Report on assisted breeding of the Nore pearl mussel 2012 - 2014

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Cover photo: Nore pearl mussel © Evelyn Moorkens

NOTE: This is a summary version for web publication of a report prepared for National Parks and Wildlife Service, Department of Arts, Heritage and the Gaeltacht. Locational information has been removed owing to the risk of pearl-fishing.

The NPWS Project Officer for this report was: Áine O'Connor

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Executive Summary

The assisted breeding projects undertaken from 2005 to 2014 are summarised in this report, including the methods trialled, changes that were made to improve conditions, and conclusions with recommendations for the future.

Key findings from the study include:

- Nore mussels did not complete gonadal development in captivity but produced good glochidial levels once transferred from the river after mid-July.
- Nore mussels encysted both native trout from non-native catchments and cross-bred trout with ease.
- Juvenile mussels survived well during early development but died when fine sediment levels built up in tanks.
- A very high level of mud was present in the intake waters and large quantities of mud settled in all tanks, resulting in both juvenile and adult mussel kills.
- Juvenile growth was average for more natural captive breeding techniques.
- Water chemistry quality was good and receiving waters were not negatively affected by the assisted breeding facility, but water chemistry was very different from native Nore waters, particularly with regard to alkalinity and hardness.
- The loss of adult mussels and their inability to breed in captivity draws the strong conclusion that assisted breeding facilities cannot act as “arks” and that even though adults are being lost in their native river, and that all adults are in the same water body, there is no benefit to the mussels in keeping some in captivity.
- A new method of assisted breeding, “short term rearing”, was developed and trialled in 2013/2014.

The results of the various trials were used to establish the feasibility of using various assisted breeding methods in the future for the Nore pearl mussel population. The most outstanding issues with assisted breeding were considered to be changes to water chemistry (likely stressors in adult transfer from the river and juvenile transfer to the river) and sedimentation of juvenile tanks.

1 Introduction

This report outlines the progress to date with the Nore pearl mussel assisted breeding project in the Republic of Ireland.

Captive breeding of *Margaritifera* is a strategy that has been attempted in a number of countries across Europe in an attempt to keep alive and propagate individuals from small populations that are in danger of extinction in the wild. It can have a number of potential functions as follows:

1) An "ark" function, to keep adult mussels in a location of higher water quality than that of its native river, if it is more likely that individual mussels would die in the wild than in captivity
2) A breeding function, in order to produce a new generation of mussels to an age where they can be placed in the wild, where the native river bed habitat cannot sustain juvenile mussels
3) A breeding function to secure a new generation while river catchment management measures are being implemented but may take more time than the lifespan of the current generation in the wild.

The Republic of Ireland has been attempting to breed the Nore pearl mussel in captivity since 2005. While the conservation strategy favoured by NPWS is habitat rehabilitation through catchment management measures, and although the taxonomic status of the Nore pearl mussel is still in question, its current listing as a separate taxon under the Habitats Directive and its very low numbers in the wild made it an exceptional case. For this reason the report refers to this population by its common name, as its correct scientific name is still under review. The estimated number of individuals in the wild was thought to be 500 adults by 2005, and there was no evidence of natural recruitment of young for 20 years prior to this. As adult mussels were found to be declining rapidly, an attempt to captive breed was considered to be a sensible approach.

The Nore pearl mussel is considered to be of very high conservation value because of 1) its rarity and restricted distribution, 2) it is the only Irish endemic species listed under the Habitat's Directive and 3) it is in such extreme danger of extinction (Moorkens & Costello, 1994).

1.1 Key causes of decline in Ireland

The key cause of decline in pearl mussel populations in Ireland, including the Nore pearl mussel population, is unsuitable habitat for juvenile mussels after they fall off the gills of host salmonids. This stage requires the safety of remaining within the river bed gravels, before growing to a size that allows the emergence of the filtering siphons into the open water body. While the juvenile mussels remain within the river bed gravels, they filter the interstitial water within the gravels. Where the gaps between the gravel stones get clogged with fine silt, the flow of water in the interstices becomes very restricted. Without adequate water movement and replacement, oxygen levels are exhausted and young mussels die. The decline in interstitial water quality in silted gravels has been detailed (Buddensiek *et al*., 1993, Buddensiek, 1995).

Fine sediments in gravels were shown to increase mortality in juvenile mussels to 100% (Buddensiek, 2001). Fine sediment can come from physical sources (erosion) and organic sources (decayed algae whose growth is caused by excessive nutrients in river).

Fine silt has become a problem in the River Nore due to excessive loading from various sources. It is currently so acute a problem as to be a cause of both adult and juvenile mortality. Thus the time scale for addressing the problem is urgent.

The Nore pearl mussel requires young native trout to carry juvenile mussels (glochidia) for the first few months of its life. The life history of pearl mussels is shown in Figure 1.

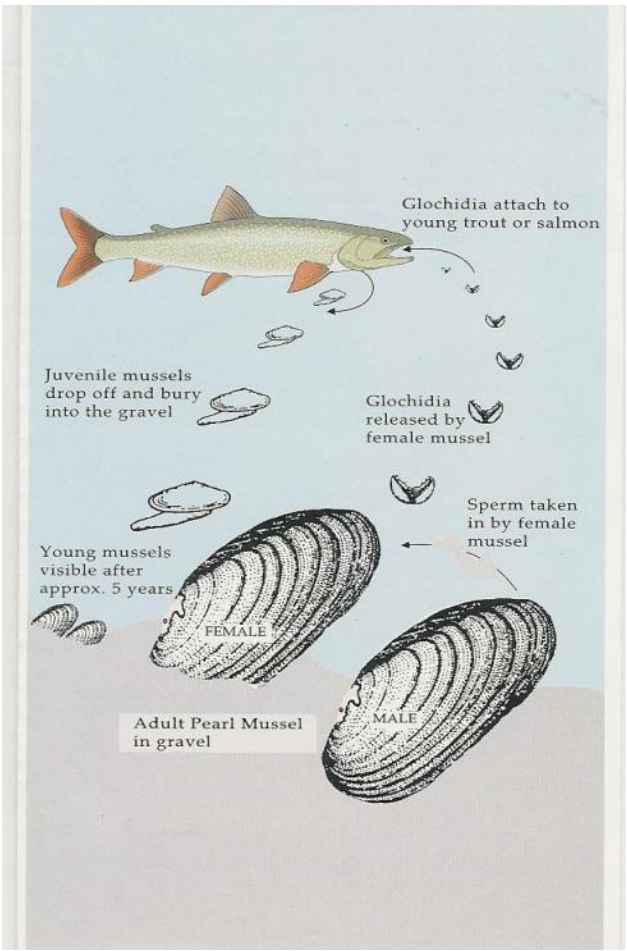


Figure 1 Life cycle of the pearl mussel.

To captive breed the Nore pearl mussel, the relationship between the mussel and its salmonid host needs to be enhanced and encouraged, by artificially bringing the mussels and fish together at the correct time of year. As the first 5 years after dropping off the fish are the most sensitive

and suitable conditions are not found in a sufficient area of river to sustain the population in the Nore River at present, it was deemed necessary to hold mussels in a captive breeding project for at least 5 years until the juvenile mussels are of sufficient size to filter feed at the surface of the sediment and to allow measures to be put in place to allow for habitat rehabilitation.

2 Background to past assisted breeding work in the Republic of Ireland

The first attempt at assisted breeding was carried out by Moorkens from 1992 to 1995, on a sample of mussels from the River Nore and the Mountain River (*Margaritifera margaritifera*).

Ten years later, assisted breeding resumed on the Nore pearl mussel population following environmental commitments that were part of a planning permission for the M7/M8 road development in May 2004 (with National Roads Authority and NPWS funding).

A feasibility study was undertaken from 2004 to 2005 and a design for assisted breeding at Site 1 was produced (Moorkens, 2005).

By October 2006 it was clear that no glochidia were attaching to Central Fishery Board/Inland Fisheries Ireland Roscrea-strain trout, so further experimentation was undertaken to assess whether the problem was caused by inappropriate fish species or genetics, the water regime (temperature or chemical constituents) or whether the mussels themselves were stressed to a level that was impeding their reproductive capabilities (Moorkens, 2006).

The experimentation included experimental breeding at three further sites (Site 2, 3 and 4) from 2007, and with a range of samples of individuals from different *Margaritifera margaritifera* populations.

By 2008 it was concluded that the most likely causes of failure at Site 1 was an incompatible water temperature regime and this was exacerbated by frequent siltation events. The immediate success of glochidial encystment in two other sites using the Roscrea-strain trout, even on mussels that were subsequently shown to utilise salmon in the wild, resulted in a recommendation to move the assisted breeding programme from Site 1 to Sites 2 and 3 which were shown to support successful encystment (Moorkens, 2007, 2008). During this period the assisted breeding programme was taken over fully by the Department of the Environment, Heritage and Local Government (NPWS).

In 2008, all Nore adult mussels were transferred from Site 1 to Site 3 and were supplemented by mussels from another river. Excellent numbers of glochidia were achieved on fish at Site 3, the first example of Nore mussel captive bred glochidiosis since efforts began in 2005. Two tanks of fish in sequence were equally encysted. There were between 500 and 600 trout present in each tank in late 2008, with approximately 225,000 glochidia.

By April 2009, there were approximately 24,000 glochidia from the Nore on fish in Site 3.

A total of 400 fish were placed over a circular tank (2m diameter) with an 8cm layer of river gravels. At the end of May, the remaining 600 fish were transferred to Site 2.

At the same time, 22 Nore mussels were transferred from Site 3 to Site 2, leaving 10 Nore mussels in Site 3.

In April and May 2009, a major upgrade to the Site 2 facility was undertaken. This consisted of excavating a lined channel from the river that had been closed for 30 years. A series of 5

wooden weirs were placed along the channel, which is approximately 200m in length. These acted as minor sediment traps which could be lifted to flush out the system on a regular basis. From the channel, the water was then piped into a pond approximately 20m by 10m in size, which also acted as a sediment trap. The pipe to the pond could be bypassed during the times when the weirs were raised to flush the system. The water from the pond then flowed into a series of tanks below through two 5cm diameter pipes. The entire system was gravity fed.

A total of 200 fish were placed over a circular tank 2m in diameter with the same design as the Site 3 tank. Following the success of semi-natural juvenile rearing in large long tanks in Ballinderry Fish Hatchery (Preston *et al*., 2007), a long tank of 8m in length and 1.5m wide was filled to a depth of 8cm of approximately 8mm commercial non-Limestone gravel from local sources. This was repeatedly washed out over 4 hours on 20th May 2009, and 12 buckets of finer gravel and sand from the river was washed and added to the gravel in both tanks. The inflow to the tanks was 2 litres per second, as used in the Ballinderry design. A total of 400 fish were transferred to this tank.

In November and December 2009, both tanks in Site 2 and the circular tank in Site 3 were found to have living juvenile mussels that had completed their first growth period. A sample of 0.1m^3 of sediment from the Site 3 tank yielded over 100 juveniles. Further history and details of this stage of the captive breeding effort is in Moorkens (2010). A further phase of captive breeding commenced from 2010 to 2012.

In March and April 2010, 420 Site 2 fish were well encysted with Nore mussel glochidia, as well as 196 fish encysted with River Licky glochidia. In May 2010, these fish were placed over gravels in two newly prepared tanks in Site 2, one circular (the 196 Licky encysted fish) and one long tank (the 420 Nore encysted fish) to the same design as in 2009. The fish in the tank with the Nore River mussels in Site 3 had low to medium levels of encystment (5 to 30 glochidia per side) in 60% of a sample of 23 fish. These were placed over a circular tank with gravel.

Glochidial attachment was attempted in Site 2 only in autumn 2010, and glochidiosis was poor (Moorkens, 2011). However, into 2011 there was good survival of 2009 and 2010 juveniles. Stress testing of adults and redox potential measurements of juvenile tanks was also part of the monitoring programme.

Due to the cost requirement of upgrading works, the Site 3 facility was abandoned in June 2011. An upgrade was carried out to the Site 2 facility, with the restructuring of pipes to provide a system where each juvenile tank was fed by two pipes of water, to ensure that in the event of a pipe blockage, a back-up flow would always be present. Concerns regarding the stress levels of adult mussels, given that the facility is attempting to act as an “ark” for endangered mussels, resulted in the design and implementation of a mussel conditioning tank, a narrow raceway where a fast flow could be provided for adult mussels (Moorkens, 2012). The velocity of the water in this tank could be adjusted by a valve on the each of the inlet pipes that can restrict the discharge through the tank. The adult mussels thereafter were placed in the conditioning tank between the period of glochidial release to close to the period of sperm release each year.

