be affected by operational forestry activities as the threatened and red-listed freshwater pearl mussel.

However, there were some considerations mentioned associated to the management and design of the riparian zone close to streams and rivers. Ten out of fifteen respondents answered that the riparian zone should preferably be deciduous dominated and seven out of fifteen responded that it should be set-aside. Six out of fifteen respondents suggested that the riparian zone should be varied and follow the nature surrounding the stream, and six out of fifteen respondents answered that they use to write in the forest management plan how the riparian zone should be managed.

Concerning practical advice and instructions for off-road driving and routines and routes for forwarding round-wood in the terrain and over or close to streams and other aquatic environments, the respondents main advice was to avoid driving in humid areas, and the respondents answered that if trespassing is absolutely needed it should be done over a bridge or at one site instead of several different sites. The respondents also suggested putting branches in the driving routes to decrease damages on the hydrology and prevent soil erosion.

The respondents suggested improvements in the forest management plan and the most frequently mentioned improvement was to develop more detailed aspects in the description codes that could be associated to aquatic environments. There were also several suggestions of more detailed maps with suggested driving routes on maps, and some of the respondents suggested a more developed oral transmission of knowledge, information and concrete
codes associated to the size and composition of riparian zones between the planner and the operational forest manager.

This study reveals severe gaps concerning knowledge, transmission of information, ability and ambition to improve operational forestry practices and considerations close to aquatic environments. More detailed information on nature values and aquatic aspects were wanted by forest management planners. Additionally, among several entrepreneurs associated to the operational forestry practices there is an attitude that the less you know the less chance to get caught if there will be any serious damages associated to driving or cutting close to aquatic environments. Knowledge, information along with time and money are often mentioned as the most important driving forces for not take considerations to associated aquatic environments (Sundin 2007).

*The freshwater pearl mussel as guide for revising forest management plans*

It has been emphasized that freshwater organisms are generally much more imperiled than terrestrial organisms (Master *et al.*, 2000; Dudgeon *et al.*, 2005; Strayer, 2006). The waters are the mirror of the watershed (Hynes, 1975), and the complex life history of the freshwater pearl mussel makes it sensitive to land use and thus an excellent indicator of the ecological integrity and sustainability of waters affected by human activities. Only by very simple knowledge concerning the status of species like the freshwater pearl mussel the forest management planning could be very much improved considering the distribution and connectivity of viable populations of the freshwater pearl mussel and associated structures and habitats and host fish species like brown trout and salmon.

Species can be used to indicate the presence of other species (umbrella species) (Fleishman *et al.* 2001), may have large effects on the distribution and abundance of other species (key-stone species), and be identified as the most sensitive to a threat in the landscape (focal species) (Lambeck 1997). The freshwater pearl mussel is classified by IUCN as "endangered". The species is also included in the EU Habitat Directive (Appendices 2 and 5) and therefore protected in the Natura 2000 system. In Sweden the fresh water pearl mussel has disappeared from 40-50% of the sites where it was observed at the beginning of the 20th century (Eriksson *et al.* 1998, Cederberg & Löfroth 2000).
Impoundments, pearl fishing, acidification, eutrophication, siltation, degraded water quality, exotic species and altered fluvial regimes have all played their role in the scenario of the freshwater pearl mussel decline (Williams et al. 1993, Young et al. 2001, Strayer et al. 2004). With an international perspective, Scandinavia, and Sweden in particular, is a core area for the fresh water pearl mussel in Europe. This implies a large responsibility for Swedish authorities concerning rehabilitation, restoration and reconstruction activities in multiple spatial scales within entire catchments for the future survival of the endangered freshwater pearl mussel (Henrikson 1995). The fresh water pearl mussel is thus a good candidate as a proxy for assessment of ecological sustainability for several reasons.

The freshwater pearl mussel is often found in oligotrophic streams, which runs on crystalline bedrock with naturally low concentrations of calcium. Delivery of nutrients like nitrogen and phosphorus and discharge of organic compounds are variables affecting water quality due to increased eutrophication, often resulting in increased freshwater pearl mussel metabolism and in general shorter life spans among freshwater pearl mussel populations (Bauer, 1988). Physical land use impact or cumulative effects at the riparian level is complex and have rarely been tested on freshwater pearl mussels. However, Björk (2004) showed a significantly decline in a freshwater pearl mussel population after the forestry in the catchment changed from deciduous forest to coniferous forest. Terrestrial landscape scale factors are rarely considered in the context of conservation of aquatic organisms in general, and of freshwater pearl mussel-populations in particular. Törnblom et al. (2008) identified four simple criteria to be robust predictors of viable freshwater pearl mussel populations (i.e., brown trout 0+ ≥ 5/100 m², water colours during spring flow ≤ 80 mg Pt/l, and turbidity as Fnu ≤ 1, phosphorous <15 ug/l). These four variables resulted in a good (79%) separation of streams harbouring viable and non-viable FPM-populations. These results suggest that the freshwater pearl mussel is an organism that requires considerations at multiple scales.

