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Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

REPORT ON PERFORMANCE OF DIFFERENT CULTURE METHODS AND TYPES OF SPAT DURING GROW-OUT FROM SPAT TO SEED

In this report the different culture methods are explained. The performance of the spat is described in the report on relative performance of triploids and diploids at the larval and spat stage (Deliverable 6).

During the two years of the Blue Seed project several batches of diploid and triploid larvae of *Mytilus galloprovincialis* and *Mytilus edulis* were obtained by the different partners involved in the project. Some of these larval batches failed but others were successfully settled on artificial collectors and reared up to seed size. To set and grow the two species (*galloprovincialis* and *edulis*) and types (2n and 3n) of mussels the same settlement and rearing methods were used.

1. SPAT SETTLEMENT

To obtain the spat two different settlement approaches were tested: settlement on ropes and settlement on nylon meshes in down-welling systems.

1.1 Settlement on ropes

The first approach tested was the settlement on collector ropes trying to mimic the system used by mussel farmers in many region of the world. This system presents the advantage that once the mussels have settled on the ropes they can be easily transferred to the nursery tanks or directly to the final grow-out areas. Different types of ropes were used: (1) polypropylene Xmas tree ropes (**XTR**), used in long-lines systems in New Zealand and the Netherlands, (2) natural coconut rope (**CCR**), used in the traditional "bouchot" system in France, (3) traditional Galician ropes (**GTR**), manufactured from used fishing nets and composed by different proportions of nylon and polyethylene strands, and (4) a new type of hairy rope called "Polipes" made "ad hoc" for this purpose (**BQR**) (Figure 1).

Figure 1 Rope collectors, from top to bottom: Coconut rope (**CCR**), Polipes rope (**BQR**), Christmas tree rope (**XTR**) and Galician traditional rope (**GTR**)

Fragments of rope (≈ 30 cm long) were deployed either suspended (straight or coiled) or laid down at the bottom of both flat- and conical-bottomed tanks. Previously to the introduction of ropes into the settlement tanks they undergo an ageing or "conditioning" process of some weeks on unfiltered seawater. Spat collector ropes were maintained within settlement tanks for a period of approximately 1-4 weeks. Settlement tanks were filled with treated seawater heated to a temperature no higher than 18ºC. Treatment varied according the quality of the incoming seawater. In some cases seawater was only filtered, using a series of several cartridge filters, up to sizes of 25 µm, 10 µm, 1 µm or 0.20 µm. In other cases, seawater was also disinfected with UV-light after filtration. Seawater in the settlement tanks was renovated every day, every other day or even once a week, depending on the facilities available for each partner. Before adding the new seawater, tanks will be cleaned by using filtered seawater or fresh water previously treated with peroxide. Setting larvae were feed a daily ration of 50000 cell·ml^{-1} of a mix of microalgae, such as *Isochrysis galbana, Chaetoceros gracilis, Skeletonema costatum* and *Tetraselmis suecica*. In the case of conical bottom tanks were agitated throughout the settlement period to both avoid decantation of larvae and microalgae and enhance attachment of spat to collectors. Agitation was done by bubbling air from the bottom of the tanks.

Although settlement intensity varied between the different experiments and partners, in general the highest densities (nº of spat per collector) were obtained with the **XTR** collector and the poorest with the **CCR** collector. The other two types of collectors, **GTR** and **BQR**, showed intermediate values, although **BQ**R performed as well as **XTR** in some experiments (for more details see BLUE SEED Activity Report 12 month part 2, pdf file). However, the most relevant result obtained in the majority of these settlement experiments was to observe that a high proportion of the spat settled not on the ropes but on the walls and bottom of the tanks. This recurrent result led the partners to try a second and simpler approach: the settlement of pediveliger larvae on nylon mesh screens in down-welling systems.

1.2 Settlement on nylon mesh in down-welling systems

In the down-welling systems pediveliger larvae were placed within the confines of nylon mesh (120-150µm) bottomed PVC cylinders suspended within a holding tank (Figure 2).

Figure 2 Down-welling settlement units within holding tank

Seawater containing microalgal food was circulated through the units by either a water pump or air-lifts. It flows in from above the water surface at a constant rate into the enclosure, moving downwards over the spat and out through the mesh base of the cylinder to return to the reservoir via an overflow which maintains the water level constant in the holding tank. This is easily undertaken in a closed recirculating system with regular water changes and addition of unicellular algae. The flow of the water encourages the larvae to settle upon the surface of the sieve (Figure 3).

Figure 3 Mussel spat settled on sieve mesh

2. REARING OF SPAT UP TO SEED

After settlement, it usually takes 2-3 months for spat to grow to a size at which they can be transferred to and seeded on the final ongrowing environments. Juvenile mussels of this size are called seed. In the Blue Seed project three different methods have been used for rearing the spat up to seed size.

2.1 Rearing on collector ropes

This was the first method of spat rearing tested in the Blue Seed project. Collector ropes containing spat were transferred to flow-through circular or rectangular tanks into which the collector were either directly hung (Figure 6, left) or introduced into down-welling units (Figure 6, right).

