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Project acronym: **BLUE SEED**

Project title: **Technology development for a reliable supply of high quality seed in blue mussel farming**

Instrument: **CRAFT – Co-operative Research**

Thematic Priority

Final Report

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BLUE SEED Final Report

1. Project execution

1.1. Project objectives

1.1.1.Mussel reproduction

Mussels are either female or male. The reproductive tissue of the mussel is located within the mantle, which extends along the inside of the shell valves, enveloping the rest of the body. The eggs and sperm are released into the water (**spawning**) where they merge and form **larvae**. After 2-6 weeks, the larvae are ready to settle. Newly settled larvae are called **spat**. Spat grows in approximately 3 months to a size at which it can be seeded on bottom plots or ropes. It is then called **seed**. Which stimulus or combination of stimuli triggers spawning in a natural population is known to be species dependent, the importance of environmental parameters like temperature, food availability, etc. is recognised, but maturation and spawning remain hard to predict.

Spawning induces low meat content in mussels (Bayne et al, 1982). The mussel spawning season in the Galician estuaries extends from February to September (Villalba, 1995), with several consecutive intense spawning events, causing considerable marketing problems during this period. The same problem exists in the other European mussel producing countries, although to a smaller extent, because the reproductive season is shorter (March-July, Seed & Brown, 1977).

1.1.2. Challenges for blue mussel producers

The existing blue mussel culture has two technical limitations. The first is the unpredictability in seed supply. Techniques for seed supply are dredging wild seed beds, scraping mussel seed from rocks and collecting seed by natural settlement on, ropes or other substrates. Success of any of these methods depends on environmental conditions, which fluctuate. The amounts of wild seed available are therefore extremely variable from year to year. Producing seed under controlled conditions in a hatchery will disconnect its production from environmental factors and provide a reliable supply of seed. The second technical limitation is a lower meat quality during and after the spawning season Preventing maturation of market-size mussels during spawning season, is a major challenge to all European blue mussel farmers. Production of triploid mussels will secure a year round high meat content.

The use of hatchery-produced seed and sterile triploids could contribute to the solution of these two pan-Atlantic challenges. To reach these goals, the appropriate hatchery and nursery technology needs to be developed. In addition, any future program aiming to strengthen mussel quality through genetic improvement techniques will require well-tuned hatchery and nursery procedures for seed production.

A more reliable seed supply, and the possibility of offering "all-season" mussels to the market will enhance competitiveness in all European Atlantic blue mussel producers. The availability of hatchery-produced seed will bring more stability to the market, long-term security for jobs, development of coastal areas and an alternative to fisheries for both workforce and products. Given the 2-year duration of the project, comparisons concentrated on methods of spat and seed production, although some market size mussels were produced as well. Commercial quantities of triploid mussels will become available approximately 1 year after the end of the project.

1.1.3. Project objectives

The **long-term goals** of the BLUE SEED project were to secure a reliable supply of hatchery produced seed and to develop techniques allowing farmers to sell high quality blue mussels all year round. To achieve the long term goals a number of scientific and technical project objectives were formulated:

1. Develop hatchery technology for a reliable blue mussel seed production, by focussing on (a) broodstock conditioning, (b) larval rearing and (c) seed production.
2. Develop a viable production method for (a) sterile triploid mussel seed and (b) tetraploid broodstock that will enable year-round marketing of high quality mussels.
3. Compare, in each of the project partner countries, the economic feasibility of producing blue mussel seed based on hatchery-produced larvae with the benefits of blue mussel seed collection methods presently in use.

1.2. Contractors involved

In the project 10 partners collaborated: five mussel farmers/sellers from France (Grainocean), The Netherlands (Neeltje Jans and Roem van Yerseke), Spain (OPMEGA) and Wales (Deepdock), an Irish based network for training and technology transfer (AquaTT), a Welsh university (University of Wales at Bangor: UWB) and three research institutes from Spain (CIMA), France (IFREMER) and the Netherlands (IMARES).

1.2.1. SME Participants

Grainocean (France)

The hatchery was founded in 1984 and specialises in the production of triploid Pacific oysters. They culture oysters from broodstock up to commercial size and thus have a wide experience in all phases of production. The organisation has 6000 m of long-lines and a 22 m boat. Nursery facilities include marsh lagoons and long-lines with lantern nets. They already have some experimental experience with *M. edulis* production.

Neeltje Jans (Netherlands)

Fish farm Neeltje Jans BV is situated on a former construction island near the Storm Surge Barrier in one of the estuaries in the SW Netherlands. It started in 1980 with fish culture, but soon also tried the long-line culture of oysters. Neeltje Jans was one of the pioneers in the development of long-line mussel culture in the Netherlands and this is now their main product. They are still improving the culture technique. At present there are 8 companies producing long-line mussels in the Netherlands with a total production of ± 1000 MT. Of the Dutch long-line companies Neeltje Jans is the largest producer.

Deepdock (UK)

Deepdock Limited began operation in 1991 (although incorporated in 1987). The principle established aim and objective is the successful cultivation of the common mussel, *M. edulis*. The methodology used to perform the task of cultivation is bottom culture. Deepdock Ltd operates on two areas within the Menai Strait and operates two other areas in Holyhead and in Swansea Bay. The Menai Strait area is responsible for between 50 –75% of the total United Kingdom production of 'farmed' mussels with some 6-11,000 tonnes being produced annually by 4 operating companies. Deepdock Ltd operates 2 shallow draft mussel dredgers: the Still Ostrea (LR111), with dimensions of 28 m length and an 8 m beam, was built in 1987; and the Mare Gratia (B932), which is 43.5m length and a 10m beam, and was launched in 2003.