A summary of the work carried out from 2005 to 2012 is shown in Table 2.1.

Table 2.1. Summary of Captive Breeding progress 2005-2012

Location	Mussel river of origin (number of individuals at start of year) (year of removal from river)	Adult mussel survival during year	Likely cause of mortality	Glochidial encystment on year of removal from river	Likely cause of failure	Glochidial encystment following 12 months of captivity	Likely cause of failure	Juvenile drop off	Juvenile survival
2005									
Site 1	Nore (32) (2005)	100%	-	None	Silt & low temp.	Not applicable	-	Not applicable	Not applicable
2006									
Site 1	Nore (32) (2005)	100%	-	Not applicable	-	None	Silt & low temperature	Not applicable	Not applicable
2007									
Site 1	Nore (32) (2005)	50%	Silt	Not applicable	-	27/200 fish encysted at approx 5 per fish	Silt & low temperature	Not applicable	Not applicable
Site 1	Caragh (26) (2007)	100%	-	None	Silt & low temp.	Not applicable	-	Not applicable	Not applicable
Site 2	Multeen (20) (2007)	100%	-	High encystment (1000 fish)	-	Not applicable	-	Not applicable	Not applicable
Site 3	Coomhola (18) (2007)	100%	-	High Encystment (1000 fish)	-	Not applicable	-	Not applicable	Not applicable
2008									
Site 1	Nore (16) 2005	50%	Silt. Transferred to Site 3	Not applicable	-	None	Silt & low temperature	Yes from Site 3 fish	5 months (intensive system)
Site 2	Multeen (20) (2007)	100%	-	Not applicable	-	None	Unknown	No, 100% fish mortality in flood	Not applicable
Site 3	Coomhola (18) (2007)	89%	Unknown	Not applicable	-	None	Unknown	Transfer to Site 1	See above
Site 3	Caragh (26) (2007) from Site 1 incl. (13) (2007) from TCD	85%	TCD mussels following stress from lab	Not applicable	-	None	Likely to have been stressed before arrival	Not applicable	Not applicable
Site 3	Nore (26) (2008) from Site 1 and river	100%	-	High Encystment (1000 fish)	-	Not applicable	-	Not applicable	Not applicable
Site 3	Owenshagh (11) (2008) From TCD	64%	TCD mussels following stress from experiment	Not applicable	-	None	-	Not applicable	Not applicable
Site 4	Licky (17) (2008)	88%	Silt	None	-	Not applicable	-	Not applicable	Not applicable
2009									
Site 1	Nore (8) (2005)	75%	Silt, 6 transferred to Site 2	Not applicable	-	Not applicable	-	Not applicable	Not applicable
Site 2	Nore (6)	57%	Hatchery	Not	-	High	-	Junenile	Survival

	(2005)(from Site 1) Nore (22) (2008) from Site 3		failure	applicable		Encystment (1000 fish)		drop off from Nore glochidia from fish transfer from Site 3	confirmed
Site 2	Licky (14) (2008) from Glanmire	93%	Unknown	Not applicable	-	High encystment (500 fish)	-	Not applicable	Not applicable
Site 3	Nore (10) (2008)	100%	-	Not applicable	-	0/5 encysted	-	Junenile drop off from Nore glochidia	Good survival after 6 months
Site 3	Coomhola (16) (2007)	94%	Unknown	Not applicable	-	0/5 encysted	-	Not applicable	Not applicable
Site 3	Caragh (33) (2007)	Returned to River	-	Not applicable	-	Not applicable	-	Not applicable	Not applicable
Site 3	Owenshagh (7) (2008)	Returned to River	-	Not applicable	-	Not applicable	-	Not applicable	Not applicable
2010									
Site 2	Nore (16) (2005 -2008)	94% (1 dead March)	Unknown	Not applicable	-	High Encystment 2009-2010	-	Junenile drop off from 2009	Survival confirmed
Site 2	Licky (13) (2008)	92% (1 dead March)	Unknown	Not applicable	-	High encystment 2009-2010	-	Junenile drop off from 2009	Survival confirmed
Site 3	Nore (10) (2008)	100%	-	Not applicable	-	40% with some encystment 2009-2010	-	Junenile drop off	Good survival after 9 months
Site 3	Coomhola (15) (2007)	93%	Unknown	Not applicable	-	10% with very poor encystment 2009-2010	Unknown	None	Not applicable
2011									
Site 2	Nore (16) (2005 -2008) 16 new from river taken July 2011	94% (1 dead March)	Unknown	Poor encystment	Frog blocked pipe to fish, fish kill and stress event	Low Encystment, poor survival 2010-2011	-	No new juveniles 2011	Survival from previous years confirmed
Site 2	Licky (13) (2008)	92% (1 dead March)	Returned to River	Not applicable	-	High encystment 2009-2010	-	Not applicable	Surviving juveniles confirmed
Site 3	Nore (10) (2008)	100% Transfer-red to Site 2	-	Not applicable	-	Not applicable	-	Not applicable	Juveniles transferred to Site 2
Site 3	Coomhola (15) (2007)	93%, returned to river.	Unknown	Not applicable	-	Not applicable	Unknown	Not applicable	Not applicable

3 Assisted breeding operations June 2012 – September 2014

3.1 Main tasks

Assisted breeding continued between June 2012 and September 2014, and was restricted to maintaining adult and juvenile Nore mussels, and encysting fish and collecting juveniles in Site 2.

The following tasks were undertaken:

Organisation and maintenance of appropriate licenses
Purchase, installation and maintenance of fish tanks, piping, disinfection facilities
The day-to-day running of each facility
Recording of operations and maintenance through site books
Communicating progress with NPWS
Fish food purchase and feeding
Disease control
Removal, humane destruction and proper disposal of fish following glochidial drop off
Monitoring condition and health of adult mussels
Monitoring glochidial attachment
Monitoring survival of juvenile mussels
Monitoring fine sediment
Monitoring substratum condition
Monitoring algal growths in tanks and water supplies

3.2 Recommendations from 2012 report

The recommendations made in Moorkens (2012) were as follows:

"*The experimental nature of captive breeding pearl mussels is such that it is difficult to anticipate circumstances that may arise in the future but have not arisen to date, and hence have not been factored in to budgets. The lessons learned from pipe blockages and iced supplies have resulted in changes to the facility, but these had to be absorbed into the contract costs. It is likely that some restoration of the Bay 1 supply pond wall will be needed in 2012/2013.*"

Recommendation 1: Include refurbishment of supply tank wall in works to do. A contingency for such unforeseen costs in future is highly recommended.

Recommendation 2: Continue to separate conditioned and unconditioned mussels and monitor glochidial success.

Recommendation 3: Continue conditioning of mussels for a longer period between October and May, monitoring stress condition following the longer period of fast flow. Monitor responses of mussels to variations in flow to assess which may be optimum.

4 Adult mussels in captivity

4.1 Conditioning tank design

The captive held mussels in Site 2 were stress tested in August 2010. The results of these tests led to concern for the long term viability of mussels brought into captivity and their ability to brood after a year or more in low flows in a hatchery situation. Successful glochidial attachment has only occurred to date from mussels taken from the wild during the gonadal development period. The normal brooding ratio in an unstressed population should be 50% (i.e. all the females), but small populations can result in an increase in hermaphroditism (Bauer, 1987). The stress testing indicates that there appears to be slow deterioration in adult mussels over time. This is likely to be due to a loss of muscle vigour due to low flows in captivity.

A "conditioning" tank was established in February 2012. The discharge from the supply tank was measured to be approximately $31.5 m^3 s^{-1}$ per 10cm diameter outflow pipe. This was based on the velocity and surface area at the 10cm pipe from the supply. Thus there was considered to be scope for better velocities through an alternative tank design that maximised the maintenance of velocity from the supply through the tank. The velocity flowing over the mussels in any tank is the level of discharge divided by the cross-sectional area where the mussels are positioned. Therefore, the highest velocity will be achieved if the mussels are in a raceway shaped tank, directly in substrate (not in a basket) with a minimal width and height of water. A design based on these requirements was installed on 16th February 2012. The tank was divided into two lengthways, each cell of 20cm width and being fed directly with a 10cm diameter pipe. The tank is undivided lengthways but has 3 supporting cross struts. This allows for two different velocity levels to be maintained, and natural dissipation of flow over the length of the tank, as can be seen in Photos 4.1, where the velocity is greater in the right side than the left side. Photo 4.2 shows the depth and substrate present in the conditioning tank.

If the captive adult mussels are also to act as a "back up population" or "ark" to the Nore wild population and provide broodstock for captivity over a number of years, they need to be maintained in good condition. Therefore the flow velocity levels were measured in each of the tanks holding adult mussels. All gave the same result as follows:

Flow velocity from pipe feeding the circular tanks: $0.4 ms^{-1}$
Flow velocity over baskets of mussels: $< 0.1 ms^{-1}$

This shows that while there is a good flow velocity emerging from the pipe, once it enters the circular tank it is reduced because of the wide area it is flowing through. The optimum flow velocity range found in the wild in Scotland was 0.25–0.75m/s (Hastie *et al*., 2000), and in Spain the majority of mussels in a study were found in flow between 0.5 and 1m/s (Outeiro *et al*., 2008). More recently Moorkens & Killeen (2014) have found optimal near-bed velocities (3cm, the height that adult mussel siphon activity occurs) in natural river conditions averaged $0.3 ms^{-1}$, rising to $0.37 ms^{-1}$ at 0.6 depth (i.e. 40% from the river bed, the standard (average) hydrological depth of velocity measurement).

For mussels to be held in captivity over the longer term (> 1 year), it should be of benefit for them to be kept in higher flow velocities for the period between October and July. In July to

September, they need to be maintained with fish at their current flow levels to ensure good glochidiosis. For the remainder of the year their flow velocity should be increased.

Photo 4.1 Conditioning tank

Photo 4.2 Upper area of conditioning tank with water switched off showing gravel and stones in the tank bottom with mussels in place.

The 31 Nore mussels in captivity were stress tested on 16th February 2012, 19 were then put into the conditioning tank and a control set of 12 was left in a basket in a circular tank. Stress testing was carried out by assessing the level of pressure needed to prise open mussel shells using tongs according to a 1-4 scale (Table 4.1).

Table 4.1 The stress assessment scale

1 - Unstressed	Mussels have high resistance to opening with tongs, and can only be prised open a small amount
2 – Slightly Stressed	Mussels show resistance to opening with tongs but under pressure the muscle resists and the surveyor could keep opening shell
3 - Stressed	Mussel shows some resistance to opening with tongs, but very little pressure needs to be exerted to open the shell
4 - Very stressed	Mussel shows poor resistance to opening with tongs, and shell opens widely with very little pressure
5 - Moribund	Mussels could be opened with fingernail, difficult to know if the individual is dead or alive

Both sets of mussels were stressed tested again on 28th May, when mussels were being removed from the conditioning tank to be placed with fish of the year, and on 15th November, when previously conditioned mussels were placed back in the conditioning tank. The control mussels were left in baskets in the circular tanks.

In 2013, the mussels were stress tested on 12th July, when mussels were being removed from the conditioning tank to be placed with fish of the year.

Table 4.2 Condition of Nore pearl mussels in captivity

	Not conditioned – left in circular tanks				Conditioned - Following period in conditioning tank			
					For conditioning-Following period in circular tanks			
Date	16th Feb 2012	28th May 2012	15th Nov 2012	12th July 2013	16th Feb 2012	28th May 2012	15th Nov 2012	12th July 2013
Stress								
1 Unstressed	3	0	0	0	3	12	8	6
2 Slightly Stressed	3	5	1	0	4	4	4	3
3 Stressed	3	4	5	0	10	1	3	4
4 Very Stressed	2	1	1	0	2	0	0	0
5 Moribund	1	0	0	0	0	0	0	0
Dead	0	2	3	7	0	2	1	3
Total number of live mussels	12	10 / 12	7 /10	0/7	19	17 / 19	16 / 17	13/16

Following the first conditioning period, the number of unstressed mussels rose from 16% to 63%, the slightly stressed level stayed the same at 21%, stressed mussels dropped from 53% to 5%, there were no very stressed mussels but 2 died during the conditioning period. Both appeared to have had small pieces of stone lodge between their valves thus keeping them open, and both had been infested by caddis larvae. The mussels that were not conditioned remained in the same status or declined, and two of these died during the 13 weeks, apparently due to wasting and slow decline.