The complex life cycle along with a long life and a rich and appealing life history with pearl fishing and as a historical archive for environmental information, the freshwater pearl mussel is a most appropriate species for storytelling. These stories of interesting species often get stuck in peoples mind and can remind them of the preconditions of a viable population of freshwater pearl mussels. This interesting species that requires consideration at multiple scales
within catchments could be a good candidate for communicating its' complex ecology and the need to work in multiple scales. We argue that this organism could even be a tool in the process of communicating and implementing the EU Water Framework Directive, which also is based on a catchment perspective with local participation at multiple scales.

*Forest management plans and species as tools for policy implementation*

To promote informed decisions associated to jurisdictional responsibility and precautionary activities in association to the EU Water Framework Directive, there is a need for including additional information into forest management plans about the composition and proportions of different land cover types and tree species within associated catchment, as well as the composition, width and management of associated riparian zones. Finally a more detailed description over aquatic and terrestrial structures and their functionality for a variety of species is needed for a more informed forest management planning.

With the freshwater pearl mussel viability as an indicator, complemented with electrofishing on trout, water chemistry and GIS-data it is possible to quantify how large-scale land use impact the freshwater pearl mussel, and further the ecological integrity of a stream system. This also implies that there is needs to start manage and restore freshwater pearl mussel-habitats in Swedish streams, not just only by protection and conservation of habitats, segments or reaches of streams. It is suggested that the thresholds indicated by freshwater pearl mussels also applies to other organisms, irrespectively of the presence of freshwater pearl mussels.

One variant of forest management plans is called “green” management plans (de Jong et al. 1999). In addition to the traditional stand data is support of efficient sustained yield wood production, four different goal classes are used. Where production goals are dominant, the codes PG or PF are used. Where environmental goals are dominating, the codes are NS and NO. Thus, the area set a side for environmental considerations, and the number of environmental trees increases from PG to PF and from PF to NS or NO (Table 4).
Table 4. Goal classification according to the Swedish National Boards initiative: “Greener Forests”.

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>Production goal with general environmental considerations. The production goal directs management activities, general considerations in the shape of voluntary conservation areas, tree groups, separate trees of nature consideration and cultural environments.</td>
</tr>
<tr>
<td>PF</td>
<td>Production goal with reinforced environmental considerations. The relationship between production and environmental aspects directs management activities. Certain current or future environmental values justify the joining of production goals with environmental considerations that go beyond the general consideration level.</td>
</tr>
<tr>
<td>NS</td>
<td>Nature conservation with management. Environmental goals direct the nature conservation oriented management. The stand has high environmental values that require recurrent management activities to be preserved, or there are conditions present that allow the stand to return to similar high environmental values.</td>
</tr>
<tr>
<td>NO</td>
<td>Nature conservation goal based on no management. The environmental goals are enhanced by free development. The stand has high environmental values that require it to be left untouched in order for it to be maintained. Or there are conditions present that allow the stand to return to similar high environmental values.</td>
</tr>
</tbody>
</table>

This study shows that the addition of different goal classes in green forest management plans was used to improve considerations in support of ecological sustainability of streams. Still, these forest management plans miss a landscape or catchment perspective that considers both ecological and social systems (e.g., Angelstam et al. 2003). There are still no incitements encouraging forest planner or forest stakeholder to consider habitat structures at different spatial scales including tree species composition or land use or land cover from instream habitats and stream segments to riparian zones and larger stands to entire catchments and landscapes. Thus, with the present planning tools it is almost impossible for actors and stakeholders to see how his or hers little piece of land in the catchment jigsaw puzzle will fit in to the larger picture for creating functional aquatic and terrestrial infrastructures at a landscape or a catchment perspective. Here, spatial analyses are needed to make priorities among subcatchments within a forest management unit.

Knowing what species’ qualitative and quantitative requirements are is a necessary but insufficient criterion for developing the hierarchical approach towards maintaining and restoring integrity of riverine landscapes. In addition, the knowledge must be in the right place, with the planners and managers. Communication with forest and river managers on the complex and often
abstract criteria for selection of individual areas or stream reaches to be part of a functional habitat network could be alleviated if the principles were dressed in simple words. The freshwater pearl mussel, along with a set of specialized species and their habitat requirements would constitute an effective tool. A suite of familiar and well-known species as the freshwater pearl mussel, but also fish and mammals can be more appreciated and more communicative and attract people’s interests (Uliczka et al. 2004). To support education, communication and public awareness in support of work toward ecological sustainability.

Local initiatives like “stream walks” where local stakeholders have a chance to gather and meet managers, civil servants, experts and researchers and discuss local stream issues as the endangered and local freshwater pearl mussel population and its’ status. The freshwater pearl mussel is in this context a perfect organism and a very communicative indicator species for local participation, involvement and engagement by local stakeholders to take part and get involved in restoration activities.

