

Report on host fish specificity and survival of FPM glochidia reared in captivity, and survival after restocking

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Background

In [Freshabit LIFE IP](#) (2016-22) we aimed, among other actions, to revive some withering populations of Freshwater Pearl Mussel (FPM) in Finland. Conservation status of the species in Finland is [endangered](#) (EN), and the recent [conservation strategy](#) proposes several measures for reviving the populations of the species, captive breeding and rearing among others. The aim of Freshabit LIFE IP was to revive at least two populations, Rivers Ähtävänjoki and Mustionjoki, in southern Finland by captive rearing, and start the measures for two more populations, Rivers Isojoki and Karvianjoki.

The initial plan of the project was to capture glochidia *in situ* in the host populations by collecting mature mussels and the glochidia they extract when taken out of the river. The glochidia were then to be delivered to Norway, Austevoll breeding station of the University of Bergen, where the nearest existing FPM breeding station had been operating successfully for several years. However, it became evident in the first project year 2016 that the mussels of both Mustionjoki and Ähtävänjoki were in too poor condition to reproduce in the nature. We then had to change the plans, and adult mussels from both populations were taken to the Konnevesi Research Station of the University of Jyväskylä, where facilities for *ex situ* rehabilitation, breeding and rearing of FPM were set up. In addition to not breeding in nature, both populations of Mustionjoki and Ähtävänjoki have been declining in numbers during the project, which highlights the urgency of producing offspring to prevent local extinction in the near future (Fig. 1).

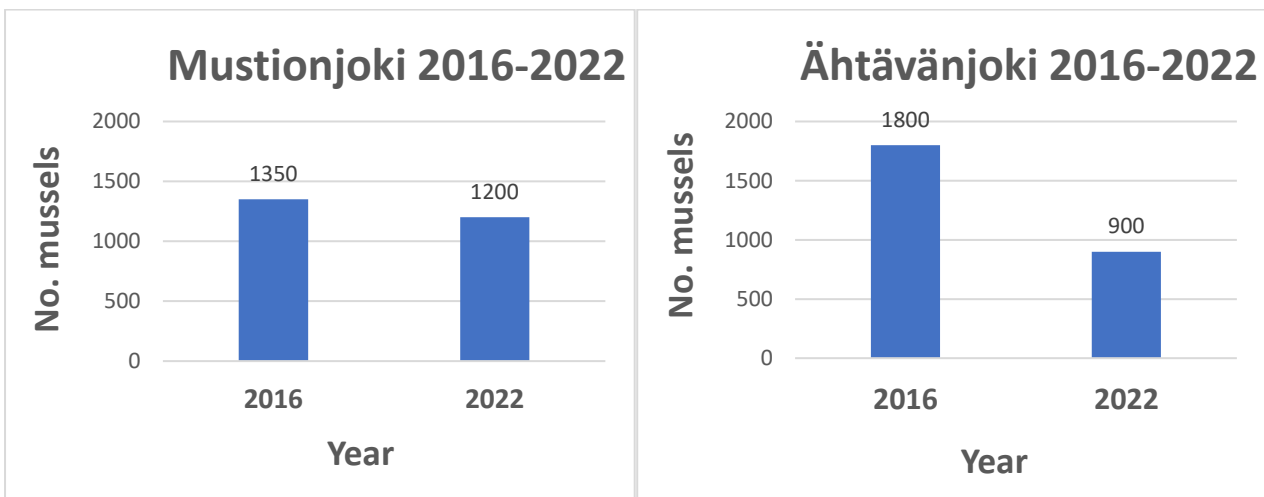


Figure 1. The estimated total number of FPM in Mustionjoki and Ähtävänjoki in 2016 and 2022, based on field surveys with standardized methodology.

This report summarizes the key measures and results of Freshabit Action C9 for reviving the FPM populations. The report is based on internal reports by the University of Jyväskylä on successes and failures experienced in captive breeding and rearing, and the reports by the ELY Centre of South Ostrobothnia and Länsi-Uudenmaan Vesi ja Ympäristö on restocking in Ähtävänjoki and Mustionjoki, respectively.

Captive breeding and rearing

Captive facilitation and breeding of adult FPM and rearing of the juvenile FPM took place in the Konnevesi Research Station (KRS) of the University of Jyväskylä, where facilities for *ex situ* facilitation, breeding and rearing of FPM were set up. Initially we planned to facilitate the adult mussels until they secrete the glochidia and ship the fresh glochidia directly to Norway for rearing. However, plans evolved, and after consulting with the FPM rearing specialists in Norway we decided to first grow all glochidia in Konnevesi in host fish over the first winter, and then ship one part of the hatched juvenile mussels to Norway, and keep the other part in Konnevesi for development of rearing methods in Finland. The facilities in Konnevesi were continuously developed, and by 2022 we have a fully operative FPM breeding and rearing station in Konnevesi, serving the rearing actions for LIFE Revives project, which continues the measures for FPM after Freshabit.