The two sets of mussels were then placed in two different circular tanks with 0+ fish to assess if there is any advantage in glochidial maturation and release between the two sets of mussels. Problems with fish disease rendered this experiment inconclusive.

The mussels were returned to the conditioning tank in November 2012. In the spring of 2013 and into the summer, a series of very severe siltation incidents occurred in the hatchery, resulting in a heavy mud release and settlement in the adult and juvenile tanks. By July 2013 all 7 mussels kept in the circular tanks were dead, and 3 of the mussels in the conditioning tank were also dead. Due to the higher flows, mud had not settled in the conditioning tank. However, the conditions upstream and downstream of the tank suggest that very high suspended solids were released in what must have been a severe pollution incident. Adult mussels are highly susceptible to damage from suspended solids. The fact that mussels were still alive in the conditioning tank, although some remained stressed, suggests it has value in maintaining vigour in adult mussels in captivity. However, these mussels did not produce glochidia (see section 5 below).

From autumn 2013 and into the spring of 2014, further severe siltation incidents occurred in the hatchery, with the same characteristic muddy releases and settlement in the adult (during the periods they were not in the conditioning tank) and juvenile tanks. Of the 24 mussels alive in September 2013, 10 had died in the 9 months up to June 2014, and another died in early July 2014. Four of these died while in the conditioning tank, suggesting that they were exposed to serious levels of suspended solids, and six died during periods in the circular tanks, where they were subjected to suspended and settled solids. Photo 4.3 shows the poor condition that the baskets are left in following a period of mud movement into the hatchery.

Water chemistry and quality is discussed in Section 7.

Photo 4.3 Mud influx to mussel basket causing kill. June 2014.

5 Glochidial attachment

5.1 Glochidial attachment 2012 – 2013 season

The fish were checked for glochidial attachment in November 2012 (destructive sampling, dissection of 10 fish). The gills of each fish were dissected out and checked under a 40X binocular microscope for presence and level of glochidial encystment.

No glochidia were found on any fish checked. The fish were all found to have developed gill rot disease, and thus the experiment to compare glochidiosus between conditioned and unconditioned mussels was inconclusive.

The fish were removed and the tanks were all cleaned, disinfected and dried in advance of the 2013/2014 season.

5.2 Glochidial attachment 2013/2014

As juvenile production has been so inconsistent at Site 2, a further experiment was undertaken in 2013 to check whether a) the Roscrea-strain trout were still fit for purpose and b) whether there was a difference between glochidial production between conditioned mussels and those taken from the wild in the same reproductive year.

To test whether the Roscrea-strain trout were still fit for purpose, two sets of fish were used – 1,000 Roscrea-strain fish were divided between Tanks 1 and 2 and 1,000 wild-strain brown trout, stripped from wild fish from Lough Owel, were divided amongst Tanks 3 and 4.

To test whether there was a difference between glochidial production between conditioned mussels maintained in the hatchery and mussels taken from the wild in the same reproductive year, a total of 11 adult mussels were removed from the river on 12th July 2013, and divided with 5 put in Tank 1 with the Roscrea-strain trout and 6 put in Tank 3 with the wild trout. On their removal a recent mussel kill was discovered in the River Nore, highlighting the vulnerable position of the remaining mussels in the river, and also indicating a possibility that the mussels removed may have already dropped their glochidia as a response to the incident that caused the kill. The conditioned mussels were also divided, with 7 placed in Tank 2 with the Roscrea-strain trout and 6 put in Tank 4 with the wild trout.

A summary of the experimental arrangement is shown in Table 5.1.

Table 5.1 Experimental arrangement of 4 different circular tanks for the 2013/2014 season

Tank	Fish	Mussels
1	Roscrea-strain trout	Mussels removed from River Nore
2	Roscrea-strain trout	Conditioned captive mussels
3	Wild trout	Mussels removed from River Nore
4	Wild trout	Conditioned captive mussels

A total of 40 fish were killed on 27th November 2013, 10 from each tank. The gills of each fish were dissected out and checked under a 40X binocular microscope for presence and level of glochidial encystment. The results are shown in Table 5.2.

Table 5.2 Check of glochidial encystment, November 2013

Tank 1	Fish length (mm)	Average glochidia per side	Tank 2	Fish length (mm)	Average glochidia per side	Tank 3	Fish length (mm)	Average glochidia per side	Tank 4	Fish length (mm)	Average glochidia per side
1	130	80	1	82	0	1	93	48	1	102	0
2	132	90	2	145	0	2	150	15	2	120	0
3	109	70	3	132	0	3	96	64	3	95	0
4	119	165	4	115	0	4	114	75	4	120	0
5	110	240	5	125	0	5	125	65	5	105	0
6	133	120	6	127	0	6	113	38	6	98	0
7	146	200	7	128	0	7	110	280	7	88	0
8	67	72	8	130	0	8	129	35	8	122	0
9	92	120	9	85	0	9	106	35	9	86	0
10	126	180	10	115	0	10	81	120	10	84	0
Average	116.4mm	133.7	Average	118.4	0	Average	111.7	77.5	Average	102	0

The results of the experiment were very clear, with no glochidial attachment found in Tanks 2 and 4, and excellent encystment in the fish of tanks 1 and 3, encystment being on average 267 per fish (Roscrea-strain) and 155 per fish (wild trout).

The results demonstrated that in spite of conditioning of mussels in captivity, and the mussel kill in the river in advance of removal of the 2013 mussels, the mussels held in captivity for more than 12 months did not produce glochidia but the recently removed mussels succeeded in releasing sufficient glochidia to give consistently good encystment. Encystment was considerably better on Roscrea-strain rather than wild trout, and Roscrea-strain trout were slightly bigger than wild trout (Average 117mm compared with 110mm).

The results suggest that it is not stress that is causing the lack of glochidial production in captivity, but more likely to be a temperature difference between the Site 2 water and the Nore water, either in cumulative degree days or in temperatures at specific times of year where cues are needed to commence part of the gonadal cycle, or possibly from an inappropriate food or water chemistry component that prevents reproductive development at key times. This aspect would require further study if 12-month captive breeding is being considered in the future.

The non-encysted fish were removed from site, leaving a total of approximately 1100 encysted fish, which were then spread between the 4 tanks, with an estimate of over 100,000 glochidia.

In the final months before drop-off, fish were examined by eye to check approximate glochidial loading, and at least 20 glochidia per side could be clearly seen by May 2014. One of the 4 tanks of fish was accidentally dewatered during May, and thus one quarter of the fish were lost.

The remaining fish were redistributed into two large circular tanks, which had been prepared to catch juvenile mussels (see Section 6 below). By late May, the fish were carrying approximately 33,000 glochidia.

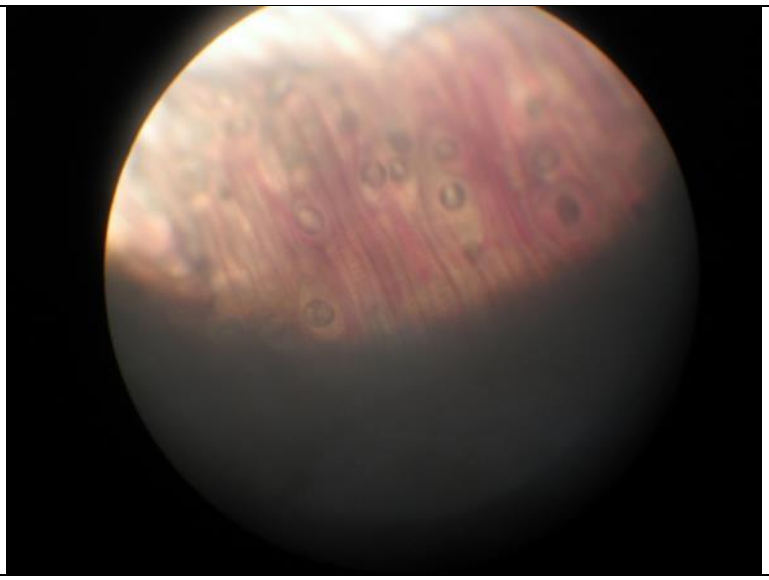


Photo 5.1 Glochidia attached to fish gills as seen under the microscope

6 Juvenile survival 2009-2014

6.1 Background to juvenile tanks held between 2009 and 2014

The tanks holding juvenile mussels are summarised in Table 6.1. The tank configuration at the Site 2 hatchery is shown in Figures 6.1-6.2.

Table 6.1 Tanks in Site 2 holding juvenile mussels 2009 – 2014

Tank name	Type of tank	Year of drop off	River of origin
A1	Long tank	2009	Nore
A2	Circular tank	2011	Nore
A3	Circular tank	2011	Nore
B1	Circular tank with 2009 Licky juveniles)	2009	Licky
B5	Circular tank	2010	Nore
B6	Long tank	2010	Nore
D1	Circular tank	2009	Nore (in Site 3 2009 – 2011, taken from Site 3 to Site 2 in June 2011)
D2	Circular tank	2014	Nore
C3	Circular tank	2014	Nore

