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Pilot project for the production of seed from commercially valuable molluscs: *Modiolus barbatus* (Bearded mussel)

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Abstract The aim of this study was to develop a pilot plant for the production of *M. barbatus* seed through artificial reproduction technology for possible use in aquaculture and repopulation of nursery areas.
The study involved various phases: setup of the pilot plant, sourcing of *M. barbatus* specimens and their transplantation to an offshore long-line farm; production of live food (phytoplankton); selection of broodstock; artificial reproduction in a controlled environment and larval and post-larval feeding in the pilot plant; refinement of the offshore farming technology and evaluation of yield on the long-lines with respect to the various production systems used and the different line depths. The specimens of *M. barbatus* raised on long-lines gave a good yield. The results of both the stimulation and larval farming confirm the applicability of the techniques described in the literature. Encouragingly, the daily mortality was lower than results reported to date (7.9%).

Keywords: *Modiolus barbatus*, bivalve molluscs, artificial reproduction, farming, long-line, mollusc culture.

1. Introduction

With an annual production of over 200,000 tonnes, mollusc culture is the main socio-economic sector in Italian aquaculture. With the exception of a few, as yet insignificant experiences of oyster culture, the entire production consists of the farming of just two bivalve species: the Mediterranean mussel (*Mytilus galloprovincialis*) and the Manila clam (*Tapes philippinarum*). While the import and export of seafood products involved in this sector is of undoubted national economic importance, this scenario does have some weak points, typical of undiversified production, and acts as an impetus for those working in the sector to take on the production of other commercially valuable molluscs. This is the background for the study described below, whose various objectives include the application of an artificial reproduction method [1,2] and the production of the seed of a new, as-yet unfarmed species: *Modiolus barbatus* (bearded mussel). Artificial reproduction is in fact a common practice in aquaculture and is closely related to the need to ensure the availability of good quality seed of the species to be farmed. Spat can sometimes be found in significant quantities in the natural environment, as in the production of Mediterranean mussels and Manila clams, which is today sustained entirely by the abundant availability of wild seed. However, in most cases the natural recruitment of juvenile stages is insufficient to satisfy the needs of the producers. In the case of commercially valuable molluscs, therefore, artificial reproduction is necessary, with the aim of ensuring the seed supply regardless of the availability of natural spat. In Italy, the introduction of the Manila clam (1983) gave rise to the first centres specialising in the artificial reproduction of bivalve molluscs - hatcheries. As there was no consolidated Italian experience in the artificial reproduction of these organisms, the first hatcheries relied on technologies previously developed abroad [3,4,5]. However, in Italy hatcheries do not have a

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decisive role in production, given that the seed of the species farmed is available in the wild [6].

Mediterranean mussel seed, for example, is so abundant in the wild as to lower the market price and render artificial reproduction financially unviable [1]. With respect to Manila clam seed, the use of artificially produced seed was probably necessary only at the beginning. Artificial seed reproduction requires considerable resources and effort. Sophisticated tools and specialised labour are essential for every phase of production; the latter accounts for 50% of the cost, while microalgae cultivation accounts for about 40% of the overall costs incurred by a hatchery. Current costs amount to a minimum of €1.50/thousand pieces (2-3 mm), making the collection of wild seed far more economical, especially if carried out directly by the producers. Moreover, the quality (both size and survival) of wild seed is significantly better than that of seed produced in the hatchery. Notwithstanding this, the activities carried out by hatcheries can have important financial, environmental and scientific implications. For example, they can provide technological support to diversify production through the use of other commercially valuable mollusc species. In addition, hatchery-produced seed can be used not only in farms but also for restocking. In many countries, the impoverishment of natural stocks due to overfishing is dealt with by seeding with spat from the hatchery. For example, along the Atlantic coast of France, banks of the king scallop, *Pecten maximus*, are continually replenished with hatchery-produced seed.

The artificial reproduction of a species can therefore be important for the conservation of resources. Knowledge of these techniques can enable the replenishment or reintroduction of a species into an ecosystem where the population has become scarce or disappeared altogether.

