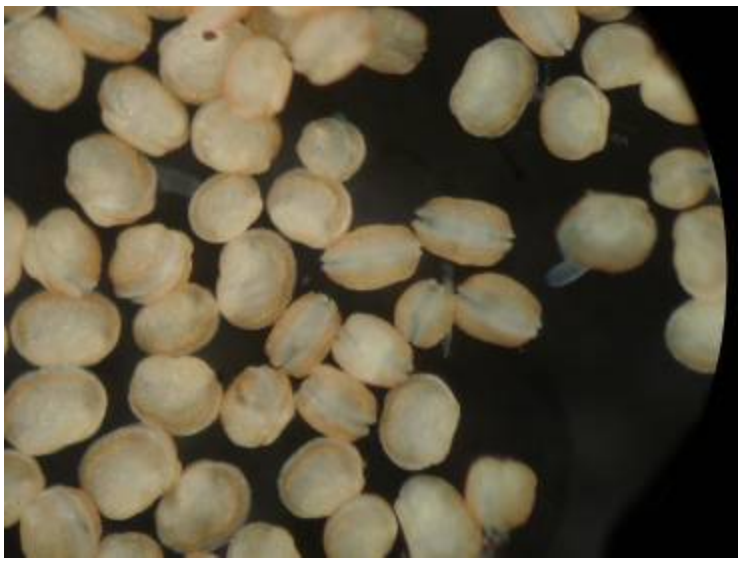
LIFE05 NAT/L/000116

« Restauration des populations de moules perlières en Ardennes »

Technical Report: Action A1 /D1 /F3
Mussel Rearing Station



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LE GOUVERNEMENT DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Intérieur et à la Grande Région

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Ministère du Développement durable et des Infrastructures
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1 Introduction

Freshwater mussels are an important component of aquatic ecosystems and their presence reflects the health status of freshwater environments. They belong to the most endangered species among freshwater organisms. Most of the mussel species in Europe are listed as endangered or threatened. Especially the freshwater pearl mussel (*Margaritifera margaritifera*) shows a dramatically decline throughout their distribution range in Europe. The species is listed in Annex II of the Habitats Directive and also on Annex V (Directive 92/43/EEC). In Luxembourg *Margaritifera margaritifera* is highly endangered and a very small remaining population still occurs in the river Our in northern Luxembourg. In the past, these mussels were common among others in a large part of the Belgian and Luxembourgian massif of the Ardennes and of the German Eifel. Now, these populations have drastically decreased due to a loss of adequate natural habitats, eutrophication, new predators, decline of fish stocks and sedimentation of the interstitial.

There are mainly four general strategies for the conservation of unionid mussels, including a) creation of protected areas, b) transfer of adult mussels from rivers with healthy populations to other rivers, c) releasing large numbers of host fish infected with glochidia and d) cultivation to produce juvenile mussels for use in restocking programs. In river systems where mussel populations are severely depleted or on the verge of extinction, which is the case in Luxembourg it is suggested that rearing of juvenile mussels for stock restoration (together with the releasing of infected host fishes) is a feasible option for species conservation.

This technical report describes the techniques of mussel rearing that were used in the mussel facility at the mill of Kalborn.

2 Technical report

Table 1: Overview of the most important details for rearing mussels

Point	**Time period**	**procedure**	**material**	**Note**
Infection of host fishes				
1	Before August	Finding mussel sites	Aqua scope	*M.m.* sites are often in the shadow with lower turbidity
2	Before August	Provide host fishes (0^{+})	Different ponds, pools and/or tanks	Fishes should be kept in different tanks
3	End of July and August	Check mussel gills for developed larvae (glochidia)	Special tool to open the shell	Shells should not be opened more than 0.5 – 0.75 cm!
4	August	Infection of host fishes	Big bucket, stereo microscope, plastic pipette, beaker	Concentration of infection solution: 2000 free larvae per fish
5	January	Winning of mussel seed	Mussel seed winning station	1 L of water for 1 cm of fish Example: 15 fishes of 10 cm need a Volume of 15 x 10 = 150 L water
Growing of juvenile mussels in the laboratory				
6	Early spring	Raise juvenile up to 1 mm	500 ml plastic boxes, detritus, commercial algae (Nanno3600 and Shellfishdiet1800), Artemia sieves, spray bottle, petri dishes, stereo microscope. Or/and: aquaria (> 16.5 L), sand, pump.	Water exchange every week (with a maximum of 8 days)
7	Summer/early autumn	Raise young mussels of ≥ 1 mm	See explanation in text below	

2.1 Explanation of the different points listed in table 1

2.1.1 Infection of host fishes (point 1-5)

Point 1, Finding mussel sites:

The sites of around 100 adult animals are known in the river Our (Belgium and Luxembourg part). Adult *Margaritifera margaritifera* are existent at some places in shallow water where the water velocity is lower. The mussels prefer shadowy places.

Point 2, Provide host fishes:

Young brown trout (*Salmo trutta fario*) were offered from the national fish hatchery in Lintgen (Luxembourg) and the fishing association in Prüm (Germany). 10000 young brown (0^+ = hatched in spring the same year) were available for the infection of glochidia at the mill of Kalborn, Prüm and in Lintgen.

If no fish hatchery can supply fishes, they can also be caught in the river by electric fishing.

It is very important to use the right fish strain that has its origin in the mussel river, because the glochidia are really adapted to the original host fishes of their rivers. Wrong fishes may lead to a loss of mussel larvae during infection.

Point 3, Check gills for developed larvae (glochidia):

Adult mussels develop larvae on the gills at late July or August. Mussels in the river Our were checked regularly at this time in order to "harvest" developed free glochidia for infection. The mussels were opened carefully (0.5 – 0.75 cm) with a special tool (see image 1) and the gills were examined for gravidity.

Gravidity can be determined by the occurrence of swollen gills. Gravid mussels were marked with nail polish and checked every day/second day in the river.

Image 1: Tool to open mussel shells

Point 4, Infection of host fishes:

When the glochidia are mature, the adult mussels can be brought to the laboratory and stay in an aerated aquarium. They should not be kept longer than one week. Water exchange must occur two times a day (30 % renewal of water per exchange). When mussels are kept in the laboratory, the aquaria water must be sucked off with a tube into a bucket every day. The larvae packets are

normally visible on the ground next to the mussels (image 2) but also the free water can contain free larvae.