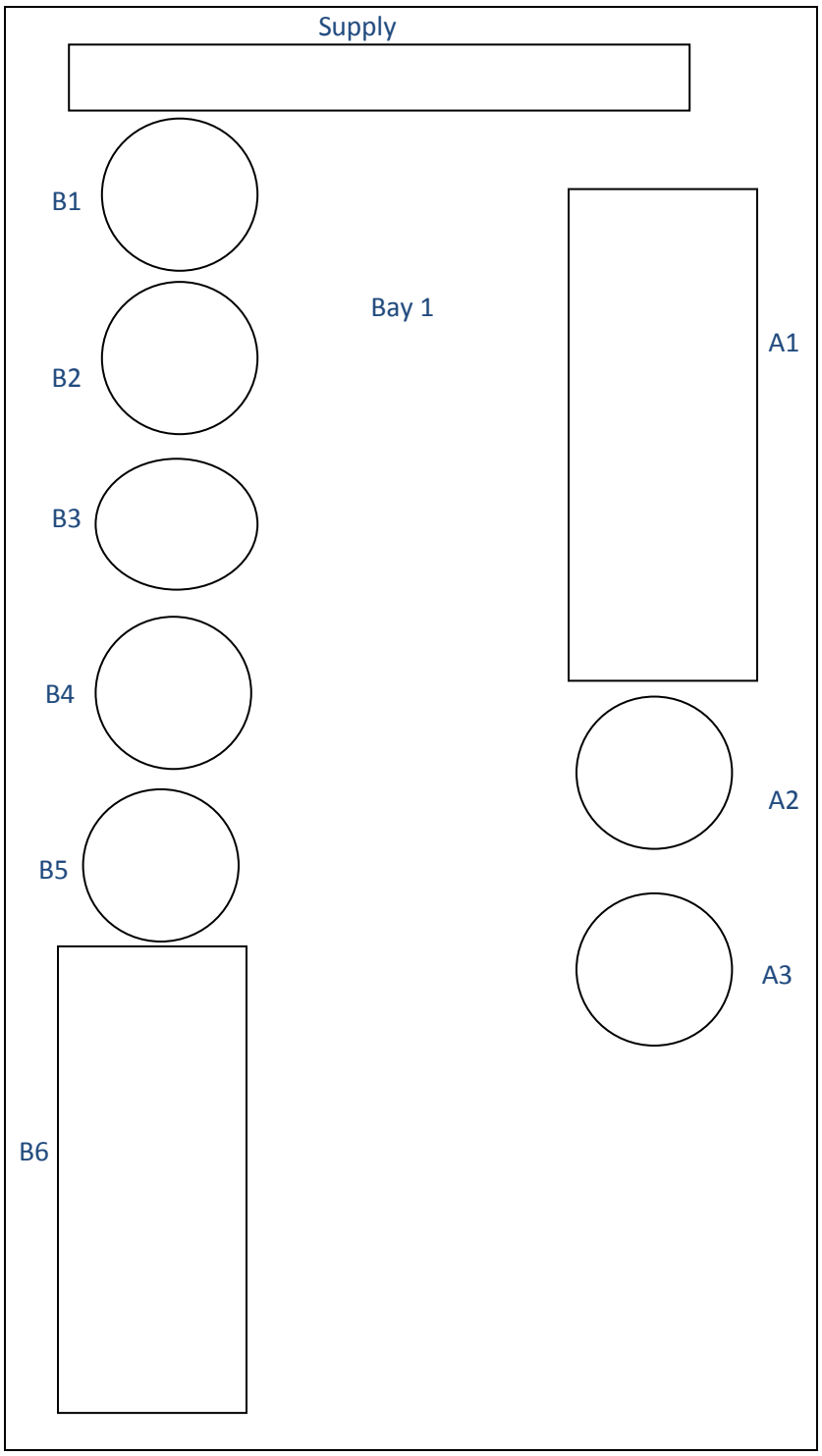


Figure 6.1 Contents of Bay 1

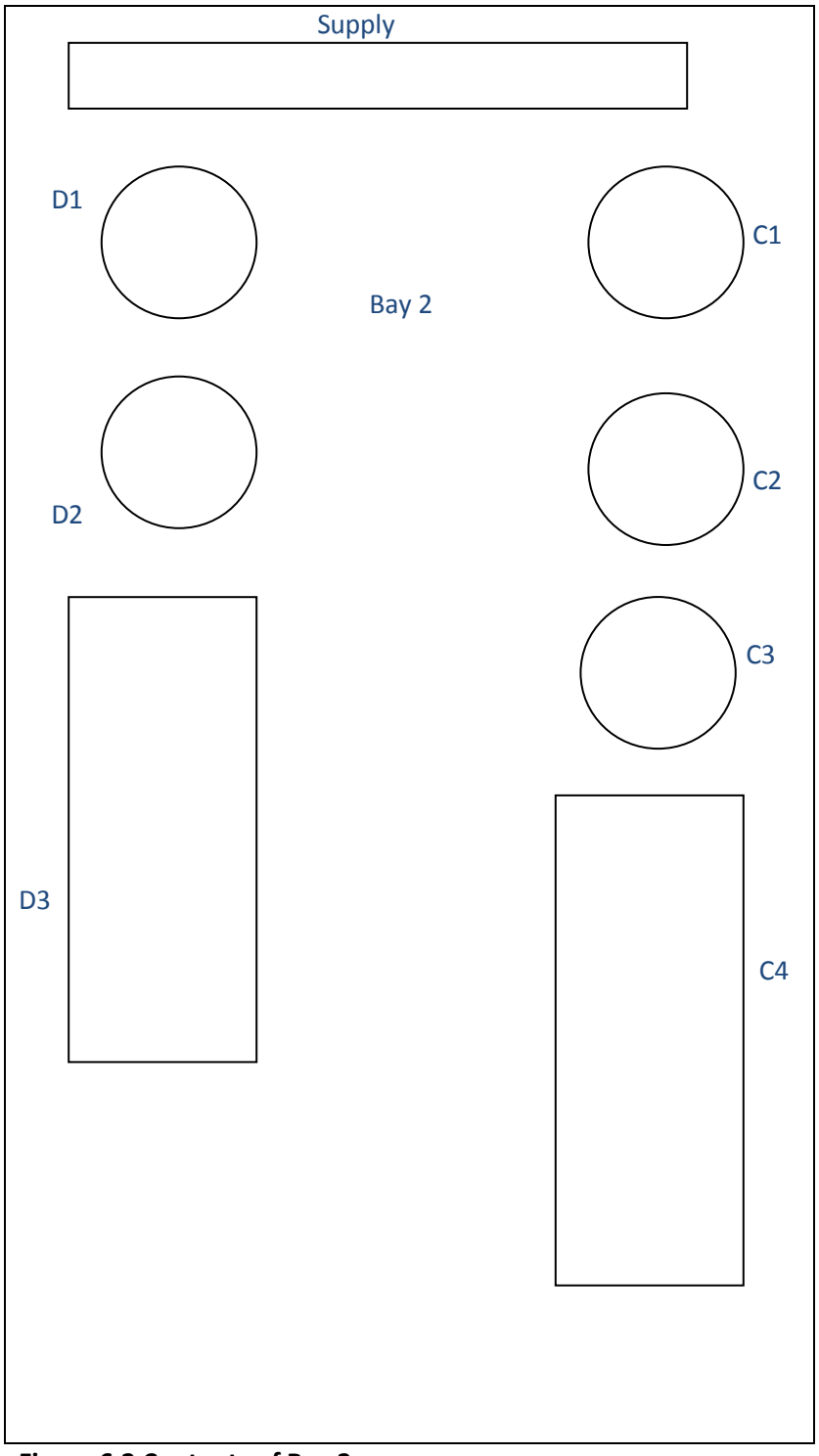


Figure 6.2 Contents of Bay 2

6.2 Methodology

6.2.1 Methodology used to prepare juvenile tanks and manage juvenile mussels

From 2009 to 2013, juvenile mussels were managed in semi-natural conditions, where encysted fish were placed in tanks with gravel beds to excyst naturally. The fish were removed when the juveniles had excysted.

The juveniles were not artificially fed, nor was the water filtered, but Site 2 river water was provided by gravity, after first letting fine sediment settle by running the water through a series of 5 wooden weirs to provide small sections of slow water over a 200m length (Photo 6.1). Sediment accumulated in these ponded areas and was cleaned regularly (water to the juveniles was bypassed to prevent silt entering the hatchery). A secondary sediment trap was provided by a tanked pond of approximately 20m by 10m in size, at the top of each bay, which also acted as a sediment trap (Photo 6.2). The water from the pond then flows into the tanks below.

The long tanks consisted of steel constructed structures 8m in length and 1.5m wide (Photo 6.3). The circular tanks were 2m in diameter and were modified cattle drinking troughs (Photo 6.4). Whereas for holding fish a side entry hole and a bottom exit hole was piped to take water in and out, for holding juveniles a side entry hole was piped, with a side exit slit made, such that the water circulated through the gravels but was not allowed to overflow.

A depth of 8cm of approximately 8mm commercial non-limestone gravel from local sources was purchased and placed into the long tank and the circular tank in Site 2. This was repeatedly washed out over 4 hours on commissioning, and finer gravel and sand was then washed and added to the gravel in the tanks. The inflow to the tanks was approximately 2 litres per second, and water was left to run through each newly prepared tank for 3 weeks before estimated juvenile drop off, to ensure a natural film of bacteria was present for the juveniles to graze from.

For the 2014 juveniles, two dried and disinfected circular tanks, into which a side entry hole was piped and a side exit slit made, were lined with a garden liner large enough to cover the base of the tank comfortably and secured to the sides of the tank with duct tape (Photo 6.5). A single layer of washed gravel was placed on top of the liner, and water was slowly added. The watered tank was left for 3 weeks before estimated juvenile drop off, to ensure a natural film of bacteria was present for the juveniles to graze from. Encysted fish were then carefully added to the prepared tank.

6.2.2 Methodology used to check for juveniles

Each year, in each tank, a total of approximately 0.003m³ gravel sediment from 6 areas was agitated with water and the elutriate concentrated and checked in approximately 50 petri dish samples for juveniles. The dishes were examined under a 20X magnification portable microscope in the field, and all live juveniles were returned to the tanks they came from.

6.3 Results

The results of the juveniles searches carried out each year is given in Table 6.2.

The average growth rates are shown in Table 6.3. Transformation from pedal feeding to filter feeding occurred at some time during the third growth period (approximately 2 years post drop-off). Growth rates were within ranges noted in other captive breeding projects (Buddensiek, 1991; Preston *et al*., 2007; Schmidt & Vendre, 2009).

Juveniles at different growth stages are shown in Photos 6.6 – 6.9. Up to 2014, all juvenile tanks were sub-sampled, but in 2014, each tank, except for the new 2014 juvenile tanks, was sampled in its entirety and emptied. In the 7 tanks that had held up to 14,000 juveniles at the end of 2010, not one remaining live juvenile was found. The process of sampling the sediment, sieving through 5mm and 0.5mm sieves, and the elutriation of the final sample is shown in Photo 6.10.

Table 6.2 Results of juvenile searches carried out each year

			Juvenile mussels found alive Yes / No						
Tank name	**Type of tank**	**Year of drop off**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Comments**
A1	Long tank	2009	Yes	Yes	No	No	No	No	Sediment conditions deteriorated in 2010
A2	Circular tank	2011	-	-	No	No	No	No	Never confirmed alive, poor glochidiosis year
A3	Circular tank	2011	-	-	No	No	No	No	Never confirmed alive, poor glochidiosis year
B1	Circular tank with 2009 Licky juveniles)	2009	Yes	Yes	Yes	No	No	No	Severe sedimentation in 2011
B5	Circular tank	2010	-	No	Yes	Yes	No	No	None found in 2010 but confirmed by subsequent sampling
B6	Long tank	2010	-	No	No	No	No	No	Never confirmed alive
D1	Circular tank	2009	Yes	Yes	Yes	Yes	No	No	Sample gave 12 live juveniles in 2012, all transformed to filter feeders
D2	Circular tank	2014	-	-	-	-	-	Yes	Confirmed alive, approx. 15,000
C3	Circular tank	2014	-	-	-	-	-	Yes	Confirmed alive, approx. 15,000

Table 6.3 Average growth rates measured each year

Growth period	At excystment	1	2	3	4
Months / Year	Following growth on fish (protoconch)	4 months post drop off	16 months post drop off	28 months post drop off	40 months post drop off
Size (mm) (Av)	0.40	0.67	1.07	1.97	None found
N	20	20	27	12	-
Min size	0.33	0.63	0.6	1.7	-
Max size	0.43	0.73	1.5	2.3	-

Photo 6.1 Channel with removable weir structures at Site 2

Photo 6.2 Settlement pond above tanks in each bay at Site 2

Photo 6.3 Long tank prepared with gravel with fish prior to juvenile drop-off

Photo 6.4 Circular juvenile tank prepared with gravel

Photo 6.5 Lined and gravelled tank being watered, 2014

Photo 6.6 2009 juvenile mussel from Site 3 tank with protoconch and ligament clearly visible

Photo 6.7 Juvenile mussel following first growth period following drop off

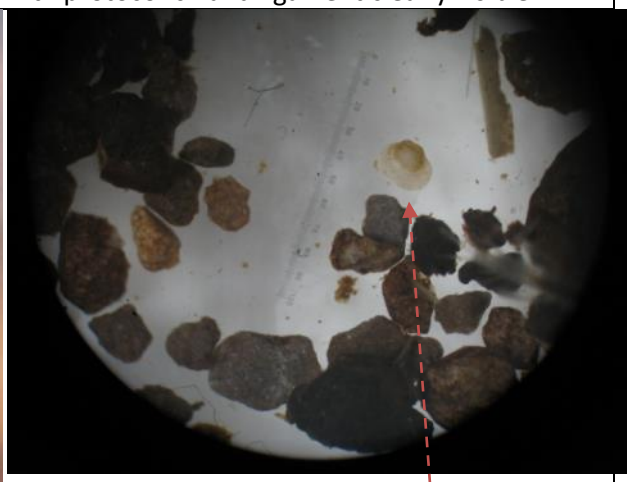


Photo 6.8 Juvenile mussel following second growth period following drop off

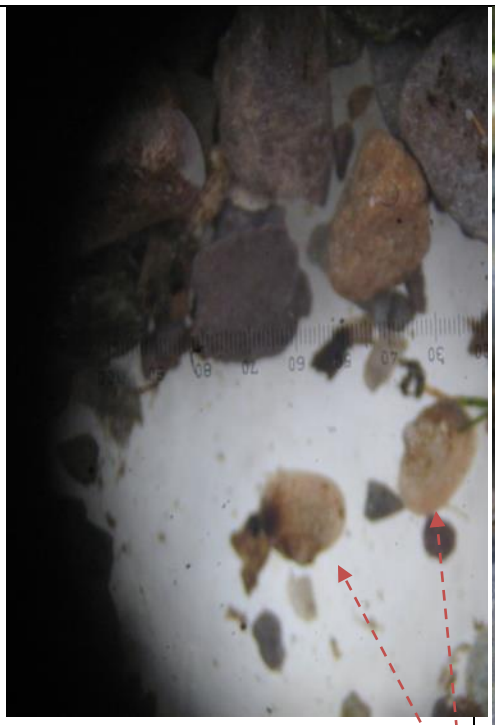


Photo 6.9 Juvenile mussels following third growth period following drop off

Photo 6.10 Process of sieving and elutriating to find juvenile mussels

7 Habitat and environmental conditions at Site 2

7.1 Introduction

Habitat conditions in semi-natural captive breeding tanks can be measured using redox potential.