Experiments in artificial reproduction techniques, especially of little-studied mollusc species, can also improve knowledge of their biology and ecology. In fact such experiments enable the identification of the factors that induce the production and emission of gametes, the ways they are emitted, their size and number, larval morphology and development, etc.

The bearded mussel, *Modiolus barbatus* (LINNAEUS, 1758), has an elongated, equivalve, asymmetrical shell. The maximum size is 90 mm (normally 40-50 mm), and so the bearded mussel is smaller than the Mediterranean mussel. The minimum length permitted for harvesting is 50 mm, which is reached at around the third year of life. This bivalve generally lives in underwater coastal areas, anchoring itself to rocks, shells or phanerogam rhizomes with its strong byssus. It has been found at depths ranging from a few metres up to 100 m. It cannot be considered euryhaline, as it does not tolerate strong changes in salinity. However, it does live in environments whose temperature changes significantly over the year: from close to 30 °C in the summer down to 2-3 °C during winter.

M. barbatus is considered very common along practically all Mediterranean coasts. Its distribution also extends to the eastern Atlantic coasts, from the English Channel to Morocco, including the islands of Cape Verde and the Azores. Literature on this bivalve is scarce. The little information available on its distribution and biology are found in an FAO publication [7]; there are also some genetic data [8], but to our knowledge there is no information available on its natural reproduction. Following the results obtained with artificial reproduction [1], breedingtrials were initiated, which have already been reported in part [9, 10, 11, 12, 13].

The commercial value of this species is 7-10 times higher that of the Mediterranean mussel (*M. galloprovincialis*). In the absence of any known natural banks, almost all the fresh (live) product sold in Italy is collected from the sea beds of Greece [14].

More information is available on similar species exploited commercially in other seas. The induced spawning of *M. capax* and the recruitment, growth and death of *M. metcalfei* were described in some articles [15, 16, 17]. The species most studied in Europe is *M. modiolus* in terms of survival strategies, population structure and reproduction [18, 19, 20, 21, 22].

2. Experimental

2.1 Set-up of pilot plant

A pilot plant was set up for the project. It consisted of a modern nursery organised for the housing of broodstock, their reproduction under controlled conditions and larval and post-larval rearing. The water circuit of the pilot plant (intake

from the sea and recirculation system) was designed to enable closed-circuit (recirculation), open circuit (with and without filtration), and semi-open circuit operation through a system of gates and pumps.

The **filtration plant** used for the study consisted of 2 high-speed sand filters; a UVC (ultraviolet) steriliser; a compact bioreactor (biological filter); a skimmer; a diatomic filter; and a titanium heat pump. The operation of the filtration equipment depended on whether the plant was running in the open, closed recirculation or semi-open circuit mode.

Open-circuit operation (with and without filtration): The system involves pre-filtration of the incoming water from the sea through two high-speed sand filters to ensure that water entering the circuit is free from any suspended particles that might impair the operation of the entire plant. The pre-filtered water is then sterilised by an ultraviolet steriliser and sent to the titanium heat pump, where it is brought to the required temperature, and then to the rearing tanks. If required, this mode can also include a full filtration phase, as with the closed-circuit operation.

Closed circuit operation: Filter 1 is not employed, so mechanical pre-filtration is assured by filter 2; after echanical filtration, the water in tank 2 is sent to the UV steriliser, the compact bioreactor (biological filter) and the skimmer, and then passes through the heat pump to the rearing tanks. From these, the return water collects in tank 3 and the overflow is returned to tank 2, from which recycling commences.

Semi-open circuit operation: Incoming seawater is pre-filtered through the two high-speed sand filters. Part of the water is then sterilised by the ultraviolet steriliser, passes through the biofilter, skimmer and titanium heat pump, which brings it to the required temperature, and is then sent to the rearing tanks, while the remainder is sent directly to the rearing tanks. The return water from the tanks collects in tank 3 and from there, part returns to the sea and part passes to tank 2 and is recycled.

