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Kyle, R., Reid, N., O'Connor, N., & Roberts, D. (2016). Development of release methods for critically endangered freshwater pearl mussels (Margaritifera margaritifera). DOI: 10.1002/aqc.2704

Published in:
Aquatic Conservation Marine and Freshwater Ecosystems

Document Version:
Peer reviewed version

Original Research

Development of release methods for captive-bred freshwater pearl mussels (*Margaritifera margaritifera*)

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Funding Information

This project was commissioned and funded by the Natural Heritage Research Partnership (NHRP) between the Northern Ireland Environment Agency (NIEA) and *Quercus*, Queen's University Belfast (QUB).

Running title

Ex situ conservation of endangered *M. margaritifera*

Keywords

Conservation, endangered species, freshwater pearl mussel, juvenile culture, mussel silo, release, survival rate

Abstract

Biodiversity loss is a global problem with freshwater bivalves considered amongst the most endangered biota. The freshwater pearl mussel, *Margaritifera margaritifera,* is declining throughout its range owing to habitat degradation and overexploitation. In most of its range, populations are regarded as reproductively non-functional which has led to the development of captive breeding programmes. A novel method of releasing *M. margaritifera* was trialled, with captive-bred juveniles being released into the rivers caged in 'mussels silos' (protective concrete domes with ventilation creating upwelling to ensure water through flow). We released 240 juvenile mussels and survival and growth rates were monitored for 18 months post-release for three size classes: A (13.01-20.00mm); B (10.01-13.00mm); and C (4.01-10.00mm). We explicitly tested two experimental treatments; one where sediment was added to each silo (allowing mussels to orientate and burrow) and one without sediment. Survival by the end of the experiment at month 18 was significantly higher for the largest size class at 97% (though growth was lowest in this cohort), and lowest for the smallest size class at 61% (though growth was highest in this cohort). Survival and growth were unaffected by the experimental treatment suggesting that adding sediment offered no advantage. Growth was positively correlated with both water temperature and the particle size of suspended solids (both of which were collinear, peaking in summer). There are a large number of *ex situ* breeding programmes for freshwater pearl mussels throughout Europe and our finding suggest that the use of 'mussel silos' could be a useful tool to protecting juvenile mussels allowing them to be released at a relatively early stage of development, minimising the risk of domestication.

1.1 Introduction

Biodiversity loss is a global problem occurring across all ecosystems and taxa (Brooks et al., 2002, 2006; Hooper et al., 2012). Freshwater ecosystems have a higher extinction rate than terrestrial systems (Saunders et al., 2002) and freshwater mussels are considered among the most globally endangered biota (Geist and Auerswald, 2007; Geist and Kuehn, 2005; Strayer et al., 2004). Ricciardi and Rasmussen (1999) estimated that at least 127 imperilled mussel species would disappear within the next 100 years, suggesting the extinction rate could be as high as 6.4% per decade. Numerous *ex situ* conservation programmes for both aquatic and terrestrial species have been developed in an attempt to help reduce predicted biodiversity losses (Bolland et al., 2010).

Across North America and Europe facilities have been developed to cultivate endangered or threatened freshwater mussels using various methods with the aim of releasing them if suitable habitat remains or is restored (Jones et al., 2006). In North America an intensive cultivation system is often used. Juveniles are propagated in laboratory conditions and kept in baskets or raceways in either flow-through or re-circulating systems with detritus added as a food resource (Gum et al., 2011; Jones et al., 2005; Thomas et al., 2010). A number of hatcheries in Europe use a similar approach (Buddensiek, 1995; Schmidt and Vandré, 2010), although a small number use less intensive approaches that involve bringing mussels and fish host into closer proximity under semi-natural environmental conditions (Gum et al., 2011; Preston et al., 2007). A number of intermediate rearing cages have been developed and trialled to release mussels at an early stage directly into the release site to avoid the risk of domestication during prolonged periods in captivity, such as Buddensiek sheet cages (Buddensiek, 1995), gauze bags (Schmidt and Vandré, 2010) and mussel silos (Barnhart et al., 2007), Table 1.