Host fish preference of the populations was eventually not specifically tested. Getting viable glochidia took two years, until autumn 2018, and as problems with fish survival occurred in Konnevesi, no host fish were killed for infection studies. However, based on observations made during the infection and hatching phases, no significant differences between salmon or trout were noted for either of the populations. For Ähtävänjoki, Atlantic salmon has been previously used in infection tests with good results. For Mustionjoki this was the first attempt.

Mortality of the adult mussels was quite high in Konnevesi. Altogether 170 adult mussels from Mustionjoki were taken to Konnevesi during 2016-19, and only 38 of the survived to 2022, when all remaining mussels were returned to their natal site in Mustionjoki. Survival rate was thus 22%. The high mortality is probably due to a combination of poor initial condition of the mussels and little previous experience in facilitating the mussels.

For example, in 2018 we added marble gravel to our FPM tanks, because this method was applied in the Austevoll FPM breeding station to increase calcium content of the water. However, the marble gravel we used contained magnesium, which is poisonous to FPM. The poisonous marble gravel was there for almost one year. This is possibly behind the fact that the mortality of FPM increased from 8 % in 2018 to 30 % in 2019 and up to 40% in 2020.

In 2019, when new water pumps were installed, rot-preventive treated wood material was used inside the FPM tanks. Wood preservatives are known to contain copper, which is very poisonous to molluscs. These poisonous wood materials were in FPM tanks for 1-2 months, before they were displaced with untreated wood, which may have had a negative impact on condition, survival and glochidia production of the mussels.

Production of glochidia and juvenile mussels turned out to be more difficult than anticipated. In 2018, after two years of rehabilitation in Konnevesi, we had the first batches of glochidia from Ähtävänjoki and Mustionjoki populations. The original number of juvenile mussels for rearing was estimated to be 10 000 - 30 000 per population. In the end we managed to rear ca. 3000 juvenile FPM for each population in Konnevesi through the infection phase in fish. Approximately 2500 of these were taken to Norway for further rearing in July 2019, and we got ca. 1500 FPM mussels back in April 2021. In addition, a few hundred FPM were left in Konnevesi to be reared there, and to be used in preliminary restocking trials in 2020. Thus the initial gain of glochidia was ca. 10% of that anticipated, and the **mortality of the juvenile mussels through the first two years was ca. 50%**.

In June 2021 all the remaining juveniles from Ähtävänjoki population, ca. 1300 individuals, were transferred to Ähtävänjoki for controlled rearing in the river. From the Mustionjoki population only 200 mussels were taken to their natal site, and the rest were retained in Konnevesi. Unfortunately, they all deceased in the station in spring 2022 due to problems in e.g. nutrition.

One of the reasons for the loss of the 2019 recruit of River Mustionjoki FPM juveniles which arrived from Norway in April 2021, as well as all juveniles at KRS in 2021-22, was that a non-functional recirculation aquaculture system was established for the juvenile mussels at KRS. The recirculation system was not working properly. Another reason was that we had continuous problems in getting mussel food, the microalgae products from USA in time. Transportation of the food to KRS took repeatedly more than 4 days – many times even 10-14 days. One of the leading FPM captive breeding experts, Dr. Ondrej Spisar for Czech Republic later informed us that using spoiled food is very harmful for the juvenile mussels.

Restocking in natal rivers

The first restocking trials were made in 2020 with one-year-old mussels kept in Konnevesi. The mussels were placed in Buddensiek -type plates, where each mussel has its' own chamber sealed with mesh, and the mussels can be monitored individually (Fig. 2). For this trial we had mostly juveniles from the Ähtävänjoki population available, and the remaining Mustionjoki juveniles were in rather poor condition. Consequently, when monitored in Mustionjoki and Vihtijoki trial sites in the Karjaanjoki region, the **survival of Ähtävänjoki juveniles from July to October was good (max. mortality 7%)**, whereas 38-71% of the Mustionjoki juveniles died during the trial (Table 1). A non-natal stream site was used in the trial to determine, if there are potential problems for juvenile survival in the natal site. Similarly, in the Ähtävänjoki area juvenile mussels were placed in River Lestijoki in addition to Ähtävänjoki, using only offspring of the Ähtävänjoki population. **Survival was good (<10% mortality)** in both sites, but the mussels grew better in the original Ähtävänjoki location. The trial was ended in Mustionjoki in October 2020 and the remaining juveniles taken back to Konnevesi. In Ähtävänjoki, the mussels were moved into different kind of containers and kept in the river over the winter 2020-21.



Figure 2. Buddensiek plates were used for the first restocking trial of one-year-old FPM. The mesh-covered chambers allow for natural food in the stream, and individual monitoring of mussel growth and survival (one mussel in each chamber).

Table 1. Survival and growth of juvenile FPM from Ähtävänjoki and Mustionjoki populations in three locations in the Karjaanjoki catchment from July to October 2020.