Acknowledgements

We would like to thank the respondents in this study for their willingness to answer our questions on their working hours and of course WWF Sweden for inviting our contribution to the International Conference: “Aquatic Conservation with Focus on the Freshwater Pearl Mussel *Margaritifera margaritifera*, Sundsvall, 12-14 of August, 2009”.
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The effects of liming on the freshwater pearl mussel 
(*Margaritifera margaritifera*) in a Norwegian river

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Abstract

The effects of liming on the freshwater pearl mussel (*Margaritifera margaritifera*) and the juvenile stocks of Atlantic salmon (*Salmo salar*) in River Ogna in southwestern Norway were assessed. From 1991 onwards, the river was limed with two main lime dosers controlled by water discharge, and liming was also performed at several of the larger lakes within the catchment area. Prior to liming (1980 – 1987) the mean annual pH level was 5.2 – 5.8. Liming resulted in a gradual increase in the mean annual pH level to 6.6 in the late 1990s. Simultaneously the density of salmon fry increased from <20 individuals to 60 - 90 individuals per 100 m². Recruitment to the freshwater pearl mussel population was completely absent during many years resulting in a predominance of older individuals (length 110 – 135 mm) in the 1980s. Mature specimens, however, have been found and the glochidia survive, attaching themselves to the gills of the salmon host fish. Finds of a few young mussels in 1997 - 1999 (4% of the total number of mussels found) indicated that recruitment was in a stage of recovery. In 2002, the number of young mussels increased to 36%, and in 2005 about two thirds of the mussels were found to be younger than 15 years (less than 95 mm). Still, only a small number of salmon were infected with mussel larvae. However, the prevalence is increasing as a higher fraction of young mussels are becoming mature. The distribution of the freshwater pearl mussel has increased from 4.0 to 4.8 km of the river. In 2008, young mussels were found at nine of the twelve study sites in the river, and the density of mussels has increased by more than 100% from 1999 to 2008.
Introduction

The freshwater pearl mussel (*Margaritifera margaritifera*) is a rare and threatened species throughout its range (Young *et al.* 2000), and is the subject of recovery programmes in many countries. The main reasons for the decline are anthropogenic influences on aquatic systems. The recruitment is failing and many populations are now without juveniles. During their life cycle the freshwater pearl mussel is dependent on the presence of salmonid fish as hosts for its larvae (Young & Williams 1984).

Atlantic salmon (*Salmo salar*) have virtually disappeared from 25 rivers in Norway due to acidification (Hesthagen & Hansen 1991), and the brown trout (*Salmo trutta*) has been seriously affected over an area of more than 50,000 km². Several of these rivers previously had considerable stocks of freshwater pearl mussels and the species has disappeared from 44 localities in southernmost Norway (Agder counties) (Dolmen & Kleiven 2004). In addition, several salmon populations in south-western and western Norway have been in various stages of decline. One of these rivers is River Ogna in the south-western part of Norway which is the present case study. In the 1980s periodical fish kills were observed in the river, usually following sudden increases in discharge, and decline in river pH (Larsen *et al.* 1992). Salmon stocks were reduced and the species was regarded as threatened during the 1980s. River Ogna has also been a well known pearl fishing river for nearly 300 years. The population of the freshwater pearl mussel was reduced to a low level and disappeared in most of the river during the 1940s and 1950s due to canalization, agricultural activities and acidification. The mussels were thought to be extinct during the 1980s (Larsen & Brors 1998). However, examination of fish gills from River Ogna revealed mussel larvae on the gills of a salmon fry in 1994 (Kvellestad & Larsen 1999), and proved that the mussels had survived in spite of the acidic condition in the river.

Liming is one of the mitigation measures listed in the Action Plan for the freshwater pearl mussel in Norway (Direktoratet for naturforvaltning 2006). The goal for the management of the threatened mussel is a presence of healthy populations within the normal distribution range of the species. All present natural populations should be maintained or strengthened. The aim of this study is to evaluate the effects of liming and the stage of recovery on the freshwater pearl mussel and juvenile stocks of Atlantic salmon in River Ogna.
Study area

The catchment area of River Ogna is 115 km², of which 37 km² is included as a result of the diversion of the Helgå water course to Hetland hydro-electric power station some 3 km from the river mouth (Direktoratet for naturforvaltning 2009, Fig. 1). The area consists mainly of slowly weathering rocks. The width of the river ranges from 15 to 30 m, and the average discharge at the river mouth is 6.6 m³ s⁻¹. Intensive farming dominates the landscape along the river. The three main species of fish in the river are Atlantic salmon, brown trout (migrant and resident) and European eel (*Anguilla anguilla*). The Atlantic salmon may ascend about 30 km, to the uppermost part of the catchment area.