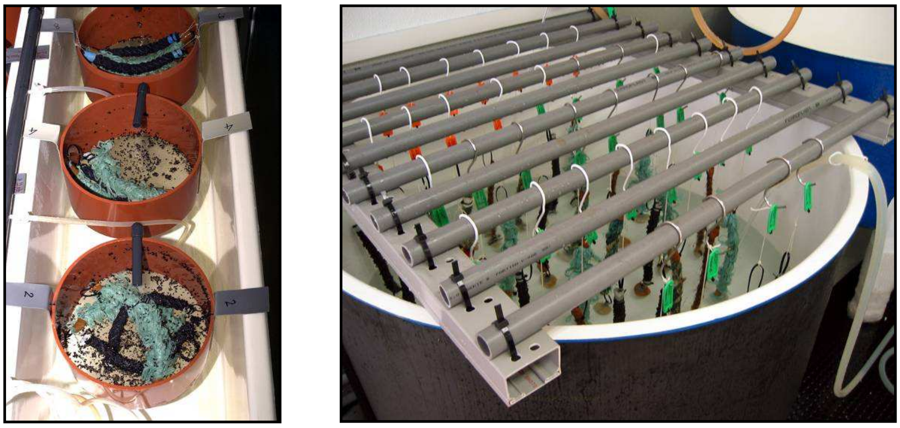

Figure 7.5 Collector ropes with mussel spat: hanging directly into a circular tank (left) and within down-welling units in a rectangular tank (right)

Always this method was used it was observed that a considerable proportion of the spat initially settled on the ropes became detached and settle again in both the walls and bottom of the tanks. This recurrent behaviour of the mussel spat generated some inconvenience in their posterior management during rearing, particularly when ropes were hung directly into flow-through tanks. This method was finally rejected.

2.2 Rearing on nylon mesh in down-welling systems

This system was used to rear the spat settled on nylon meshes (Figure 4, left). Settlement down-welling units were moved inside flow-through rectangular tanks (Figure 4).

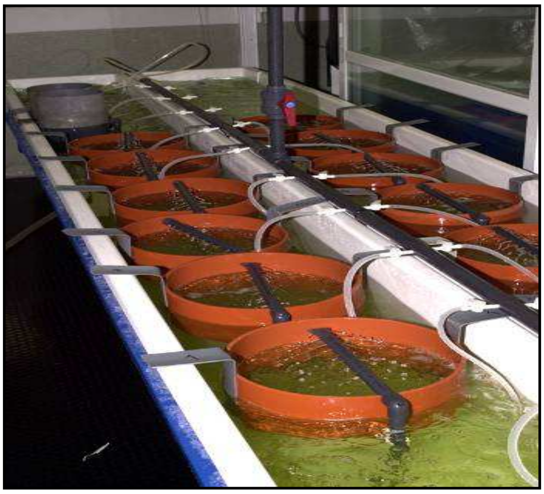

Figure 4 Spat settled on nylon mesh (left) and down-welling units in flow-through rectangular tank (right)

Each down-welling unit consisted of a plastic or PVC cylinder with a 150 µm nylon screen mesh fitted to one of their ends. A continuous circulation of filtered seawater was maintained through each unit by a pump or an air-lift tube. Spat was fed an initial daily ration which varied from 40000 to 100000 cell·ml^{-1} of mix of the microalgae such as *Isochrysis galbana, Chaetoceros gracilis, Skeletonema costatum* and *Tetraselmis suecica*. This ration was progressively increased depending on the requirements of the seed. In most of the cases tanks were cleaned only with filtered seawater without using any type of chemical treatment but in one case fresh water treated with peroxide was used. Cleaning operations were carried out every day, every other day or once a week, depending on facilities and experimental conditions.

It was observed that spat reared using this method was much more easily handled that the spat attached to collector ropes. Once the spat reached seed size, it was easily removed from the nylon mesh without damage and re-attached onto new substrates (ropes, bags or nets) to undertake the posterior phases of cultivation.

2.3 Rearing on square-frame screens

This system could be considered a variation of the previous one. It is a more industry-focused approach developed by one of the SME partners of the project (Grainocean). The mussel spat, previously detached from the settlement substrates, was spread over square-frame polyester screens of 250 µm mesh size which were horizontally introduced into rectangular tanks in an indoor micro-nursery (Figure 5)

Figure 5 Spreading of mussel spat on square-frame polyester screens in a micro-nursery

After this micro-nursery phase, the mussel seed attached to the polyester frames screens was transferred to a large outdoor raceways tank in a land-based nursery for continuing their pre-ongrowing (Figure 6). Naturally produced microalgae were pumped to the raceway tank to feed the seed.