	Äminne	Menestyminen (kuollut)					kasvu, koko (mm)				
		istutettu	16.7.2020	28.8.2020	13.10.2020		istutettu	16.7.2020	28.8.2020		
Mustionjoki											
	Mustionjoki	13	11	8	8	-38 %	1,4	1,8	2,2	2,4	70 %
	Ähtävä	15	15	15	15	0 %	2,2	3,1	4,1	4,2	91 %
Junkarsborg											
	Mustionjoki	14	12	4	3	-71 %	1,4	1,6	2,1	2,2	52 %
	Ähtävä	15	15	14	14	-7 %	2,4	3,4	3,7	3,6	52 %
Vihtijoki											
	Ähtävä	15	15	15	15	0 %	2,2	2,9	3,9	4,2	88 %

The second controlled restocking trial took place in June 2021 with juvenile mussels received from Norway. In Ähtävänjoki, ca. 1200 juvenile mussels (all remained from the batch reared in Norway), were taken back and stocked in the river in 4-liter plastic boxes (16 pcs. in two original FPM locations) with mesh-covered openings and lid, half filled with natural gravel. In Mustionjoki, 200 juvenile mussels were stocked in similar boxes (3 pcs. in the primary remaining FPM location). This kind of in-situ rearing box provides natural-like conditions for the mussels and still enables monitoring at the box level (i.e. survival rate and mean growth per box).

Survival and growth in these gravel boxes have been remarkably good in both rivers. During the first year the **mortality in Ähtävänjoki trial was <6%** (June 2021 - September 2022) and in **Mustionjoki <8%** (June 2021 - June 2022). Mussels in both sites have grown from ca. 0,5 cm to ca. 2,5 cm during their third year after detaching from the host fish. Therefore, it can be concluded, that the method for in-situ rearing of juvenile mussels after the ex-situ rearing phase seems very promising. Thus far boxes have been used for monitoring purposes, but most likely the juveniles would thrive as well if restocked freely in their natal locations.

Continuity

In general, the LIFE Revives project [LIFE revives project](#) (2021-27) continues the captive breeding measures for FPM in Finland. Based on the results and experiences in Freshabit, the project has functioning rearing and breeding facilities in the Konnevesi station at the beginning of the project. The facilities and resources for keeping the mussels are far better than during Freshabit.

Approximately 12 000 juvenile mussels (< 1 year, length ca. 0,5 mm) from the latest batch produced from the Mustionjoki adult FPM were restocked in the primary remaining FPM location in Mustionjoki in September 2022. They were restocked in wooden mesh boxes, which allow for later monitoring of the survival. Additional funding needs to be raised for monitoring the Mustionjoki population, and this is not secured by any ongoing project. The Ähtävänjoki Fund can be utilized for monitoring and additional conservation measures for the Ähtävänjoki FPM population. Funding can in addition be applied from nature conservation funds, and the funding for conservation of threatened species by the Ministry of Environment.

Lessons learned

1. It took at least 2 years to rehabilitate the poor quality, old FPM so that they resumed reproductive functions, spawned and produced glochidium larvae in all trial rivers (Ähtävänjoki, Mustionjoki, Isojoki, Karvianjok).
2. Collection of River Mustionjoki FPM and transportation to KRS took place in November in 2016 and in September in 2020. Maybe September is seasonally a worse timing for such activity as compared to November, as the September 2020 group faced a higher mortality at KRS. September is supposedly close to the natural glochidia release period of River Mustionjoki FPM, which is in general assumed to be energetically demanding and stressful period for FPM.
3. Results from KRS indicate that the production of glochidium larvae is not continuous but there are gaps. Probably the mussels do not produce glochidia every year.
4. Rearing and breeding of FPM is very complicated (adult mussels, fish, juveniles, released juveniles; success in all stages is needed) and resource demanding. One has to be prepared for whatever surprises and availability of sufficient workforce and funding is requested.
5. It is possible to control the development of glochidia, and the seasonal timing of juvenile production, by increasing the temperature of host fish. This will enable staggering the detachment and collection of juveniles during the course of year in the future.

6. Keeping adult (parental) mussels at the facility for extended periods is maybe not a good idea. If the mussels are in poor condition, they may require even two years to rehabilitate. However, there are risks associated with the laboratory maintenance of adult FPM, and the mortality of FPM increased after 3 years in captivity. Therefore, the parental mussels should be returned after a maximum of 3 years captivity and replaced with new individuals, if possible or needed. This would also widen the genetic base of the bred juveniles.
7. Finally, we may have found a way to get mussel food so that shipping does not take too long. A French re-seller is selling only once in every two months, but surprisingly, a UK re-seller can sell the products whenever so that the products will be shipped to us by the French re-seller. Very strange system and price is high, but if it works, the double price does not matter.
8. To avoid the problems with survival of fish die to water mold (*Saprolegnia*) and *Flavobacterium* infections, efficient UV-treatment and filter of the incoming water was installed to KRS in April 2021. After that the survival of fish has improved substantially, being practically close to 100% now. This is the main reason why even a low number of fish infested with FPM glochidia in autumn 2021 (20 +20 ind.) produced even more than 12 000 River Mustionjoki FPM juveniles in summer 2022. The very high survival of fish at KRS is one of the key factors making it very probable that captive breeding of FPM at KRS is successful in the future.