Liming project

In an attempt to restore the Atlantic salmon stock in River Ogna, a liming project was initiated in 1991. The river was limed from two lime dosers controlled by river discharge (Fig. 1). The upper site for liming was at Laksesvela bridge, and the lower site was at Hetland power station where water passing through the power plant was limed. In addition, a smaller tributary at Eikeland was limed from a doser, and liming occurs at several of the lakes within the catchment area.

The aim of the liming programme in Ogna is to restore the water chemistry and restore the aquatic bio-communities. The restored communities are, however, usually more unstable than those in undisturbed areas. A monitoring programme aims to evaluate the impact of the liming project and is a necessary basis for the assessment of the applied liming strategies. Monitoring of the freshwater pearl mussel was added to the programme in 1999.
Water quality

Prior to liming the pH level in River Ogna was lower than 5.5 for some time every year (Fig. 2), and the mean annual pH was 5.2 – 5.8 (Fig. 3). In the winter of 1981/82 the river reached a minimum pH level of 4.7 during a period of snow-melt (cf. Skogheim et al. 1984). The liming project from 1991 resulted in a quite abrupt increase in mean annual pH to 6.2 and a subsequent gradual increase to a present mean annual pH of approximately 6.4. During the last years pH has never been below 6.0. In some years the mean annual pH has been higher than 6.5. An increase in calcium content was also evident after liming, and the annual average exceeded 2.2 mg/l in the first years of liming (Fig. 3). From 1998, however, the calcium concentration has decreased to a mean annual value below 2.0 mg/l.
The discharge from the Helgå basin was previously seriously acidified with average pH values between 4.7 and 4.8 throughout the year in the period 1982 - 1990. Even with the liming programme, occasional short periods of low pH are still recorded.

**Figure 2.** Data on pH from the monitoring programme in River Ogna above Hetland power station in 1971 - 2008. Permanent liming was started in 1991 (marked with the arrow). From Saksgård & Schartau (2009).

**Figure 3.** Annual mean value for pH and calcium (Ca, mg/l) in River Ogna above Hetland power station in 1971 - 2008. From Larsen (2009).
Fish

The density of juvenile of Atlantic salmon and brown trout in River Ogna has been recorded for six years before liming (1983 - 1988) and 18 years after treatment (1991 - 2008) (Larsen et al. 1992, Larsen et al. 2006b, Saltveit et al. 2009). Fish densities were estimated by the removal method (Bohlin et al. 1989), and separately for 0+ (fry) and ≥1+ (older specimens) age group. In spite of fish mortality in the 1980s, the river Ogna sustained a low level of recruitment of salmon throughout the period. From 1994 there was a notable increase in the density of salmon fry to more than 60 individuals per 100 m² (Fig. 4). This resulted simultaneously in an increase in the density of older salmon parr.

The density of brown trout fry has declined after liming was initiated. In 1983 - 1988 the density was 10 - 20 individuals per 100 m², while more recently density has fallen to less than 5 individuals. The density of older trout specimens was already low before liming, but has declined notably in recent years.

Figure 4. Mean densities per 100 m² for 0+ and ≥1+ age groups of Atlantic salmon (Salmo salar) in River Ogna before liming, 1983 - 1988, and after liming, 1991 - 2008. Data from Larsen et al. (1992), Larsen et al. (2006b) and Saltveit et al. (2009).
Freshwater pearl mussels

An initial mussel survey was undertaken in 1997 - 1998 in the whole catchment area of River Ogna. This included both examinations of fish gills of young Atlantic salmon and brown trout for mussel larvae, as well as direct observations and counting of live mussels on the river bed using an aquascope. The distribution of the freshwater pearl mussel was limited to 4 km, as opposed to 20 - 25 km before acidification (Larsen & Brors 1998). Recruitment had been completely absent during many years resulting in a predominance of older individuals with a shell length larger than 110 mm. All mussels were found in the lower part of the river, and in 1999, 12 study sites were established in the lower 8 km of the river to study the effects of liming on the mussel population in River Ogna (Fig. 5).

Figure 5. The monitoring programme on the freshwater pearl mussel includes 12 study sites in the lower part of River Ogna.
Methods

Young Atlantic salmon were sampled quantitatively with an electrofisher in spring (April). Normally 15 - 20 one year old salmon parr (age 1+) and 10 two or three year old salmon parr (age 2+/3+) were sampled on each of the 12 study sites. The fish were examined under a microscope to count the total number of mussel larvae on all gills. Brown trout occurred only in small numbers and less than five individuals on average were examined at each study site.

The infection of mussel larvae are presented as prevalence (the percentage of fish infected of the total number of fish examined), abundance (mean number of mussel larvae on all fish examined) and intensity (mean number of mussel larvae on infected fish).