If it is not possible to bring the mussels to the lab, the larvae can also be collected in the field. However it is necessary to collect the larvae on the right day, in order to get free infective larvae. In the field, adult mussels can be brought to release their larvae by placing them in a beaker with a little bit warmer river water (2-3˚C more than river water, for example heated up by the sun).

In our case in 2009 and 2010 most of the adult mussels released their larvae in the laboratory. The larvae containing water in the bucket after removing it from the aquarium, was stirred intensively but carefully (in order to free glochidia from their egg cover). The maturity and concentration of larvae was checked under a stereo microscope. When there are mostly free glochidia without egg cover that move (flap), the infection of fishes should start directly. If this is not possible, the glochidia can stay in aerated river water over night (≤ 24 h). To calculate the concentration of glochidia that are needed for infection, 200 µl of the larvae solution was pipetted on a microscope slide and counted.
Depending on the size of the infection bucket about 250-500 fishes were placed in a big plastic bucket. Glochidia were added with an amount of 2000 free larvae/fish. The water in the fish buckets was agitated every 5 - 10 minutes. This process lasted around half an hour to 45 min. The mean intensity of infection and prevalence of infection are normally around 1000 mussels/fish and 90-95%. After the infection the fishes were held in different ponds, pools and tanks over the winter.

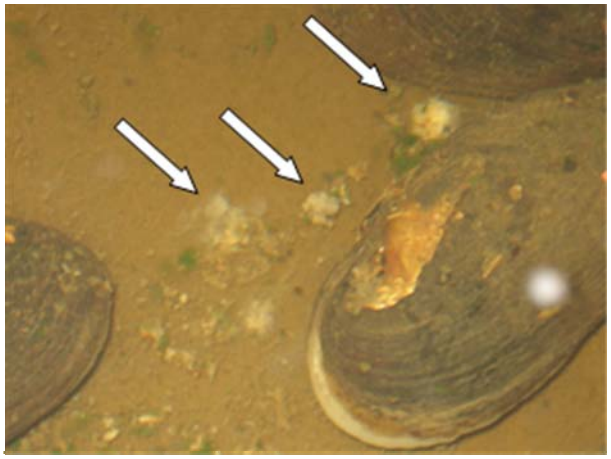

Image 2: Released packets of glochidia (white slime)

Point 5, 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 (image 3). 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 river water.

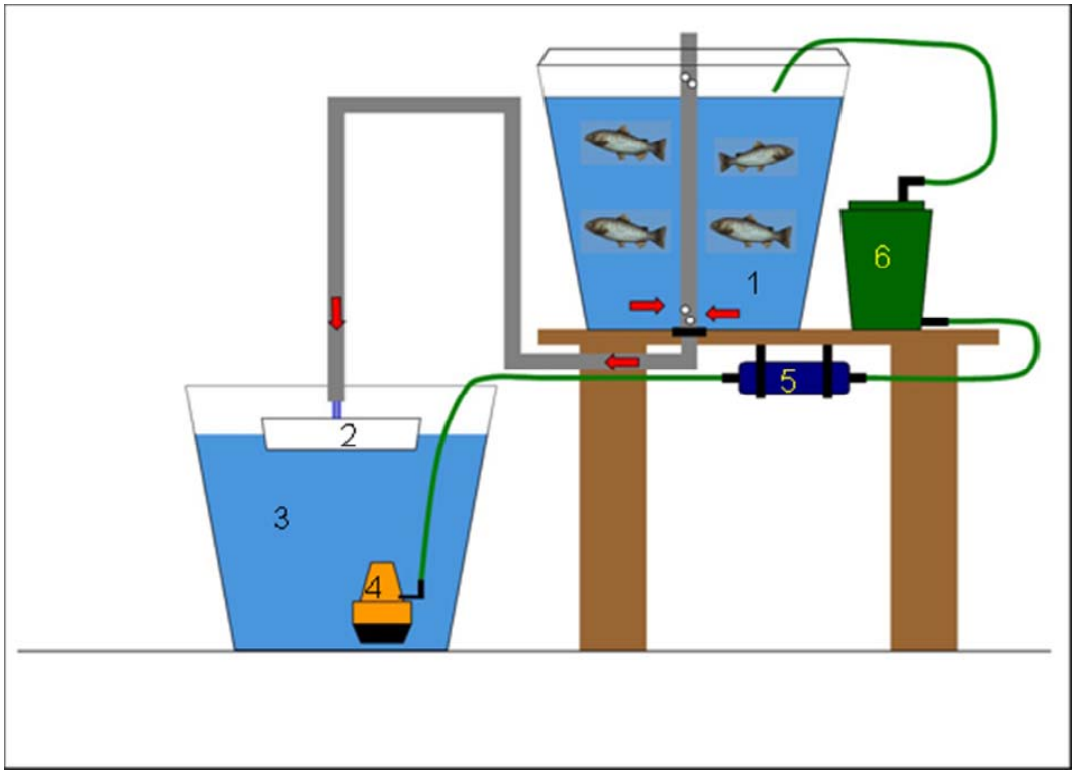

Image 3: Schematic drawing of the mussel seed winning station

1: Tank 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 by opening carefully the gill cover in order to choose only well infected individuals. Once the fish were in the winning station (in January) 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. 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 before setting them in plastic boxes or aquaria.

