Conservation of the Freshwater Pearl Mussel

1. Captive Breeding Techniques

Conserving Natura 2000 Rivers

Conservation Techniques Series No. 2

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I: Captive Breeding Techniques

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Conserving Natura 2000 Rivers

This report on developing techniques for captive breeding of the freshwater pearl mussel (*Margaritifera margaritifera*) has been produced as part of **Life in UK Rivers** – a project to develop methods for conserving the wildlife and habitats of rivers within the Natura 2000 network of protected European sites. The project's focus has been the conservation of rivers identified as Special Areas of Conservation (SACs) and of relevant habitats and species listed in annexes I and II of the European Union Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (92/43/EEC) (the Habitats Directive).

One of the main products is a set of reports collating the best available information on the ecological requirements of each species and habitat, while a complementary series contains advice on monitoring and assessment techniques. Each report has been compiled by ecologists who are studying these species and habitats in the UK, and has been subject to peer review, including scrutiny by a Technical Advisory Group established by the project partners. In the case of the monitoring techniques, further refinement has been accomplished by field-testing and by workshops involving experts and conservation practitioners.

Life in UK Rivers is very much a demonstration project, and although the reports have no official status in the implementation of the directive, they are intended as a helpful source of information for organisations trying to set 'conservation objectives' and to monitor for 'favourable conservation status' for these habitats and species. They can also be used to help assess plans and projects affecting Natura 2000 sites, as required by Article 6.3 of the directive.

As part of the project, conservation strategies have been produced for seven different SAC rivers in the UK. In these, you can see how the statutory conservation and environment agencies have developed objectives for the conservation of the habitats and species, and drawn up action plans with their local partners for achieving 'favourable conservation status'.

The project has also developed new conservation techniques for practical management of key species.

For each of the 13 riverine species and for the *Ranunculus* habitat, the project has also published tables setting out what can be considered as 'favourable condition' for attributes such as water quality and nutrient levels, flow conditions, river channel and riparian habitat, substrate, access for migratory fish, and level of disturbance. 'Favourable condition' is taken to be the status required of Annex I habitats and Annex II species on each Natura 2000 site to contribute adequately to 'favourable conservation status' across their natural range.

Titles in the Conserving Natura 2000 Rivers ecology and monitoring series are listed inside the back cover of this report, and copies of these, together with other project publications, are available via the project website: www.riverlife.org.uk.

Contents

1 Background

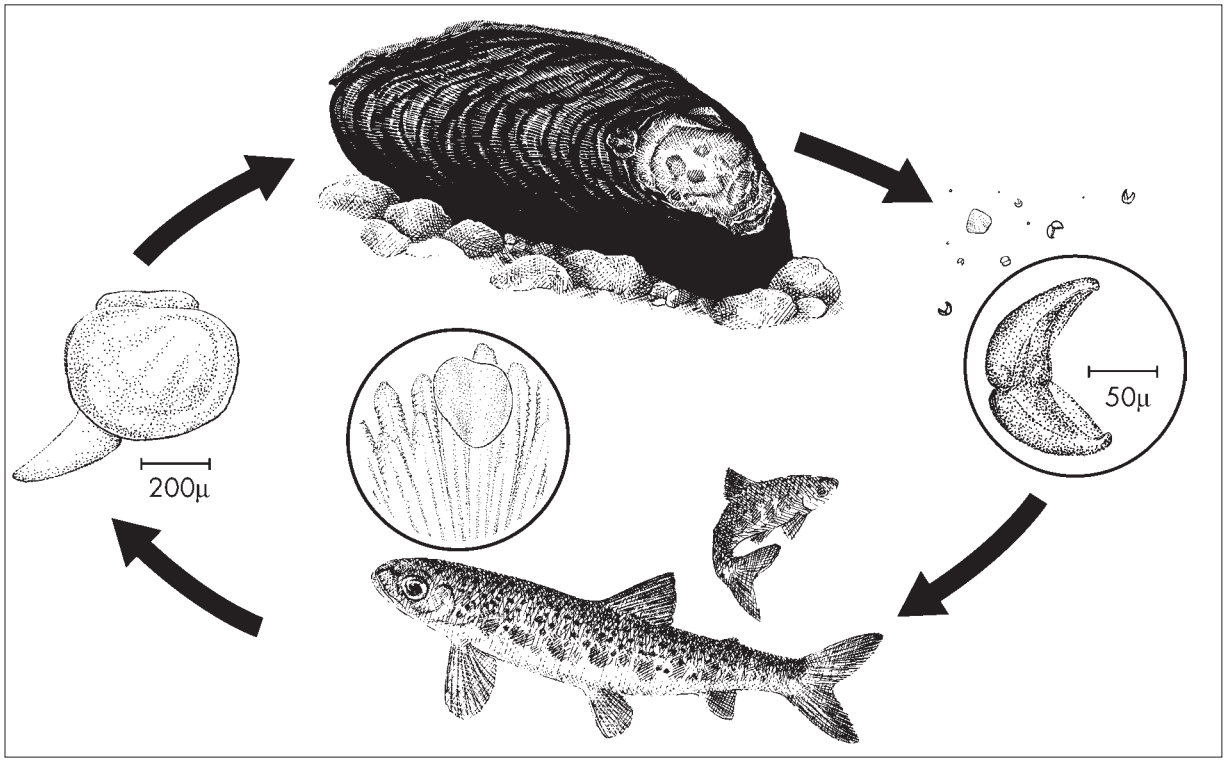
1.1 General introduction

The endangered freshwater pearl mussel (*Margaritifera margaritifera* L.) is presently under serious threat throughout most of its holarctic range (Bauer 1986; Ziuganov *et al.* 1994; Young *et al.* 2001; Skinner *et al.* 2003). Several reasons for the observed decline of *M. margaritifera* populations in Europe and North America have been suggested, all of which relate to changes in human activity. These include the detrimental effects of destructive pearl fishing, industrial and agricultural pollution, physical habitat degradation due to river engineering and the recent decline of migratory salmonid host stocks (Young 1995; Cosgrove *et al.* 2000; Cosgrove & Hastie 2001; Hastie & Cosgrove 2001). Throughout continental Europe, many pearl mussel populations have been in serious decline or have disappeared completely during the past 50–100 years (Baer 1969; Jungbluth 1971; Valovirta 1977; Bauer 1980, 1986; Bauer *et al.* 1980; von Dettmer 1982; Young & Williams 1983; Wachtler *et al.* 1987; Young 1995; Cosgrove *et al.* 2000; Young *et al.* 2001).

1.2 The freshwater pearl mussel life cycle

The slow-growing *M. margaritifera* is one of the longest-lived invertebrates known, capable of reaching ages greater than 100 years (Bauer 1992). In common with other freshwater bivalves, the sexes are separate; both sexes mature at age 12–20 years (Young & Williams 1984a).

An annual cycle of gametogenesis is apparent (Ross 1992). Up to 3 million unfertilised eggs pass out of the ovary into the mantle cavity and collect in brood pouches in the modified gills (marsupium), where they are fertilised in early summer. The female mussels inhale sperm by normal filtering action, in which a stream of water (containing food particles) enters the mantle cavity via the inhalant siphon. In mid- to



Sarah Wroot

The freshwater pearl mussel has a unique association with juvenile salmonids. Mussel larvae, known as glochidia, are released in summer and attach themselves to the gill filaments of host fish. Here they encyst until the following spring, when they drop off and begin to mature.

