



### LIFE05 NAT/L/000116

# « Restauration des populations de moules perlières en Ardennes »

## Technical Report: Action D1 and F3 Mussel Rearing Station



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### Part D1: Management and maintenance of the mussel rearing station

The following report describes the first year of mussel seed wining at the provisional station at the mill of Kalborn. The rearing of the mussels follows several steps:

#### 1 Semi natural infection of Brown Trout (Salmo trutta fario)

In August 2006 32 *Margaritifera margaritifera* were collected in the river Our and kept in aquaria with Our water until the release of the glochidia. The water was changed partly or completely daily and aerated continuously (see Figure 1)



Figure 1: *Margaritifera margaritifera* in a aquarium

Furthermore the water was checked daily for the presence and development of larvae (Glochidia). In the week from the 18.08.2006-26.08.2006 the larvae were fully developed, free and ready for the fish infection (Figure 2).

The water of the aquarium with the infectious larvae was collected in plastic tanks and transported to the national pisciculture in Lintgen where most of the fish were infected. The number of fish that could be infected was depending on the number of glochidia/ml in the infection solution. The fish were placed in tanks with a low water level and the infection solution was added (Figure 4). After half an hour the infection was finished and the fish were placed back in the pond.

Overall 4720 2+ trout (trout in their third year, length between 15-25 cm) were infected. Most if the trout (3900) were kept in ponds at the national pisciculture in Lintgen and the rest (820) was kept during the winter in ponds from the angler organization in Willwerath (Germany). To check the infection success a few trout were killed immediately after the infection and the attached larvae were counted. Between 500 and nearly 12000 mussel could be counted on the different fish analyzed. The prevalence turned out to 100 % and a mean abundance of 4053 +/- 3493 was calculated. In spring the glochidia load of another 12 Brown Trout from Lintgen was analyzed. The infection parameters were the following: Prevalence 92 %, mean abundance 1435 +/- 1169.



Figure 2: Semi natural infection of Brown Trout (Salmo trutta fario) with larvae of Margaritifera margaritifera



Figure 3: Heavily infected gill of a Brown Trout (*Salmo trutta fario*)



Figure 4: Adding the infection solution to the fish

### 2. Winning of mussel seed.

In order to collect the young mussel from the gills of the Brown Trout a mussel seed winning station was constructed at the mill in Kalborn in February 2007 (Figure 5).

This station consists of two 500 liter plastic tanks where one (1) contains the infected fish and the other (3) the sieve (2) to collect the mussels. The water is circulating with the help of a water pump (4) and cleaned by a UV-clearer (5) and water filter (6). In the water reservoir (3) the water can be heated in order to accelerate the development of the mussels on the gills of the fish. Furthermore the water is aerated in this tank. During 24h the whole water is changed once with new spring water.



Figure 5: Schematic drawing of the mussel seed winning station

- 1: Tank to with the infected fish
- 2: Sieve to collect the mussels dropped from the fish
- 3: Water reservoir and tank where the water is heated
- 4: Water pump, pumping the water back to the fish reservoir
- 5: UV-clearer (killing algae and pathogens)
- 6: Water filter (biological and mechanical cleaning of the water)

Previous to placing the fish into the station, the gills of the fish were checked in order to choose only well infected individuals (Figure 6). Once the fish were in the winning station (19.03.2007, 12 individuals) the water temperature was increased daily by one degree until 17-18°C was reached. After a few days at this optimum temperature the first mussels started to drop. The number of mussels falling from the fish increased day by day over the next weeks and decreased again after five weeks. This first cycle was finished after six weeks (see Figure 7).



Figure 6: Collecting of young mussels with the mussel seed winning station



Figure 7: Dropping of young mussels during the first mussel winning cycle

The mussels were collected each day from the sieve and transferred into a petri dish. Here they were cleaned and dead mussels were separated out (Figure 6).

Another two cycles, one with fish from Willwerath (6 individuals) and one with fish from Lintgen (12 individuals) started on the  $5^{\text{th}}$  of May. The falling of the mussels is illustrated in Figure 8.