OPMEGA (Spain)

The "Organización de Productores Mejilloneros de Galicia", OPMEGA, is the main mussel producers organisation of Galicia. It was legally established in 1996 as successor to the former organisation OPMAR. At present, it includes 19 smaller lower-level associations distributed throughout the Galician Rías (Vigo, Pontevedra, Arosa, Muros-Noia and Ares-Betanzos). OPMEGA controls the production of nearly 1900 rafts belonging to 1300 mussel farmers, which yielded, in 2002, 150,000 metric tonnes of mussels with a approximate market value of 60 million €.

AquaTT (Ireland)

AquaTT was founded in 1992 under the EU COMETT programme. AquaTT is a European network for Education, Training and Technology Transfer in Aquaculture sciences. The core business is to support the strategic goals of the aquaculture industry by facilitating education, training and technology transfer. Activities in the project included assisting with an anti-fouling survey and dissemination of the project results. AquaTT has excellent links with producers, researchers, policy makers and markets and are key facilitators of meetings and training events at the European level. In addition they have the infrastructure (eg. PISCES website) and networks to quickly and efficiently disseminate project results from the short to long term.

1.2.2. Other enterprises and end-users

RVY (Netherlands)

Roem van Yerseke B.V. was established in 1942. They are located in Yerseke, (the Netherlands), the centre of the Dutch mussel and oyster industry. RVY has 3 plants in Yerseke where they pack mussels for the fresh market, cook and preserve mussels, and where they pack oysters. Their markets are mainly European for the fresh products, but worldwide for frozen and preserved products. In coastal areas of the Oosterschelde and the Wadden Sea, RVY has culture plots for farming mussels. In the Oosterschelde and Lake Grevelingen they have culture plots for farming oysters. The annual turnover is +/- 50 million €, with around 120 employees. In addition, RVY has 2 daughter companies. 1 in Germany where they have a factory and culture plots for mussels, and 1 in Denmark, where they have a processing plant.

1.2.3. RTD Performers

IMARES (Netherlands)

Wageningen IMARES – the Institute for Marine Resources and Ecosystem Studies – specializes in strategic and applied marine ecological research. The institute was established in mid 2006 and is the result of a cooperation between RIVO (the Netherlands Institute for Fisheries Research), elements of Alterra, and the Department of Ecological Risks within the TNO. IMARES is part of Wageningen University and Research Centre (Wageningen UR). IMARES has a total staff of 170, divided over four locations in the Netherlands, of whom 25 work in Yerseke. Yerseke represents the heart of the Dutch mussel industry. where 95% of associated activities are based; including ships, processing plants, distribution centres and a special livestock auction for bivalves. Through the location in Yerseke, IMARES has already been involved in mussel research for more than 30 years and is the only research institute in the Netherlands with in depth knowledge of the blue mussel sector. IMARES monitors the size of shellfish populations in coastal and estuarine waters, predicts seed production, monitors algal blooms and quality of mussels, predicts production based on the food availability, studies spat collection methods and provides advice to the mussel industry.

IFREMER (France)

IFREMER, the French Research Institute for the Exploitation of the Sea is an industrial and commercial public company. Founded in 1984, it is the only French organization exclusively devoted to maritime interests. It operates under the joint auspices of the National Ministry of Education, Research and Technology, the Ministry of Agriculture and Fisheries and the Ministry of Equipment, Transport and Housing. The Genetics and Pathology Laboratory of IFREMER is internationally recognized for its work in genetics and pathology of marine bivalves. In genetics, the work is dedicated to marine bivalves, covering selective breeding, polyploidy, aneuploidy and development and use of molecular markers. Facilities include a hatchery which is fully equipped to

carry out experimental reproduction and rearing of marine bivalves (phytoplankton production, 2 larval rearing rooms with 55 larval rearing tanks). The laboratory is equipped to perform DNA analyses (sequencing, RFLPs, microsatellites, SSCP), ploidy analyses (flow cytometry, image analysis, chromosome counting), histology, transmission electron microscopy, in situ hybridization. The research team includes 14 researchers and 7 technicians.

UWB (UK)

The Centre for Applied Marine Sciences is the division of the School of Ocean Sciences, University of Wales, Bangor (UWB) that carries out commercial and applied research for industry and governments. CAMS is a multidisciplinary centre, with staff working in the areas of marine biology (including aquaculture), marine chemistry, oceanography, coastal zone management, survey and instrumentation and geosciences. Currently CAMS has a dedicated staff of 15 people, with a further 5 people being taken on in the next 3 months. In addition, the 100 members of staff of the School of Ocean Sciences act as associate members, adding greatly to the expertise available to the Centre. The School is one of the largest marine biology departments in Europe and the main UK research centre for coastal seas. The laboratories, aquaria, equipment, ICT facilities, library, boats and research vessel of SOS are also available for CAMS contracts. The School of Ocean Sciences has a 30-year history of aquaculture research, and has developed an international reputation in shellfish aquaculture. Commercial aquaculture work within CAMS is a significant proportion of the portfolio, with close connections to industry and a good European and international network of contacts.

CIMA (Spain)

The Marine Research Centre (CIMA) is part of the Department of Fisheries and Marine Affairs of the Regional Government of Galicia. There are more than 50 staff members including researchers, technicians and administrative staff. Research is mainly directed and applied to the reasonable and effective management of the renewable marine resources existing along the coast of the Autonomous Community of Galicia. Studies include factors affecting the production of cultivated mussels, the patterns of recruitment of mussel post-larvae on both rope collectors and intertidal beds. temporal and spatial distribution of mussel larvae along the Galician coastline, selective breeding of European flat oysters *Ostrea edulis* for resistance to *Bonamia ostreae*, optimised production of resistant *Ostrea edulis* spat, and studies of oyster (*Ostrea edulis*) immune mechanisms".