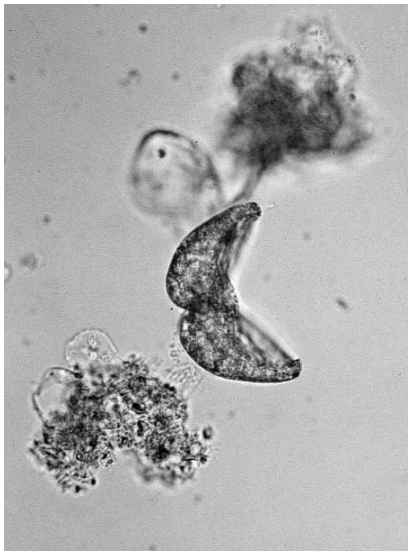
late summer, following an incubation period of several weeks, the females discharge their glochidia into the river (Hastie 1999). Glochidia resemble miniature mussels, measuring 0.06–0.08 mm across (Buddenseik 1991). They are obligate parasites of fish and are usually found encysted on the gills and/or fins of their hosts. Of the many glochidia released by pearl mussels, only a few that are ingested or inhaled by host fish become attached to and encyst on their gills.

The parasitic phase of *M. margaritifera*, which does not appear to harm wild fish, lasts for several months before the glochidia metamorphose into tiny mussel 'seed' (by then about 0.4 mm across), excyst from the host gills and drop off and settle onto the riverbed (Young & Williams 1984a). Those that settle in clean, stable sand may survive to adulthood. The sizes of the early developmental stages are presented in Table 1.

Table 1. Comparative sizes of the different developmental stages of *M. margaritifera* (from Buddensiek 1991; Young 1995; Hastie 1999).

Stage	Size (mm)
Unattached glochidium	0.06–0.08
Encysted glochidium	< 0.1–0.4
Newly-settled seed mussel	~0.4
1 year post-settlement	>0.4
3-year-old mussel	~12
4- to 5-year-old mussel	~20
Mature adult (~12 years old)	≥65

1.3 Recruitment in *M. margaritifera* populations



Mark Young

Losses of unattached glochidia may be over 99% of production.

At all early stages of the pearl mussel life cycle, between the production of glochidia and the establishment of young mussels in the riverbed, there are heavy mortalities. Losses as high as 99.9996% for unattached glochidia (failing to attach and encyst), 95% for encysted glochidia (failing to complete development on host fish) and 95% for newly settled mussels (failing to establish themselves on the riverbed) have been reported for *M. margaritifera* (Young & Williams 1984a, Bauer 1987c).

These losses are balanced by a great reproductive lifespan (30–60 years), and vast numbers of glochidia are produced by spawning mussels each year (Bauer 1987a). Each gravid female can produce 1 million to 4 million glochidia (Young & Williams 1984a, Bauer 1987a) and the proportion of adult mussels producing glochidia varies from 30–60%, even in sparse populations (Young & Williams 1983; Bauer 1987a; Ross 1992). The early post-settlement phase, when mussel seed must establish themselves on the riverbed, is particularly critical, and it appears that this stage is usually particularly affected (by degradation of the riverbed habitat) in declining populations.

A number of ageing *M. margaritifera* populations have large numbers of healthy adult mussels but no signs of recruitment during several decades (Young *et al.* 2001). There are still some viable populations, particularly in Scotland where many rivers still provide suitable habitat, but here some local *M. margaritifera* populations have been wiped out by destructive pearl fishing.

1.4 Special conservation projects

A considerable amount of effort has been directed towards maintaining and enhancing *M. margaritifera* populations, but mostly through uncoordinated and poorly planned activities. Conservation schemes have included adult mussel transfers, release of fish infected with glochidia, culture of juvenile mussels and freshwater habitat restoration projects. Translocation of adult mussels has never been shown to be effective in the long term, and could in any case reduce already depleted donor stocks (Young 1995).

Valovirta (1990) has reported short-term survival rates of 90% for within-river transfers, but only 50% for between-river transfers.

Birgit Heninnger

The freshwater pearl mussel lives for 30–60 years and some individuals may live for over 100 years. The adult and juveniles above are from a stable population in Scotland.

It is difficult to decide on the best strategy for re-introductions, because growth and reproduction are so slow and erratic, and little is known of the habitat requirements of newly-released mussels (Young 1991). There has been much interest in the release of host fish artificially infected with glochidia, as a means of maintaining or restoring natural mussel beds, and several attempts have been made, particularly in Germany.

In the course of an ongoing project in Germany, juvenile trout have been infected with glochidia and then released into a variety of small rivers. However, survival of the farm-reared fish has been very poor, suggesting that they do not compete well with wild fish. Furthermore, there has been no evidence of the establishment of juvenile mussels following these releases, probably because the factors that initially led to the failure of wild juvenile mussels have not been fully remediated (Schmidt and Wenz pers. comm.).

The only similar scheme to have shown evidence of success is that in the River Lutter, near Hanover, where measures have been put in place to restore water quality by diverting sediment-rich drainage ditches. Native trout have been trapped in the river, immediately infected with glochidia and then released again. Some juvenile mussels have since been recovered, the first in the River Lutter for many years (Hochwald & Altmuller, pers. comm.).

An alternative strategy is to infect fish in the laboratory and to collect fully developed mussel seed from them. These may then either be placed directly into a stream, or an attempt made to rear them to a size when their survival in a stream is more assured. As Young & Williams (1983) pointed out, it is technically easy, although laborious, to infect host fish with glochidia and retrieve viable mussels from them. The difficulty arises in rearing the young mussels after their release from the fish.

Since juvenile *M. margaritifera* appear to be more sensitive than adults (Buddensiek *et al.* 1993, Hastie *et al.* 2000b), any practice that increases juvenile survival during the critical first year or two prior to release is worthy of further consideration.

Recently, the feasibility of culturing *M. margaritifera* (post-parasitic phase) as a conservation tool has been studied by Buddensiek (1991, 1995), whose pioneering work has resulted in juveniles being raised to 52 months of age in small plastic cages buried in the sediment of riverbeds (Hochwald 1995). However, the rate of survival is usually so low that such schemes do not result in the rearing of sufficient mussels to be worthwhile. Typical survival values of less than 1% are reported by various workers, although published data are not available. It is crucial to develop techniques that are more successful.

Even though it may be possible in the future to release mussels at a size at which subsequent mortality is reasonably low, any re-stocking program would only succeed in the long term if conditions for release were suitable and the cause of the previous decline was known and removed (Young & Williams 1983). Various pollutants and even mild eutrophication are detrimental to the successful reproduction

of *M. margaritifera* (Bauer 1983, 1988a, b). Thus it is necessary to ensure that pollution is controlled rigorously in rivers that support mussel populations (Young 1995).