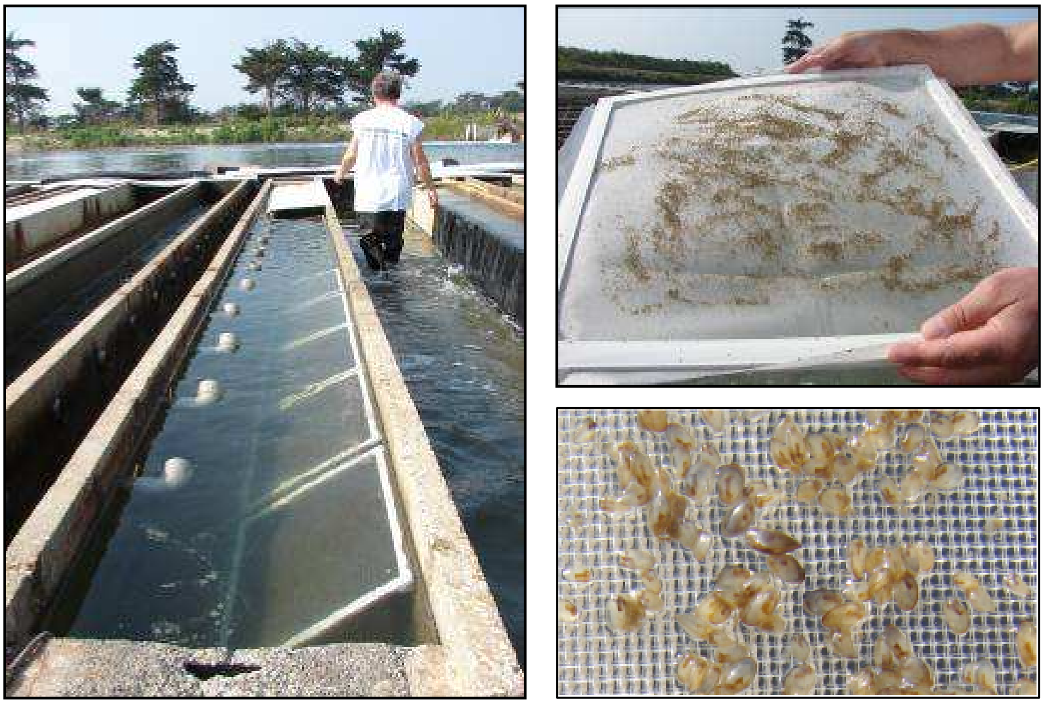

Figure 6 Polyester frames screens in an outdoor raceway tank in a land-based nursery (left)
Frames showing attached mussel seed (right)

To promote a less clumped and more homogeneous distribution of the mussel seed on the frames screens (Figure 7, right) they were grown in darkness by covering the raceways with a light-reflecting material (Figure 7, left)

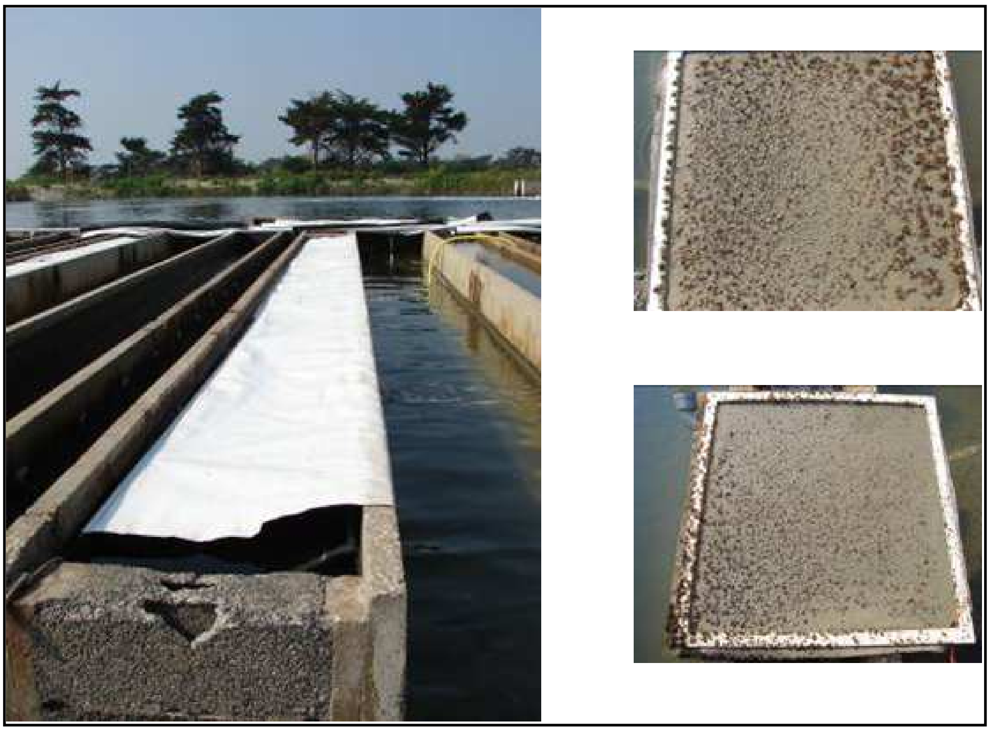

Figure 7 Covered raceway (left) and frames with seed (right) showing an in darkness homogeneous distribution (above) and an in light clumped distribution (below)

After a month under these rearing conditions mussel seed between 4 and 10 mm long was separated from the frames and transferred within lantern nets to a long-line system situated in an offshore ongrowing environment.