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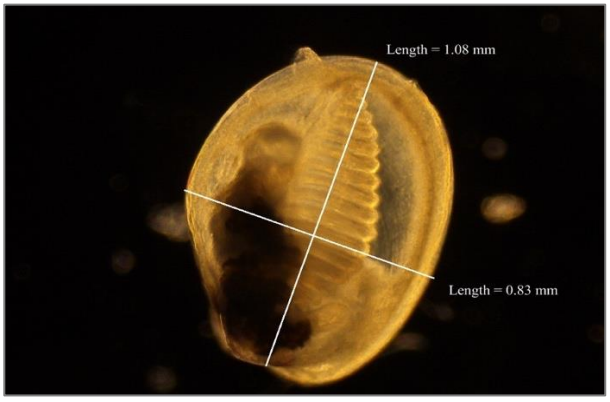
Hatchery protocols for production of blue mussel seeds

Task 9.1 of the Case Study 9

Camille Saurel, Clarie Ng, Pascal Barreau, Iarfhlaith Connellan, Colin Hannon, Adam Hughes, Pernille Nielsen



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Executive summary

Mytilus edulis, commonly known as the blue mussel, is the second most economically important mussel cultivated in Europe. Faced with increasing spatial limitations inshore, researchers and industry are looking at distant offshore sites for longline cultivation with potential failure and lack of recruitment. Presently, low, inconsistent seed availability and suboptimal systems not adapted to exposed conditions present obstacles for successful expansion. Hatchery production of blue mussel seeds could be a solution to ensure reliable supply and introduce opportunities for selective breeding and triploid production. However, due to the low cost of natural spat and final sale value of mussels, it remains underdeveloped. To overcome the prohibitively expensive cost of hatchery reared spat, optimisation is necessary.

The extant Irish protocol for blue mussel hatchery seed production developed within AquaVitae, the Atlantic research consortium for sustainable aquaculture, is not operational for mussel farmers without a traditional shellfish hatchery. This report aims to report an adaptation of this protocol that requires less technology, and labour. *Mytilus edulis* larvae were grown under two rearing systems in Denmark, the first in specialised conical tanks optimised for larval rearing and the second, in large square tanks that have not been tested before. This protocol details the processes starting from broodstock conditioning and spawning to larval rearing, and finally, to larval settlement and the production of spat. The report demonstrates that the high technological protocol developed in Ireland could be adapted to require less labour through a flow-through water change system and a cheaper, low- technological method developed in Denmark could be a viable alternative.

Danish Resume

Mytilus edulis er det latinske navn for blåmusling, som er den næstmest økonomisk vigtige musling, der dyrkes i Europa. Med de stigende udfordringer med at finde egnede områder tæt på kysten, er forskere og muslingeopdrættere begyndt at kigge på områder, der ligger længere fra kysten i forhold til opdræt af muslinger. Etablering i mere offshore forhold kan potentielt øge risikoen for manglende rekruttering fra naturlige blåmuslingebestande. Det er således ikke uden risiko at flytte eksisterende produktion ”offshore”, da risikoen for lavere og uregelmæssig yngeltilgang potentielt er større, ligesom eksisterende produktionssystemer ikke er tilpasset de mere eksponerede forhold i offshore områder. En løsning til at sikre en stabil tilgang af yngel i offshore områder kunne således være at producere blåmuslingeyngel i klækkeriet, hvilket også åbner mulighed for selektiv avl og triploid produktion på længere sigt. Imidlertid er de nuværende omkostninger ved eksisterende metoder til yngelindsamling fra naturligt forekomster af muslingelarver betydelig mindre end ved klækkeriproduktion, hvilket sammenholdt med den relative lave salgsværdi af blåmuslinger gør at der er behov for, at klækkeriproduktionsomkostningerne for blåmuslingeyngel reduceret før klækkeribaseret blåmuslingeyngelproduktion bliver rentabel.