There have been few attempts to restore pearl mussel habitats. In the Fichtelgebirge, Germany, an extensive interception canal was built to divert potentially harmful agricultural effluent downstream of the mussel beds that harbour the last remaining healthy population in the area (Bauer & Eicke 1986, Hochwald 1995). The Water Board of Hof, Germany, was involved in cleaning up the muddy sediment of a mill stream (contaminated with ferric oxide) without harming the mussels (Hochwald 1995).

However, the long-term success of these expensive measures is not guaranteed because little is known of the microhabitat requirements of juvenile *M. margaritifera*. The mussel beds in this river have declined further in the last 15 years, with no sign that success can be achieved.

The **Life in UK Rivers** project on which this report is based aimed to develop more successful rearing techniques to improve the chances of establishing a conservation programme for the mussel in rivers where environmental quality has been restored.

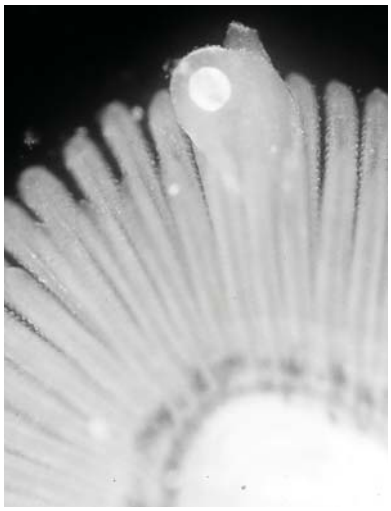
Section 2 provides technical and quantitative information on infecting host fish (juvenile salmonid) stocks with *M. margaritifera* glochidia and maintaining them until the glochidia metamorphose, in order to produce mussel seed. The work was carried out at the Lochailort and Kinlochmoidart hatcheries in north-west Scotland, and the Dinnet hatchery in north-east Scotland.

Section 3 provides technical and quantitative information on intensive mussel cultivation using small baskets of sediment in a raceway. The work was carried out at the Lochailort and Kinlochmoidart hatcheries in north-west Scotland, and the Dinnet hatchery in north-east Scotland.

Section 4 provides technical and quantitative information on semi-intensive mussel cultivation, using plastic cages stationed in the river. The work was carried out in the River Moidart candidate Special Area of Conservation (cSAC) in north-west and River Dee cSAC in north-east Scotland.

Section 5 provides a review of extensive mussel cultivation techniques, involving the release of artificially infected host fish into rivers. It is based on work carried out in a number of rivers in Scotland and elsewhere.

The results of these studies and their implications for the conservation management of *M. margaritifera* are discussed briefly in each section. Based on these, conclusions and recommendations are provided in Section 6. Finally, a general summary of the entire report is provided in Section 7.



Mark Young

Glochidia attach themselves to the gill filaments of host fish.

2 Glochidial infection of host fish

2.1 Summary

During 2000–2003, stocks of juvenile salmon infected with freshwater pearl mussel glochidia were maintained at Lochailort hatchery (north-west Scotland) and Dinnet hatchery (north-east Scotland). The purpose of these operations was to obtain mussel seed for cultivation trials. At Lochailort, 500 fish produced 3,500 mussel seed (2000–2001).

At Dinnet, 400 fish produced 115 seed (2001–2002) and 20 seed (2002–2003). The lower than expected numbers of seed collected at Dinnet in 2002 and 2003 indicate that the glochidia may have metamorphosed and excysted from their hosts far earlier than expected, possibly due to elevated water temperature.

2.2 Introduction

In this section, a technique for infecting host fish (juvenile salmonid) stocks, with M. margaritifera glochidia and maintaining them until the glochidia metamorphose, in order to produce mussel seed, is described.

2.3 Methodology

2.3.1 Site selection

The fieldwork was carried out in the River Dee cSAC and the River Moidart cSAC in northern Scotland. The Dee is a relatively large north-east river (overall length >100 km) while the smaller Moidart (<20 km) is located in the north-west of the country. Both rivers support large, functioning *M. margaritifera* populations of international importance. Recent conservative estimates of population size are >500,000 mussels in the River Dee (Cosgrove *et al.* 2003) and >100,000 mussels in the River Moidart (Hastie *et al.* 2000a). The River Dee *M. margaritifera* population extends for >70 km (Aberdeen to Braemar) whereas the River Moidart population is restricted to a lower 5 km reach. Laboratory work involving Dee and Moidart mussels was carried out at the Dinnet (Dee Salmon Fishery Trust) and Lochailort (Marine Harvest Ltd.) salmon hatcheries, respectively.

2.3.2 Examination of mussels

Samples of live adult mussels (shell length L >70 mm, n = 100) were taken from the Rivers Dee (July–August) and Moidart (June–July). These were examined non-destructively for gravidity by carefully opening the shell valves with special opening tongs, and checking for the presence of glochidia in the modified gill structures (marsupia) of the female mussels. The developing glochidia can be easily seen as a cream-coloured mass within the translucent brown gills (Young & Williams 1983). Small samples of selected gravid female mussels (n = 20) were transferred to the fish hatcheries and maintained in tanks with running river water. The other examined mussels were returned safely to the riverbed.

2.3.3 Infection of host fish

Lochailort hatchery

During July 2000, a sample of hatchery-reared salmon fry (age 0+, n = 500) was infected with glochidia from River Moidart mussels. The fish were kept in a 2 m diameter circular tank with running (untreated) river water. Gravid female mussels (n = 20) were left to spat (release glochidia) naturally in the bottom of the tank for two weeks. A sub-sample of mussels with mature glochidia (n = 5) was induced to spat artificially by leaving them in a bucket of river water for 30 min.

A sample of water from the bucket was examined under the microscope to determine that the glochidia were active (snapping their valves together) (Young & Williams 1984b)). The mussels were then removed and the bucket containing glochidia was agitated. The contents were then added to the fish tank. During this operation, the main water supply was turned off for 10 minutes to ensure that a large number of fish were exposed to glochidia. The infected fish were maintained at Lochailort hatchery for one year (July 2000–July 2001).

Dinnet hatchery

During August 2001, a stock of hatchery-reared salmon fry (stock 1, age 0+, n = 400) was infected with glochidia from River Dee mussels. The fish were kept in a 2 m circular tank with running (untreated) river water. Gravid female mussels (n = 20) were left to spat naturally in the bottom of the tank for two weeks. A sub-sample of mussels with mature glochidia (n = 3) was induced to spat artificially, and the fish were exposed to the glochidia as previously described. The infected fish were maintained at Dinnet hatchery for 10 months (August 2001–June 2002).

Another stock of fish (stock 2, age 0+, n = 500) was infected during August 2002 in order to produce mussel seed if required for continuation of the project. In the event these were not used in 2003 and the fish were later released in the River Dee catchment.

2.3.4 Examination of host fish

A small sample of fish was examined immediately following infection to ensure that this process had taken place. Thereafter, during the infection periods, small opportunistic samples (n = 5–10) of the artificially infected fish were taken at regular intervals for examination. Fish were killed (overdosed) in a 30 ppm benzocain anaesthetic solution and measured (fork length FL to nearest mm). The fish were then examined for glochidia. The opercula were carefully removed using a scalpel and scissors, and the excised gills were placed between two glass slides. In some samples, the numbers of glochidia were counted using a low-power microscope (x50) and a tally counter.