The freshwater pearl mussel, *Margaritifera margaritifera*, is a long-lived, globally endangered bivalve with populations having declined by up to 90% throughout its Holarctic range over the course of the 20th century (Bauer, 1988; Cosgrove et al., 2000;Bolland et al., 2010; Reid et al., 2013). These declines have primarily been linked to pollution driven by nutrient enrichment and habitat degradation (Beasley and Roberts, 1999; Hastie et al., 2003; Reis, 2003; Österling et al., 2010;Österling and Högberg, 2014), the lack of suitable host fish (Geist et al., 2006; Arvidsson et al., 2012; Österling and Larsen, 2013), and historically pearl fishing on a local scale (Cosgrove et al., 2000). The freshwater pearl mussel has a complex lifecycle, during which the parasitic glochidia (larvae released from the gills of the female mussel) spend up to nine months on the gills of a host fish, normally salmon, *Salmo salar*, or trout, *Salmo trutta* (Geist et al., 2006). *M. margaritifera* is regarded as an indicator species because it is sensitive to environmental change and is regarded as a keystone species due to its filtering because of its filtering capacity (Geist, 2010).

M. margaritifera is generally found in clean, moderate-fast flowing waters and is associated with a stable cobble-boulder substratum including sand for burrowing (Hastie *et al.*, 2000, 2003; Wilson *et al.*, 2011). Juvenile mussels spend the first four to five years completely burrowed in the riverbed and require high rates of oxygen exchange between free moving and interstitial water (Buddensiek, 1995). If conditions are muddy or silty the juveniles will suffocate as a result of clogging of the interstitial spaces inhibiting the exchange of oxygen (Hastie et al., 2000; Geist and Auerswald 2007).

In Northern Ireland, the freshwater pearl mussel, which anecdotal evidence suggests was once found abundantly throughout the country (Beasley et al., 1998),is recently extinct from ten rivers and its range is now restricted to short stretches of just six rivers (Fig. 1). Remaining remnant populations can be separated into three genetically distinct metapopulations which should be managed separately in any proposed captive breeding

programmes (Wilson *et al.* 2012). Recent surveys suggest there approximately 22,000 mussels remain throughout their range in Northern Ireland but that all populations are regarded as reproductively "non-functional" owing to a lack of recruitment (Reid et al., 2013). Total species extirpation in Northern Ireland has been predicted by the year 2098 (Wilson and Roberts, 2011). Surveys have revealed there are fewer than 1000 wild mussels in the Ballinderry River (Reid et al., 2013); these declines are linked primarily to habitat degradation and declines in host fish (Horton et al., 2015).

Consequently, an *ex situ* captive breeding programme was established in 1998 with 100 adults taken as broodstock for a custom-built facility on the Ballinderry River (Fig. 1; Preston *et al.*, 2007). This system uses a semi-natural approach to cultivation, allowing natural fertilisation of mussels, with water from mussel tanks draining into tanks containing suitable host fish for infection. Infected fish are then transferred a vivarium (a tank set up to mimic the natural river with substratum and controlled flow) where glochidia to fall off (excyst) the gills of the fish and burrow into river substratum about nine months later (Preston et al., 2007). Previously, 350 hatchery-reared juvenile mussels (*ca.* 8-10 years old) were released directly into the Ballinderry River at three sites in February and August 2009 with subsequent survival monitored by passive integrated transponders (PIT) tags (Wilson 2010). Recovered shells show mortality rates during the first 18 months post-release ranging from 4.3 - 14.3% with the estimated maximum mortality calculated from mussels which were not recovered again ranging from 33.6-36.6% suggesting hatchery-reared mussels could survive at least initial release into the river.