1.3. Work performed

The work was divided into five workpackages: Internal project management was taken care of in Workpackage 1. Workpackage 2 concerned broodstock conditioning and larval rearing. Workpackage 3 dealt with production of triploid larvae and tetraploid broodstock. Workpackage 4 concentrated on spat settlement and on rearing of diploid and triploid spat to seed size. Workpackage 5 compared the costs entailed in the production of hatchery seed with those of conventional wild-caught seed. In addition, within Workpackage 5 project results were communicated within the consortium and to end-users, through workshops, meetings with producer associations and a website

(www.blueseedproject.com). Considering that the normal production cycle for blue mussel in Europe is 2 to 3 years and the BLUE SEED project was only 2 years long, its focus was on spat and seed production.

1.3.1. Broodstock conditioning & spawning

In year 1 of the BLUE SEED project, broodstock was collected and spawned at all locations involved in broodstock husbandry. A protocol for the different tests was developed and the tests were carried out. The best success in obtaining good larvae from these spawnings was during the first 6 months of the year. Cold temperature conditioning worked well for both species (*Mytilus edulis* and *M. galloprovincialis*) with larvae still being obtained during August. Warm water conditioning was carried out successfully with *M. galloprovincialis* and tests were subsequently carried out with *M. edulis*. In spite of significant efforts - many spawning trials at all institutions involving more than six thousand mussels overall - only a small proportion of spawning trials produced veliger larvae. This problem was present at all four locations (Wales-United Kingom, The Netherlands, France and Galicia-Spain) and did not correspond to past experiences of some partners in earlier years. It may have been related to the unusually warm weather during July.

In year 2, all partners (UWB, CIMA, IFREMER and IMARES) have undertaken conditioning trials in which temperature and food levels were manipulated (heat and treat). Most of these trials succeeded in enhancing the ripening process and enabled earlier spawning than natural populations. Successful enhancement of mussel spawning condition was achieved at IFREMER (*M. edulis*), IMARES (*M. edulis*) and CIMA (*M. galloprovincialis*) in conditioning trials using live algae. No conditioning trials were successful at UWB where trials employed artificial diets, and only low success was achieved at IMARES with a *Chlorella* paste diet used in the heat-and-treat conditioning. The project therefore indicates the superiority of live food over artificial formulae tested in these experiments. Using a fixed temperature (either cool [8 °C] or warm [18°C]) did not seem to be critical but the most successful trials used a gradual rise in temperature or a compressed “winter-to-spring” temperature profile. Duration of conditioning is important, but the role of photoperiod remains unclear. Success was achieved under constant illumination (IFREMER, *M. edulis*) or under a number of chosen photoperiods (CIMA, *M. galloprovincialis* and IMARES, *M. edulis*). Naturally ripened *M. edulis* and *M. galloprovincialis* can be held in spawning condition at low temperature with minimal input of live feed (cold-and-hold) from March until August. A suitable low temperature is 6 ºC for *M. edulis* and 9 ºC for *M. galloprovincialis*. Although spawning effort was similar, results were better than the spawning trials conducted in year 1. In this second reporting period 87% of spawning trials produced gametes compared with 65% in previous period and 58% of the trials produced normal D-larvae after 3 d (43% in previous period). A total of 60 spawning trials using 5946 mussels were carried out at the partner Institutions in year 2 of the BLUE SEED project. Optimal egg density during the first 2 days is 200 eggs. cm^{-2}. Safe-to-handle D larvae can be collected as soon as 48 hours after fertilisation. Continued high effort was directed towards optimisation of larval

rearing. Comparison with the previous year demonstrates similar effort by all partners but better success due to availability of ripe mussels combined with a lengthening of the spawning season by cold-and-hold conditioning.

Table 1. Spawning success during BLUE SEED project.

month	# trials	% mussels spawning	% D-larvae
2006			
Jan	6	7	4
Feb	3	15	73
Mar	11	9	64
Apr	11	11	40
May	11	19	51
Jun	5	23	85
Jul	3	1	0
Aug	10	5	32
Sept	8	3	0
Oct	0	0	0
Nov	2	3	0
Dec	3	1	0
2007			
Jan	14	21	55
Feb	8	30	14
Mar	9	30	91
Apr	5	33	56
May	8	16	28
Jun	5	6	12
Jul	6	17	66
Aug	3	39	42
Sept	0	0	0
Oct	4	0	0

Results on broodstock conditioning and spawning show that mussels can produce viable larvae throughout the year in a hatchery situation (see table 1). Thus, the goal of broodstock conditioning as part of the development of hatchery technology for a reliable blue mussel seed production was reached.

1.3.2. Triploid and tetraploid induction

In year 1, a course in triploid induction protocols was given to all partners involved in triploid mussel production at UWB. Trials were than performed using two chemical (CB and 6-DMAP) and one physical (heat) 3n induction method. The chemical inductions showed good 3n yield percentages in the larval stage but poor larval survival, usually inferior to 2n controls. Heat shock induction of 3n showed a definite partial induction of 3n though its proportion declined over larval rearing and was low after settlement. Settled 3n mussels were therefore few in number in the first year and methods not yet commercially viable. Both heat shock and chemical induction were pursued at Ifremer whilst other partners concentrated on 6-DMAP induction in the second year. Viable tetraploid mussels were not produced in year 1 of the project, as the method of 4n

induction investigated was found not to function as expected and to be highly toxic. A modified method of direct induction was sought and tested successfully in the second year. .