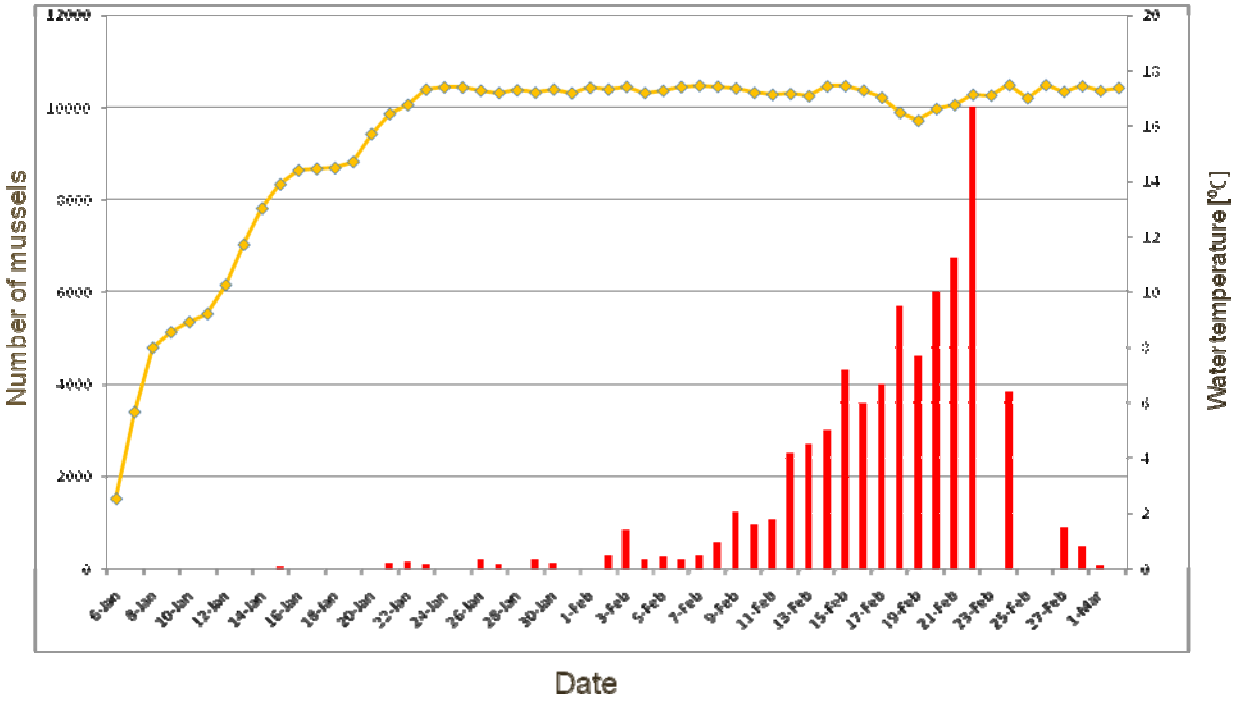

Image 4: Dropping of juvenile mussels during a winning sample

2.1.2 Growing of juvenile mussels in the laboratory (point 6 – 7)

Point 6, Raise juvenile up to 1 mm:

The next step is to grow the young mussels in the laboratory up to approximately 1 mm. This is a size when the mussels are easier to handle and less sensible. Two different methods were performed in the laboratory at the Mill of Kalborn: the rearing in plastic boxes and the rearing in aquaria.

Rearing in plastic boxes (first 100 days):

About 300 -500 cleaned young mussels were kept in small plastic boxes of 500 ml at a constant temperature of 17-18˚C in a conditioning cabinet (image 5). At this stage the mussels were fed on fine organic material (detritus) and algae. The algae fed to the mussels were a mixture of Nanno3600 and Shellfishdiet1800 (image 7) (both from ReedMariculture Inc., USA, http://www.reedmariculture.com). Nanno3600 (Nanno) consists of Nannochloropsis with a diameter of 1-2 µm and Shellfishdiet1800 (SFD) is a mixture of different algae (Isochriysis, Pavlova, Thalassiosira weissflogii, Tetraselmis) with a diameter of 5-20 µm. Water change occurred weekly (with a maximum of 8 days) by the help of artemia sieves (180 µm mesh size for juveniles) (Dohse Aquaristik, http://www.dohse-aquaristik.com/EN/product/21630/Artemia-Sieve-Combination-85x8-cm) (image 8).

The mussels must be checked under a stereo microscope for predators (flat worms, annelids). Dead mussels must be removed in order to keep the other mussels healthy. The juvenile mussels were fed with a single concentration of algae the first month and then with a double concentration of algae until they were grown up to a size of 1 mm (approximately 100 days): The mixture of algae was prepared by filling a bucket with 10 L of river water. To obtain a single concentration of algae, 120 µl of SFD and 4 drops of Nanno were added to the water. To obtain a double concentration of algae, 240 µl of SFD and 8 drops of Nanno were added (the water must be stirred regularly because algae drop over the time). 500 ml of new river water that was enriched with algae was added after checking the mussels and also 25 ml of detritus. This detritus was collected in the area around sources where the ground is saturated with water, which contains a lot of organic material from plants, some algae and bacteria (see image 9). Detritus works as biological filter and reduces harmful ions. In order to find out, how ammonium, nitrite and nitrite are reduced

in plastic boxes with detritus, an experiment was conducted by analyzing the concentration of the three ions in mussel containing plastic boxes with and without 25 ml of detritus for one week (see results: 3.1.1 Measurement of ammonia, nitrite and nitrate in plastic boxes).

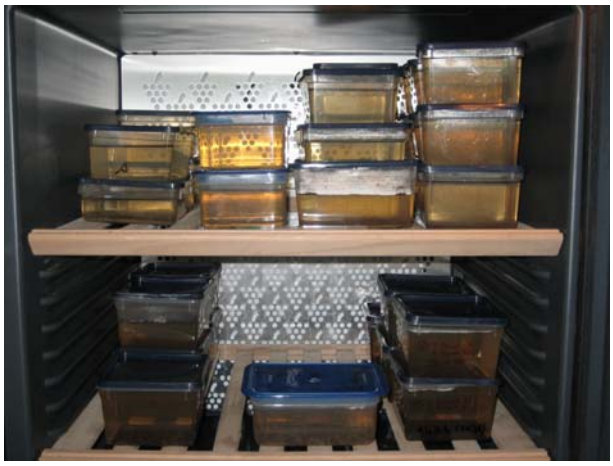

Image 5: plastic boxes with juvenile mussels in the conditioning cabinet

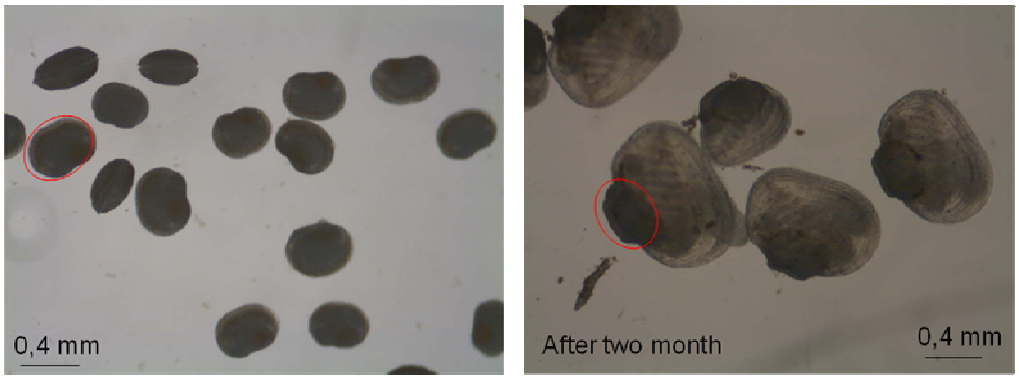

Image 6: Growth of juvenile mussels

Image 7: Commercial algae (Nanno3600 and Shellfishdiet1800)

Image 8: Artemia sieves


Image 9: Detritus

Rearing in aquaria (first 100 days):
Plastic aquaria (see image 10) were equipped with a pump (Swordfish internal Filter, Aqualitiy 200 l/h). The filter parts like carbon filter and filter mat were removed in order to allow added algae to flow through the aquaria. Sand or sieved gravel (size 4-6mm) from the Our river was added with a layer thickness of 0.5 respectively 2 cm. A minimum of 16.5 L of Our water was filled in the aquaria. The aquaria were placed in the cellar of the mussel facility. 1000 juvenile mussels were placed in the aquaria and water exchange occurred every week (minimum of 10 L were replaced by fresh Our water). The mussels were fed to algae every day: 175 µl SFD and 2 drops Nanno.