Juvenile mussels normally live in open gravels amongst larger clast size stones, where oxygen is freely exchanged. In low flow conditions, fine sediments fall to the river bed in a higher percentage of habitat than in high flow conditions. When fine sediments infiltrate the open coarse gravels associated with juvenile mussels, oxygen exchange is impaired. In the absence of oxygen exchange, a reducing environment exists where a microbially facilitated process of nitrate reduction occurs, and nitrate is transferred to nitrite and ammonium, both toxic to juvenile mussels (Augspurger *et al*., 2003). Redox potential is a very useful measurement of this potential for reduction in the bed sediment, and thus provides a correlation with likely oxygen loss, and the continued loss of oxygen from oxidised nitrogen molecules. A drop in value of less than 20% between the measured open water and the sediment at 5cm depth is considered to be appropriate conditions for the survival of juvenile mussels (Geist & Auerswald, 2007).

7.2 Redox methodology

The equipment comprises a 0.7m long probe fitted with a platinum tipped electrode, a reference potassium chloride electrode and a meter with a millivolt display. A reading is obtained by holding both electrodes in the water column until a stable reading is obtained (typically this would be 500-540mV). With the KCl electrode remaining in the water column, the platinum electrode is then inserted into the substrate to a depth of 5cm and a reading taken immediately.

The redox potential was measured in both circular tanks and long tanks that have gravel beds and into which juvenile mussels dropped. Redox potential was measured at 5cms only, with 10 readings taken in each circular tank and 15 readings taken in each long tank, 5 each at the top, middle and bottom of each long tank.

7.3 Redox results

For the first two years following drop off, conditions in both long and circular tanks appeared to be benign, with relatively low levels of surface or infiltrated silt and with redox potential values between 10% and 14%, which is equivalent to the best of natural juvenile habitat. By 2014, when sediment conditions had gone from silted to muddy, redox values could no longer be taken, as the reduction potential was so high the readings kept dropping and did not stabilize. The results are given in Table 7.1. The condition of the tanks by 2014 can be seen in Photo 7.1, where the flow is only keeping the gravel surface clean in places, and the entire sediment has been infiltrated by a depth of mud. When the surface gravels were removed, the extent of anoxic mud beneath could be seen (Photo 7.2).

Table 7.1 Redox potential measurements in juvenile tanks

Tank	Location	Juveniles present	Loss of redox potential at 5cm April 2011	Loss of redox potential at 5cm April 2012	Loss of redox potential at 5cm April 2014	Comments
A1	Long tank with 2009 juvenile Nore mussels	2009-2010	14%	42% (min 24%, max 53%)	Kept dropping, anoxic	Cleaner in upper section with surface silt more obvious towards the end of the tank. Muddy by 2014.
A2	Circular tank with 2011 Nore mussel juveniles (poor glochidiosis)	No juveniles found	N/A	43% (min 34%, max 55%)	Kept dropping, anoxic	Visibly silted in centre but Infiltrated throughout. Very muddy by 2014.
A3	Circular tank with 2011 Nore mussel juveniles (poor glochidiosis)	No juveniles found	N/A	42% (min 32%, max 51%)	Kept dropping, anoxic	Visibly silted in centre but Infiltrated throughout. Very muddy by 2014.
B1	Circular tank with 2009 Licky juveniles	2009-2011	10%	50% (min 42%, max 58.5%)	Kept dropping, anoxic	Heavily silted with overlying debris and mud by 2012.
B5	Circular tank with 2010 Nore mussel (Site 2) juveniles	2010-2012	10%	32% (min 31%, max 33.4%)	Kept dropping, anoxic	Relatively clean in earlier years, muddy by 2013.
B6	Long tank with 2010 juvenile Nore mussels	No juveniles found	14%	49% (min 29%, max 59%)	Kept dropping, anoxic	Overlying silt in places, chironomid casts, some litter, debris and some fluffy algae, cleaner near inlet pipes. Very muddy by 2014.
D1	Circular tank with 2009 Nore juveniles (taken from Site 3 in June 2011)	2009- Feb 2012	N/A	41% (min 24%, max 50%)	Kept dropping, anoxic	Clean except for centre up to 2012, went from silted to muddy by 2014.

	
Photo 7.1 Long tank following mud infiltration over time	Photo 7.2 Mud layer in long tank A following gravel removal, note anoxic black conditions underneath

7.4 Water quality

Water quality testing was carried out upstream and downstream of the assisted breeding activities, and analysed by City Analysts Limited for the project. The results were sent to the local authority to comply with the discharge license for the project. The local authority also carried out their own independent sampling.

The results were found to be satisfactory and confirmed that the water body can be classified in the highest quality range i.e. high status as per the EC European Objectives (Surface Waters) Regulations, 2009. Surface waters with Ortho-p levels $\leq$ 0.025 mg/l P and ammonia levels $\leq$ 0.04 mg/l N can generally be classified as high status. The results are shown in table 7.2.

Comparing these results with River Nore (EPA) data, the pH of Site 2 is within the range of the native source waters, but the alkalinity and hardness levels are much lower in Site 2 than in the Nore (Moorkens, 1996). A change of environment for mussel to and from a hardness level of over 400 (Nore) to less than 100 (at Site 2) may be a stressor for mussel following translocation, as the mussels may have genetic or developmental adaptation to manage calcium from their own water.

The facility has not resulted in any environmental impact on the receiving water below.

Table 7.2 Average water quality measured upstream and downstream of assisted breeding facility (Site 2) 2012 – 2013 (BDL = below detection limit)

Parameter	Ortho – phosphate as PO_4	Ortho – phosphate as P	Dissolved Oxygen	Nitrate as NO_3	Nitrate as N	pH	Hardness as $CaCO_3$	Alkalinity as $CaCO_3$	Ammonia as N	Ammonia as NH_3	$CBOD_5$
Average upstream	<.075	<.025	11.5	<8.9		8.07	69.96	83	<.01	<.0121	<2
Average downstream	<.075	<.025	10.7	<8.9		8.10	70.87	77	<.01	<.0121	<2
Average local authority upstream		0.0126	10.13			7.71			BLD		0.625
Average local authority downstream		0.024	10.15			8.17			BLD		0.575

8 Work carried out in summer 2014

8.1 Programme of work for Summer 2014

The following were the agreed work activities to be undertaken from June to September 2014 (From Moorkens, 2014):

1) Normal day to day running of the hatchery while fish and mussels still present.

2) Kill and remove all fish from the hatchery to a licensed facility following juvenile drop off (June).

3) Label all captive mussels by plastic tags attached with superglue (method of Young & Williams, 1983) (June).

4) Carry out a velocity and redox survey in riffle areas within the current adult population area of the Nore River. Plot the most likely survival zones for juvenile mussels. Recommend at least 5 quadrat places for juvenile transfer (July), and identify areas for captive adult transfer back to the wild.

5) Return all captive adult mussels to the River Nore within area of current occupancy (July).

6) Transfer the 2014 juveniles from the hatchery with their sediment to the Nore in a series of net bags sitting in open topped “Esky” coolers with hatchery water (July).

7) In each of the 5 or more quadrat receptor sites, excavate the quadrat to 5cm, agitate and clean the sediment further, partially rebuild the quadrat, insert the 2014 juveniles with their sediment from the net bag using a large funnel. Finish rebuilding the quadrat to ensure it is stable (July).

8) Clean and disinfect all tanks at the hatchery, disconnect and tidy all piping and leave in a manner that would facilitate complete decommission with ease.

9) Disconnect water to bays in the hatchery by taking out the backboards from the river outflow channel – this will ensure the water does not reach the level that allows entry into the bays. Leave a flow through the channel system that feeds the bays to keep clean and functional (July).

10) Check of adult mussels in River Nore one month post transfer (August).

11) Finish 2014 final report and long term recommendations.

8.2 Results of work undertaken in Summer 2014

The following describes the results of the ten agreed tasks from the list of agreed work activities above.

1. Normal day to day running of the hatchery while fish and mussels still present.

The management of the hatchery included feeding of fish until their removal following juvenile drop-off, and the management of flow through the tank systems until all adult and juvenile mussels were removed, and all other juvenile tanks were systematically checked for living juvenile mussels. Following the end of this process (July 2014), day to day management of the facility was no longer needed, and cleaning and decommissioning could begin.

2. Kill and remove all fish from the hatchery to a licensed facility following juvenile drop off.

At the end of June 2014, fish had a final check for glochidia, and all gills were found to be clear of any glochidia. The fish were killed and removed to a licensed facility for disposal.

The process of removing the fish from the tanks had to be carried out in a very slow manner, removing one or two fish at a time, in order to prevent the fish from getting agitated, as this would have disturbed the thin sediment layer with all the juveniles present.

3. Label all captive mussels by plastic tags attached with superglue.

Of the 24 mussels alive in September 2013, 10 had died in the 9 months up to June 2014. On 23rd June, 8 of the 14 remaining adult mussels were labelled from 1 to 8 according to the methodology of Young & Williams (1983). Rotex embossing tape labels were created with numbers 1 to 9. These were cut by hand with small scissors to circle shapes of approximately 8mm diameter to prevent sharp edges that would be vulnerable to peeling away.

Mussels were labelled one at a time, the complete process was completed and the mussel individual returned to water before the next mussel was removed. In each case, the backing plastic of the label was removed to reveal the self-adhesive backing, the mussel was removed from the water, the right valve below the umbo was dried with tissue, and a small amount of glue to fit 8mm diameter (Loctite ™ Gel 235495) was placed on the shell, and the label immediately pushed on top of it and pressed in place firmly for 45 seconds. The mussel was then returned to its basket in water (Photo 8.1).

On 20th July the 8 labelled mussels were checked. Seven were filtering well but one (Number 5) had died. The remaining 6 mussels were labelled (5, 9 -13) in the same manner.

Photo 8.1 Mussels following the labelling process

4. Identification of receptor sites.

A survey to identify receptor sites for juvenile Nore mussels was carried out on 17th July 2014.

Sustainable levels of juvenile mussels are not surviving in the River Nore due to infiltration of fine sediment into the river bed gravels resulting in insufficient oxygen supply to the juveniles. The majority of the River Nore habitat has been negatively impacted, and therefore the chance of newly excysted juvenile mussels falling into the remaining areas of cleaner gravels is very low. Therefore, placing newly excysted juveniles in areas of higher gradient should provide the best chance of their survival. These areas are characterised by tops and tails of riffles where flow is significant but the river bed habitat remains stable. All tops and tails of riffles were assessed between the steps at Dunmore to New Bridge.

A velocity and redox survey in potentially suitable areas was carried out.

Measurements of velocity were taken in 3 quadrats at each potentially suitable site. This is carried out using an OTT C2 Small Current Meter. Measurements are taken where the flow is not impeded by large boulders or dense weed. The full water depth was measured and then velocities are measured at near-bed level (i.e. 3 cm above the substrate surface), and at 60% depth (i.e 40% from the substrate surface) – the latter in accordance with widely used techniques for measuring river velocities. The equipment was set to measure over 50 seconds duration. The number of pulses in 50 seconds was then converted to ms^{-1} using the factors appropriate for the size of the impeller used.

The redox potential was measured as described in Section 7.2.

Area A.

This area has good riparian woodland buffering both banks. The river bed is characterised by angular boulders and cobbles, interspersed with sand and silt (Photo 8.2). The interstitial habitat was found to be very compacted, and a recently dead mussel was found *in situ*. The left bank edges were very muddy in the low flow conditions. The better habitat is on the right bank, but this had declined considerably since the last survey (Moorkens report to NS Share for sub-basin plans, 2009). This is at the top of the riffle, where there was a mix of live mussels and dead shells *in situ.* The gravel areas had calcium deposits and the habitat was compacted and lithified in places. Not only would juveniles be unlikely to survive here, they would be difficult to place in these conditions. However, one quadrat was considered to have some potential (Photo 8.3), named Site 1. Redox and flow measurements are shown in Table 8.1.

Photo 8.2 River at Area A

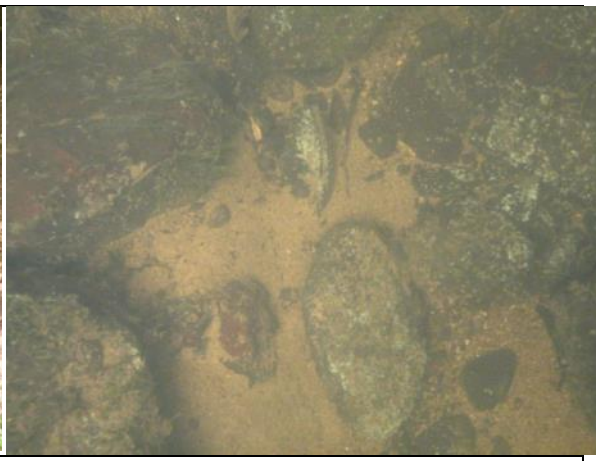


Photo 8.3 Habitat at Site 1, Area A with dead shell *in situ*

Area B.