In year 2 of the BLUE SEED project significant progress has been made with the objective to develop a viable production method for triploid mussel seed and tetraploid broodstock that will enable year-round marketing of high quality mussels. A total of 24 chemical triploid inductions were carried out at UWB, IMARES, IFREMER, CIMA and at the Technology Transfer Workshop where participants from UWB, IMARES, IFREMER, CIMA and RvY collaborated to make hatchery–scale 3n inductions. 6-DMAP was used to inhibit expulsion of the 2nd polar body (see Fig. 1). The technique employed is successful in producing up to 100% triploidy in treated embryos. Several tests were carried out to optimise details of this method. These included tests on the effects of length of chemical treatment (UWB) and the possible advantages of reducing the use of a sieve during induction (IMARES). Other elements of the technique that were examined represent the natural extension of parameters explored under WP2 to the induction treatment: different gamete handling techniques such as egg density during the first 2 days following induction and use of different water types (UWB). The effects of individual progenitors on 3n progenies were also examined (UWB). Diploids and triploids were compared for larval yield, normality and growth and survival during larval rearing. Results indicate a lower yield of larvae in the triploid treatment compared to the diploid treatment. But good trials demonstrate that this need not be a barrier to production of ready-to-settle triploid larvae. This illustrates the importance of having a reliable supply of ripe broodstock for successive inductions and the need for overall sound hatchery technique to ensure the best rearing conditions. The Technology Transfer Workshop showed that triploid *M. edulis* larvae can be grown successfully at a semi-commercial scale. Results obtained suggest that there could be a relationship between triploidy and larger size of larvae within batches. In many trials triploid mussel larvae grew significantly faster than their diploid counterparts and in none of the trials did diploid larval growth performance exceed that of triploids. However, most of these trials were compromised by either lack of replication or uncontrolled density. Nevertheless, it is safe to conclude that triploid mussel larval growth performance is similar to or better than that of diploid larvae during culture. Water source may influence normality in 6-DMAP treated embryos, although no significant differences were observed. There are maternal effects on normality and yield of 6-DMAP treated embryos, and there is a paternal effect on the yield of 6-DMAP treated embryos. Ready to settle triploid mussel larvae were provided to WP4 and were grown to seed at four different locations. Heat shock induction showed promising results for both triploidy and tetraploidy percentages but incurred high abnormality and/or reduced survival. Progress on tetraploidy induction was also made at IFREMER with a series of experiments to find a suitable method. Tetraploid larvae were reared beyond the settlement stage. Tests were made by flow cytometry at 4 and 6 months old spat, which confirmed tetraploidy. It is beyond the scope of the project to know whether the tetraploid route will ease triploid production, as the production of this next generation must await the reproductive maturity of such tetraploids. Scaling up of the method using a large sieve resulted in some tetraploid

larvae, although these tended only to be dominant in the smaller size classes and were not found post-settlement.

Fig. 1. Transfer of fertilised eggs on to a mesh sieve submerged in a tray holding 6-DMAP.

Overall therefore, the method in which 6-DMAP was used to inhibit expulsion of the 2nd polar body proved to be a viable production method for triploid mussel seed. In addition, tetraploid spat was produced as a first step towards tetraploid broodstock that will enable year-round marketing of high quality mussels.

1.3.3. Larval growth and survival

At all locations involved in larval rearing, larvae were reared up to the ready to settle stage (see Fig. 2). A protocol for the different tests was developed and several tests were carried out. Parameters included temperature, larval density, algal food species and combinations, container shape, aeration, broodstock origin and water quality. All institutions produced ready to settle diploid larvae, but mortality rates were higher than expected, possibly also related to the atypical weather conditions.

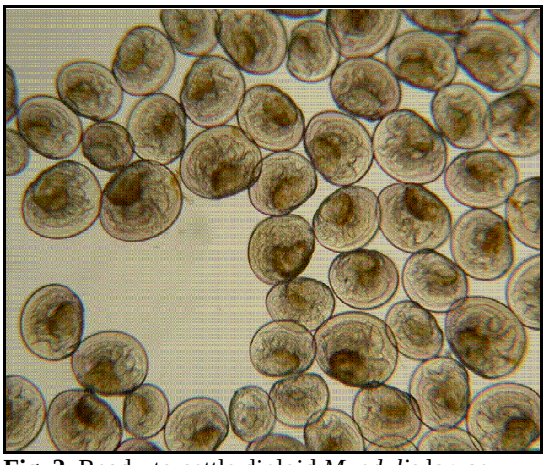

Fig. 2. Ready-to-settle diploid *M. edulis* larvae.

In year 2, it was determined that particular algal species of *Chaetoceros* have different feed value for the two species of mussel larvae, and 50 cell μl^{-1} of algal food produces significantly faster growth than concentrations of 25 and 100 cell μl^{-1} in *M. edulis* diploid larvae up to 10 days old. As with 2006, occasional unexplained high larval mortalities continue to thwart efforts to regularly rear a high proportion of veliger larvae through to metamorphosis. Nevertheless, the fact that some batches of larvae did succeed in reaching metamorphosis without heavy losses does indicate that most aspects of the rearing method have been optimised at the laboratory scale. Hatchery-scale triploid induction trials at Roem van Yerseke were successful in producing good survival and growth of diploid larvae as controls. This demonstrates that the most suitable conditions for rearing diploid mussel larvae have been identified and optimised at the hatchery-scale, even if problems remain at the laboratory-scale. Significant numbers of ready-to-settle diploid mussel larvae were produced during 2007 at each institution and also following hatchery-scale triploid induction trials at Roem van Yerseke in March 2007. These ready-to-settle larvae were used in WP4 to investigate on-growing systems.

Average larval growth rates varied from 2.1 to 7.5 $\mu m\ d^{-1}$. This is in the same range as reported elsewhere (Sprung, 1984; Helm et al, 2004). The objective of rearing mussel larvae to settlement within similar time frame and with a similar survival rate to other hatchery reared bivalve species has been achieved. Thus, the goal of larval rearing as part of the development of hatchery technology for a reliable blue mussel seed production was reached.