Image 10: Young mussels raised in plastic aquaria with a current of water from a pump

Point 7, Raise young mussels of ≥ 1 mm:

To raise young mussels of ≥ 1 mm, different systems were constructed: Aquaria, down-wellers (= "artemiea sieve-boxes"), plates with mesh covered holes kept in a closed artificial stream and kept in an open artificial stream, plastic boxes and a gravel filled channel in a closed system.

Aquaria

In October 2009, 700 mussels of 1 mm were placed in plastic aquaria that were similar to the aquaria for juvenile mussels for the first 100 days. The mussels were fed to 300 µl of SFD and 3 drops of Nanno every day. The mussels were raised at room temperature (20°C) in the laboratory.

Down-wellers

200 mussels of 1 mm were placed between 2 artemia sieves (560µm mesh size). 8 artemia sieves were placed in one down-weller (image 11 and 12). Water was pumped (Pump used: Ferplast / Blu-Power / Germany 350l/h) from the bottom of the boxes to the surface and had to pass the sieves with the mussels. 650 µl of SFD and 6 drops of Nanno were fed every day.

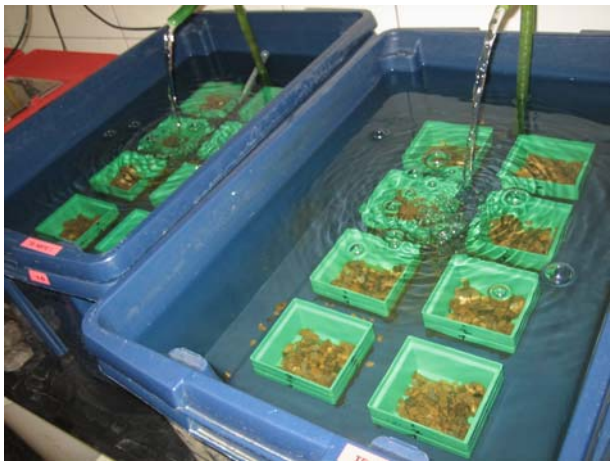

Image 11: Down-wellers (artemia sieve-boxes)

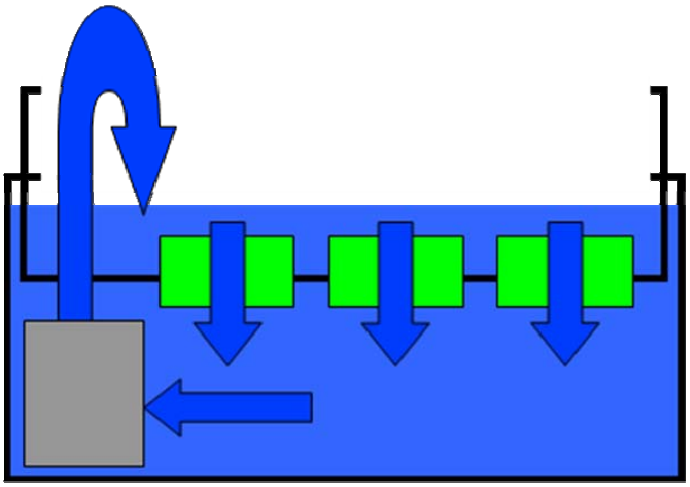

Image 12: Schematic view of down-wellers ("artemia sieve-boxes")

Mesh covered hole-plates (closed and open system)

100 mussels of 1 mm were placed in one hole-plate. 20 hole-plates were inserted in a closed artificial stream system holding approximately 400 liter of water (see image 13). 25 drops of Nanno and 2500µl of SFD were added to the system everyday as food source.

Another 37 hole-plates containing 100 mussels each were kept in an open artificial stream continuously supplied with river water.


Image 13: Mesh covered hole-plates (5 mussels/hole)

Plastic boxes
400 mussels of 1 mm were placed in 1.5 L-plastic boxes (image 14) that were stored in a conditioning cabinet at 17-18°C in October 2009. Water exchange occurred every week (with a maximum of 8 days) by the help of artemia sieves. Food was added to the fresh water. The mussels were fed to a triple concentration of algae: 360 µl of SFD and 12 drops of Nanno were added to a bucked of 10 L of river water that was used as stock. The water was stirred regularly before adding to the boxes.


Image 14: 1.5-L plastic boxes

Gravel filled channel
200 mussels were counted to one gravel filled plastic box each (image 15) that stood in a channel (flower box for balconies). The water was pumped (Pump used: Heissener Smart line / HSP 1600 / Germany / 1600 l/h) from the basin to two empty plastic boxes from which it ran in two cascades through the gravel filled boxes with mussels. The mussels were fed to 750 µl of SFD and 8 drops of Nanno every day.

Image 15: Two gravel filled channels (closed system)

3 Results

3.1 Rearing juvenile mussels in plastic boxes (first 100 days)

After 100 days the survival of juveniles in plastic boxes was 79.8 % and the growth 189.47 %.