2.3.5 Collection of the mussel seed

Regular examination of infected fish allows estimation to be made of the date at which the fully developed mussel seed drop from their host fish. Just before this time, plankton nets were placed over the tank outlets. Newly released mussels are around 0.4 mm diameter and the mesh of the nets was chosen to ensure that the mussel seed would be captured. However, this mesh inhibits water flow from the tank and accumulates debris quickly, so it was planned to use the nets only at the exact time of mussel release. Each day the nets were removed and their contents tipped into a dish, from which the young mussels were retrieved. A binocular microscope was required for this process.

2.4 Results

Examinations carried out on Lochailort and Dinnet fish shortly after exposure indicated that at both sites most fish had been successfully infected with significant numbers of glochidia. Individual loads ranged from 10–800 glochidia per fish. Table 2 shows the numbers of glochidia recorded in samples taken at the Dinnet hatchery (2001–2003).

Table 2. Observed glochidial loads on two stocks of River Dee juvenile salmon kept at the Dinnet hatchery (2001–2003).

Date	Stock	No.	Post- infection period (months)	Glochidia per fish			
				N	Mean	Standard deviation	Range
18-08-01	1	400	0	10	130.0	170.24	15–500
20-02-02	1	400	+6	5	40.4	68.87	0–162
31-05-02	1	400	+9	8	3.9	10.56	0–30
17-10-02	2	500	+2	10	53.9	73.33	0–218
04-03-03	2	500	+5	10	37.7	72.40	0–228

During June-July 2001, approximately 3,500 live mussel seed were collected from the Lochailort hatchery. These were transferred to Kinlochmoidart hatchery (River Moidart) for cultivation trials (section 3–4).

During June 2002, 115 live mussel seed were collected from the Dinnet hatchery. These were kept at the hatchery for mussel cultivations trials. Numbers of expected and collected mussel seed are provided in Table 3.

Table 3. Expected numbers of *M. margaritifera* seed produced and actual numbers collected at Lochailort (LA) and Dinnet (D) hatcheries.

Hatchery	Period	Mean infection glochidia per fish	No. fish infected	Number of mussel seed		
				Overall no. encysted glochidia	Expected	Collected (%)
LA	2001	162	500	81,000	4,050	3,500 (86.4)
D	2002	130	400	52,000	2,600	115 (4.4)

2.5 Discussion

The relatively large number of *M. margaritifera* seed collected at Lochailort hatchery in 2001 (3,500 mussels from 500 host fish) indicate that it is not difficult to produce substantial numbers of freshwater pearl mussels using small stocks of farmed salmon. The numbers here were required for post-settlement cultivation trials (sections 3–4). For conservation purposes, perhaps 10,000–100,000 mussel seed may be required.

However, it should be easily possible to scale-up production to these numbers, since Scottish freshwater salmon farms typically hold stocks of 100,000–500,000 0+ fish. Based on the numbers of *M. margaritifera* seed produced per fish at Lochailort hatchery, potentially several million seed could be produced at a large freshwater salmon farm in northern Scotland.

The small number of mussel seed collected at Dinnet hatchery in 2002 (115 mussels from 400 host fish) was disappointing. Based on numbers of encysted glochidia observed on samples of host fish, several thousand mussel seed were expected. It is thought that an error was made estimating the date when the mussels were about to drop off the host fish. In 2002, the mussels excysted far earlier than expected, probably before the collecting nets were in place. As a result, most of the mussel seed (90–95%) was lost before the nets were in place.

The rapid development and early excystment of the River Dee mussels at Dinnet hatchery may be explained by water temperature. In unionid mussels, the duration of the parasitic period is inversely related to temperature (Lefevre & Curtis 1912, Woody & Holland-Bartels 1993). According to Ziuganov *et al.* (1994), *M. margaritifera* glochidia develop faster in warm conditions. The Dee is a colder river than the Lochailort and Moidart, and Dee mussels typically spawn (and probably excyst) 1–2 months later than Moidart mussels (Hastie 1999, Hastie & Young 2003).

However, at Dinnet hatchery, the water supply is taken from the Dinnet Burn, a small tributary of the River Dee. In summer, this stream is often several degrees warmer than the main stem of the Dee (M. Patterson, pers. comm.). Therefore, relatively high water temperatures may have resulted in shorter encysted periods of the Dee mussels in the hatchery.

In future it would be prudent to take more frequent samples of infected fish, as the time of excystment approaches, so that the time of release is not missed.

Overall, there are no technical reasons why mussel seed should not be produced in numbers sufficient for a conservation programme, using typical fish farming techniques. It is essential that the water used is from a suitable source, in which mussels thrive, and that host fish are susceptible. In this study water was drawn from natural mussel streams and fish stock were also locally native.

3 Intensive culture system – mussel baskets

3.1 Summary

During 2001 and 2003, freshwater pearl mussel seed collected at Lochailort hatchery (north-west Scotland) and Dinnet hatchery (north-east Scotland) were cultured in small sediment baskets supplied with flowing river water (the Lochailort mussels were transferred to Kinlochmoidart hatchery nearby). At Kinlochmoidart, 2000 seed were introduced to the baskets but none were found at 8 months and 11 months post-settlement. The difficulty in sampling the sediment from the baskets made the negative results inconclusive. Following the experience gained at Kinlochmoidart, 100 seed were introduced to sediment baskets at Dinnet and these produced an estimated 40 mussels at 10 months post-settlement.

3.2 Introduction

In this section, the feasibility of using an intensive cultivation technique in order to increase survival

through the critical early post-settlement phase of the *M. margaritifera* life cycle is assessed. Mussel seed were cultivated in small baskets of sediment in the carefully managed flow regime of a hatchery raceway or tank.

3.3 Methodology

3.3.1 Site selection

The work was carried out in the River Dee cSAC and the River Moidart cSAC in northern Scotland. Full details of the sites used are provided in Section 2.3.1.

3.3.2 Collection of mussel seed

Mussel seed were obtained from hatchery salmon infected with glochidia. Fully metamorphosed mussels that dropped off the fish were collected in small plankton nets (25 cm diameter, 100 mm mesh) placed below the tank drains. All the material collected (including uneaten fish-food, algae, invertebrates, sand particles) in the nets was removed daily and checked under a low-power stereo microscope (x 10). Live mussels (shell length L < 500 mm) were carefully removed using a plastic dropper and transferred into a jar of clean (river) water. The holding jars were kept cool by immersion in a tank of running water.

3.3.3 Cultivation of mussels

Kinlochmoidart hatchery

Collected mussel seed were transferred from the holding jars into small 200 µm mesh baskets (10 cm diameter x 5 cm), each containing approximately 275 cm^3 sediment. Baskets were filled with one of four different types of sediment:

- Washed riverbed sediment from the River Moidart (gravel).
- Unwashed riverbed sediment.
- Builder's sand.
- A 50:50 sand-gravel mixture.

These were submerged and stationed in alternating series in a 5 m x 0.5 m x 0.3 m raceway with flowing (gravity-fed) unfiltered river water. Each basket was supplied individually with 0.1–0.2 litres of water per minute (from an overhead pipeline). A minimum density of 100 mussels/basket was achieved.