1.3.4. Spat growth and survival

In year 1 of the BLUE SEED project, a protocol for tests with spat was formulated and experiments were carried out. Only small amounts of seed were produced because of limited availability of broodstock in spawning condition and low larval survival. The effects of type of settlement rope, algal species diet, amount of food and culture density on spat settlement and growth rate were tested. The quantities and quality of spat were insufficient to allow large scale experimental studies of settlement or on-growing, or the comparison between triploid and diploid seed. However, grow-out in the field was successful (see Fig. 3).

Fig. 3. Rope collectors with hatchery produced diploid *Mytilus galloprovincialis* seed when retrieved from a raft.

In year two of the BLUE SEED project, spat settlement was tested at IMARES and IFREMER. Work at IMARES showed that that good larval growth is correlated with good settlement. Work at IFREMER suggests that the addition of Epinephrine (Adrenaline) speeds up settlement when added at the end of larval rearing. Experiments at IMARES determined the optimal feeding regime for spat based on the pseudofeaces threshold, uptake efficiency and comparison of different algal species. CIMA and OPMEGA determined the relative recruitment of *Mytilus galloprovincialis* seed obtained from larval cultures carried out under different experimental conditions and showed a density-dependent effect of recruitment on growth rate of spat suggest a possible effect of the moment of the season when induction of spawning or/and larval culture are carried out, on the recruitment of spat. An important milestone was reached when triploid spat was transferred to the field. Four comparative trials of diploid and triploid spat growth were conducted, each using a different on-growing system. In one trial, diploid spat appeared to grow faster than triploid spat in a down-welling system although this could not be demonstrated statistically. In another trial, one particular cohort of triploids grew significantly faster than all other spat, but other triploid cohorts in the same trial were not significantly different from diploid controls. Survival of spat appeared to be more dependent on their genetic background than on their ploidy. There was no significant difference in length, width, thickness or shell weight of triploid and diploid spat. It is safe to conclude that growth performance of triploid spat/seed is probably similar to that of diploid spat/seed. However, 2n individuals were significantly heavier than their 3n counterparts. One batch of triploids and diploid controls that was produced in February at IFREMER and grown at Grainocean was large enough to determine possible gonad development. Results showed that almost no mature individuals were detected within the

triploid group, while the diploid group contained many mussels with gonads. During the final meeting of the BLUE SEED project, an evaluation was conducted to compare the sensory properties (colour/ appearance, odour, taste/flavour and texture) of this diploid and triploid *M. edulis* batch (see Fig. 4 and 5). As a whole, both types of mussels had a good acceptance by the panellists. Diploid and triploid mussels obtained very high total scores: 33.0 and 30.9, respectively, of a maximum of 36. It was agreed by most of the panelists that diploid mussels had a slightly stronger marine flavour than triploid mussels grown under the same conditions. However, some of the panel considered that the less intense and more neutral flavour of the triploid mussel could favour their acceptance by the consumers not accustomed to eating mussels or marine products. Although to rear mussels up to market size was initially outside the scope of the project, it is important to highlight that a little before of the end the second year of the project (September 2007) CIMA and OPMEGA were able to harvest market-size mussels (≈ 7 cm long) from diploid seed hatchery-produced during the first year. When this seed was on-grown under a raft cultivation system it showed similar mortality and growth parameters to diploid seed from wild origin.

Fig. 4 2n and 3n mussels in plastic pans

Fig. 5 Tasting of mussels by BS panellists

The goal that spat can be cultured to seed size within a time frame and with mortalities similar to other hatchery reared bivalves species has been achieved, and thus, the goal of seed production as part of the development of hatchery technology for a reliable blue mussel seed production was reached. In addition, batches of diploid and triploid seed were produced in parallel in order to compare the performance of diploid and triploid seed.

1.3.5. Seed production costs

In year 1 of the project, the first accounting data on production costs for hatchery seed were collected. A first draft of the bio-economic model was made. The figures provided

in this preliminary estimation of economic costs however did not make economic sense. Some areas were identified where economies could be made to bring production costs more into line with potential sale value of mussel seed: (1) Use low tech algal culture, (2) Restrict activities to the natural season, (3) Scale up volumes of culture during this restricted period of activity.

In year 2 of the project, the model was expanded and updated with more accounting data. Production costs of hatchery seed, seed collected with ropes and fished seed of approximately 5 mm were estimated. These are: € 0.31 per kg mussel seed for fished seed, € 1.35 per kg mussel seed for collector seed and € 430 per kg mussel seed for hatchery seed. This shows a very high price for hatchery seed compared with the other seed sources available. Sales prices of mussel seed from hatcheries in Canada and Australia are lower, but still considerable: € 126 per kg 8-mm seed in Canada and €162 per kg 4-mm seed in Australia. In these countries rope culture is the grow out system in use. Rope culture gives a better yield than bottom culture, which is the main system used in the Netherlands and United Kingdom. In addition, there are no alternative sources of mussel seed available in the hatchery areas in Canada and Australia and the market price is much higher: around € 7.00 per kg in these countries.

The economic feasibility of producing blue mussel seed based on hatchery-produced larvae was compared with the benefits of blue mussel seed collection methods presently in use. From this it can be concluded that hatchery production of mussel seed in Europe is only economically feasible when the product has an added value such as triploidy.

1.4. Achievements of the project related to the state-of-the-art

1.4.1. Hatchery and nursery technology for blue mussels

Methods for culturing bivalves in a hatchery are well established for oysters and clams (Utting & Spencer, 1991, Helm et al, 2004). There are 22 commercial shellfish hatcheries present along the European Atlantic coast that produced a total of at least 2 billion individuals in 2003 (ICES WGMASC Report 2003). However, there are no commercial blue mussel hatcheries or nurseries in Europe, although controlled reproduction and subsequent spat and seed production is known to be technically feasible. A few hatcheries exist outside Europe. For example, the North American company Taylor Shellfish produces *M. galloprovincialis* at a price of €1.50 per 1000 seed of 1 mm. This seed is sold in an area where there is no large natural supply of blue mussel seed.