I AquaVitae projektet, er der således blevet udviklet en klækkeriprotokol i Irland til produktion af blåmuslingyngel, men denne metode kræver tilgang til et traditionelt klækkeri, og er derfor ikke umiddelbart operationel for muslingeopdrættere. Den irske protokol blev således yderligere tilpasset til danske forhold med fokus på mindre teknologiske forhold og en optimering af arbejdsgangene. *Mytilus edulis* larver blev produceret i to opdrætssystemer, det første i specialiserede koniske tanke optimeret til larveopdræt og det andet i store firkantede tanke, hvilke ikke er blevet testet før. Protokollen beskriver alle processerne ved klækkeriproduktion af muslingeyngel, startende med konditionering og gydning af muslingelarver til larvevækst og endelig til larvenedslag og produktionen af muslingeyngel. Den irske protokol udviklet for klækkeribaseret produktion af blåmuslingeyngel blev således modificeret til optimerede arbejdsgange via anvendelse af et gennemstrømnings-vandskiftesystem samt en billigere og mere lavteknologisk metode, hvilket er et mere levedygtigt alternativ til klassiske klækkeriproduktion af blåmuslingeyngel.

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Abbreviations

DTU Aqua	Technical University of Denmark, National Institute of Aquatic Resources
Chl *a*	Chlorophyll *a*

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1. Introduction

The blue mussel (*Mytilus sp.*) fishery and aquaculture productions have declined in Europe over the past decades (Avdelas et al. 2021) despite an overall increase of mussel aquaculture globally. Marine aquaculture of low trophic species (LTS) such as bivalves are increasingly recognized as a potential source of healthy marine protein and is in the list of future food poised to fulfil some of UN sustainable development goals (Costello et al., 2020; Parodi et al., 2018). In addition, LTS are important from an ecological point of view, such as keystone species preserving important ecosystems, exerting top-down control on phytoplankton, forming key elements in the circulation of organic matter and nutrients, as well as acting as ecosystem engineers to support biodiversity.

One significant bottleneck of mussel production is the lack of seeds. In order to expand mussel aquaculture to areas with poor or no wild seed recruitment (e.g. offshore), as well as areas with extensive biofouling of non-valuable species on spat collectors (e.g. *M. trossulus*, starfish, ascidians), mussels spats produced in hatchery could be a solution. Previous studies (Kamermans et al. 2013 and ref. within) have concluded that the biggest barrier to hatchery produced mussel spats is cost. Current hatchery practices are too expensive and non-profitable to produce mussel spats. For instance, the price of mussel seeds collected from a spat collector versus seeds produced in hatchery were 1.35 euros vs 430 euros kg^{-1} (Kamermans et al. 2013).

In Europe, there remains no commercial operational mussel hatcheries (Mero et al., 2019), nonetheless, protocols for producing spats exists (e.g. Pronker et al. 2008, Alfaro et al. 2010), and have been further tested and developed within the EU H2020 AquaVitae project, together with a few experiments regarding the grow-out phase of hatchery produced mussel spat. In this document, we report protocols with an industry-centred approach with the goal of reducing the costs and consequently, becoming economically viable and attractive to the industry. Ultimately, it is intended for one of the protocols to be tested and applied by the industry itself, as opposed to professional hatcheries.

The aim of this report is to present two tested protocols for hatchery production of mussel seeds by DTU Aqua within the AquaVitae project: i) the first protocol is directed to professional hatcheries, ii) the second protocol is developed for large-scale hatchery production by the farming industry, particularly for suspended culture farmers (Figure 1). The first protocol is an adaptation of the initial protocol developed and tested by Cartron Point Shellfish Ltd. in Ireland, who in turn provided training to DTU Aqua and transferred this knowledge within the AquaVitae project framework.