The baskets were rotated every two weeks to avoid position effects and cleaned by brushing the sides of the baskets and gently removing layers of silt and debris from the surface of the sediment using a plastic dropper. The raceway was cleaned every month. After 6–9 months post-settlement, the baskets were sampled systematically by searching for mussels in small quantities of sediment under a stereo microscope (x 10).

Dinnet hatchery

Conditions were as described previously, with the following exceptions:

- Maximum density of 10 mussels/basket.
- Only washed riverbed sediment was used, in smaller volumes (approximately 16 cm^3 per basket).
- Baskets were hung around a 2 m diameter circular tank through which flowed approximately 10 litres of unfiltered river water per minute.
- Basket rim tops were kept above the water level to prevent water entering from the top and to prevent mussels escaping.

Searches for mussels were carried out as previously described, with the exception that only sub-

samples of sediment were removed from the baskets and searched on each occasion. Mussel size (maximum shell length, in mm) was measured using a calibrated eye-piece graticule.

3.4 Results

Kinlochmoidart hatchery

During June and July 2001, approximately 3,500 live mussel seed were collected from the Lochailort hatchery. Of these, 2,000 were transferred to 20 baskets in a raceway at Kinlochmoidart (100 mussels per basket). In February 2002, the total contents of three baskets were checked, but no mussels were found. A fourth basket was checked in May 2002, but again, no mussels were found.

It was extremely difficult to sample the baskets due to the amount of sediment and the expected small size of the mussels. It was decided to leave the remaining baskets in the raceway until 2003 and beyond, in the hope that any surviving mussels would become large enough to find. However, by the time of this report's publication, none had been discovered.

Dinnet hatchery

In June 2002, 115 live mussels were collected from the fish tank. Of these, 100 mussels were transferred to 10 sediment baskets (10 mussels per basket) and maintained over the winter. In April 2003, a 0.8 cm^3 sample of sediment (5% by volume) was taken from each basket and searched for mussels. Overall, two live mussels and one empty shell were found (in the 5% sediment samples), giving an estimated total of 40 live mussels in the 10 baskets. In other words, an estimated 40% post-settlement survival was achieved in 10 months. Two live mussel shell sizes of 1.13 mm and 1.25 mm and an empty shell size of 1.20 mm were recorded. The results of the River Dee basket trials are summarised in Table 4.

Table. 4. Descriptive statistics for cultured River Dee mussels (June 2002 – April 2003).

			Shell length L (µm)		
Date	**Age (months)**	**N**	**Mean**	**Standard deviation**	**Range**
05.06.02	0	50	420	55	313–500
07.04.03	10	2	1190	85	1113–1250

3.5 Discussion

These results demonstrate that it is possible to grow newly-settled *M. margaritifera* in sediment baskets supplied with flowing river water for at least 10 months. The negative results at Kinlochmoidart hatchery were inconclusive because of the difficulties in sampling the sediment in each basket. It was very difficult to search for mussels 1–2 mm in size. Too much sediment was added to each basket, and even if there were mussels still surviving, they could not be found.

Based on the experience gained at Kinlochmoidart hatchery (2001), much smaller quantities of sediment were added to the baskets at Dinnet hatchery (2002), in order to facilitate sampling. The fact that two live mussels were found (10 months post-settlement) despite the small number of seed initially introduced, indicates that this change in approach was successful.

The sizes of the Dee mussels (1.2 mm at 10 months) were comparable to those reported elsewhere (Buddensiek 1995; Hruska 2001; Schmidt & Wenz 2001; Preston *et al.* 2002). Previous attempts to intensively cultivate *M. margaritifera* have generally involved maintaining artificially-infected fish in special raceway tanks with sediment traps to collect excysting mussels (eg. Preston *et al.* 2002). It is far easier to simply allow excysting mussels to drop off onto prepared sediment than to physically collect them. However, it is quite difficult to maintain sediment quality and search for mussels (Preston *et al.* 2002).

By contrast, the method using baskets described here is initially more intensive, but allows for greater control and monitoring in the long term. In retrospect, the trials would have been more easily controlled at Dinnet, near to Aberdeen University, allowing more regular attention to be paid to them. Furthermore, the design using smaller sediment volumes permitted much easier searching for developing mussels. At Kinlochmoidart water entered into the top of the baskets and a 'pool' of water was always present, some of which escaped over the basket rims. This carried the risk that young mussels might be lost.

The improved results at Dinnet indicate that there is much to be gained from further development of the method, with realistic hope that success can be achieved.

4 Semi-intensive culture system – mussel cages

4.1 Summary

During 2001 and 2003, freshwater pearl mussel seed collected at Lochailort hatchery (north-west Scotland) and Dinnet hatchery (north-east Scotland) were cultured in plastic cages in the River Moidart and a hatchery tank at Dinnet, respectively. (The Lochailort mussels were initially transferred to Kinlochmoidart hatchery). Most of the cages (8-10) were lost during a 10-year return-flood on the River Moidart in 2001. However, in two remaining cages, survival estimates of 11%, 3% and 1% were achieved at 7 months, 12 months and 16 months post-settlement. In one cage at the Dinnet hatchery, a survival estimate of 7% was observed, over a 10-month period.

4.2 Introduction

Here, a technique for infecting host fish (juvenile salmonid) stocks, with M. margaritifera glochidia and maintaining them until the glochidia metamorphose, in order to produce mussel seed, is described.

4.3 Methodology

4.3.1 Site selection

The work was carried out in the River Dee cSAC and the River Moidart cSAC in northern Scotland. Full details of the sites used are provided in Section 2.3.1.

4.3.2 Collection of mussel seed

Mussel seed were obtained from hatchery salmon infected with glochidia. Details of the protocol used for collecting mussel seed from host fish are available in Section 2.3.2.

Temporary raceway set up at hatchery for maintenance of sediment pots.

Sediment pots (filter baskets) containing mussel seed (close-up).

4.3.3 Cultivation of mussels

Kinlochmoidart hatchery

Mussel seed were collected and transferred from the initial holding jars into small perspex 'cages' (125 x 85 x 12 mm), each of which consisted of a flat perspex block, into which were drilled 90 small open-ended wells (5 x 7 mm diameter) for holding newly-settled mussels. The cages were based closely on an original design for *M. margaritifera* developed by Buddenseik (1995). A selection of the wells in each cage (n <50) was used, and the mussels were prevented from escaping by securing a 100 mm mesh cover above and below each well. A maximum of two mussels were kept in each well.

Small amounts of riverbed sediment (0.1 mg) were placed in half of the wells containing mussels, and these alternated with 'empty' wells. The sealed cages were secured by non-toxic wire to stainless steel pins pushed into the riverbed and left in the River Moidart for several months. They were checked at regular intervals of approximately two months. At each visit the sides of the cages were cleaned, by gentle brushing, to ensure a through-flow of water.

Dinnet hatchery

The same procedure was used at Dinnet, with the following exceptions:

- All wells had small amounts of sediment placed in them.
- A maximum of one mussel was kept in each well.
- Cages were kept in a large tank at the hatchery, through which untreated stream water flowed at a rate of 10 litres per minute. The cages were also checked and cleaned regularly.