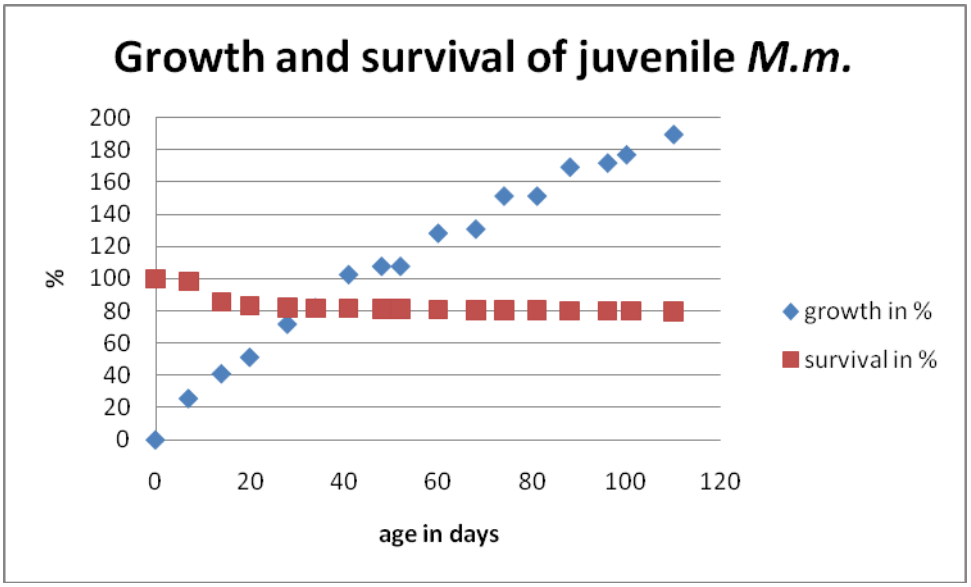

Figure 1: Growth and survival of juvenile *M.m.* in plastic boxes, fed to a single concentration of algae the first month and then a doubled concentration of algae.

3. 1. 1 Measurement of ammonia, nitrite and nitrate in plastic boxes

In boxes with mussels, where detritus was existent, the concentration of the harmful ions nitrite ($NO2^-$) and ammonia ($NH4^+$) sinks continuously over the eight days. Ammonia is already reduced to the half after one day.

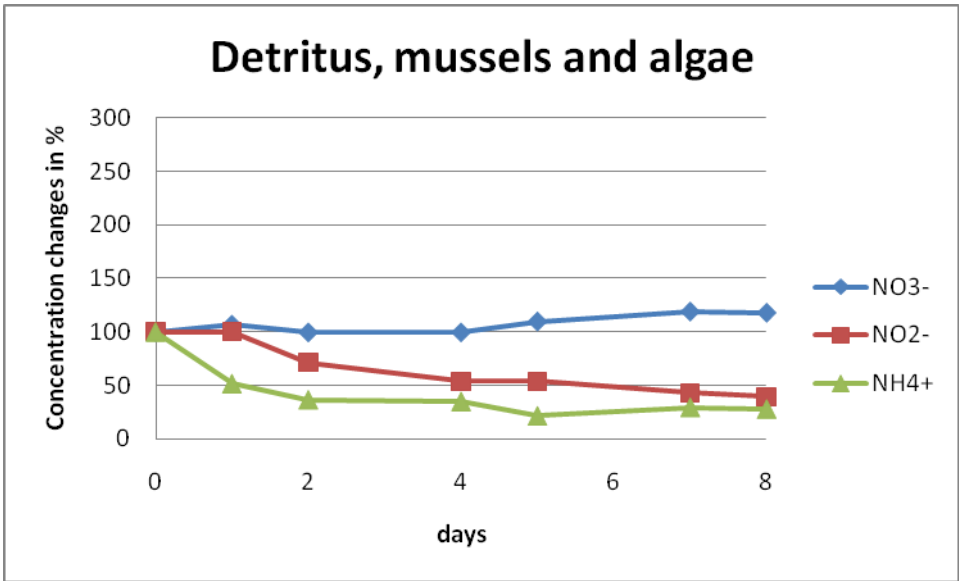

Figure 2: Concentration changes from the ions nitrate, nitrite and ammonia over 8 days in boxes with mussels, algae and detritus

In the absence of detritus, the two ions ammonia and nitrite start to increase after half a week. Especially the nitrite concentration shows an increase of more than 250 %. Mussels stay in high concentrations of the ions over the eight days.

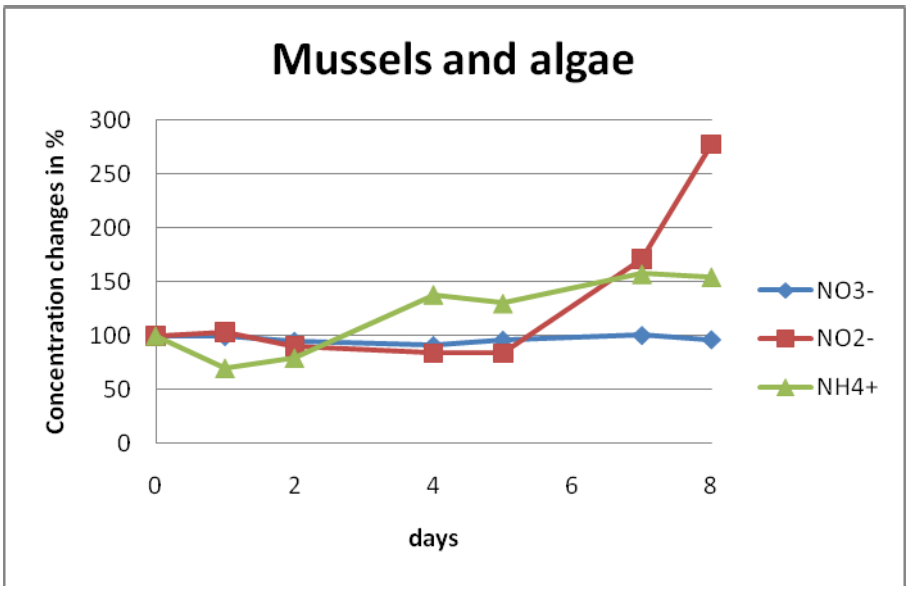

Figure 3: Concentration changes from the ions nitrate, nitrite and ammonia over 8 days in boxes with mussels, algae but without detritus

3.2 Rearing juvenile mussels in aquaria (first 100 days)

The survival in both, sand or gravel was the same (62.7 %). The mussels kept in sand grew up to 1.99 mm (452 %) compared to the mussels kept in gravel that grew up to 1.36 mm (277,78 %).

The use of aquaria showed a higher growth compared to the use of plastic boxes. Although the highest survival could be obtained in plastic boxes (79.8 %) (see 3.1 Rearing juvenile mussels in plastic boxes).