The mussel monitoring programme is based on a survey using transects and “15 minutes counts” during late summer (August). A survey of five transects (160 - 246 m²) was carried out where mussels were present and/or environmental conditions appeared suitable. In addition time-restricted counts of mussels was added upstream and downstream of all transects and at seven separate study sites. At least two “15 minutes counts” was carried out at each of the 12 study sites.

The number of individuals found when counting continuously for 15 minutes is inaccurate, but gives a relative density of mussels expressed in number found per minute (cf. Larsen & Hartvigsen 1999).

Since few mussels were present in the river it was considered inappropriate to search quadrates to provide mussels for an age profile. It was decided that normally all individuals on all study sites should be included in the length frequency. The maximum length of the shells was measured to the nearest 0.1 mm using callipers. This allowed comparisons of the size profile of the population between years. Growth rings in the shell of some of the small mussels were measured to establish a growth curve and age determination of the youngest mussels.
Results

Mussel larvae were found only on the young Atlantic salmon in the river – never on brown trout (Table 1). In some years and at least in some parts of the river nearly half of the young salmon (age 1+) were infected by mussel larvae. The mean value for all 12 study sites has increased from 10% in 1999 to 15% in later years (Table 1). The increase in percentage of fish infected by mussel larvae is due to increased numbers of mussels reaching reproductive age.

The number of mussel larvae per fish has ranged from 1 to 90 individuals and the average number was normally less than 10 mussel larvae at the different sampling sites. In 2008 the average intensity for all 12 study sites was 7 mussel larvae on the infected fish (both 1+ and ≥2+ salmon, Table 1). This is considered to be a very low infection rate.

Table 1. Mussel larvae on the gills of young Atlantic salmon and brown trout in River Ogna in April 1999, 2002, 2005 and 2008. Prevalence, abundance and intensity values are given as mean numbers of all 12 study sites. N = total number of fish examined, Max = maximum number of mussel larvae found on single fish, SD = standard deviation. From Larsen (2009).

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Year</th>
<th>N</th>
<th>Prevalence</th>
<th>Abundance</th>
<th>Intensity</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>Atlantic salmon</td>
<td>1+</td>
<td>1999</td>
<td>232</td>
<td>9.5</td>
<td>0.9 ± 5.0</td>
<td>9.5 ± 13.6</td>
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<tr>
<td></td>
<td></td>
<td>2002</td>
<td>197</td>
<td>13.7</td>
<td>0.7 ± 3.6</td>
<td>5.1 ± 8.6</td>
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<tr>
<td></td>
<td></td>
<td>2005</td>
<td>199</td>
<td>17.1</td>
<td>0.6 ± 4.2</td>
<td>3.7 ± 9.7</td>
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<tr>
<td></td>
<td></td>
<td>2008</td>
<td>209</td>
<td>14.8</td>
<td>1.0 ± 5.4</td>
<td>6.6 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>≥2+</td>
<td>1999</td>
<td>52</td>
<td>7.7</td>
<td>0.3 ± 1.3</td>
<td>4.5 ± 2.4</td>
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<td></td>
<td></td>
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<td>119</td>
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<td>1.7 ± 10.0</td>
<td>12.7 ± 25.1</td>
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<tr>
<td></td>
<td></td>
<td>2005</td>
<td>120</td>
<td>35.8</td>
<td>3.1 ± 8.9</td>
<td>8.6 ± 13.2</td>
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<tr>
<td></td>
<td></td>
<td>2008</td>
<td>129</td>
<td>13.2</td>
<td>0.9 ± 4.2</td>
<td>7.2 ± 9.5</td>
</tr>
<tr>
<td>Brown trout</td>
<td>≥1+</td>
<td>1999</td>
<td>34</td>
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<td></td>
<td></td>
<td>2005</td>
<td>47</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2008</td>
<td>41</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The density of freshwater pearl mussels has increased at most study sites from 1999 to 2008 (Fig. 6). The mussels have re-established at three of the study sites in the lower part of the river. They have expanded their distribution from 4.0 to 4.8 km of the river. The main distribution area in 1999 was at the study sites 9 and 10. Later the number of old mussels has decreased in that area and young mussels
have re-established further downstream due to a more favourable substrate and a high number of young host fish.

**Figure 6.** Relative density of living freshwater pearl mussel in River Ogna based on time-restricted counts (given as number of mussels per minute) in 1999 - 2008. From Larsen (2009).

The average density of mussels has increased from 0.10 individuals per minute in 1999 to 0.24 individuals in 2005, with a slight increase also in 2008 (Fig. 7). Thus, the density of freshwater pearl mussels has more than doubled over the last ten years. The average density in transects has increased from 0.09 individuals per m² in 1999 to 0.16 and 0.14 individuals in 2005 and 2008, respectively. The increase in density is more precisely an increase in density of visible mussels. Mussels added to the density in 2005 were not visible in 2002 but buried in the substrate or hidden under the stones in the river.