4.4 Results

Kinlochmoidart hatchery

During June-July 2001, approximately 3,500 live mussel seed were collected from the Lochailort hatchery. Of these, 1,200 were transferred to 10 cages (at Kinlochmoidart) at different sites in the River Moidart. Unfortunately, during November 2001, a 10-year return-flood occurred in the River Moidart and eight of the cages were lost.

In January 2002, the remaining two cages were taken to Kinlochmoidart hatchery and examined. A total of 27 live mussels were found, providing a seven-month post-settlement survival estimate of 27/240 = 11%. The cages were returned to the river and re-examined in June 2002, when 7 live mussels were found, providing a 12-month survival estimate of 7/240 = 3%. In October 2002 the cages were again checked and then returned to the river. At this time three live mussels were found, providing a 16-month survival estimate of 3/240 = 1%. The sizes (L) of the three surviving mussels aged 16 months were 2.11 mm, 2.25 mm and 1.75 mm. The cages were last checked in January 2003, but no mussels were found. The results of the River Moidart cage trials are summarised in tables 5 and 6, and a plot of post-settlement survival of the caged mussel seed is shown in Figure 1.

Dinnet hatchery

In June 2002, 115 live mussels were collected from the fish tank. Of these, 15 mussels were transferred to a cage and maintained in a tank of running water over the winter. In April 2003, the cage was checked and one recently dead mussel shell (size L = 1.13 mm) was found, providing a 10-month post-settlement survival estimate of 1/15, or 7%.

4.5 Discussion

Plastic mussel cages were originally designed to cultivate *M. margaritifera* seed in German rivers by Buddensiek (1995). He found a small percentage survival over two years but his results were inconsistent and he was unable to guarantee success. The results presented here mirror his closely and do indicate that cages could also be used in Scottish rivers, in order to produce significant numbers of

Table 5. Numbers of live mussels found in cage wells at 7, 12 and 16 months post-settlement, River Moidart 2000-2001 (% survival rates in parentheses).

	Jan 2001 (7 mo.)			Jun 2001 (12 mo.)			Oct 2001 (16 mo.)		
Cage	Sediment wells	'Empty' wells	All wells	Sediment wells	'Empty' wells	All wells	Sediment wells	'Empty' wells	All wells
1	15/60 (25)	3/60 (5)	18/120 (15)	6/60 (10)	0/60 (0)	6/120	3/60 (5)	0/60 (0)	3/120 (2.5)
2	7/60 (18.3)	2/60 (3.3)	9/120 (7.5)	0/60 (0)	1/60 (1.7)	1/120 (0.8)	0/60 0	0/60 (0)	0/120 (10)
Overall	22/120 (18.3)	5/120 (4.2)	27/240 (11.3)	6/240 (2.5)	1/120 (0.8)	7/240 (2.9)	3/120 (2.5)	0/120 (0)	3/240 (1.3)

ND = no data (mussel seed not measured).

Table 6. Descriptive statistics for cultured River Moidart mussels (June 2001–October 2002).

			Shell length L (µm)		
Date	**Age (months)**	**N**	**Mean**	**Standard deviation**	**Range**
06-01	0	50	412	42	325–475
01-02	7	27	767	71	625–875
06-02	12	7	ND	ND	ND
10-02	16	3	2037	258	1750–2110

ND = no data (mussel seed not measured).

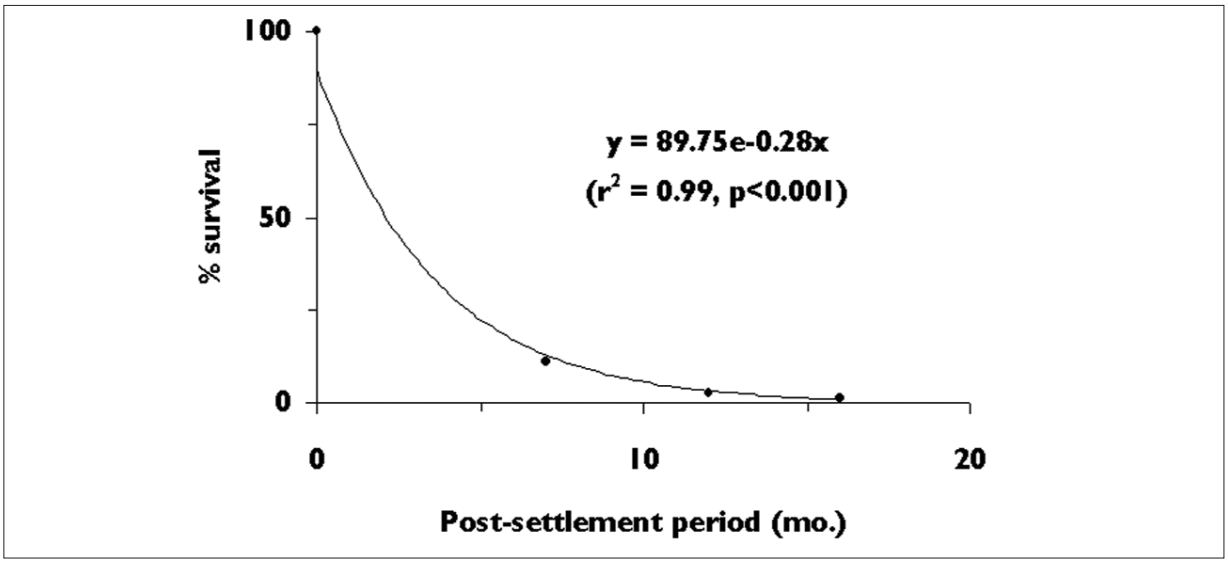


Figure 1. Plot of % survival of caged mussel seed in River Moidart (2001-2002). Fitted regression line and correlation coefficient displayed.

young mussels for conservation purposes. It is more time-consuming to prepare the cages initially, than it is to load the cages discussed in Section 3, but the advantage of this technique is that there is no requirement to maintain a raceway supplied with running river water. However, some checking and cleaning of the cages, particularly after flood events is required.

In Scotland, many rivers with *M. margaritifera* populations are highly dynamic systems and so some equipment losses due to spates would be expected. However, the 80% loss experienced during this study was exceptional and unfortunate. With hindsight, more robust stainless steel spikes should have been buried more securely into the riverbed, and with more careful consideration of site location, it should be have been possible to reduce operating cage losses to <10% per annum. In most years all cages should remain secure.

Most of the caged mussels that survived in the River Moidart were found in the cage wells with sediment. This corroborates the work of Buddensiek (1995), who also reported increased survival in cages with small amounts of sediment added. Newly-settled *M. margaritifera* are thought to deposit-feed briefly, by scraping the bacterial film from sediment particles using the tip of their muscular foot, before commencing filter-feeding (Wachtler *et al.* 2001). Thus, sediment particles may be essential for the nutrition of *M. margaritifera* seed before they switch to filter-feeding.

If filter feeding is to be successful, the mesh used on the cages must be of such a size that organic food particles can pass in and reach the mussels. More success might be achieved if the initial fine mesh was replaced by progressively larger mesh as the mussels grow.