The semi-natural cultivation system used in Northern Ireland means that when individuals are large enough to be collected from the vivarium, they are too large for Buddensiek cages (Buddensiek, 1995). This study aimed to test the use of mussel silos (Fig. 2 and 3), designed by Barnhart *et al.* (2007), as an alternative release method facilitating the release of larger

juvenile mussels than are used by other programmes. We hypothesised that releasing larger individuals will result in greater survival and thus success for the captive breeding and release programme. Mussel silos use the Bernoulli effect (Barnhart et al., 2007), to draw a continuous supply of water through a hole in the centre of a concrete dome (Barnhart *pers. comm.*). The main objectives of this study were to test: 1) The efficacy of mussel silos at improving juvenile *M. margaritifera* survival; 2) the minimum size at which juvenile mussels can be released with comparable (or better) survival to other methods; 3) the variation in survival and growth rate among different size classes, treatments and release sites.

1.2 Materials and Methods

1.2.1 Site selection

Extensive surveys were carried out along the Ballinderry River catchment to identify two suitable sites to trial the release of juvenile *M. margaritifera* into mussel silos. Site 1 was a tributary of the main Ballinderry River channel selected because of high water quality and the presence of suitable habitat, such as cobbles with gravel for burrowing and bankside vegetation (following Wilson *et al.*, 2011; Horton *et al.*, 2015). Site 2 was on the main Ballinderry River channel with suitable habitat (following Wilson *et al.* 2011) and a nearby extant remnant, non-functional, adult mussel population.

1.2.2 Selection and tagging of mussels

Juvenile mussels were collected from the *ex situ* culturing vivarium and divided into three size classes, with 80 individuals in each; Class A = 13.01 - 20.00 mm (mean 15 mm), Class B = 10.01 - 13.00 mm (mean 11 mm) and Class C = 4.00 - 10.00 mm (mean 8 mm). Prior to release, each mussel was quantified and tagged with a bee tag (EH Thorne (Beehives) Ltd., Lincolnshire, UK) for identification purposes. Each mussel was swabbed with alcohol, lightly sanded and had alcohol applied again until dry. The bee tag was then attached using Loctite Precision Super Glue.

1.2.3 Experimental design

Four individuals from each size class were included in each silo (12 individuals per silo). At each of the two sites 10 silos were deployed, five of which had the sediment treatment and five of which had the no sediment treatment. Mussels in the sediment treatment had a small amount of river substrate gravel included within the chamber (which was filled up to 2.5 cm from the top of the chamber) where mussels were held. The no sediment treatment had

nothing included with the mussels which is what had been trialled previously (Barnhart et al., 2007). Sediment was included as a treatment to test the hypothesis that mussels exposed to sediment should have a higher survival and growth than mussels not exposed to sediment as they would be able to orientate and anchor themselves within the sediment rather than being vulnerable to the water flow.

1.2.4 Maintenance and monitoring

Site visits were carried out once a week when conditions permitted to ensure there were no blockages interrupting water flow through the mussel silo chamber. Mussel silo chambers were opened once a month and survival (0/1) was recorded for each individual over an 18 month period (from September 2013 to March 2015). During the course of the experiment, one mussel chamber, which was part of the sediment treatment, was lost at Site 2 either by being washed out or stolen, and was excluded from analysis.

Temperature and siltation data were collected each month to provide descriptions of background conditions and help interpret results. An eleventh silo containing gravel without mussels was included at each site as a sediment trap to quantify the settlement of suspended solids; this was emptied and refilled each month. Sediment analysis was carried out with the Department of the Environment Marine Division. HOBO Pendant® Temp/Light, 64K data loggers (Tempcon Instrumentation Ltd., West Sussex, UK) were deployed at each site, logging temperature every two hours.