The BLUE SEED project optimised existing hatchery and nursery technologies for commercial blue mussel seed production. Important achievements were made in optimising the production and use of hatchery-produced mussel spat and seed are discussed below.

Broodstock conditioning

The parents that produce larvae in a hatchery are called **broodstock**. The ideal broodstock of any aquaculture species provides sufficient ripe individuals at any time to enable year-round reproduction. The essential method of "**broodstock conditioning**" is to hold adults under conditions in which gametogenesis and spawning can be induced any time of the year. It is challenging to be able to prevent mature individuals from spawning spontaneously. Artificial conditioning of broodstocks has been achieved for a number of commercially important bivalves, notably oysters, scallops and clams, but there are no published data on conditioning of mussels (Loosanoff and Davis, 1963; Walne, 1970; Lannan, 1980; Lannan et al., 1980; Leal, 1994; Millican, 1997). Reproductive activity in bivalves is controlled largely by two factors: temperature and food supply. Holding broodstocks at appropriately fixed or rising temperatures and food levels ("heat and treat") involves simple technology, but determining the precise temperature and food supply at which gametogenesis will proceed and the time-period required at that temperature is critical. An alternative to conditioning broodstock from a "spent", or early gametogenic status is to collect ripe individuals from the wild and hold them at a low temperature until required for spawning. This "cold and hold" method has been used routinely in the UWB laboratory to provide mussel broodstock for larval culture from April until August each year (Beaumont et al., 1988). Within the BLUE SEED project the hold and cold method has been optimised and the heat and treat method has been developed for blue mussels (Dominguez et al, in prep.).

Larval growth and survival

For any new species being brought into commercial culture, there are a number of factors that require optimisation. The earliest embryonic stages are non-feeding and unshelled – in the case of mussels this lasts for approximately 48 h at 14 ^{o}C – and care is required to prevent the development of abnormalities (Beaumont et al., 1992). Veliger growth is dependent on temperature, salinity and food species (Helm and Millican, 1977) and these factors need to be optimised for use at pilot, and then commercial scale. The BLUE SEED project showed that the larvae of the different mussel species require different algal species for optimal growth (Beaumont et al, in prep). In addition, optimal egg density, concentration of foods and size and shape of the rearing vessels were determined (Galley et al, in prep).

Spat growth and survival

Effective production of spat in a nursery requires knowledge of the optimal diet, algal concentration and supply rate. Since mussels are not routinely cultured in nurseries these data were not available for mussels until now. Within the BLUE SEED project the parameters were determined in the laboratory for *Mytilus edulis* (Kamermans et al, in prep.). Other work in BLUE SEED suggests that the addition of Epinephrine (Adrenaline) speeds up settlement when added at the end of larval rearing. Furthermore, the relative recruitment of *Mytilus galloprovincialis* seed obtained from larval cultures carried out under different experimental conditions showed a density-dependent effect of recruitment on growth rate of spat. This suggest a possible effect of the moment of the season when induction of spawning or/and larval culture are carried out, on the recruitment of spat.

1.4.2. Triploid and tetraploid induction

The main reason for interest in triploidy in bivalves is that triploids are generally sterile, or have very under-developed gonads. As a result, energy is invested into somatic growth rather than gonad growth as the animal matures and a better product can be marketed. In addition, some species are unmarketable at certain times of the year due to reproductive activity and triploids can overcome this. A second advantage is higher growth. Significantly increased growth of triploids compared with diploids has been reported for 2 year old *C. virginica* (Stanley, et al., 1984), 15 month old *O. edulis* (Hawkins et al., 1994) and veliger larvae of *C. gigas* and *M. edulis* (Yamamoto et al., 1988; Beaumont & Kelly, 1989).

There are two methods to produce triploid bivalves: (1) treat embryos with (a) chemicals, or (b) physical shock, or (2) cross diploids adults with tetraploid adults created by (a) chemical treatment, or by (b) using gametes from triploids. Before the BLUE SEED project, triploid mussels had only been produced on a small scale using embryo treatment with chemicals or temperature shock in several laboratories worldwide (Beaumont and Kelly, 1989; Yamamoto and Sugawara, 1988; Scarpa, et al., 1994; Toro and Sastre, 1995; Brake el al., 2002). The success rate (i.e. proportion of triploids in treated batches) was not always high.

In recent years, new interesting advances were made by John Brake and co-workers in PEI (Canada), who have shown that triploids *M. edulis*, obtained by induction with 6-DMAP (Brake el al., 2002), had a greater growth rate than diploids in field evaluations (Brake et al., 2004). Results of the BLUE SEED project did not confirm this (Kamermans et al, in prep).

Tetraploids, when mature, will develop diploid gametes, and therefore crossing tetraploids with ordinary diploids will produce 100% triploid offspring (Chourrout et al., 1986). Crossing tetraploids with ordinary diploids will produce 100% triploid offspring. In oysters, crosses between tetraploids and diploids are currently used to produce 100 % triploids (Guo et al., 1996) and the bulk of production of *Crassostrea gigas* spat from hatcheries in the USA and in France consists of triploids (Nell, 2002). The BLUE SEED project produced tetraploid spat by direct induction from diploids (McCombie et al, in prep).

Because normal (diploid) blue mussel meat contains gonads, their absence could influence taste, texture and palatability. However, for the oyster *Crassostrea gigas* it was established that consumers and experts favoured the firmer meat of triploids over the softer meat of diploids (Allen & Downing, 1991), and market-sized diploid and triploid *Saccostrea commercialis* were also given equivalent scores (Korac et al 1996). The evaluation to compare the sensory properties of diploid and triploid mussels at the end of the BLUE SEED project showed both types of mussels had a good acceptance by the panellists (Kamermans et al, in prep).