The detection of a few young mussels in 1997 - 1999 indicated that recruitment was in a stage of recovery (Larsen & Hårsaker 2000, Fig. 8). Young mussels constituted 4% of the total number of mussels found. In 2002, the number of young mussels had increased to 36% (Larsen & Berger 2003). Aged mussels showed that these individuals belonged to the age classes 1991 onwards. Most were present in the river in 1999 but they were not visible at that time. A study of freshwater pearl mussel in eight Norwegian watercourses found that on average
34% of the individuals in a mussel population are buried (Larsen et al. 2007, Degerman et al. 2009). Only half of the mussels were visible even at a length of 50 mm.

Figure 7. Mean annual density of freshwater pearl mussel in River Ogna in 1999 – 2008.
Figure 8a. Length frequency diagram of living freshwater pearl mussels in River Ogna in August 1997 – 1999 (N = 154), 2002 (N = 194.)
In 2005 the frequency of young mussels increased even more and about two thirds of the individuals were found to be younger than 15 years or less than 95 mm at that time (Larsen et al. 2006a). However, no mussels were less than 20 mm.

In 2008 the dominating number of shells was in the length groups 95-105 mm and no mussels less than 50 mm were found (Larsen 2009). This was even the
case when two areas were examined more thoroughly by digging in the substrate. Even so, because two thirds of the population were younger than 20 years and some mussels were younger than 10 years, the population can still be said to be in a favourable condition in 2008 (cf. Young et al. 2001).

Conclusion

Liming has been an important measure in River Ogna. The development has been positive for the water chemistry, fish and freshwater pearl mussels in the first years of liming. The survey indicated that populations of the freshwater pearl mussel have the potential to recover if the conditions are improved. But the watercourse is still sensitive to acid water and continues to be dependent upon a continual supply of lime. It is of concern that no mussels were smaller than 50 mm in 2008. This may be due to lower liming activity and periods of suboptimal water quality (Saksgård & Schartau 2009). To create a favourable environment for mussels, pH should be at least 6.2 all year round; not only during spring time. It may be important to include analyzes of heavy metal concentrations during the year in the monitoring programme. The content of calcium has been decreasing for some years and it may become necessary to raise the concentration back to 2.5 mg/l. Finally, all actions to reduce the leakage of nutrients from farm land and agricultural activities will be beneficial.

Nevertheless, the recovery and re-establishment of the freshwater pearl mussel in River Ogna has so far been a success story.

Acknowledgement

The monitoring programme on the freshwater pearl mussel in River Ogna is financed by the Directorate for Nature Management. Thanks are due to H.M. Berger, S. Brørs, K. Hårsaker and J.H. Simonsen for field assistance, R. Saksgård for valuable help at the laboratory, and O.T. Sandlund for comments on the manuscript and improving the language.
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Young, M., Hastie, L. & Al-Mousawi, B. (2001). What represents an "ideal" population profile for *Margaritifera margaritifera" p. 35-44 in:
Do signal crayfish *Pacifastacus leniusculus* harm freshwater pearl mussels? Some field observations

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Abstract

More than 90 % of the freshwater pearl mussel populations in Bavaria (Germany) are strongly overaged. In the course of managing adults of *Margaritifera margaritifera* in a small population during the spawning season in August 2007, individuals with injured shells were found. It was clear that the damage was not done by muskrat. Therefore we suspected that non-native signal crayfish *Pacifastacus leniusculus* were gnawing at the mussels.

Starting in June 2008, an inventory of the damaged mussel population and of signal crayfish was done. *P. leniusculus* specimens were sampled by setting traps at intervals of 100 meters. Within the stretch populated by pearl mussels a capture-recapture study was carried out in two 100 meter sections. Trapped crayfish were marked by cutting notches on their uropod. The data were analysed with a closed-population model using the statistics program MARK.

A total of 2,249 *Pacifastacus leniusculus* were captured in a river section of 6 km. The upper distribution is limited by low water temperature. Data loggers recorded a mean summer temperature of 12 °C upstream of the crayfish population, as compared to 13.6 °C within. For the 1.1 km-section populated by mussels the capture-recapture analysis estimated mean densities of 5.4 signal crayfish per m² corresponding to 13,000 individuals.

35 % of the pearl mussels showed varying degrees of shell damage. Damage exclusively occurred on the points of the shell and looked like abrasions. Badly damaged shells could not be closed properly. We suggest that the omnivorous signal crayfish might locally become an additional threat to declining pearl mussel populations.
Introduction

The freshwater pearl mussel *Margaritifera margaritifera* (L.) is undergoing substantial decline throughout its range from western Russia to the eastern seaboard of North America (Young *et al.* 2001). Particularly the deteriorations of habitat quality by e.g. eutrophication (Bauer 1983, 1988) and physical degradation (Cosgrove *et al.* 2000, Geist & Auerswald 2007) has led to increased mortality of all age classes and to a lack of juvenile mussels.