Figure 8: Dropping of young mussels during the second mussel winning cycle

A third cycle again with fish from Lintgen started at the beginning of June. But due to a power breakdown the fish died and the cycle was finished earlier. Overall around 14000 young mussels were collected during the three cycles.

### **3** Growing of the young mussels in the laboratory

The third step is to grow the young mussels in the laboratory. Therefore about 250-300 cleaned young mussels were kept in small plastic boxes (12cm x 12cm) at a constant temperature of 17-18°C in a conditioning cabinet (Figure 9). At this stage the mussels feed on fine organic material (detritus). This food was collected in the area around sources where the ground is saturated with water, which contains a lot of organic material from plants (see Figure 10). At the beginning the boxes were cleaned each day and dead mussels were removed. The boxes were than filled up with 80% water and 20% detritus. As protein source a few drops of ornamental fish food was added. Later the boxes were cleaned every third day.

The mussels were fed with food from three different sources and with detritus directly collected in the river Our and the Mill channel. About every 30 days the mussel length was measured to control the growth (see below F3).

In this phase still a lot of mussels die and a complete box can be lost due to mycosis. Therefore it is important to check the boxes regularly and to remove dead mussels.



Figure 9: Conditioning cabinet with boxes containing young mussels



Figure 10: Organic plant detritus serving as mussel food

#### 4 Growing of mussels in the natural environment

In the next step the young mussels having reached around 1 mm are transferred into multiple cages (transformed after Buddensiek, 1995). This cage system consists of three plates of plastic (polyacryl) into which 20 holes are drilled (10.0 mm diameter). The young mussels were enclosed within the 20 holes of the 9 mm thick central plate by two sheets of plastic gauze (315  $\mu$ m mesh) held in place by 2 mm thick outer plates using four stainless steel screws (Figure 11). With these cages the young mussel can be transferred into the river under controlled conditions. During the project it is planned to install these cages in the mussel rearing channel which has already been constructed (Figure 12). This small channel with a large contact zone with plant roots will produce enough organic food for the young mussels. The constructions to supply the channel with water are however not finished so that the channel is not yet in use. The first cage systems were installed in the environment in the middle of August. Four young mussels were placed in each cell giving a total of 80 for each

plate. Plates were transferred to the river Our and the mill channel where they were fastened above the bottom, facing the current (Figure 13).



Figure 11: Cage system for young mussels



Figure 12: Mussel rearing channel



Figure 13: Cage systems for young mussels installed in the Mill channel

### 5 Growing of mussels in the natural environment in gravel boxes

In the last step, before the young mussels are reintroduced to the wild, is to keep them under controlled conditions in gravel boxes (Figure 14). The mussels are placed in these boxes having reached a length of 2-3 mm. This step will start at the earliest in 2008.



Figure 14: Gravel box

All steps to collect and grow the mussels under laboratory and semi natural conditions presented here were tested and developed by Dipl.-Ing. (FH) Michael Lange during the Interreg IIIA Project "Flussperlmuschel Dreiländereck" (SN-01-I1-3-C0203-EEV) based on the Method of Hurska. The methods were slightly transformed and adapted to our needs.

#### Preparation for the semi natural infection in 2007

On the  $10^{th}$  of July 27 adult mussels of *Margaritifera margaritifera* were transformed into two 100 liter aquaria at the mill of Kalborn. The location of these mussels in the river Our was known from the year before. The next two weeks another 9 mussels were collected in the stretch between Tintesmillen and Kalbermillen. On the  $24^{th}$  of July Dipl. Biol. Rainer Dettmer controlled the gills of the mussels to check if the mussels were mature. However only two mussels were found to be mature and another four mussels were slightly mature. These mature mussels were separated into one aquarium. After an intensive search nineteen further mussels were also checked and finally 21 mussels were kept at the station. However virtually no eggs were visible on the gills of most of these mussels. The mussels were kept in two well aerated aquaria. The bottom of the aquaria was cleaned daily and checked for released larvae. Furthermore about 1200 0+ Brown trout were directly kept at the mill in order to have immediately fish that can be infected if larvae appear.