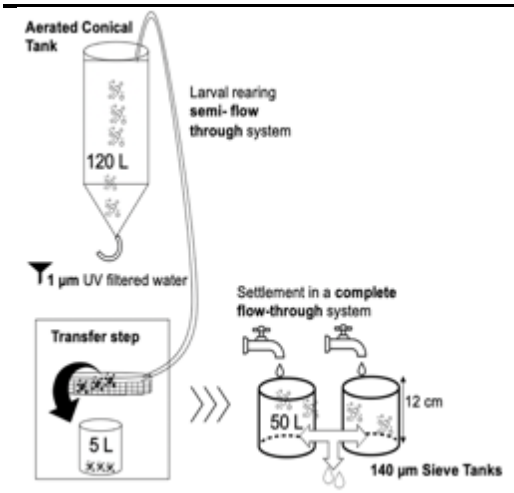
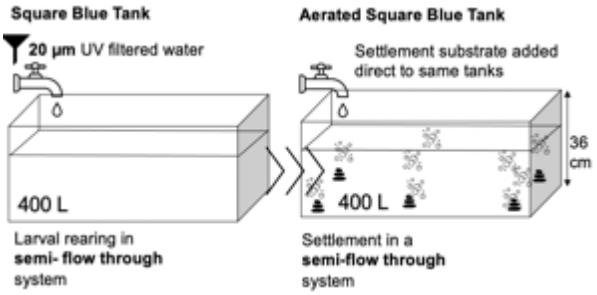
Hatchery small-scale	Hatchery large-scale
	
• High technological method • Medium labor • Small production volume • 1 µm UV filtered water • + 1 larval transfer step	• Low technological method • Low labor • Large production volume • 20 µm UV filtered water • No larval transfer step

Figure 1. Summary of two protocols tested at DTU Aqua based on the initial protocol developed by Cartron Point Shellfish Ltd. Graphic: Clarie Ng

2. Protocols for blue mussel seed production in hatchery

This protocol was initially developed in Ireland at Cartron Point Shellfish in corporation with Galway-Mayo Institute of Technology, transferred to DTU Aqua and adapted to the DTU Aqua's facilities hatchery in Nykøbing Mors. DTU Aqua received training from Cartron Point Shellfish prior to the testing of the protocol. The DTU Aqua protocols focuses on the optimisation of hatchery procedures in terms of water quality and exchange, continuous feed with monitoring and removal of handling steps to reduce labour cost. For instance, after fertilisation, the larvae rearing can be done either at small scale in two steps, or at large scale with only one step for the mussel industry.

From 2022, DTU Aqua has a unique low trophic aquaculture hatchery producing shellfish, macroalgae and crustacean with a focus on the production of European flat oyster (*Ostrea edulis*) and macroalgae dulse (*Palmaria palmata*). Several species can be produced at the same time, thanks for the modularity of the water system and the different compartments of the hatchery, allowing no contamination between the setups and independent water temperature regulation adapted to the needs of each species.

The small-scale protocol derived from Cartron Point Shellfish and the new large-scale protocol were adapted, tested and developed between March-April 2021.

2.1. Water quality and flow

DTU Aqua hatchery in 2021 had a different water treatment system than the hatchery in 2022. The protocol in this report was tested in the hatchery 2021 as detailed below. DTU Aqua hatchery 2021 pumped water from the surrounding Limfjorden. The water passed through settlement tanks, followed by a sand filter (400 µm) and then through a series of cartridge filters up to 1 µm with UV-treatment. There was an extra cartridge filtration of the water for the microalgae production down to 0.2 µm as well as UV-treatment to avoid any contaminations.

Average input water temperatures during the time of testing (19/03/2021-30/04/2021) ranged from 4.5-9.8°C and salinities were 26.3-29.4 psu. The temperature was increased in the hatchery and automatically regulated between 17-20ºC for the production of mussel larvae.

A water recirculation system was used for settlement and the micronursery with a flow rate of 20-30L h^{-1} with a 10% daily renewal of water was applied in the small-scale hatchery setup. The water was not recirculated for the large-scale setup, but six times a week, during a period of 3h water exchange at a flow rate of 60 L h^{-1} was realised.

2.2. Algal culture

The four species traditionally used at DTU Aqua for conditioning and larval rearing of European flat oysters come from 50L batch culture. For convenience and cost reduction, the same species were used for *M. edulis*. There is one diatom species: *Chaetoceros muelleri* (CCAP 1010/3), and three flagellate species of different size and nutritional composition: *Rhodomonas salina* (CCAP 978/27), *Tisochrysis lutea* (CCAP 927/14) and *Pavlova gyrans* (CCAP 940/1C). The concentration of the different algae in the 50L bags vary between 3-9 million cells ml^{-1}.

2.3. Mussel broodstock

2.3.1. Broodstock conditioning

The mussels can be collected either from the wild or from mussel farms and it is recommended to collect medium sized individuals of 35-45 mm in shell length. The number of individual mussels needed depends on the desired number of larvae to be produced. A single well-conditioned female can produce up to 5.0 x 10^6 eggs per spawning event.