The water temperature is regulated by a titanium heat pump which provides complete control over both incoming and recycled water, whether separately or in combination, in order to assure the desired temperature in all tanks and for the entire test duration.

The pilot plant was supplied by a gravity-powered inlet which collected seawater via a subterranean pipe into a 9 m^3 collection tank. The seawater was then pumped through a highspeed sand filter to tank 1; the overflow collected in tank 2 and was then pumped to the filtration system and to the rearing tanks or, if required, to the phytoplankton production facility and thermostatic chamber.

To enable the production of live food (phytoplankton), the Termoli site has a thermostatic room with a usable volume of about 23.6 m^3 for the maintenance of mother cultures and preparation of cultures for use (bottles) and a complete facility for the mass production of phytoplankton to feed the bivalve mollusc larvae. The water used to prepare the culture was filtered through a diatomic filter (2-5 µm) beforehand.

The rearing tanks consisted of: 3 square tanks (3.5 m^3); 3 flat-bottomed cylindrical tanks, (3 m^3); 9 round-bottomed cylindrical tanks (3 of 4 m^3, 3 of 0.06 m^3 and 3 of 0.25 m^3); 2 rectangular incubation units (horizontal troughs) (0.2 m^3), each fitted with 7 baskets; 4 flatbottomed rectangular tanks (0.3 m^3); 2 round-bottomed rectangular tanks (0.6 m^3); 1 complete bivalve mollusc seed production unit (about 2 m^3).

All artificial reproduction trials were carried out at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise's Termoli site and the Istituto Delta Ecologia Applicata's C.Ri.M (Mollusc Research Centre) hatchery in Goro (Fe). The same methods were used for all the sites.

2.2 Production of live food for Modiolus barbatus larvae

To meet the nutritional needs of mussels in the larval stage during the trial, phytoplankton cultures produced in the algae room of the same hatchery were administered daily. The microalgae species most commonly used as the basis of the food chain in the hatcheries were two diatoms, *Chaetoceros calcitrans* and *Phaeodactylum tricornutum*, and three flagellates, *Tetraselmis suecica*, *Isochrysis galbana* and *Dunaliella tertiolecta*. These species were chosen as they are relatively easy to raise, the starting strains were immediately available and the cells are of a suitable size.

Various algal species were used as food in this study because a multi-specific diet has numerous nutritional and productive benefits.

Certified strains of the algal species used for the test were purchased from the Dunstaffnage Marine Laboratory (Oban, Argyll, UK). Cultures were stored in growth medium containing seawater enriched with phosphates, nitrates, silicates and micronutrients (trace metals and vitamins) and kept under carefully controlled light and temperature conditions (thermostatic room at 20-22 °C, with exposure to artificial light: 24 h, 5000-6000 lux). The maintenance cultures were kept in 250 mL of culture medium in autoclaved 500 mL flat-bottom glass flasks stoppered with sterile cotton wool. They were not ventilated and no carbon dioxide was introduced. The strain quality and algal growth stage was evaluated regularly by microscope. The growth curves of the various algal cultures were also evaluated to determine the interval possible between one subculture and another, the times necessary for mass food production, and the growth medium and the environmental conditions to be used in the strain maintenance process. The batch culture method was used for the algae in this study, following methods similar to those reported in literature [23, 24]. This method permits relatively restricted daily volumes of algae (from 2 to 10 L) produced in conical and flat-bottomed flasks kept under controlled environmental conditions.

The choice of culture system was based on the following factors: culture medium, water quantity, nutrients, temperature, energy consumption, laboriousness, and the final product to be obtained. The algal culture media necessary for the production of phytoplankton were prepared by enriching seawater with nutrients (Guillard's f/2 formula: N 878 µmol/L, P 36.45 µmol/L). The salinity of the seawater used was carefully controlled to between 25 and 30 PSU for flagellate cultures and between 20 and 25 PSU for diatoms.

2.3 Stimulation tests

The simultaneous release of gametes by both sexes can be induced by numerous types of stimuli, generally depending on the species in question. The methods most commonly used in hatcheries can be classified by the type of stimulus: physical (temperature rise, thermal or saline shock, UV, etc), chemical (administration of active ingredients), biological (addition of gonad products or phytoplankton), mechanical (vibrations), etc.