Mussel growth was quantified over three consecutive six monthly periods (0-6, 6-12 and 12-18 months) after release. Instantaneous growth rate (r) was calculated as:

$$r = ln(Sl_t) - ln(Sl_{t-1})$$

where *Sl* was shell length in millimetres (mm) at time period *t* (current measurements) or *t-1* (previous measurement) expressed as a natural logarithm.

1.2.5 Data analysis

Survival and growth of juvenile *M. margaritifera* post-release were examined using General Linear Mixed Models (GLMMs). Survival (0/1) was fitted using a binomial logistic distribution whilst growth was fitted using a gamma distribution (i.e. data were highly left skewed). Individual mussel identity was included as a nested Random Factor within Site i.e. Site (Individual_ID) fitted with an autoregressive error structure (AR1) to account for multiple measurements per individual per site. Site (1/2), Size Class (A, B and C), and Treatment (Sediment/No Sediment) were fitted as fixed factors. For the survival model, the effect of time was fitted as eighteen Months (0-18 inclusive) whilst for the growth model; time was fitted as three Periods (0-6, 6-12 and 12-18 months). Two-way interactions were initially fitted but subsequently dropped as they were not significant. *Post-hoc* pairwise differences in survival between size classes by the end of the experiment at month 18 were tested by *t*-tests. The relationship between growth rate and water temperature and sediment particle size was examined using Pearson's correlation whilst differences in monthly water temperature and sediment particle size was tested between site using paired *t*-tests. Graphs show combined data from sites 1 and 2. Results examine 228 mussels rather than 240 as one cell was lost before mortality and growth could be monitored. All statistical analysis was performed using IBM SPSS v21 and all plots were drawn in SigmaPlot v12.

1.3 Results

Post-release survival varied significantly ($F_{df=21,4082}$= 8.950, p<0.001) among size classes, site and month but not between experimental sediment treatments (Table 2a). Survival by the end of the experiment at month 18 differed significantly between all size classes (*post-hoc* pairwise contrasts p<0.020) where the largest size class A (~15 mm) had the greatest survival (97%), followed by the intermediate size class B (~11 mm; 86% survival) and the lowest survival was for the smallest size class C (~8 mm; 61% survival). Survival rates in the smallest size class C stabilised after 9-10 months (Fig. 4). Survival (mean percentage ± 1SD) was lower at Site 1 (76 ± 43% survival) compared to Site 2 (87 ± 34% survival). Survival in silos without the sediment treatment was 82 ± 39% compared to 81 ± 40% for those with the sediment treatment 18 months post release.

Growth rates varied significantly ($F_{df=6,455}$ = 82.989, p<0.001) among size classes, site and time periods (three six monthly periods), but not between experimental sediment treatments (Table 2b). The instantaneous growth rate (mean ± 1SD) was similar between the largest size class A (0.014 ± 0.009) and the intermediate size class B (0.015 ± 0.010) but substantially higher (+38%) in the smallest size class C (0.020 ± 0.032) 18 months post release. Growth rate was 53% lower at Site 1 (0.023 ± 0.027) than at Site 2 (0.050 ± 0.055). Growth was 4.3 fold greater during the summer period (6-12 months post release) than the winter period (0-6 and 12-18 months post release), but did not vary between sediment treatments (Fig 5) despite mussels exposed to sediment having a 9% higher growth rate (0.038 ± 0.050) than those in the no sediment treatment (0.035 ± 0.041). Growth was positively correlated with both water temperature (r_p = 0.733, p<0.001) and sediment particle size (r_p = 0.217, p<0.001). Particle size was weakly correlated with water temperature (r_p = 0.100, p=0.009) with sediment deposition being greatest during summer low water flow (Kyle *pers. obs.*). Particle size was significantly smaller (paired $t_{df=12}$ = 3.269, p=0.007) at Site 1 (592.1 ± 114.4µm) than Site 2

1 (713.6 ± 154.1µm) whilst water temperature was lower but not significantly so (paired $t_{df=17}$ =

2 0.318, p=0.754) at Site 1 (8.9 ± 3.0^{o}C) than Site 2 (9.1 ± 3.4^{o}C).