In September and December 2020, a collection of broodstock with approximately 2x100 adult blue mussels from suspended culture and from wild bed are kept in 2x 50L tanks at the recommended temperature of 6 ± 1°C until they were needed for spawning (Beaumont et al. 2004). Temperature acclimation can be done by increasing the temperature by 1°C every two days until reaching 17°C. In areas where different species of mussels occur e.g., *M. edulis* and *M. trossulus* genetic screening of the mussels might be needed before initiating the hatchery work (Penney et al., 2007, Dias et al., 2019).

The broodstock mussels should be cleaned free of epi-fouling organisms and debris before placing them in a single layer in the conditioning tanks to reduce food competition. Tanks were kept aerated and with a constant food supply by a peristaltic pump providing a mix of the four algae (*C. muelleri*, *R. salina*, *T. isochrysis* and *P. gyrans*) at a ratio of 3:1:1:1.

The period for conditioning depends on the animals collected but can generally be reached within 6-10 weeks (Kamermans et al., 2013), where fecundity (ripeness) is assessed weekly. Mussels are ready to spawn when gonads with ripe gametes can be detected macroscopically: the mantle containing the female gametes are orange, while the males are creamy-white; while opening a ripe mussel, a milky liquid are visible for the males.

2.3.2. Spawning (1 day)

The broodstock mussels were collected from the conditioning tanks and were cleaned thoroughly with freshwater to remove byssus and faeces.

The spawning was induced by thermal shock (Utting and Spencer 1991) using two buckets of filtered sea water at 10°C and 25°C. Batches of 10-12 mussels per run are recommended for producing gametes to assure sufficient genetic diversity.

The first batch of mussels was submerged in 10 °C cold bucket with 5L seawater for 20-minutes, if either orange eggs or white sperm were released (Figure 2), the females and males were separated immediately in two other buckets labelled males and females holding 5L of 20°C treated seawater. If no spawning occurred, the mussels were transferred to the 25°C bucket for a further 20-minutes, and the process repeated every 20-minutes until spawning.

When the mussels stopped or slowed down the release of gametes, they were removed from the buckets.

Figure 2. Separation of female (left) and male (right) mussels. Photo: Clarie Ng

2.3.3. Fertilization (72h)

The eggs were sieved and rinsed through a 40 µm sieve and put into a clean 5L bucket of treated seawater at 20°C. The eggs were counted under an inverted microscope before being fertilized. The eggs needed to be fertilised at an egg to sperm ratio of 1:5 to avoid polyspermy. Previous study recommended a ratio of one egg to 100-200 sperm ratio (Beaumont et al. 2004). Around 50 mL of sperm was added to the buckets with sieved eggs until the ratio was reached. The eggs were sampled every 15-minutes to assess fertilisation. If there were less than five sperm per egg observed, 50 mL of sperm needed to be added again, with another 15-minute interval before sampling again. If there are five sperm visible per egg in the sample after mixing every 15-minutes, indications of successful fertilisation should be visible after one hour. Indications of successful fertilisation are polar body expulsion or cellular division (Figure 3). The eggs and sperm should be used within 30-minutes to one hour according to Kamermans et al. (2013), or up to 2-hours of spawning (Galley et al, 2010). However, gametes can be kept at low temperature in the fridge overnight if needed.

Once fertilisation was confirmed the eggs were rinsed once more in a 40 µm sieve. It is recommended that the eggs are incubated on flat-bottomed trays for 72-hours during the initial stages of larval rearing to maximize survivorship and proper egg development with no aeration and little disturbance (Galley et al. 2010). After 72-hours the eggs were then transferred to larval rearing tanks.