This area was found to be damaged by cattle entry and poaching along the left bank area (Photo 8.4). Due to the muddy conditions in the river in a wide area of damage, Area B was not considered further as a receptor site.

Photo 8.4 Cattle poaching and entry into river at Area B

Area C.

This area has good riparian woodland buffering both banks. The river bed in from the centre channel to the left bank has reasonable flow and a good mix of small boulders, black cobble, and a smaller clast mix from pebbles to sand, dominated by substrate in the 2-8mm size range. The habitat is towards the centre of the channel, as the bed becomes dominated by finer sediment towards the left bank (Photo 8.5), and sand movement may be problematic in this area at different times of the year. There was much less calcium deposit on the substrate in this area. The best quadrats were stable and relatively clean, maintained by good flows at the tow of the riffle. Redox and flows were measured here, and referred to as Site 2 (Photo 8.6).

Towards the right bank, some areas of clean gravel were evident, but a number of dead shells were present. Some buried boulders were interspersed in the river bed away from the bank, some with remnants of *Fontinalis* growing on them. Sand was deep and widespread across the river, but fine gravel was also present (Photo 8.7). Larger stones had calcareous growth present. The substrate was severely compacted in places, but physically there were areas with a suitable substrate mix. Redox and flows were measured in the most suitable quadrats, and referred to as Site 3 (Photo 8.8).

Photo 8.5 Area C left bank habitat towards centre channel, too sandy towards bank

Photo 8.6 Habitat at Site 2

Photo 8.7 Area C right bank Substrate composition

Photo 8.8 Habitat at Site 3

Area D.

The area from mid channel to the right bank in this area supported low numbers of mussels throughout the period of survey of the River Nore. The river bed substrate towards the bank edge is muddy, and then a mixed substrate bed of small boulders, cobble, gravel and sand is present from approximately 2m from the bank. The flow here is much better, but *Ranunculus* is luxuriant over a wide area (Photo 8.9). The best quadrats were stable and relatively clean, maintained by good flows, and most evident where there were gaps in the *Ranunculus* growth (Photos 8.10 and 8.11). The area of potential juvenile habitat was larger here, up to 4m^2. Redox and flows were measured here, and referred to as Site 4.

Towards the left bank from mid channel, areas of loose rock with calcareous growth were present under the shade of very large trees with a wide overhang. From 2-3m out from the bank small boulder sized rocks with some *Fontinalis* growth were present, with reasonably clean

gravels in their lee. Quadrats in this area were considered to have good potential as receptor sites. Redox and flows were measured here, and referred to as Site 5.

Photo 8.9 Area D left right bank habitat towards centre channel, too muddy towards bank

Photo 8.10 Centre channel habitat at Area D

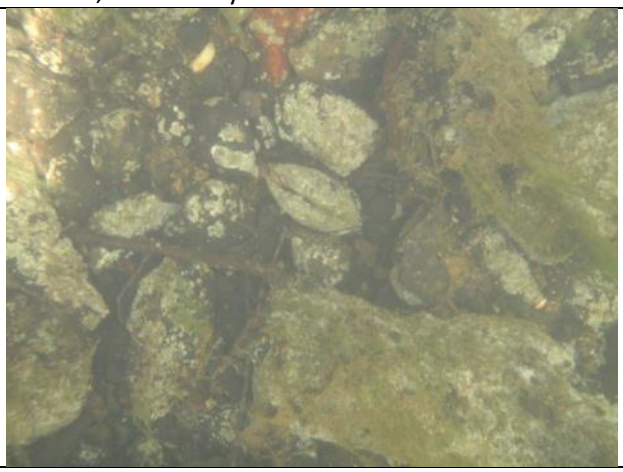


Photo 8.11 Site 4 substrate composition with living mussel visible

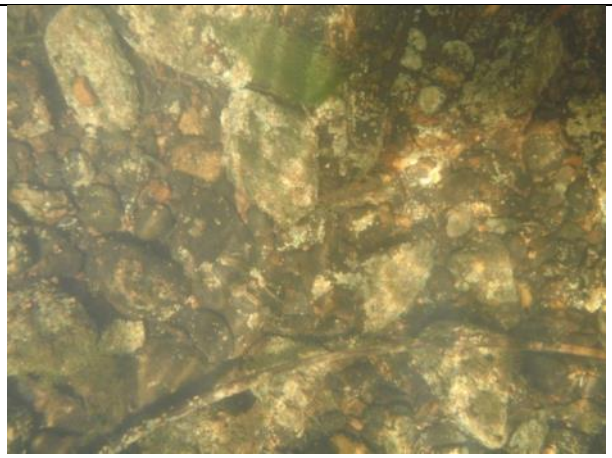
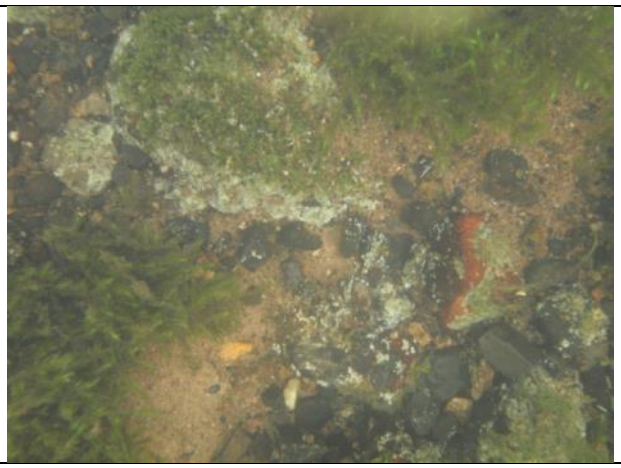


Photo 8.12 Habitat at Site 4

	
Photo 8.13 Looking across from centre channel to right bank at Area D.	Photo 8.14 Habitat at Site 5

Area E

There was difficulty in accessing this potential site, due to a road closure in Durrow. This road was due to be closed for the next week, including the proposed date for translocation (21st July). The area was then accessed from Castlewood crossroads, but the distance from vehicular access to the river could result in stress to the adult and juvenile mussels so this area was discounted.

This resulted in a total of 5 potential sites to be further assessed as for receptor potential. At each site 10 redox measurements were taken and 3 velocities measured, all within a 2 to 4 metre square area depending on the size of potential habitat. The results are shown in Table 8.1. These results, along with the substrate composition and condition from the survey, were used to identify the best potential for receiving juvenile mussels (Table 8.2). Riparian habitat and surrounding landuse would normally be important in the decision process but in this case similar banks and woodland were present and thus these characteristics were not discriminating.

Redox measurements are given in percentage loss of redox potential from open water. Velocities were measured at near bed level (3cm) and at 0.6 level (60% below surface) according to the methodology of Moorkens & Killeen (2014).

Table 8.1 Redox and velocity measurements from 5 potential receptor sites.

Site	1	2	3	4	5	Average
Redox (N=10/site)						
Redox Average % loss at 5cm	26%	25%	20%	20%	21%	22%
Redox Min % loss	20%	21%	17%	16%	17%	18%
Redox Max % loss	34%	32%	24%	23%	29%	28%
Flow (3 locations / site)						
Depth 1	33	33	38	34	35	
Velocity near bed ms^{-1}	0.18	0.17	0.32	0.44	0.19	
Velocity 60% depth ms^{-1}	0.28	0.24	0.42	0.51	0.16	
Depth 2	42	25	50	36	37	
Velocity near bed ms^{-1}	0.21	0.2	0.22	0.28	0.13	
Velocity 60% depth ms^{-1}	0.26	0.29	0.37	0.33	0.3	
Depth 3	32	38	45	39	35	
Velocity near bed ms^{-1}	0.21	0.17	0.29	0.16	0.18	
Velocity 60% depth ms^{-1}	0.38	0.21	0.4	0.27	0.4	
Averages per site						
Average Depth (cm)	36	32	44	36	36	36.8
Average Velocity near bed ms^{-1}	0.2	0.18	0.28	0.29	0.17	0.223
Average Velocity 60% depth ms^{-1}	0.31	0.25	0.4	0.37	0.29	0.321

Table 8.2 Summary of receptor habitat condition.

Each site is weighted from 1 (worst) to 5 (best) for each characteristic. The order of preference is from 1 (best) to 5 (worst).

Site	1	2	3	4	5
Physical substrate composition	1	2	3	4	5
Level of compaction	1	3	2	4	5
Redox Av	1	2	5	5	3
Redox Min	2	1	4	5	4
Redox Max	1	2	4	5	3
Velocity near bed	3	2	4	5	1
Velocity 60%	3	1	4	5	2
Ease of access	1	2	4	5	3
Marks out of 40	13	16	29	38	26
Order of preference	5	4	2	1	3

A total of seven quadrats were chosen as receptor sites, to be placed in the river on the same day, in the order 1 and 2 (Site 4), 3 and 4 (Site 5) (as these two sites are closely located), 5 and 6 (Site 3) and 7 (Site 2). It was decided that Site 1 should not be used. The locations are shown in Figures 8.1 to 8.3, and are summarised in Table 8.3.

Table 8.3 Summary of translocation quadrat locations.

Quadrat Number	Site Number	Area	Approx. Grid Reference	Map	Number of adult mussels translocated
1	4	D	S–(detail removed to protect mussels)	Figure 8.3	3
2	4	D	S–(detail removed to protect mussels)	Figure 8.3	4
3	5	D	S–(detail removed to protect mussels)	Figure 8.3	1
4	5	D	S–(detail removed to protect mussels)	Figure 8.3	1
5	3	C	S–(detail removed to protect mussels)	Figure 8.2	0
6	3	C	S–(detail removed to protect mussels)	Figure 8.2	1
7	2	C	S–(detail removed to protect mussels)	Figure 8.2	3

Map removed to protected resident and translocated mussels

Figure 8.1 Site 1 of potential receptor site survey.

Map removed to protected resident and translocated mussels

Figure 8.2 Sites 2 and 3 of potential receptor site survey, showing receptor quadrats 5 to 7.

Map removed to protected resident and translocated mussels

Figure 8.3 Sites 4 and 5 of potential receptor site survey, showing receptor quadrats 1 to 4.

5. Return all captive adult mussels to the River Nore within area of current occupancy.

The 13 labelled adult mussels were transferred to the River Nore on 21st July 2014, and placed within and at the periphery of where juvenile mussels were translocated (Photo 8.15) . This should assist future location of both these mussels and the juvenile areas. Three were placed in Site 2 (Q7), one in Site 3 (Q6), 7 in Site 4 (contiguous within Q1 and Q2) and two in Site 5 (1 in Q3, 1 in Q4).

Photo 8.15 Example of adult mussel placement in a juvenile receptor site

6. Transfer the 2014 juveniles from the hatchery with their sediment to the Nore in a series of net bags sitting in open topped "Esky" coolers with hatchery water (July).

Very early on the morning of the 21st July 2014, the sediment containing juveniles was transferred from the circular tanks to individual net bags of approximately 35cm X 45cm, kept in a circular tank with flowing water, and then transferred into to 2 "Esky" cooler boxes and 2 large buckets. The samples were driven directly to the River Nore.

Photo 8.16 Juveniles and sediment before transfer

Photo 8.17 Sediment with juveniles scooped into net bags

7. Transfer the 2014 juveniles from the hatchery with their sediment to the Nore.

A total of 7 quadrats were used as translocation habitats at the locations identified in Figures 8.1 to 8.3.

In each of the 7 quadrat receptors, approximately 0.5m x 0.5m was excavated to a depth of 5cm with a sharp trowel, and the removed sediment was agitated and further cleaned and replaced, partially rebuilt, and the contents of one of the juvenile-rich sediment bags added. The quadrat was then further built up until it was considered to be stable. The translocated adult mussels were added to the quadrat. To protect the quadrat area from the upstream flow, the rebuilding and adding of the juvenile sediment was carried out within a bucket that had its base removed. The process is shown in Photos 8.18 to 8.21.

A small sample of sediment from one of the bags was taken back to the laboratory to check for juveniles. This confirmed that the juvenile mussels had survived the journey alive. Photo 8.22 shows juvenile mussels pedal feeding (under a microscope).

Photo 8.18 Cleaning the receptor quadrat.