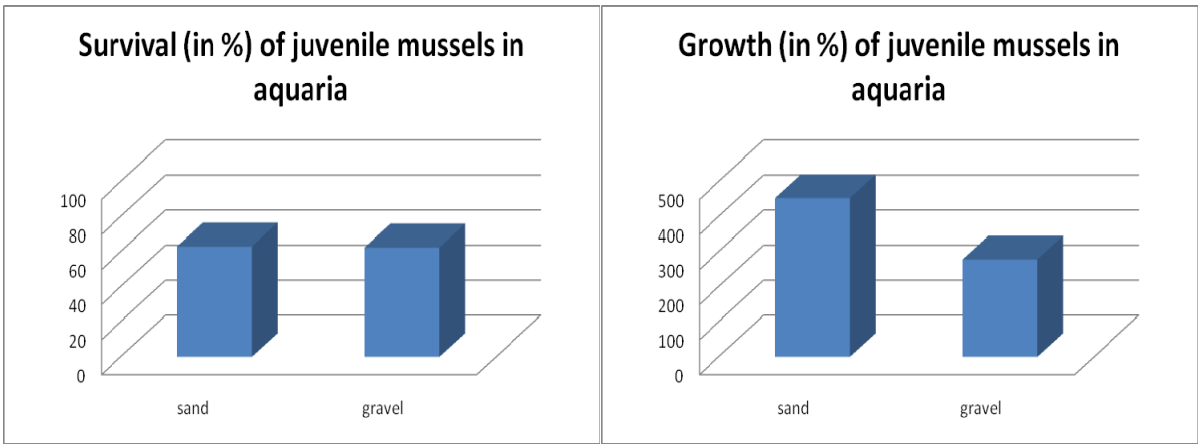

Figure 4: Survival and growth of juvenile mussels in aquaria filled with sand or gravel.

3.3 Rearing 1 mm mussels in plastic boxes, down-wellers, aquaria, mesh covered hole-plates and gravel filled channel

The survival was highest in plastic boxes (59 %) followed by down-wellers (48 %), gravel filled channel (33 %) and aquaria (13 %). Worst survival occurred in hole-plates kept in the close artificial stream (1.6 %). The survival of the mussels kept in the open artificial stream, supplied continuously with river water (not presented in the diagram), showed a survival of 24%

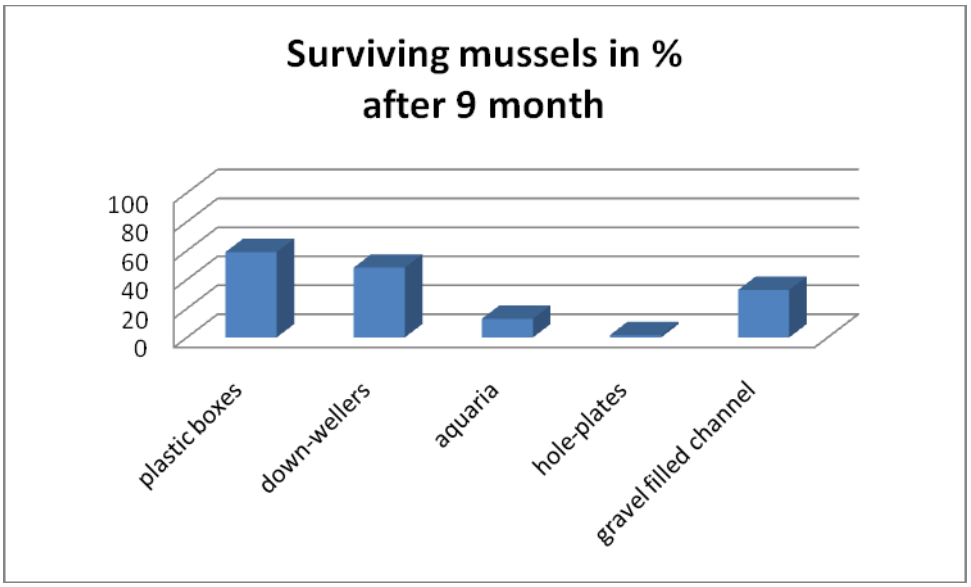

Figure 5: Surviving mussels after nine month in diffent systems

Growth was best in aquaria (454.55 %) followed by plastic boxes (126 %), down-wellers (102 %) and gravel filled channel (95 %). Worst growth occurred in hole plates (88.98 %). The growth of the mussels kept in the open artificial stream, supplied continuously with river water (not presented in the diagram), turned out to be 206%.

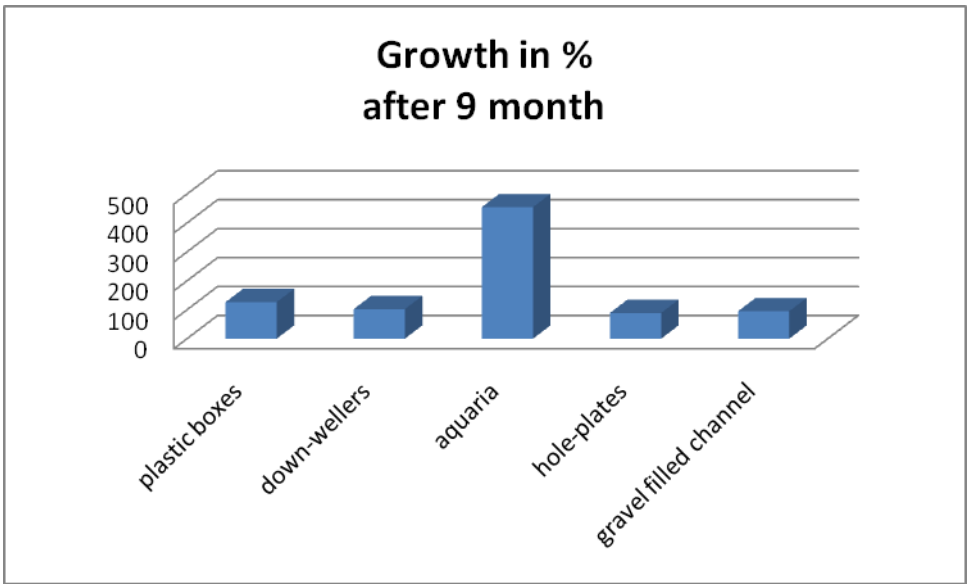

Figure 6: Growth of mussels after nine month in different systems

Mussels that were grown in plastic boxes at 17-18 °C showed a steady growth over the nine month time period of the experiment. The same occurred to the mussels that were grown in aquaria at 20 °C room temperature. Compared to that, the mussels from down-wellers that were kept in the cellar of the rearing facility showed a time of no growth during the cold winter month (Temperature < 10˚C). Mussels in the aquaria grew fastest.