A chemical stimulus, hydrogen peroxide (H_2O_2), was used for this study. This method was based on a study who described the effects of this substance on the gastropod *Haliotis rufescens* and other molluscs [25]. The method was modified in some fundamental aspects: broodstock specimens were stimulated en masse rather than individually and exposure to hydrogen peroxide was reduced from 2.5 hours to just 15 minutes [1]. The concentration of the stimulant (5 mM) was unchanged. Each broodstock group was divided into two batches. One was subjected to stimulation and the other acted as a control, and was left in the maintenance tank. The alkalinity of the water was increased to pH 9.1 by adding sodium hydroxide (NaOH).

A total of 4 stimulation tests involving 400 specimens were carried out in total: 200 (50x4) for the stimulations + 200 for the controls. The temperature was the same as used in maintenance conditions, to avoid producing any stimulus other than those required. The temperature varied between 19.5 and 26 °C in the 4 stimulation tests.

The tests were carried out in rectangular tanks (60 x 40 x 15 cm) with a black bottom, to enable the easy identification of emitting specimens through the colour difference between the gamete and the tank.

The stimulation protocol can be divided into three phases, as reported in the **Table 1.**

Wild broodstock kept on a long-line system off the coast of Termoli was used for the stimulation tests. Its suitability was determined, first in situ and subsequently in the laboratory, by visual and microscopic observation of gonad maturity on representative samples of the stock.

Before beginning the stimulation tests, the broodstock pool was carefully selected according to some essential rules. Sources of stress were minimised in the period between mussel collection and their arrival at the hatchery, as these can affect their viability. Before making the final choice of broodstock, the entire batch was acclimatised, immediately discarding any dead or damaged specimens (all deformed animals and any with obviously broken shells). The specimens used all had a length of at least 4-5 cm.

As the dense periostracum of *M. barbatus* normally hosts numerous animal and/or vegetable

epibionts, it was necessary to clean the selected broodstock. This was carried out by manually brushing the outer surfaces. The suspension of food 24 hours before stimulation was to reduce the expulsion of faeces and/or pseudofaeces, thus reducing the risk of contaminating the gametes.

Table 1. - Stimulation protocol for *M. barbatus*

PHASE 1: PRE-TREATMENT	
1	Select and clean broodstock
2	Stop feeding 24 hours before treatment
3	Keep broodstock out of water (damp environment) for 4-6 hours
4	Place broodstock in seawater at pH 9.1
PHASE 2: TREATMENT (STIMULATION)	
5	Add H_2O_2 to a concentration of 5 mM
6	Maintain in treated water for 15 minutes
7	Remove broodstock and rinse carefully with fresh water
PHASE 3: POST-TREATMENT	
8	Replace broodstock in the same tank after replacing the water (without stimulant)
9	Monitor broodstock for the next 4 hours

Keeping the broodstock out of water does not affect the number or extent of gamete emissions but encourages the rapid reopening of the valves and restarting of filtration by the broodstock after their introduction into the treated water.

In the 4 hours after treatment, the tanks were monitored to identify any specimens releasing gametes, which were promptly removed to individual 500 mL tanks to continue their release.

2.4 Raising the larvae

After release, eggs were fertilised by the addition of a sperm suspension from a number of males, so as to favour genetic variability. Successful fertilisation was verified by optical microscope and eggs were then placed in an incubator for at least 24 hours at a concentration of up to 50 eggs/mL.

The next phase consisted of the initial selection of larvae at the first veliger stage (D-shaped) 24-36 hours after fertilisation. This was performed by siphoning the water in the egg incubator through a cylindrical sieve with a 36 µ mesh. To avoid excess pressure, which might have damaged the larvae, the sieve was located no lower than 30-40 cm above the bottom of the incubator. For the same reason, the selection was carried out with the sieve kept constantly wet within a tank. An overflow ensured the drainage of excess water.