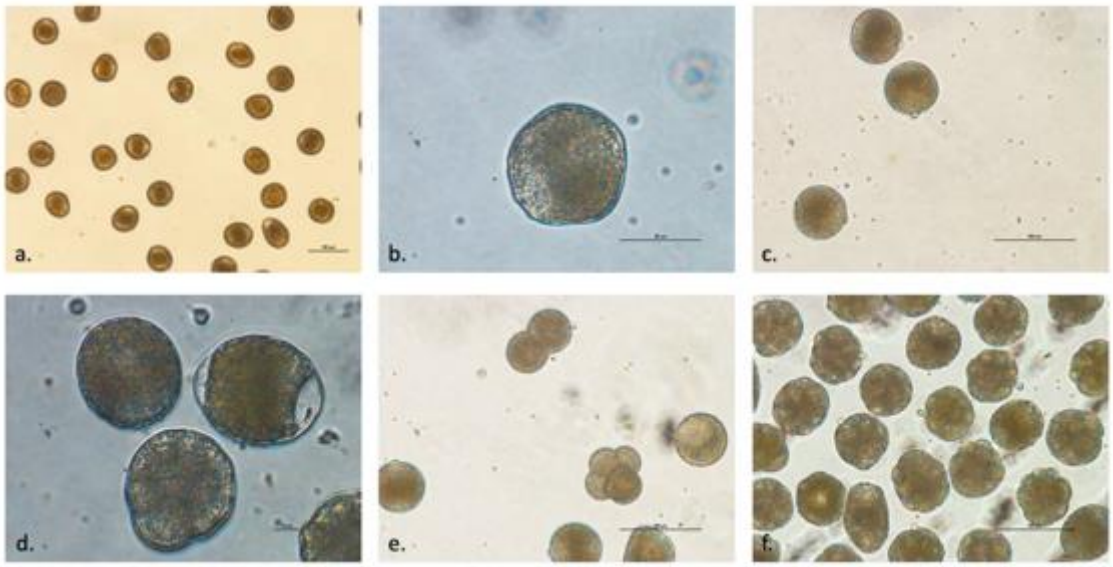


Figure 3. (a) Unfertilised eggs (b) Fertilization at a sperm to egg ratio of 5:1 to prevent polyspermy (c) Polar bodies indicating successful fertilisation (d) Gastrulation (e) Early cell division with 2-4 cell stages (f) Late cell division. Photos: Clarie Ng.

3. Larvae to micronursery for small-scale hatchery production

The water quality in the small-scale hatchery production was 1 µm and UV filtered seawater.

3.1. Larvae rearing (3-4 weeks)

An approximate density of 10 fertilised eggs mL^{-1} was placed in 120L conical tanks at 20°C and with a light airflow (Figure 4). There was no food for 24-hours and no water-flow for 36-hours. After 48-hours post fertilisation, the feeding of the larvae began.

Figure 4. Larval rearing system consisting of 120 L conical tanks and aeration from the bottom. Photos: Clarie Ng

Six times a week, the full water volume was changed at a low flow of 10-25 L h^{-1} to avoid potential mechanical damage to the larvae. A 30 µm filter was attached to the outflow pipe to prevent loss of larvae.

The various steps from larvae rearing to micro-nursery are summarized in figure 5.