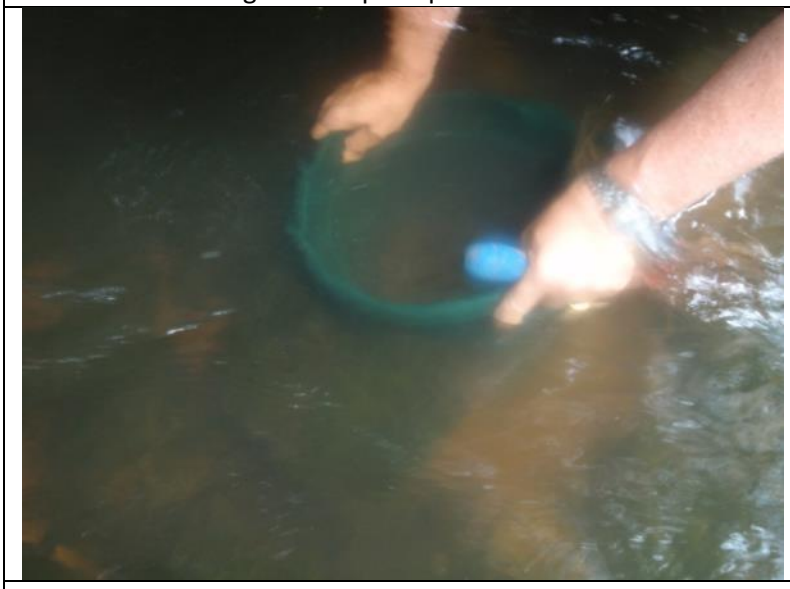


Photo 8.19 Rebuilding the quadrat within the bottomless bucket.

Photo 8.20 Adding the juvenile rich sediment.

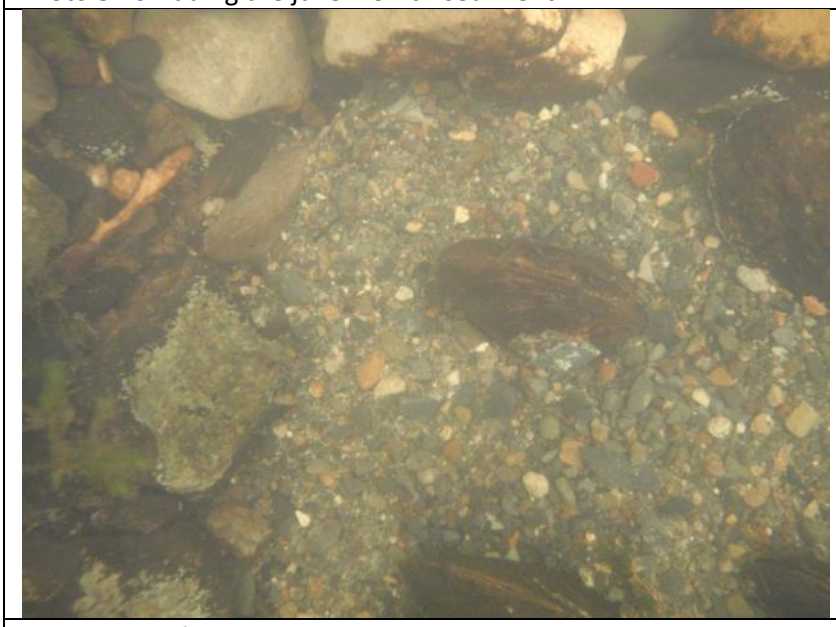


Photo 8.21 A finished quadrat.

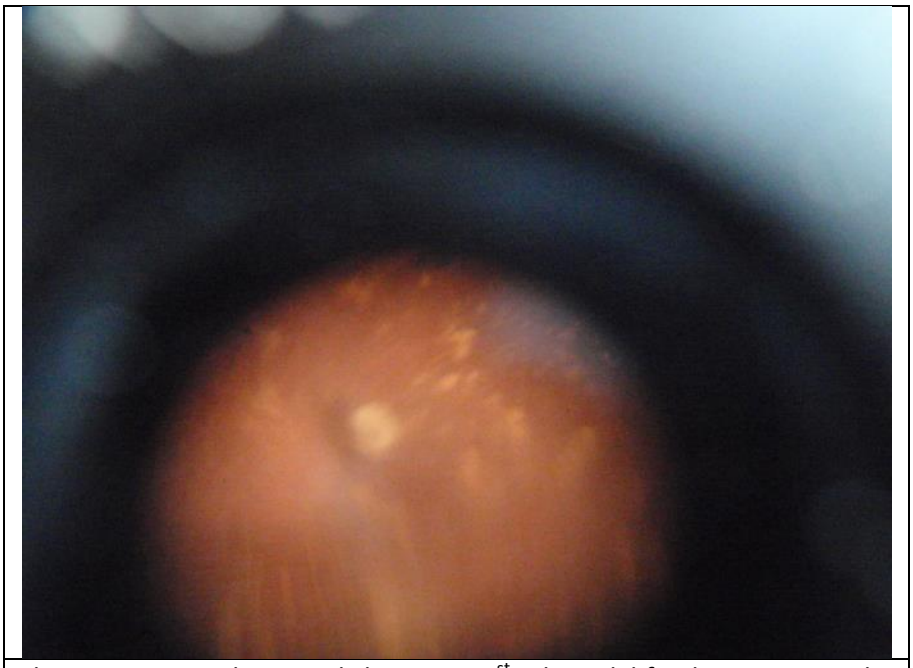


Photo 8.22 Juvenile mussels later on 21st July pedal feeding amongst the sediment.

8. Clean and disinfect all tanks at the hatchery, disconnect and tidy all piping and leave in a manner that would facilitate complete decommission with ease.

The cleaning and disinfection of the two bays at Site 2 was carried out over two weeks in August 2014. The site was inspected on 3rd September. All tanks had been cleaned out, all mud and sediment disposed of to a licensed facility. All tanks had been thoroughly disinfected. The circular tanks were left dry and on their sides to prevent filling with water (Photo 8.23). All pipes had been disassembled and stacked neatly along with all other equipment and netting.

Photo 8.23 the cleaned site at Site 2.

9. Disconnect water to bays in the hatchery by taking out the backboards from the river outflow channel.

In conjunction with the drying and cleaning of tanks, the wooden weirs that raised the water levels along the feeder channel were removed. This resulted in the water remaining below the entry level into the captive breeding bays, but keeps the water flowing through the channel system itself in order to keep it clean and functional.

As a result, the concrete tanks above each bay are slowly emptying and are reaching a position to be dredged of the layer of mud that has collected in them during the operation of the assisted breeding programme in Site 2 (Photos 8.24 and 8.25).

Photo 8.24 Bay 1 header tank at Site 2.

Photo 8.25 Bay 2 header tank at Site 2 is slower to empty.

10. Check of adult mussels in River Nore one month post transfer.

A survey of the translocation sites was undertaken on 2nd September 2014, one month after the adult and juvenile translocations.

At each of the 7 translocation quadrats, the adults were checked for filtering and the juvenile area was inspected to ensure it had not scoured out or coated with fine sediments.

Three of the 13 translocated adult mussels had died in the month, and another 3 were missing (Table 8.4, Photos 8.26 to 8.32). Just over half the adult mussels were known to have survived the first month back in the river.

The juvenile habitat in all cases looked in very good condition, with no fine sediment or algae visible, and in all cases the quadrats had remained stable, with no scouring of the introduced sediments. However, the conditions between the end of July and the start of September were extremely calm and dry, with very low flows in the river throughout this period. In high flows considerable mobilisation and movement of sediment can take place, as evidenced by the deep layer of river sands that have been pushed over the high banks and on to the paths by the river bank (Photo 8.33).

Table 8.4 Results of post translocation survey

Site	Quadrat	Mussels alive	Mussels dead	Mussels missing	Juvenile habitat condition
4	1	1/3	1/3	1/3	Very clean
4	2	3/4	1/4	0/4	Very clean
5	3	1/1	0/1	0/1	
5	4	0/1	0/1	1/1	
3	5	-	-	-	Relatively undisturbed
3	6	0/1	0/1	1/1	Relatively undisturbed
2	7	2/3	1/3	0/3	Very clean, good condition
Total		**7/13**	**3/13**	**3/13**	

Photo 8.26 Q5 Relatively undisturbed habitat

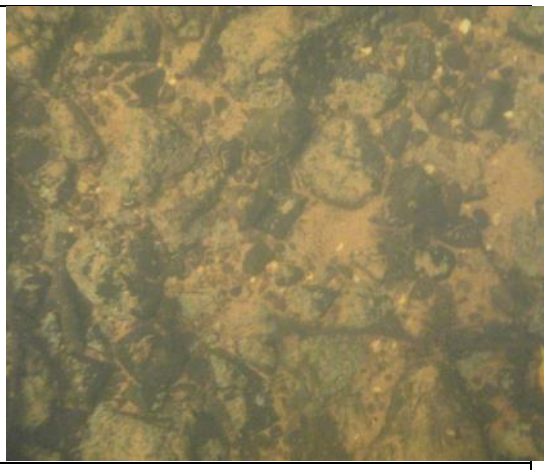


Photo 8.27 Q6 Relatively undisturbed habitat but no adult mussel found

Photo 8.28 Q7 Juvenile habitat very clean but 1 recently dead translocated adult mussel with flesh present

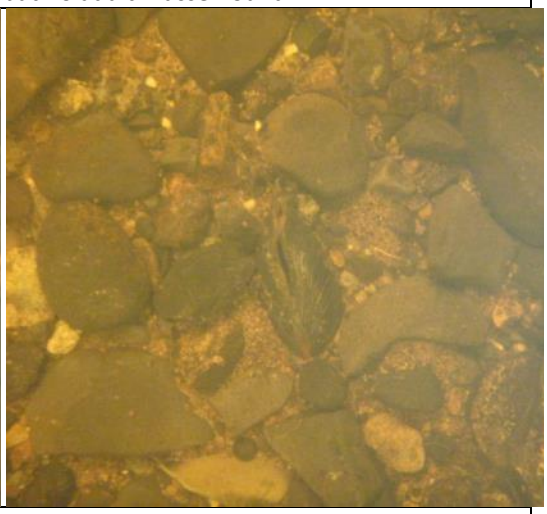


Photo 8.29 Q7 Clean juvenile habitat with other live mussel

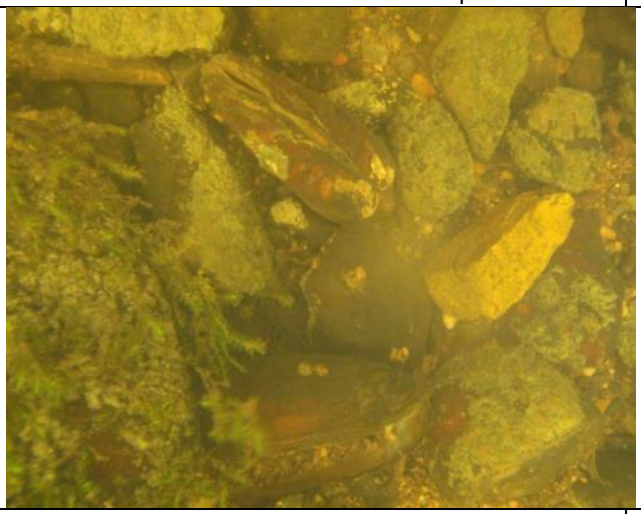


Photo 8.30 Q1 Juvenile habitat very clean but 1 dead translocated adult mussel (empty shell)

Photo 8.31 Q2 Juvenile habitat very clean but 1 dead translocated adult mussel (empty shell)

Photo 8.32 Q3 Juvenile habitat in good condition

Photo 8.33 Path by banks of the Nore shows how high flows result in river bed sands high on the banks

9 Discussion

The studies to date show that assisted breeding resulted in juveniles that were able to survive well in their early years, but not in the longer term under the semi-natural methodology used to date. Early survival is not considered to be due to better resilience of younger juveniles but most likely due to their placement in clean substrate. The condition of the substrate deteriorated over time, with the resultant loss of juveniles.

The conditions in the tanks over the last 2 years cannot be explained simply by "normal" expected sedimentation levels or by slow filling and loss of function of the header tanks. The level of mud coming into the system is too extreme, and became evident once the upper layer of gravel was removed for examination and the depth of mud was revealed.

When the assisted breeding operation system was designed in 2007 a system of weirs and bypasses were incorporated to allow cleaning of the intake channel. Donal Golden and I walked the lands upstream of the hatchery area, and Donal spoke with the local fisheries inspector to assess potential risks from farming and forestry upstream. Subsequent to this, a small waterbody coming from a nearby farm was channelled under the hatchery intake channel to prevent pollution entering the channel. The remaining risk was mainly from forestry, but this did not seem to be causing excessive siltation problem in the first two years.