After filtering, the larvae trapped by the sieve were carefully washed with a weak jet of water at the same salinity and temperature. They were then counted before being resuspended in a tank to continue their growth [1].

Subsequent selections were carried out every 2 days, increasing the sieve mesh size each time as follows (**Table 2**).

Table 2 - Selection steps

Day	Selection	Sieve (µm)
1	1	36
3	2	53
5	3	60
7	4	73
9	5	85
11	6	100
13	7	124
15	8	150
17	9	175

The water was changed completely at each step and the tank walls carefully washed with a dilute sodium hypochlorite solution. During this stage it is particularly important to keep the water under the same conditions, in order not to further disturb the larvae. The number of larvae was estimated at the end of each selection, to determine the mortality rate.

The development of the veliger ends with the formation of larvae with a foot (pediveligers) which gradually reabsorbs the velum. This phase ends in metamorphosis, following which the mollusc takes on the form and habits of the adult organism. The larva's metamorphic competence is demonstrated by the appearance of light-sensitive spots, as well as behavioural factors (the larva extends its foot to look for a substrate). These sensory organs enable the animal to orientate itself with respect to the surface of the sea.

At this stage of rearing (day 20) water filtration is no longer necessary: the use of untreated water in

fact helps replenish and diversify the food supply. Post-larval rearing in open-cycle systems supplied with temperature- and salinity-controlled water is an ideal practice [1,2]. After about 70 days the spat reaches a mean length of 2.4 ± 0.5 mm, with a linear growth rate of 30 µm/day. During this period mortality is under 10% [1,2].

2.5 Management of broodstock

Specimens for the broodstock were sourced from the coast facing Margherita di Savoia (FG), from a rocky/sandy bed. The specimens, 18 kg of bearded clams of a size between 1 and 5.5 cm, were taken to the Torelli mussel dispatch centre in Traini (BA). Specimens under 3 cm in length accounted for a total weight of about 500 g, while the remainder had a mean length of 4 cm. The mussels were then taken to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" seafood farm in Termoli and housed in an aquarium at a temperature of 15±1 °C and a salinity of 36‰. 3 days after they were first taken, specimens were transported to the Lagmar mussel culture plant off the north coast of Termoli, with depths of between 10 and 16 m. Specimens were selected by size and then inserted into 4 lines of different mesh sizes, depending on the size of the mollusc.

Evaluation of yield in the offshore farm

This test aimed to ascertain the production potential of *M. barbatus* farmed in an offshore plant. Two 4 m lines were prepared for specimens between 3 and 4.5 cm (medium), two lines of 3.5 and 3.8 m were prepared for specimens between 4.5 and 5.5 cm (large) and a remaining 0.6 m line was used for specimens of under 3 cm. The latter was positioned at a mean depth of 7 m, while the other four were placed at depths of 3.5, 7.5, 8 and 12 m. This differentiation aimed to evaluate the long-term effects of rearing depth on the growth and general condition of the test specimens.

The first sampling of the four long-lines was carried out after about a month and revealed the excellent general state of health of the molluscs, regardless of their depth, and a mortality of almost zero (> 1%). Sampling was carried out every two months thereafter.

After 70 days, some changes were made to the layout of the long-lines: specimens on lines 3 and 4 were transferred to two Ostriga ® baskets placed at the same depth as the original line, in order to compare mussel growth between an innovative rearing method like the Ostriga® baskets (**Fig. 1**) and the traditional long-line (**Fig. 2**), and evaluate any time and productivity benefits determined by the use of the different methods.

Fig.1. Mussel transfer to the Ostriga® baskets

Fig.2. Long-lines prepared with the mussels

The bimonthly sampling for biometric tests coincided with routine maintenance of the production systems. This involved cleaning the lines and baskets of Mediterranean mussel seed (*Mytilus edulis)*, which is abundant in the area; cleaning the production systems of the mud residues that built up between the specimens, especially following heavy seas; and the replacement as necessary of the plant ropes supporting the lines and baskets.

The total duration of the growth test was 16 months.