In this paper we report a study investigating some curious field observations that might indicate an additional threat to pearl mussel populations: In August 2007 we had found some mussels with injured shells in the Biberbach, a stream near the border between Germany and the Czech Republic (Schmidt *et al.* 2007). The shell abrasions look quite different from the damage caused by muskrat (Figure 1). We suspected the non-native signal crayfish *Pacifastacus leniusculus* caused the damage. This species had been introduced in 1996 by a fish farmer in some ponds draining towards the Biberbach. In the stream signal crayfish were first observed two years later in one single location (Schmidt & Wenz 1998).

![Figure 1. Damaged pearl mussel shell (left), found in the Biberbach in August 2007, as compared to an intact shell (right).](image-url)
Approximately 1.1 km of the Biberbach is currently inhabited by freshwater pearl mussels. The actual population size is about 370 individuals, compared to about 1,000 in 1990 (Schmidt & Wenz 1990). The population is overaged as well: The youngest specimens are between 40 and 50 years old. The main causes for the population decline are inadequate sewage treatment, pollution from fish farms and intensive agriculture. However, over the last decade water quality has improved due to conservation measures, for example the establishment of buffer zones.

The present study started in June 2008. The purpose was 1) to investigate the spatial distribution of signal crayfish in the river; 2) to calculate the total number of crayfish in the section inhabited by pearl mussels; 3) to survey the damage to the mussels; and 4) to look for evidence that signal crayfish were causing the damage.

Methods

To capture crayfish traps with wide openings at both ends were used, supplied with fresh ox liver as a lure (Figure 2).

Figure 2. Trap (model PIRAT) for the capture of crayfish.

The range and distribution of crayfish in the Biberbach was investigated by a longitudinal inventory. From June to September 2008, traps were set at intervals of 100 meters, covering a stream section of about 6 km and extending
to both upstream and downstream of the stretch inhabited by pearl mussels. All traps were installed in the afternoon and examined the next morning. For each trap the number, sexes and sizes of the captured crayfish were recorded. Additionally at each trap site the physical stream structure was described according to Hahner (2002). Water temperature was recorded by data loggers (Syntec HOBO Water Temp Pro) exposed in the upper, middle and lower part of the investigated stream section.

In the stream section inhabited by mussels, the absolute number and density of crayfish was estimated by a capture-recapture study in August and September 2008. Two stretches of 100 meters were investigated, one in open meadow, one in forest. 80 traps were set simultaneously within each stretch. The catch was repeated 4 times at intervals of one week. All captured crayfish were marked by a punch mark at the uropod (Figure 3) and then put back into the stream. The population size of each of the two investigated stream stretches was estimated from the resulting crayfish encounter histories, using the closed-population approach (Otis et al. 1978) of the statistics program MARK (White & Burnham 1999).

Figure 3. Male signal crayfish, marked by a punch on the uropod.

Between July 2008 and July 2009 a complete survey of the pearl mussel population in the Biberbach was done. All mussels were measured and examined for shell damages.
Results

Although in former times the Biberbach was famous for its stock of noble crayfish *Astacus astacus*, in the longitudinal crayfish inventory only signal crayfish but no native species were caught. This was to be expected, as *Pacifastacus leniusculus* is highly competitive (Söderbeck 1995 and 1991) and a vector of the crayfish plague (Alderman 1997). A total of 2,249 *Pacifastacus leniusculus* were captured, with the highest numbers of crayfish recorded about 300 meters downstream of the fish farm that originally introduced the crayfish in 1996 (Figure 4).

![Figure 4. Number of crayfish per trap caught in the longitudinal inventory. Diamonds = main stream. Squares = millstream and tributary. x = location of the fish farm that introduced signal crayfish. Arrows = locations of the temperature data loggers.](image)

Although certain morphological criteria of the river are essential for crayfish settlement (Bohl 1987), in this study no correlation was found between the number of crayfish caught and the recorded parameters of physical stream structure (e.g., shelter structures; Figure 5). However, the signal crayfish distribution is probably limited by water temperature: In the upper part of the investigated stream section, where no crayfish were caught, the mean day temperature did not reach 14 °C in the summer of 2008 (overall mean 12.0 ± 1.2 °C), whereas mean day temperatures of up to 17 °C were measured further downstream in the sections inhabited by the signal crayfish (middle part: overall mean 13.6 ± 1.2 °C; lower part: overall mean 14.4 ± 1.6 °C; Figure 6). This corresponds well with observations from other authors (Strätz et al. 2004,
Capurro et al. 2007), who stated that 15 °C might be a critical threshold for signal crayfish.

Figure 5. Scatter plot of the index of shelter structures on trap sites versus number of crayfish caught.

Figure 6. Mean day temperatures of stream water calculated from data logger measurements at the three sites indicated in figure 4, from June to September 2008.