1.4 Discussion

Larger mussels were shown to have the greatest survival post-release (size class A; 13.01-20.00 mm) and were virtually all alive after 18 months but had the lowest growth rate. Small mussels had the highest mortality of almost 40% (size class C; 4.01 – 10.00 mm) after 18 months post-release but had the highest growth rate. Post-parasitic *M. margaritifera* spend the first four to five years of their life completely submerged in the sediment pedal feeding before making the transition to filter feeding (Bauer and Vogel, 1987; Geist and Auerswald, 2007). Mussel size varies across its range (Miguel et al., 2004; Ziuganov, 2004) and age determination of the shell is most accurate when the mussel is sacrificed (Helama and Valovirta, 2008), therefore, very little is understood about when exactly the pedal-filter feeding transition takes place and whether it is a gradual change or sudden. It is possible that a least a proportion of the mussels in size class C were still in the pedal feeding stage and food availability was a limiting resource contributing to their higher mortality rates. They would also be more vulnerable to being physically covered by silt.

This study had a mean mussel survival of 81%, which was relatively high compared to similar release studies (Table 1). A previous study examining *Villosa iris* using mussel silos designed by Barnhart *et al.*, (2007) showed a similarly high survival rate ranging from 81-88% (Johnson et al., 2014). Studies using various other cage release methods of *M. margaritifera* suggest a large degree of variation in survival from 0.21 - 82 % (Hastie and Young, 2003; Johnson et al., 2014; Schmidt and Vandré, 2010; Wilson, 2010). Buddensiek cages are widely used as an intermediate release method for juvenile *M. margaritifera* (Schmidt and Vandré, 2010; Eybe et al., 2013). However, mussels released in Buddensiek cages have been shown to have highly variable survival rates. Buddensiek (1995) released juveniles in Buddensiek cages ranging from <500 - >900 µm and found 100% of individuals less than <700 µm died. Wilson (2010) carried out direct releases into the sediment of

mussels, which were *ca.* 10 years old and had a high survival rate, suggesting size (age) at release is a strong indicator of survival. Based on these findings it would be recommended that mussels in size class C (4.00 - 10.00 mm) should not be released using mussel silos but should be maintained within the hatchery facility and released when they attain a minimum of size class B (10.01 - 13.00 mm).

Whilst there was no significant difference in water temperature between sites, highest survival and growth were coincident with highest water temperature (i.e. at Site 2). Growth was positively correlated with water temperature and it is well known that many important *M. margaritifera* life stages are achieved only when a certain threshold of degree days have been experienced (Scheder et al., 2011), highlighting the importance of site selection before release (Bolland et al., 2010). Sediment deposition was greatest during low summer flow when temperatures were highest. Thus, particle size was correlated with water temperature but this probably reflects total levels of sediment deposition rather than a skew in sediment particle size distribution with temperature. Site 1 had a higher level of sediment deposition than Site 2 which could have interrupted water flow through mussel silos, therefore, limiting food causing lower growth and higher mortality at Site 1.

Experimental sediment treatment had no effect on survival or growth rates of juvenile *M. margaritifera* in mussel silos. Previous investigations by Barnhart *et al.* (2007) didn't include sediment, however in this experiment sediment was added to allow mussels to orientate themselves within the cage system.