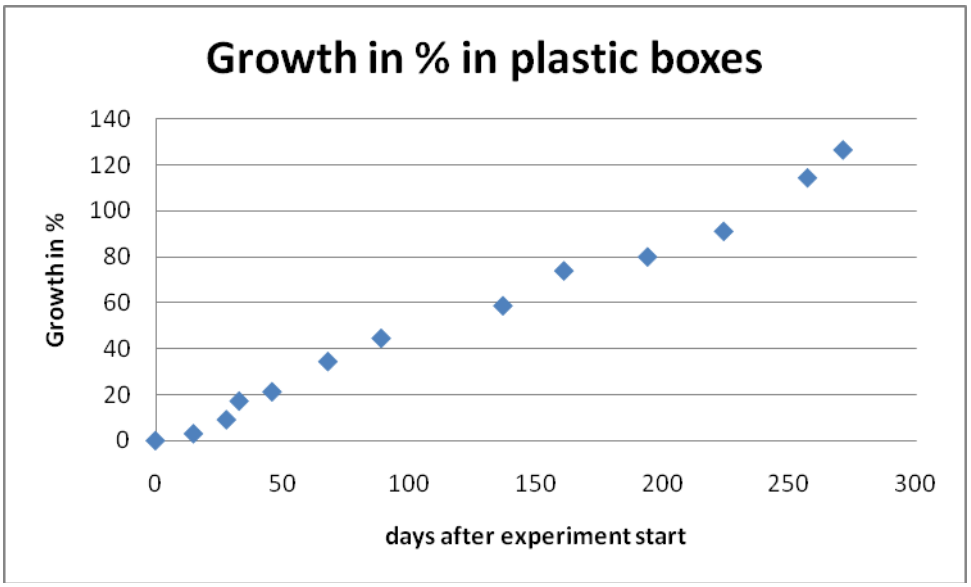

Figure 7: Growth line of 1 mm mussels in 1L-plastic boxes for 9 month

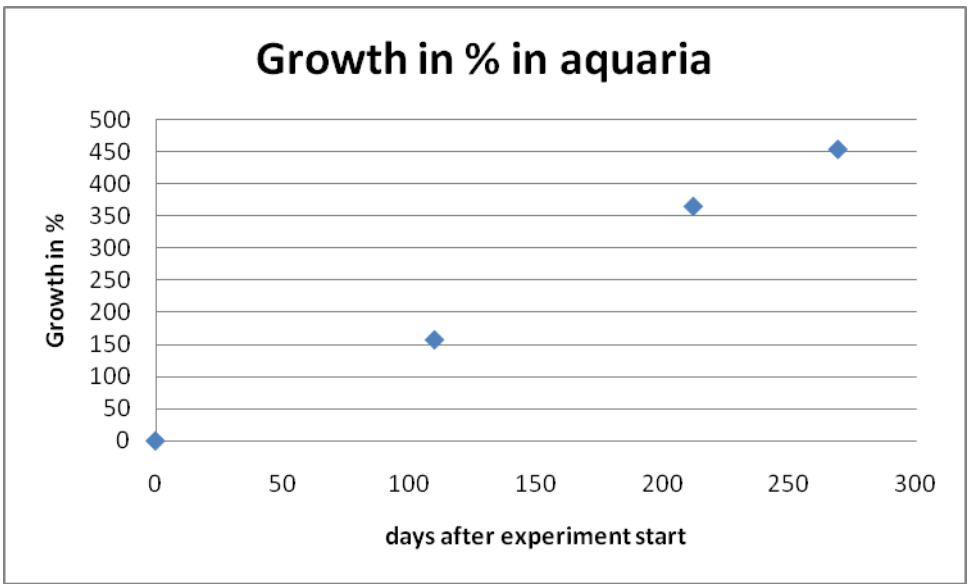

Figure 8: Growth line of 1 mm mussels in aquaria for 9 month

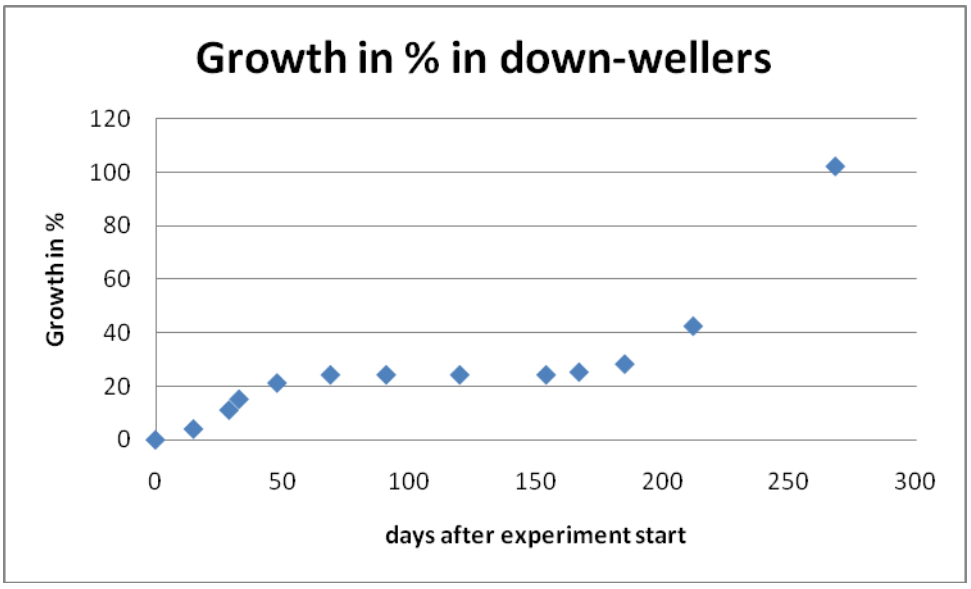

Figure 9: Growth line of 1 mm mussels in artemia sieve boxes for 9 month

4 Discussion

4.1 Rearing juvenile mussels in boxes (first 100 days)

The rearing of juvenile mussels in plastic boxes shows a very high survival of 79.8 %. The mussels reach a size of 1 mm after approximately 100 days. At this size they are easier to handle and less sensible. The boxes have the advantage that mussels can be removed easily to control the growth, the survival and the presence of predators. When mussels move, instead of laying around half opened, it is a simple indicator that they are fit. The mussels were fed a single concentration of algae in the first month and a double concentration of algae in the second. Preexperiments showed that the survival is better than by feeding a double concentration of algae already from the beginning and the growth was better than feeding a single concentration of algae for 110 days. 25 ml of detritus were added to each box directly after the weekly water exchange. Detritus is a food source (bacteria, algae) for young mussels. Preexperiments showed that juvenile mussels can survive in boxes without algae in 25 ml detritus, although their growth and survival is worse (79.48 and 43.88 % after 110 days).
Detritus also works as a natural filter and helps to reduce harmful ions like ammonium and nitrite in the boxes that would increase significantly over one week without detritus (see next point: 4.1.1).