1.4.3. Seed production costs

The minimum price at which the hatchery production method for blue mussel seed will be economically profitable was determined. In general, hatchery seed is used in areas where other sources of seed are scarce. In the BLUE SEED project a comparison with fishing for seed was made. This showed that hatchery seed is economically

interesting only when added value is present, or when other sources become less available.

1.5. Impact of the project on its industry or research sector

1.5.1. Improved competitiveness of the European blue mussel industry

The new technologies developed in this project are applicable in all European Atlantic blue mussel producing countries, making it possible to reduce the fluctuations in annual production, and sell high quality blue mussels year round. The latter improvement will also lead to an increase in total demand for locally produced mussels, and enable farmers to compete with imports from outside Europe (mainly from Chile and New Zealand). Stability in the supply of spat and seed will lead to a more stable production from year to year, which will allow farmers to better balance production output to input and production assets. The blue mussel industry could obtain strong stimulation from the application of results from this project, making the industry as a whole more robust and competitive. The more stable supply of high quality blue mussels will allow for better promotion among European consumers, and thus contribute to an increased demand.

1.5.2. European dimension

The SMEs had the capacity to innovate, but did not have the required research facilities and skills. Thus, they greatly benefited from a consortium with partners from all over Europe. The linkage of the SME-partners to RTDs gave further impetus to the dissemination of knowledge and expertise among project partners. All partners made optimal use of each others facilities and skills. The jointly formulated goals ensured optimal involvement and support. This resulted in a joint responsibility and interest.

Each individual SME did not have the necessary economic strength to translate new rearing techniques from the experimental laboratory scale to the industrial scale. In contrast, the RTDs involved in the project were able to develop new experimental rearing techniques but did not have the resources to implement them beyond the pilot scale. Therefore, a CRAFT consortium, in which SMEs worked together with RTDs, was an ideal vehicle to take new rearing techniques from lab scale directly to implementation at industrial level. The major reason the SMEs participated in the project is because they are convinced that a more secure year round supply of high quality blue mussel seed will strengthen their competitiveness. The SMEs at individual level and the SME-RTD networks at national level, were too small to support such a development. When it comes to technologies for the production of triploid blue mussel seed, and the development of sustainable production protocols, a market at European scale is necessary. No member state harbours all the knowledge that will be applied in the project. Only working at a European scale could secure the success of the project. In the BLUE SEED project, expertise on production of tetraploid oyster broodstock from France was combined with expertise on production of triploid mussel larvae from Wales (UK). In addition, hatchery technology was available from France and expertise on rearing spat came from the two main mussel producing countries, Spain and the Netherlands. The benefit of the project is that now the network has broadened to the European level. It is expected that the

relationships developed during this 2-year project will remain functional long after the termination of the project.

1.5.3. Community societal benefits

Blue mussels are a traditional product in the European Community. The many hundreds of recipes for mussels found in the European Community prove that the product is highly regarded and well accepted by consumers. In addition, mussels are healthy, tasty and produced in a sustainable way. Blue mussels are a “pure” biological product, with no need for fertilizers or high-energy feeds to enhance productivity during the grow-out phase, practices which are so typical with other types of aquatic or terrestrial meat production. Mussel meat contains only 1% fat, mostly (healthy) polyunsaturated fatty acids (PUFAs), 10% protein, and very few carbohydrates. Therefore, mussels can be regarded as a “light” product among the different types of meats and seafood available on the market. The project aims to make blue mussel more available to consumers on a year round basis, and at a stable price, providing more consumers with the health benefits of regular mussel consumption.

Experience has shown that women are the main working force in marine hatcheries for finfish, shrimp or molluscs, apparently because women put more care into the handling of the delicate broodstock and larvae. A similar development can be expected in blue mussel hatcheries.

1.5.4. Estimated time to market and economic benefit

The average production cycle in blue mussel is 1-3 years. Given the 2-year duration of the project, it concentrated on spat and seed production, though some market size triploid mussels were produced as well showing that hatchery production may well shorten this average. Commercial quantities of triploid mussels will become available approximately 1 year after the end of the project. Hatchery techniques to produce triploid blue mussel spat and seed, broodstock conditioning protocols, and a comparison of the performance of hatchery and conventionally produced spat and seed, were all realised during the project. In consequence, the SMEs were able to (a) take up the technology during project execution, (b) take the first steps to put the hatchery reared, triploid lines on the market, and (c) benefit from more stable high quality annual productions within their home and international markets.

The culture of mussel seed in hatcheries and nurseries is more controlled than the use of seed collected in the field. This provides opportunities for a tracking and tracing system. Such a system will ensure better protection of the consumer. The controlled culture cycle provided by hatcheries will allow producers to meet the requirements of supermarkets more easily. Supermarkets want a regular supply, availability and standard quality of products. Use of local broodstock will avoid the risk of mixing different species or stocks of blue mussel and reduce the risk of spreading diseases between different blue mussel producing countries.

Exploitation of the project results, a reliable supply of hatchery produced seed and year-round selling of high quality blue mussels, will enhance European competitiveness. The availability of hatchery-produced seed will bring more stability to the market, long-term security in jobs, development of coastal areas and alternative jobs and products to fisheries.

Development of the hatchery/nursery technique for blue mussel seed fits into the EU strategy for sustainable development of aquaculture (COM (2002), 511). Improving technology for the culture of shellfish and increasing production by genetic improvement, both of which were addressed directly in BLUE SEED, are among the aims and suggested actions of this strategy

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2. Dissemination and use

2.1. Exploitable knowledge and its Use

Below a description is given of the publishable exploitable results that came out of the BLUE SEED project.

Website for end users (www.blueseedproject.com)

Through this website the dissemination of the outcomes and project deliverables to its end users is ensured. This information can be beneficial to potential producers and consumers. In addition, the information can be used for educational purposes. The website is public and will be kept on line by AquaTT until five years after the end of the project (30 November 2012).