The problem may be linked to recent fellings in the upstream forests. The hatchery owners have noted felling and replanting in coupes on a regular basis in recent years. The Coillte Management Plans for the sites indicate 50 Ha of clearfell planned from 2011-2015 in one forest, and 9Ha of thinning for a second.

The use of semi-natural juvenile growth tanks seems unlikely to remain sediment free in the near future. While the design of the faster flowing conditioning tank produced a cleaner bed environment than the slower long tanks or circular tanks, the flow levels may be too high to ensure stable juvenile conditions. Indeed, the more consistent nature of the captive breeding tank flow conditions may result in overall poorer conditions with regard to sedimentation than the rivers themselves. In the more intensively managed assisted breeding centres (England, Luxembourg, Austria), the main concentration of work is in cleaning juvenile trays.

The other important information learned from the work at Site 2, and at Site 3 and Site 1 in earlier years, is that captive held adult mussels can become severely stressed and do not have good survival rates either in the hatchery or in the wild following a period in the hatchery. Poor survival of *Margaritifera* in captivity has also been evident in other captive breeding facilities, for example in two Welsh facilities (now closed). Loss of adult mussels shortly after their return to the wild was disappointing in the current work, and was also recently documented in England (Ian Killeen, pers. comm.). This suggests that for the purposes of assisted breeding, mussels should be removed and returned to their native river in as short a time scale as possible, and therefore captive breeding sites cannot act as "Arks" as a back-up to the wild population. It is likely that because this sedentary animal lives for many years with very little change of environment, particularly with regard to water chemistry, food sources, and micro-habitat (shelter and flow direction etc.), facing the multiple changes a mussel encounters in a new environment is likely to be stressful. This also raises concerns regarding movement of juveniles

from captivity to river bed after years in captivity and consequential adaptation to the conditions in the captive breeding hatchery environment. It is likely that the greater the differences between the two environments, the more stress may be caused by movement to a different environment. Thus captive breeding within the same catchment (such as in the Ballinderry case) is likely to lead to the best results.

With regard to host fish, the ability to transfer from mussel to trout was easier than expected, once the fish were in good health. Both native trout from a different catchment and genetically manipulated brown trout (including some non-native genetics) were good hosts for the Nore pearl mussel. Specialist handling and early treatment for furunculosis in advance of placing with mussels was determined to be essential in order to have healthy fish that needed no treatment over the following 11 months with the mussels. Fish were fed a low protein diet to keep fish closer to natural growth sizes for age, as this kept gill filament sizes to levels that allows for good encystment and cyst maintenance, and kept faeces to a minimum when the fish were transferred to the juvenile tanks.

Other key findings from the assisted breeding project were that the assisted breeding project did not negatively affect the water quality downstream and met with the terms of its discharge licence, but the water chemistry at Site 2 was considerably different from that of the Nore, particularly with respect to hardness.

Assisted breeding falls into 6 general design options, which are summarised in Table 9.1. The different options need to be considered with regard to the timescale of recovery needed, the current state of the native river, the budget available, the facilities and manpower available. The captive breeding system in Site 2 to date has been Option 4, a semi-natural flow-through system. The system trialled in the summer of 2014 is option 5.

Option 1 is a high end, expensive approach that is used in the USA (Mummert *et al.,* 2006), where it is funded through “polluter pays” schemes. It is by necessity expensive, as it involves a wide range of species, some of which have unknown glochidial host species. The controlled temperature, feeding and host maintenance allows for very many individually operated small aquaria (Ahab Units) to serve the wide variation involved (Photos 9.1, 9.2).

Photo 9.1 Algal rearing process in White Sulphur Springs breeding facility, West Virginia, USA.

Photo 9.2 Example of "Ahab" unit rack, White Sulphur Springs breeding facility

Option 2 is also highly intensive, although the stages following juvenile drop off can be completed in any building with room for an incubator (Eybe *et al*., 2013). It involves rearing early stage juveniles in petri dishes with no oxygen replenishment but with artificial feed and very regular changes of water. Later stages of juveniles are moved to oxygenated systems. This system removes the need for winter periods of rest from growth, as the temperature is artificially controlled (Photos 9.3, 9.4). However, the very artificial nature of the rearing has not yet been proven to be successful in supporting the development of healthy adult mussels in the long term.

Photo 9.3 Temperature controlled units in Kalborn Mill hatchery, Heinerscheid, Luxembourg

Photo 9.4 Early juveniles from incubated petri dish, Plauen laboratory, Vogtland, Germany

Option 3 is where juveniles are reared in shallow trays in flowing river water with no additional feeding. The trays, bowls or sieves have very little substrate and are regularly cleaned to prevent detritus build-up, sedimentation and any loss of oxygen or build-up of ammonia (Photo 9.5 – 9.8). Water can be dripped downwards or upwelled to the trays/bowls. This system has the disadvantages of requiring large volumes of water as well as full time intensive management with regular cleaning required, and is really only suitable for a larger hatchery facility with full time staff involved in a range of projects.

Photo 9.5 Shallow tray system in FBA, Windermere, UK

Photo 9.6 Drip fed shallow tray system in FBA, Windermere, UK

Photo 9.7 Sediment build up in shallow tray system if not cleaned

Photo 9.8 Upwelling shallow substrate bowls with juvenile mussels, Marion Hatchery, West Virginia, USA.

Option 4 is the system that has been used at Site 2, and is the only system that has a proven record of success – albeit in the short term so far - with juveniles returned to the river, i.e. the Ballinderry project (Preston *et al*., 2007). It requires a flow through system of water that is either naturally free of fine sediment, or can have fine sediment removed, or where the sediment can be changed when a build-up of sediment has occurred (Photos 9.9, 9.10). At Site 2, in spite of designing a system with 6 weirs to slow and trap sediment, followed by a large header tank to further remove sediment, concentration of mud particles in the water have overwhelmed both systems and rendered the system unsuitable for semi-natural rearing.

9.9 The long tank, Ballinderry Fish Hatchery, where juvenile mussels were first reared in the semi-natural method

9.10 Juvenile mussels approximately 4 years old from the long tank, Ballinderry, 2004.

This option is not considered to be suitable for ongoing assisted breeding for the Nore mussel population at Site 2 due to the high level of fine sediment in the water. It could be possible at another hatchery, such as Site 3, which has previously grown juveniles up to transformation stage.

Options 5 and 6 rely on juvenile rearing in the native river.

Option 5, called "short term rearing", mixes the benefits of in-river juvenile rearing with the large scale benefits of juvenile production that can be achieved through hatchery encystment. This is a new methodology that has been trialled at Site 2 and has been described in detail with photographs in Section 8. It delivers tanks of up to 10,000 juvenile mussels without the need for either intensive management of the resultant juveniles, or the risk of sedimentation of semi-natural tanks. The success of juvenile survival with this system in any river that currently has poor juvenile survival rates would be dependent on choosing the right receptor sites. The choice of receptor site is based on preferential flows in the natural river, such that in every river with deteriorated bed conditions, there are pockets of habitat that remain suitable due to their gradient and the direction of flow at that point. The requirement for good velocities and the interaction of near-bed velocity with in-combination effects from sediment and nutrient inputs has been described by Moorkens & Killeen (2014). Thus if the correct habitat can be targeted and seeded directly with newly excysted juvenile mussels, there should be sufficient survival to maintain a living population until longer term catchment management improvements take effect and a wider habitat area becomes suitable for juvenile survival. The potential for this technique to be successful is also based on evidence from a number of rivers where a) juveniles are only found in the fastest flow areas (Killeen & Moorkens, 2013; Moorkens & Killeen, 2009), and b) fish are encysted in the river but juveniles are very rare (such as found in the Eske, Clady, Glaskeelan surveys). The hypothesis is that in the absence of this seeding option, fish carrying glochidia will be spread over all habitat areas, and the chances of juveniles dropping off fish in these suitable but localised pockets would be very low, and would decrease further as adult mussels get rarer in the wider river bed environment. This option provides two stages of amplification of survival chance, firstly by increasing the numbers of juveniles through hatchery rearing large numbers of fish, and secondly by placing juveniles where they have a much better chance of survival.

Option 6 is bank-side encystment, where electrofishing is organised for a time that mature glochidia are being released from female mussels, and mussels and fish are held together in tanks in order to ensure good encystment on fish gills (around 20-30 minutes), and then released back into the water. A proven technique, bank-side encystment has worked very well in the Lütter River in Germany (Altmüler & Dettmer, 2006). However, the success only followed after large scale catchment management measures had been implemented and had, over time, become successful in rehabilitating the river bed to a level of cleanliness that was supportive of the young mussels. No other rivers have had such a level of rehabilitation to date, but it is the best method for large scale revitalisation of populations when catchment improvements have been achieved.

Bankside encystment is time consuming and involves intensive work at key times of year. It involves the co-operation (and licensing) of electrofishing teams with mussel ecologists. The

period of glochidial development is long and unpredictable, but the window of opportunity of ripe glochidial release is short. It is a matter of luck as to whether the timing would be right if the various teams need to be organised for specific dates in advance. To be more confident of a successful result, mussels need to be monitored for glochidial development on a regular basis, either by regularly checking at the river, or by taking a small number into captivity and using them to check for glochidial progress (assuming they are maintained at the same temperature as the river) (Photos 9.11 – 9.14). In this case, an electrofishing team would need to be on standby.

Photo 9.11 Fish holding tank for bankside encystment, Lütter River, Germany

Photo 9.12 Checking lab-held mussels for brooding stage, Lütter River, Germany

Photo 9.13 Checking glochidia for development stage, Lütter River, Germany

Photo 9.14 Checking mussels from river for glochidial development, Esk River, Yorkshire, UK.

Table 9.1 Design options for assisted breeding of Unionids.

Option number	Type of assisted breeding	Description	Example of current usage	Advantages	Disadvantages
1	Indoor intensive recirculating system	Juveniles are reared in recirculating, artificially fed systems in small sieves	Sulphur Springs National Fish Hatchery, West Virginia, USA.	Many different populations and species can be reared in a small site. Good control of environmental variables.	Very expensive to run. Very labour intensive.
2	Indoor intensive incubation system	Juveniles are reared in standing water in petri dishes, artificially fed with collected detritus	LIFE project, Clervaux Mill, Luxembourg. Planungsbüro, Landes- und Denkmalpflege Vogtland, Plauen, Germany	Good control of environmental variables.	Very labour intensive. Long term effects of development under unnatural conditions unknown.
3	Indoor or outdoor intensive flow through system	Juveniles are reared in flowing river water with no additional feeding, rearing in very shallow substrate in trays or sieves that are regularly cleaned.	FBA laboratory, Windermere, England.	Many different populations and species can be reared in a small site.	Expensive to run. Very labour intensive.
4	Indoor or outdoor semi-natural flow through system	Juveniles are reared in flowing river water with no additional feeding, rearing in large tanks with substrate to mimic a close to natural environment.	This project, Site 2, 2007 - 2014. Ballinderry Fish Hatchery, Northern Ireland.	Low maintenance. Relatively low cost. Close to natural growth rates may confer advantage over intensively reared juveniles.	Low numbers of juveniles produced. Sedimentation incident can cause large losses. Difficult to upscale to juvenile numbers that may be needed.

5	Short term rearing	This involves the encystment and maintenance of fish and the movement of newly excysted juveniles to suitable places in the river	Present Site 2 project 2014	Low maintenance. Relatively low cost. Suitable for rivers with pockets of good habitat.	Unsuitable for rivers with no juvenile habitat.
6	Bank side encystment	This involves the electrofishing of native salmonid hosts and providing enhanced encystment through leaving them in a bucket with adult mussels that are releasing glochidia. The fish are then returned to the river.	Lutter River, Germany.	This system works well in rivers where large areas of habitat have been restored to a high level.	This system works will not work in rivers with poor juvenile habitat conditions. Glochidial release can be between July and September. The system needs a high level of intensive survey to ensure the timing is correct. The system needs an on-call electrofishing team for the time period around glochidial release.

Recent survey of the Nore River indicates that the decline towards extinction is continuing, and that catchment practices are still contributing to poor water and river bed quality. It is a matter of grave concern that the likely time needed to improve the habitat for the mussels will not be achieved within the timeline of the current population in the river. For this reason, and in spite of the poor results to date, assisted breeding is recommended to be continued at some level, because in its absence extinction of the population is inevitable.

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