Measurement of hydrological parameters

Alongside the above operations, the rearing site was characterised using a multi-parametric portable probe to measure various hydrological

parameters: pH, salinity, temperature, and dissolved oxygen.

Sample preparation

Specimens taken for biometric tests and weighing were differentiated by the line or basket of origin and the rearing depth. The length, thickness and weight of each specimen was measured by calliper and electronic balance (Mettler BB300).

Unless prevented by adverse weather or sea conditions, the utmost care was taken to ensure that no more than 1-2 days passed between the removal and replacement of the mussels. Specimens used for the biometric tests were kept alive in a specially prepared 200 L aquarium fitted with mechanical and biological filtration, UV, oxygenator and cooling system. The aquarium water was kept at a constant salinity, equivalent to that of the seawater at the time of removal, and a temperature of 15 ± 1 °C, to keep the specimens' metabolism low. *Dunaliella tertiolecta, Phaeodactylum tricornutum* and *Tetraselmis suecica* were provided occasionally as food in variable quantities while the sampled specimens were awaiting return to the sea.

About 100 specimens were taken at each sampling. They were first subjected to the following procedures: preliminary washing with seawater; shelling, with removal of part of the byssus by scalpel; careful washing of the periostracum with seawater; drying with absorbent paper; screening of specimens, discarding any dead mussels or those with damaged and/or broken valves.

The samples then underwent biometric analysis and weighing in the laboratory.

3. Results and Discussions

3.1 Stimulation

The presence of H_2O_2 is perceived immediately by *M. barbatus*, which reacts by closing its valves and then reopening them. This action can be repeated up to 5-6 times a minute. This behaviour continues for around 10-15 minutes, after which the animal closes up. Mature *Mytilus galloprovincialis* specimens do not react in the same way to this treatment: a few seconds after the stimulant is added, *M. galloprovincialis* broodstock specimens close their valves, and reopen them to release gametes or to return to normal behaviour only after the medium is replaced with untreated water. In contrast, the Pacific oyster *Crassostrea gigas* shows similar behaviour to *M. barbatus* [26]. Of the 16 bivalve pieces stimulated with hydrogen peroxide to date at the Mollusc Research Centre, only those of the Mytilidae family and the Pacific oyster have reacted by releasing gametes. In *M. barbatus*, the first gametes are released about 30 minutes after the treated water is replaced with untreated seawater. The eggs are normally released slowly and a female may spawn up to 7.5 x 10^6 egg cells, in the form of grains. The eggs are orange, with a more intense tone than those of M. galloprovincialis, and have a diameter of about 64 µm. Males release their sperm more quickly, over just a few hours; it has a milky appearance and the cells cannot be distinguished by the naked eye. The results of the stimulation tests (number of emitting specimens after 4 hours) are reported in **Fig. 3.**

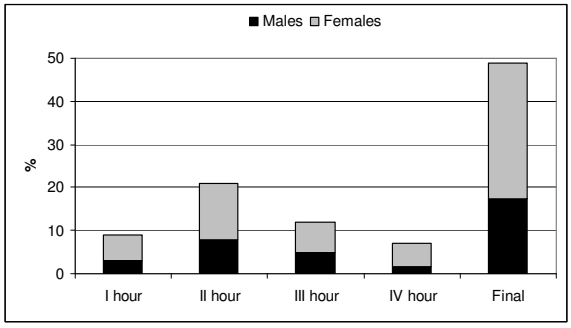

Fig. 3.Emission results

No emissions were observed during the 15 minutes' exposure to the stimulant. The results can also be expressed as the number of emitting specimens against the number treated (**Table 3**).

Table 3 – Number of emitting specimens (%) against the total number of treated broodstock

N	Day	1° h	2° h	3° h	4° h	Tot.
1	18/06/2007	8	24	8	6	46
2	20/06/2007	6	16	16	10	48
3	27/06/2008	12	22	6	10	50
4	03/07/2008	10	22	18	2	52
	Mean	9.0 ± 2.6	21.0 ± 3.5	12.0 ± 5.9	7.0 ± 3.8	49.0 ± 2.6

This enables comparison of the results of this study with those obtained from other stimulation tests on the same and other species using the same treatment.