Schmidt and Wenz have also recently been using cages based on Buddenseik's design to try to cultivate young mussels. They also achieved inconsistent results, with adjacent cages in one river having very different success rates. Moreover, they have never found survival above 1% over two or more years' cultivation, indicating that some development must be carried out in cage design (C Schmidt and G Wenz, pers. com.). Overall, the results suggest that there is something to be gained from continuing to develop the cage design, but that at present they are not sufficiently successful to be an effective way of obtaining young mussels.

5 Extensive culture system – release of artificially infected fish

5.1 Introduction

Numerous attempts have been made to restore depleted freshwater pearl mussel populations by releasing large numbers of host fish artificially infected with glochidia (Jungbluth 1971; Wachtler *et al.* 1987; Bauer 1988b; Hsruska 1992, 2001; Schmidt & Wenz 2001; Preston *et al.* 2002). This strategy is particularly popular with conservation managers because it is relatively inexpensive and widely applicable (Preston *et al.* 2002). However, it is very difficult to assess the effectiveness of this strategy in terms of its impact on mussel recruitment success.

A considerable amount of research is required in order to determine mortality rates during the parasitic (encysted) and early post-settlement (excysted) phases of the *M. margaritifera* life cycle. Preliminary field studies have provided estimates of approximately 95% mortality for each of these phases, even in successfully recruiting populations (Young & Williams 1984a, 1984b, Hastie & Young 2001). The release of infected host fish could be a feasible option based on these estimates, as long as very large numbers of fish, each heavily infected with glochidia, are used. However, the figures were calculated for near-optimal river habitat conditions, and under these circumstances, intervention would not be required, unless the adult mussels had been effectively wiped out, for example by destructive pearl fishing, without reducing the intrinsic quality of the habitat.

The only example of an apparently successful attempt to boost the numbers of young mussels present in a river is that of the conservation authorities in Lower Saxony, Germany. There the habitat of a mussel river, the River Lutter, was improved by the diversion of drains that had been producing heavy sedimentation in the river. Thereafter, juvenile trout were collected from the river, immediately infected with glochidia, and promptly released into the same sections from which they had been removed. A small number of young mussels have been found in the river in the last two years, but so far not in numbers sufficient to ensure survival of the population (Altmuller, pers. com.).
In Scotland a number of *M. margaritifera* populations have been wiped out by pearl fishing (Cosgrove *et al.* 2000), so some of the extinct and non-viable historical sites are still thought to contain suitable riverbed habitat conditions for optimal recruitment (Young 1995, Hastie 1999). These would certainly be suitable candidate sites for extensive mussel cultivation trials. The suitability of other sites, where the habitat is degraded and recruitment is currently inadequate to maintain the population in the long term, would depend on the introduction of effective habitat improvement measures. In many cases, the

river habitat has been degraded to such an extent that the release of infected fish would be a complete waste of resources (Young 1991). However, there are also likely to be a number of sites where the natural habitat is currently sub-optimal or 'marginal' for *M. margaritifera*, and some recruitment occurs, albeit at a very low level, and these may benefit from direct intervention. Releases of the infected host fish should be preceded and accompanied by measures to protect and (where appropriate) improve the riverbed habitat of *M. margaritifera* in order to ensure their effectiveness in the long term.

5.2 Methodology – technical options

5.2.1 Release of hatchery-reared fish

To date, most attempts to boost *M. margaritifera* populations using artificially-infected host fish have involved the release of hatchery-reared fish, usually trout/salmon fry (0+ fish) or parr (1++ fish). This is because it is generally perceived to be the least-expensive and least-difficult method available. However, hatchery-reared fish are less able to survive in wild conditions, and it is unclear how many fish carrying glochidia are likely to survive long enough to produce mussel seed. Pearl mussel workers Schmidt and Wenz report that six weeks after the release of 1000 marked farmed trout into a mussel river, they were only able to recapture seven fish, and they speculate that farmed fish are out-competed by native fish (C Schmidt and G Wenz, pers. com.). The effect of such losses could be minimised by releasing the infected fish shortly before the mussels are expected to excyst and drop off, onto the riverbed.

According to Hruska (2001), hatchery-reared fish have no immunity to glochidia, so greater numbers of glochidia can be raised initially. However, these fish may lack sufficient vigour as hosts, adversely affecting young mussel growth and metamorphosis and subsequent post-settlement survival of the mussels (Hruska 2001). Field studies are urgently required in order to determine the survival of infected hatchery-reared fish and newly-settled mussels in the wild.

5.2.2 Infection of wild fish

An alternative approach is to infect the resident host fish in a river with *M. margaritifera* glochidia (Hastie 1999), as reported above for the River Lutter. It is easy to obtain large numbers of viable glochidia from gravid female mussels (Young & Williams 1983). The infection of wild fish *in situ* could be achieved by either releasing glochidia directly into the river (Jansen *et al.* 2001) or by capturing and infecting wild fish before releasing them immediately back into the river. An advantage of the latter technique would be that a controlled number of wild fish could be infected. Overall estimates of numbers of encysted glochidia could be monitored for both techniques by examining re-captured wild fish (electro-fishing surveys).

An advantage of artificially infecting wild fish (either *in situ* or in a more controlled manner) over using hatchery-reared fish, is that there is no requirement to maintain wild fish (except possibly for a very brief period of time). Furthermore, wild fish are better adapted and more likely to survive river conditions, so fitness and post-settlement survival of excysted mussels may be more ensured.

An exceptional circumstance would be where wild host fish numbers are considered to be too low to support *M. margaritifera* populations in the long term. For example, in north-west Scotland, migratory salmonid stocks have collapsed, and as a result, host fish densities in small streams may now be insufficient to support *M. margaritifera* populations (Hastie & Cosgrove 2001). In such a scenario, the release of hatchery-reared fish would be a more appropriate option. However, this would only be advantageous where the reasons for the low fish numbers lay with external factors, such as over-fishing at sea.

The relative success of the River Lutter scheme, taken together with the existence of rivers in Scotland where the habitat apparently remains in good condition, but where adult mussels have been dramatically reduced by pearl fishing, indicates that regeneration of some mussel populations may be achieved by the use of artificially infected fish. However, it is clearly important that the glochidia used to infect fish originate from the river into which the young mussels are to be released. If no or very

few mussels can be found in a river, following over-fishing, then an alternative source of glochidia must be considered very carefully. We recommend that glochidia from a nearby river should only be considered for use if it is certain that no donor mussels remain. This decision should follow careful discussion by the relevant authorities.

6 Conclusions and recommendations

The results of this project demonstrate that it is not difficult to infect stocks of juvenile salmon or trout with large numbers of *M. margaritifera* glochidia in order to produce mussel seed. Based on the numbers of glochidia released by each female mussel, and the seed produced per fish, potentially several million young mussels could be produced at a large freshwater salmon farm in northern Scotland. Workers in Europe have already established that it is just about possible to cultivate *M. margaritifera* through the critical early post-settlement phase, and our own results are similar.