The greater survival of larger size classes suggest that there are benefits to rearing juvenile mussels to larger sizes before being released with silos, with little effect of domestication. Mussel silos provide a high survival rate in release studies (Barnhart et al., 2007; Johnson et al., 2014), and can be securely fitted within a river to ensure they are not washed away during flood events. Although sediment was included to allow mussels to orientate themselves, it

was not found to be of benefit, with no sediment included the silos require relatively little husbandry whereas Buddensiek cages need regular cleaning (Scheder et al., 2014). Recent surveys have shown that the *M. margaritifera* population in the Upper Ballinderry River catchment is < 1,000 individuals and is regarded as reproductively non-functional (Reid et al., 2013), with extinction estimated to be in the year 2098 (Wilson and Roberts, 2011). Thus, the successful release of juvenile mussels via mussel silos coupled with catchment restoration work nearing completion (Horton et al., 2015) should improve the chances of recreating a reproductively functional population with a varied age structure. This work has included bank revetment works, erecting stock proof fencing along the length of the river and closing open cattle drinkers (Horton et al., 2015). Findings reported in the present paper are widely applicable to other small populations of *M. margaritifera* which are reproductively non-functional (i.e. not recruiting). A number of *ex situ* captive breeding programmes are producing large numbers of juveniles (Preston *et al.*, 2007; McIvor and Aldridge, 2008, and references therein; Scheder and Gumpinger, 2008). Such mussels could be grown in silos, as described above, to a size when they can be released into restored natal rivers. Future work should therefore establish the size at which mussels can be transferred from silos to natural sediments show survival levels comparable to those reported by Wilson (2010) for mussels which have recently reached sexual maturity. An advantage of this approach is the mussels in the silos also serve as biological indicators that test whether water quality is suitable for mussel release.

Acknowledgements

This project was commissioned and funded by the Natural Heritage Research Partnership (NHRP) between the Northern Ireland Environment Agency (NIEA) and *Quercus*, Queen's University Belfast (QUB). The Client Officer was Tony Waterman. Special thanks go to Ballinderry Rivers Trust (David Bell, Katie Bruce, Emma Downie, Mark Horton, Alan Keys, Lisa Kirkwood and Frank Mitchell) for the provision of animals, building mussel silos and fieldwork and to Mike Allen (DoE Marine Division) for help with sediment analysis.

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Table 1 Average survival rates using different methods of caged release for freshwater mussels.

Species	Method	Cage Mechanism	Survival	Reference
Villosa iris	Mussel silos	See method	81-88%	Johnson *et al.*, 2014
Margaritifera margaritifera	Buddensiek sheet cages	Acrylic plate with drilled holes which are surrounded by a fine mesh. Each hole houses one mussel (~1mm). The box is placed upright in the river to allow constant flow of fresh water through the mesh	0.21-80%	Schmidt and Vandré, 2010; Eybe et al., 2013
	Mussel cages	Modified Buddensiek sheet cages	1-7%	Hastie and Young, 2003
	Gauze bags	Gauze bags are filled with sieved gravel collected from mussel rivers. Mussels are placed in the bag which is then embedded in the river bed.	0.7-6.0%	Schmidt and Vandré, 2010
	Sediment mussel baskets	Plastic bowls are placed under a water outlet. Plastic colanders lined with gauze and filled with sieved gravel are set into the water filled bowl. Juveniles are placed in the gravel in the colander.	2.9-82%	Hastie and Young, 2003; Schmidt and Vandré, 2010

Table 2 Generalised Linear Mixed Model (GLMM) results for **a)** survival and **b)** growth rates of juvenile *Margaritifera margaritifera*. The function used to fit each model is shown in parentheses. Two-way interactions were initially fitted by subsequently dropped as they were not significant. (n.df = nominator and d.df = denominator degrees of freedom respectively).

Explanatory variable	***F***	**n.df**	**d.df**	***p***
a) Survival (binomial logistic)				
Site	4.059	1	4082	**0.044**
Size class	13.382	2	4082	**<0.001**
Month (0-18 inclusive)	10.389	17	4082	**<0.001**
Treatment	0.016	1	4082	0.898
b) Growth rate (gamma)				
Site	48.011	1	455	**<0.001**
Size class	3.235	2	455	**0.040**
Period (0-6, 6-12, 12-18)	195.425	2	455	**<0.001**
Treatment	0.581	1	455	0.446