The results of these stimulation tests are similar to those obtained in other study [1] when applying the same method to the same species. Here too, the number of emitting specimens peaked (21%) during the second hour after treatment. In the first and fourth hour, less than 10% of specimens released any gametes. After 4 hours, 49.0 ± 2.6% of the total number of animals treated had released gametes. Of these, a clear majority were females (1.8:1), in line with the results of the above-cited authors, albeit in slightly different proportions. Given that the sex distribution of wild populations of *M. barbatus* is 1:1, it can be asserted that the stimulation method adopted has a preferential effect on females. Favourable results have also been seen with the application of this method to other bivalves: the percentage of specimens issuing gametes was 42.1% for *Crassostrea gigas* [27], 56.3% for *Mytilus galloprovincialis* [2] and 29.2% for *Musculista senhousia* [28].

3.2 Rearing of larvae

The eggs produced during the second stimulation test were fertilised and the resulting larvae were reared as described. Incubation of the fertilised eggs at 25 °C determined the formation of 47.8% of veliger larvae (D-shaped) 24 hours after fertilisation. 8 million of these were washed and used for the larval rearing test.

In other trials [29], 24 hours' incubation at 20 °C gave rise almost exclusively to trochophores, which developed into D-shaped larvae after about 36 hours. This highlights the importance of temperature during embryonal development, where a temperature of 23 °C was used.

The cumulative mortality over the next 15 days was 70.21%, while the instantaneous mortality calculated over 2 days was 15.75 ± 5.02% (**Table 4**). The cumulative mortality at the end of the larval phase also favours the theory that a higher temperature permits faster development, thus lowering the mortality rate. In fact, a previous study reported a cumulative mortality rate of 83% at 20 °C [29].

The first competent larvae (pediveligers) appeared after 15 days. The larval phase is the most hazardous for survival in the life of bivalves. In the hatchery, where high temperatures and high larval density are maintained, bacteriosis is one of the main causes of death [30].

Table 4 - Results of larval rearing test

Day	Sieve (µm)	Larvae (x 10^6)	Instantaneous mortality (%)	Cumulative mortality (%)
1	36	8.00		0
3	53	6.90	13.75	13.75
5	60	5.98	13.29	25.21
7	73	5.23	12.53	34.58
9	85	4.52	13.69	43.54
11	100	3.98	11.81	50.21
13	124	3.20	19.67	60.00
15	150	2.38	25.52	70.21

To combat this, use of antibiotics such as streptomycin-sulphate and/or penicillin G is a common practice in commercial hatcheries. This possibility, which was not considered in this study, is certainly worth evaluating in future trials. In mussel larvae, the mortality rate from fertilisation to metamorphosis is 15%/day [31]. This means that a prolongation of the larvae's pelagic life will reduce the number of individuals that undergo metamorphosis. It is estimated that from a starting number of 107 fertilised eggs, at a mortality rate of 15%/day only a single larva would survive beyond 13 weeks [31]. Such a long pelagic period may even occur in nature, but in hatchery conditions, this phase concludes within 15-20 days. The daily mortality rate of Modiolus in this study was 7.9%, the lowest ever recorded for this species.

3.3 Yield from the long-lines

Modiolus barbatus specimens suspended in offshore farms between July 2007 and November 2008 showed an encouraging yield (**Fig. 4, 5, 6,** and **7**). Although there was no great difference in the growth of specimens in this trial, it did reveal some differences between the type of support and the

rearing depth. Mussels kept at a mean depth of 5.5 m showed a better yield than those reared at a mean depth of 10 m.