However, the mixed results obtained using the sediment baskets in raceways indicate that more research is required to improve performance, but also that it would be worthwhile to continue this work. The cages performed reasonably well in a Scottish river, with good survival rates recorded for the young mussels. However, 80% of the cages were lost due to a large spate, indicating that they must be positioned in less-exposed areas and very tightly secured. It should be possible to reduce cage loss due to floods to <10%. The advantage of using sediment baskets over cages is that they allow for greater control and monitoring of mussels. The cages, on the other hand, are more time-consuming to prepare but, once in the river, they require less attention and require lower operating costs.

The release of large numbers of infected fish into rivers is also a feasible option, but steps must be taken in order to ensure that the riverbed habitat is suitable to support *M. margaritifera* during the early post-settlement phase.

All of this refers to the increase in numbers of mussels up to a maximum of two years after excystment from the fish host. It is presumed that the major mortality in young mussels occurs during the immediate post-settlement stage, and that even two-year-old mussels are by then subject to much lower mortality rates. However, this is speculative, and it may be that it is necessary to help mussels survive to an even greater age, before their subsequent survival can be assured.

It is therefore possible to culture young *M. margaritifera* in order to try to restore depleted populations in Scotland and elsewhere. However, is clear that more research is required to achieve significant survival post-settlement rates to ensure effective conservation. Northern Scotland is perhaps one of the best locations to continue research on *M. margaritifera* cultivation for a number of reasons. These include the following:

- Scotland is a stronghold of *M. margaritifera*, with approximately 50% of the world's known viable populations.
- There are a significant number of Scottish rivers with *M. margaritifera* populations depleted or extinct due to pearl fishing that still have suitable riverbed habitat conditions for effective restoration work.
- There are a considerable number of *M. margaritifera* experts in Scotland, where research has been carried out on this species for >25 years.
- There are numerous commercial freshwater salmon farms in Scotland, where large numbers of mussel seed could be produced.

Freshwater pearl mussel cultivation is relatively expensive and labour-intensive. The experimental work carried out during this project was dependent on the co-operation and kindness of three Scottish commercial organisations (fish farm companies and a fishery trust). During the project, the work was often disrupted because of staffing and operational changes made at the different fish farm sites. Both mussels and fish had to be moved around the hatcheries several times, and proper maintenance of the raceways and husbandry was not ideal at times. For continuity and effective cultivation work to be

carried out at the scale required for *M. margaritifera* conservation in Scotland, it is recommended that proper contracts or binding agreements with companies operating salmonid hatcheries are made. This will be more costly, but it should result in acceptable (mussel seed) production levels. In this respect, determining how much it costs to produce an adult freshwater pearl mussel, by cost-benefit analysis, based on operating costs and predicted mussel survival rates, would be very worthwhile. The ideal approach would be to set up a dedicated mussel cultivation unit in Scotland, using a converted salmon hatchery (employing two or three trained staff) on a river that supports a viable mussel population. There remains an open question as to whether each river needs a separate rearing unit, so that local water and local mussel stocks can be used.

Another consideration is that freshwater pearl mussels live for more than 100 years and take 12–20 years to mature. Only limited success in mussel cultivation could reasonably be expected within the three-year time period of the present project. It is therefore recommended that further research in this area should be planned over at least a five- to 10-year period. Young mussels need this length of time to grow to a size where they can be found and handled safely.

An appropriate follow-up to this work would be to continue to experiment with mussel cultivation, and also to attempt the restoration of a small number (n = 1–5) of depleted *M. margaritifera* populations in Scotland where the riverbed habitat is still considered to be suitable. Scotland is probably unique in that a large proportion (70%) of *M. margaritifera* populations have been affected by destructive pearl-fishing, and a considerable amount of suitable but unused habitat is still present. In most other countries where *M. margaritifera* conservation projects are being carried out, the habitat is largely degraded and therefore attempts to restore populations will be far more difficult.

7 Summary

The feasibility of cultivating endangered freshwater pearl mussels (*Margaritifera margaritifera*) as a conservation tool for the restoration of depleted populations was investigated. During 2000 and 2003, stocks of juvenile salmon infected with *M. margaritifera* glochidia were maintained at two hatcheries in northern Scotland in order to produce mussel seed. At Lochailort, 500 fish produced 3500 mussel seed (2000–2001). At Dinnet, 400 fish produced 115 seed (2001–2002) and 20 seed (2002–2003). The mussel seed were cultivated in small sediment baskets supplied with flowing river water (the Lochailort mussels were transferred to Kinlochmoidart hatchery nearby). At Kinlochmoidart, 2,000 seed were introduced to the baskets but none was found at eight months and 11 months post-settlement. The difficulty in sampling made the negative results inconclusive. At Dinnet, 100 seed were introduced to similar sediment baskets (but with an improved technique) and produced an estimated 40 mussels at 10 months post-settlement. Samples of seed were also cultivated in plastic cages in the River Moidart and a hatchery tank at Dinnet. Most of the cages (8/10) were lost during a 10-year return-flood of the River Moidart in 2001. However, in two remaining cages, survival estimates of 11%, 3% and 1% were achieved at 7 months, 12 months and 16 months post-settlement. In one cage at Dinnet hatchery, a survival estimate of 7% was observed.

It has been demonstrated that it is possible to grow young *M. margaritifera* to restore depleted populations in Scotland and elsewhere. However, is clear that more research is required in order to achieve the post-settlement survival rates needed for effective conservation. Northern Scotland is perhaps one of the best locations to continue research on *M. margaritifera* cultivation for a number of reasons:

- Scotland is a stronghold of *M. margaritifera*, with approximately 50% of the world's known viable populations.
- There are a number of Scottish rivers with *M. margaritifera* populations depleted or extinct due to pearl fishing that still have suitable riverbed habitat conditions for effective restoration work.

- There are a considerable number of *M. margaritifera* experts in Scotland, where research has been carried out on this species for >25 years.
- There are many commercial freshwater salmon farms in Scotland where large numbers of mussel seed could be produced.

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The Life in UK Rivers project was established to develop methods for conserving the wildlife and habitats of rivers within the Natura 2000 network of protected European sites.

Set up by the UK statutory conservation bodies and the European Commission's LIFE Nature programme, the project has sought to identify the ecological requirements of key plants and animals supported by river Special Areas of Conservation.

In addition, monitoring techniques and conservation strategies have been developed as practical tools for assessing and maintaining these internationally important species and habitats.

The freshwater pearl mussel (*Margaritifera margaritifera*) is one of the most endangered invertebrates in Europe. In the UK, viable populations are only found in Scotland, and these sites hold almost half the world's remaining populations with active recruitment.

The freshwater pearl mussel has a unique association with juvenile trout and salmon, so any management efforts designed to conserve the pearl mussel must also consider the needs of these fish. Reintroduction of captive-bred pearl mussels has so far been ineffective in the long term, particularly where their habitat has not been conserved or restored.

This publication reports on a project to develop more successful rearing techniques for the freshwater pearl mussel, so as to improve the chances of using these to establish a conservation programme for the mussel in rivers where environmental quality has been restored.

Information on Conserving Natura 2000 Rivers and the Life in UK Rivers project can be found at **www.riverlife.org.uk**

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