Reports on (1) performance of diploid and triploid blue mussel larvae and seed and (2) performance of different culture methods and types of spat during grow-out from spat to seed

These reports summarize the results of the comparative studies on diploid and triploid mussel larvae and seed. They give the companies an overview of what to expect regarding growth and survival when starting commercial production of triploid mussel seed. They are public documents that can be used by researchers, teachers, people involved in the aquaculture industry and environmental agencies.

Bio-economic model for calculations regarding costs of blue mussel seed
The model developed during the BLUE SEED project provides a framework for some basic bio-economic analysis of the costs and benefits associated with the various sources and methods of securing seed mussel. This can include seed from wild ephemeral beds, seed settled on deliberately placed collectors, hatchery produced diploids and triploids and indeed seed from any other origin; all providing of course that the costs associated with the gathering or production of the seed are known. If this is the case, the costs can be inserted into the model in the appropriate place. Then, levels of required break-even-production can be estimated. The model will be public and can be used by industry, researchers and teachers involved in aquaculture.

Report on production costs for different blue mussel seed production methods
This report summarizes the outcomes of the model calculations concerning the costs of mussel seed production. It will be a public document that can be used by other researchers, teachers, people involved in the aquaculture industry. With the information contained in this report, companies can take decisions on starting commercial production of hatchery seed.

Scientific manuscripts
Eight scientific papers are planned on outcomes of the BLUE SEED project. Drafts will be shown to the for SME's approval. Publication is free from approval 3 years after the end date of the project. The results to publish should not have an intensive description of the method which may reveal the information protected until 2013. Once published, the results are public and can be used by other researchers, teachers, and people involved in the aquaculture industry.

2.2. Dissemination of knowledge

1) The main portal for information has been the blue seed website, www.blueseedproject.com. On this site you can find a complete overview of the project objectives, the partnership and the tasks within the WP. Contact details are also provided if viewers require more information.
2) A generic project presentation has been created for all partners to use as and when required. The presentation gives a good overview of the project and was updated as the project progressed. The presentation is also downloadable from the project website.
3) RTD partners with the assistance of their respective SMEs have promoted the project at their national producer events.
4) AquaTT helped promote the project using its established news service "Training News," a free monthly electronic newsletter disseminated to over 3500 subscribers.
5) AquaTT attended the biggest European aquaculture event in 2007. They promoted the project with a poster and obtained feedback from a variety of stakeholders.
6) The project coordinator IMARES has hosted a large international shellfish symposium and presented the project during the farmers day at the event. This event coincided with the last partner meeting and thus ensured project participants from IFREMER and

CIMA were present to help promote the project with one oral presentation and two posters.

7) Based on the results obtained, 8 scientific articles are planned.

Overview table

Activity	Date	Partner responsible	Venue	Dissemination Method
Dissemination Activities				
Project Website	**Full project period**	**AquaTT**	**online**	**A website giving general information on Blue Seed**
AquaTT Website www.aquatt.ie	**Full project period**	**AquaTT**	**online**	**General information on Blue Seed and link to project website**
Wageningen IMARES Website www.wageningenimares.wur.nl	**Full project period**	**IMARES**	**online**	**General information on Blue Seed and link to project website**
Project Presentation	**Revised on ongoing basis**	**AquaTT/IMARES**	**Online and used at events**	**A general presentation summarising the project**
EAS Show, Turkey	**24/10/07**	**AquaTT/IMARES**	**Istanbul, Turkey**	**A scientific poster presenting the project to over 500 participants**
Presentation at FP7 information Day	**3/10/07**	**AquaTT**	**Galway, Ireland**	**David Murphy presented to Irish researchers on experience in FP6 projects. Included mention of Blue Seed project**
Seafood Health Symposium	**4-6/10/07**	**AquaTT**	**Granville, France**	**Mark Norman, AquaTT director presented on AquaTT role**

				in the European Aquaculture Sector. Blue Seed was mentioned as one of the projects we are working on.
Electronic Newsletter – Training News	**2007**	**AquaTT**	**Electronic**	**Articles promoting website and project in newsletter sent to over 3500 users in the aquaculture sector.**
Publication in Provinciale Zeeuwse Countant	**22/03/07**	**IMARES**	**Zeeland, Netherlands**	**Publication in newspaper of Province of Zeeland**
Publication in newsletter of EG-Liason	**June 2007**	**IMARES**	**Den Haag, Netherlands**	**Publication in newsletter of Dutch EU connection office**
Publication in Bevelander	**26/06/07**	**Roem van Yerseke**	**Zeeland, Netherlands**	**Publication in newspaper of one of the islands of the Province of Zeeland**
Publication in BN De Stem	**07/04/07**	**Roem van Yerseke**	**Brabant, Netherlands**	**Publication in newspaper of Province of Brabant**
Project Presentation	**June 2007**	**CIMA/OPMEGA**	**Vilanova de Arousa, Spain**	**Power Point presentation of BLUE SEED project to OPMEGA partners**
Project leaflet	**August 2007**	**IMARES**	**Den Haag, Netherlands**	**Publication in newsletter of Dutch EU connection office**

Project Summary	**October 2007**	**CIMA**	**Vilanova de Arousa, Spain**	**Summary of the CIMA activities within BLUE SEED. Included in the CIMA Annual Activity Report (2006)**
10th International Conference on Shellfish Restoration (www.icsr2007.wur.nl)	**15/11/07**	**IMARES/IFREMER /CIMA**	**Vlissingen, Netherlands**	**One oral presentation to industry representatives and one oral presentation and two poster presentations to scientific audience on the progress of Blue Seed**
Publication in SenterNovem EG liaison Magazine	**January 2008**	**IMARES**	**Den Haag, Netherlands**	**General article about the BLUE SEED project in magazine of Dutch EU connection office**