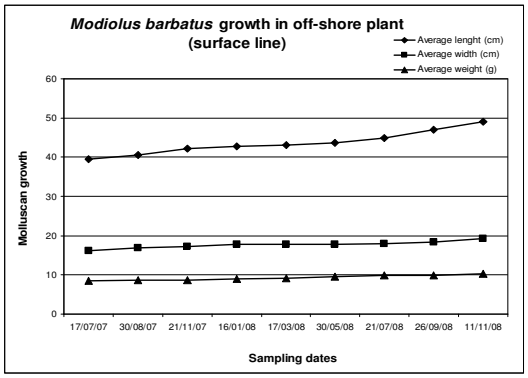

Fig. 4. Growth on surface line (July 2007 - November 2008)

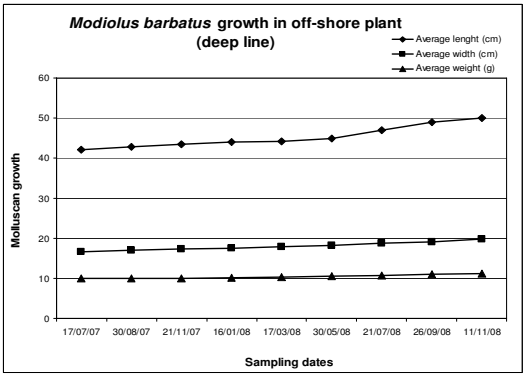

Fig. 5. Growth on deep line (July 2007 – November 2008)

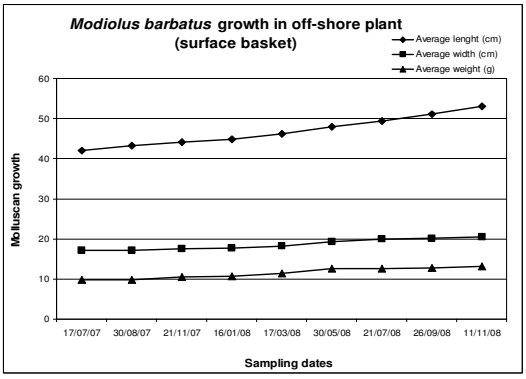

Fig. 6 - Growth in surface basket (July 2007 - November 2008)

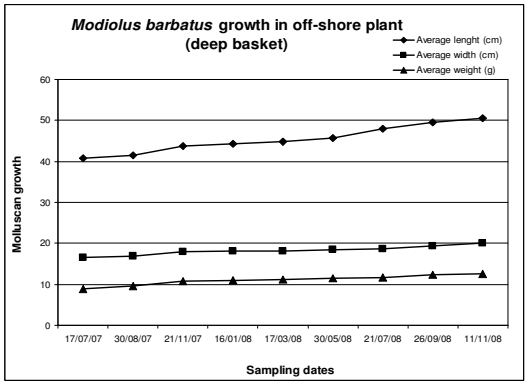

Fig. 7. Growth in deep basket (July 2007 - November 2008)

The depth also determined some variations in the final size and weight of the molluscs. The best results were obtained with specimens housed in the new Ostriga® production systems, with the traditional lines providing a slightly worse growth performance.

4. Conclusions

M. barbatus was found to be particularly "easy" and its maintenance under laboratory conditions did not require any substantial modifications to the plants. Control of chemical and physical parameters was particularly important: the salinity must be close to 35‰, and the temperature between 10 and 28 °C.

The stimulation and larval rearing tests carried out in this study confirmed the applicability of the techniques described in the literature. The larval rearing results demonstrate a further improvement over previous studies, with a reduced mortality rate at 25 °C.

The growth seen in specimens on offshore long-lines is encouraging, despite the numerous epibionts (*M. galloprovincialis*, barnacle, and oyster seed; algae, etc) found on the production systems used during the growth tests. Notwithstanding the good standardisation of the artificial reproduction techniques refined for *Modiolus barbatus*, knowledge of the species and production yields in the hatchery could be further improved by investigating the following aspects: diet, conditioning phase (off-season maturation), use of bactericides in the larval phase and use of chemical inducers for metamorphosis.

This study is an encouraging example of innovation in the mollusc culture sector, from the point of view of product diversification and rearing of autochthonous species. In fact, many countries are ever more inclined to raise allochthonous species, which can cause significant problems for the community structure and have a serious impact on the ecosystem. To improve the management and conservation of resources and the environment, the increased use of autochthonous species is desirable.

5. References

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