In the capture-recapture study again *Pacifastacus leniusculus* was the only crayfish species caught. In the catch the number of males was higher than that of females. Females are less active and hide for longer periods, especially when
they are carrying eggs or offspring (Bohl 1987). In the course of the repeated captures the number of unmarked individuals in the traps did not decline, indicating that the real number of crayfish by far exceeds the number of caught specimen (Figure 7). Accordingly, from the encounter histories of 826 crayfish caught in the meadow-stream stretch a population of 1,462 was estimated with MARK (95 % confidence limits: 1.196 to 1.943). In the forested investigation

Figure 7. Capture - recapture inventory: Total number of crayfish caught at each of the 4 catches, and number of marked, that is, recaptured specimen.
site from 557 caught specimens a population of 890 animals was estimated (95% confidence limits: 738 to 1,178). From these figures for the 1.1 kilometre of the stream inhabited by pearl mussels a crayfish population of 13,000 is extrapolated, corresponding to 5.4 individuals per m². Some signal crayfish showed snapped claw points (Figure 8). This was exclusively found in the river section populated by mussels.

Figure 8. Crayfish claw with broken point.

The examination of the pearl mussel population revealed that 35% of the animals show shell injuries. No correlation was found between mussel size and the occurrence of injuries (Figure 9). However, as no mussels with total length smaller than 80 mm are present in this population, the potential violability of young mussels is not known. Curiously, the damage was observed more often at the leading end of the mussels that normally is buried in the substrate (Figure

Figure 9. Cumulative distribution of the length of damaged and intact pearl mussel shells.
10). The reason might be that displaced or moving mussels will stretch out their foot at the leading end, which might attract predators. Some of the damage was so severe that the mussels were no longer able to close their shells (Figure 11). This is likely to be lethal in the long term.

Figure 10. Dissected pearl mussel. Location of shell damages are marked black. From Korschelt 1926, supplemented.

Figure 11. Severely damaged mussel, showing shell injuries at the leading end.
Discussion

Considering the already depleted size of the freshwater pearl mussel population and its ongoing decline, the observed damage poses a serious threat to the remaining individuals. The question is, can the signal crayfish be held responsible for the observed damage to the mussels?

In a Swedish laboratory study Hylander (2004) found that signal crayfish attack juveniles of the mussel *Unio tumidus*. The crayfish used their mouth parts to gnaw on the edge of the shells to get inside. In the present study there was no possibility to directly observe the action that injured the mussels. The snapped claw points might however be interpreted as evidence for crayfish involvement. Possibly the claw breaks when a disturbed mussel shuts its shell.

There is no doubt that the main impairment of freshwater pearl mussel populations is habitat degradation. The major task for mussel conservation therefore is the restoration of rivers, including the river catchments. Locally invasive species like muskrat (Zahner-Meike & Hanson 2001, Hochwald 1990) and signal crayfish seem to be an additional issue.

Irrespective of the possible impairment of mussels, signal crayfish were shown to have a strong negative impact on the stocks of native crayfish (Söderbeck 1991, Huber & Schubart 2005). They can also cause the degradation of macrophytes, aquatic insects and benthic fishes (Crawford *et al.* 2006, Guan & Wiles 1997, Nyström 1999).

For all these reasons precautions and alertness are needed to prevent further dispersal and spread of *Pacifastacus leniusculus*, particularly as no effective measure has been found to eradicate this non-native species from running waters (Peay *et al.* 2006, Hyatt 2008).

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Aquatic Conservation with Focus on
*Margaritifera margaritifera*

The freshwater pearl mussel *Margaritifera margaritifera* (L.) has attracted a large human interest, since the mussel is fascinating from biological, cultural and environmental perspectives. The mussel has a complicated life cycle depending on host fish, and has great demands on its habitat. Furthermore, the mussel is an environmental indicator, and is used as an umbrella and flagship species. Pearl fishing has been described in Sweden as early as in the 16th century and by Carl Linnaeus in the 18th century.

Many freshwater pearl mussel populations have insufficient recruitment and therefore decrease in numbers, and many populations are even extinct. Therefore, conservation work on the freshwater pearl mussel is going on all over Europe. Actions to preserve the freshwater pearl mussel will also favour other aquatic species – freshwater pearl mussel conservation is aquatic biodiversity conservation!

WWF (World Wide Fund for Nature) Sweden implemented the project “The Freshwater Pearl Mussel and its habitats in Sweden” during 2004-2009 (LIFE04NAT/SE/000231). The overall objective was to improve the habitats of juvenile freshwater pearl mussels and the host fish brown trout *Salmo trutta* in 21 streams. The actions were improvements of the biotopes, re-introduction of mussels, information to the stakeholders, and development of planning methods. An international conference “Aquatic Conservation with Focus on the Freshwater Pearl Mussel *Margaritifera margaritifera*” was held in Sundsvall 12–14 August, 2009. In these proceedings, presentations from the conference are published.