4.1.1 Measurement of ammonia, nitrite and nitrate in plastic boxes

In spring 2010 many young mussels died in the laboratory at the mill of Kalborn. This was probably caused by very high concentrations of the ions ammonium, nitrite and nitrate in the river water. The plastic boxes with juvenile mussels that were fed 25 ml of detritus were not affected by this. This experiment was started in order to find out, if bacteria in the detritus can reduce these harmful ions, what could have led to the better survival of the juveniles in plastic boxes.
In boxes with detritus, the ions ammonium and nitrite were reduced significantly to nitrate by bacterial nitrification. Nitrate is less harmful to organisms than the other two ions.
In boxes without detritus, no decrease of nitrite and ammonia occurred, but the concentration of both ions increased dramatically which can lead to a chronic intoxication and to a higher death rate of juvenile mussels. Therefore the adding of 25 ml detritus to the boxes should be followed every time the water is changed.

4.2 Rearing juvenile mussels in aquaria (first 100 days)

Rearing mussels in aquaria is, compared to raise mussels in plastic boxes, less time consuming and the mussels grow faster. The disadvantage is that the survival rate is lower and that it is not possible to check the growth and survival of the mussels every week because they are hidden in the sand or gravel. During the weekly water exchange, the water must be added very carefully in order that the sand or gravel will not be moved and buries or sqweezes mussels.

4.3 Rearing 1 mm mussels in plastic boxes, down-wellers, aquaria, mesh covered hole-plates and gravel filled channel

The survival of the mussels in this experiment is very difficult to evaluate, because in spring/summer 2010 the river Our contained high amounts of nitrite and ammonia and could have caused the high mortality in rearing systems without biological filters!
The installation of a water tank with a good filter system (carbon and UV filter + aeration of the water) was mandatory after becoming aware of this problem (that was realized by a high mortality of mussels). This tank supplies all mussel raising systems since summer 2010.

The survival of the mussels in this experiment is also difficult to evaluate because dying can be caused by not enough food but also by too much food. For example too much nutrients in the water can increase the concentration of nitrite and ammonium. There is no experience in feeding young freshwater pearl mussels (≥ 1 mm) and neither the kind of food nor the amount of food they need is already known.

All mussels had an average size of 1 mm at the beginning of the experiment. This is a size when they are already easy to handle and their survival is more stable than in the first month after dropping from the fish.
The fastest growth showed the mussels in the aquaria: Although mussels in this age are known to live some centimeter deep in the interstitial of a river, the mussels in this experiment stood nearly under the surface of the sand and the siphons were visible as dark spots. The pump in the aquaria caused a current of water and the mussels could be supplied by algae easily without moving.
It can be excluded that these mussels died by starvation because of the high growth rate visible in this system. Presumably the previously described high concentrations of ammonium and nitrite in the water caused the high mortality. Suddenly, within 2 weeks, all mussels were lying on their sides instead of standing upright without moving and after some weeks they died. Two month ago, the mussels were still very mobile, which could be observed by traces in the sand from moving around and there were at the least 245 mussels more alive, which was counted during a check of the mussels. This would have been a survival of ≥ 48 %.
Because the growth in aquaria was very promising, future experiments are planned in order to optimize this system.

Mussels in systems that were placed in the cellar (down-wellers) of the mussel facility, showed a period of no growth during the cold winter month compared to systems that were placed in the conditioning cabinet (plastic boxes) or under room temperature (aquaria). Future experiments are planned to show if mussels need this natural cold winter period or if they can stay under continuous warm temperatures which lead to a faster growth in the same time period.

The mussels in the mesh covered hole plates showed a much better growth and survival in the open system, continuously supplied with river water than in the closed system. Although the mussels in the closed system were fed with algae, it seems that the food concentration in this system was not high enough for the mussels to grow and survive well or that it was too high and the water quality became bad. Adapting the food concentration and automated, continuously feeding would probably increase both, growth and survival in this system. Harmful ions like ammonium and nitrite should also be measured regularly.

Table 2 shows the advantages and disadvantages of all rearing systems. The survival was not disregarded due to the water quality (ammonium, nitrite) in the river water which makes the survival difficult to evaluate. Table 3 shows a summary of all systems used so far with the respective growth- and survival-rates, regardless the problems with the water quality occurring in spring.

Table 2: Advantages and disadvantages of different mussel growing systems

	advantages	disadvantages
Plastic boxes	- Good growth (126.26 %) -regular control of mussels (growth, survival, predators) easy -boxes are easy to handle (light weight) and can be transported	-labor intensive when checked regularly
Down-wellers	-already established system in other mussel facilities -regular control of mussels (growth, survival, predators) possible	-Bad growth (102.22 %) -labor intensive when mussels are checked regularly -sieves can clog and must be sprayed regularly to remove waste -squeezing of mussels possible (between artemia sieves)
Aquaria	-Very good growth (454.55 %) -not time consuming	-regular control of mussels (growth, survival, predators) not possible -water change must occur carefully (not cover all mussels with a thick sand layer)
Mesh covered hole-plates	-not time consuming	-medium growth (88.89 % -206%) -meshes can clog and must be sprayed regularly from waste -regular control of mussels (growth, survival, predators) not possible
Gravel filled channel	-not time consuming	-Bad growth (94.95 %) -regular control of mussels (growth, survival, predators) not possible -squeezing of mussels possible (by gravel)

Table 3: Summary of all systems used so far (Growth and survival in %)

Keeping System	Mussels <1mm Growth [%] after 110 days	Mussels <1mm Survival [%] after 110 days	Mussels>1mm Growth [%] after 110 days	Mussels >1mm Survival [%] after 110 days
Mesh covered hole-plates closed system	/	/	89	1,6
Mesh covered hole-plates open system	/	/	206	24
Down-wellers	/	/	102	48
Gravel filled channel	/	/	95	33
Plastic boxes	190	80	126	59
Aquaria (Sand)	452	63	455	13
Aquaria (Gravel)	278	63	/	/