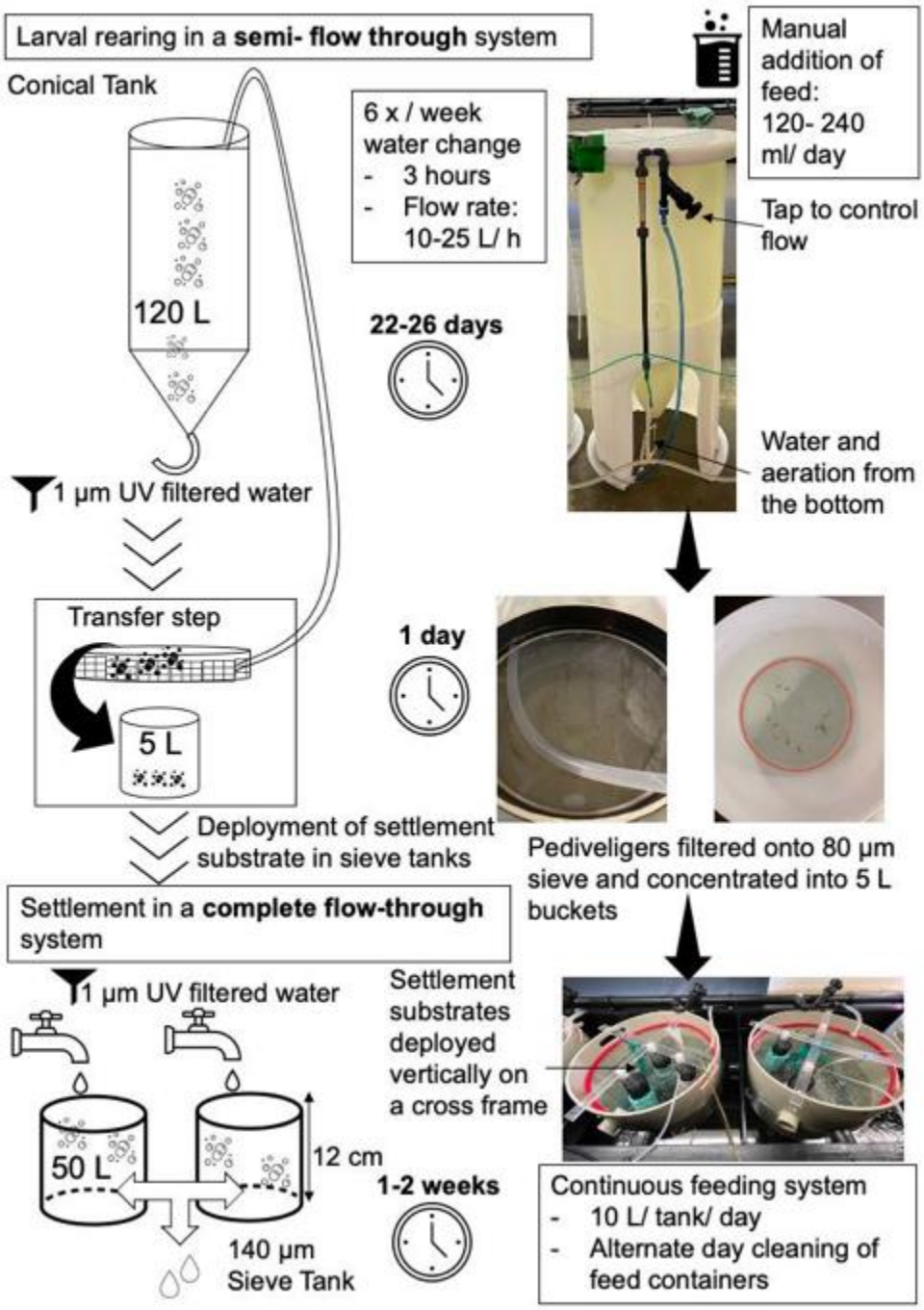


Figure 5. Graphical overview of protocol from larval rearing to micronursery. Fertilized eggs are loaded into semi-flow-through 120 L conical tanks that are gently aerated from the bottom. When 50% of the larvae become eyed, the conical tanks are emptied, and all larvae are transferred to 50L 140 µm sieve downweller flow-through tanks for settlement. During the entire process 1 µm UV filtered seawater is used. Design: Clarie Ng

3.1.1. Microalgal Feeding of the larvae

Feeding occurred once a day after water changes to avoid clogging the filters during water changes.

A mixed feed consisting of three microalgae species was used throughout larval rearing and spat settlement. One diatom species, *C. muelleri*, and two marine flagellates, *T. lutea* and *P. gyrans* were fed in the ratio detailed in Table 1 according to the time after fertilisation.

Table 1. Ratio of three different species of algae and volume given per tank and per day during the larvae rearing phase.

Time after fertilization	*Chaetoceros muelleri*	*Tetraselmis lutea*	*Pavlova gyrans*	Volume per tank/day
Week 1	1	0.5	0.5	120-240 ml
Week 2	1	0.5	0.5	120-240 ml
Week 3 +	3	0.5	0.5	120-240 ml

The larval period prior to settlement typical lasts 20-30 days at 20°C. To ensure the amount of food was appropriate to the larvae, fluorescence was checked twice a day, once in the morning to monitor food depletion overnight, and once more 1-2 hours after feeding to monitor food sufficiency. A handheld fluorometer such as AquaFluor® Turner can be used. Given that microalgae batch cultures have variable concentrations from week to week, fluorometer measurements allows flexible tailoring of food volumes for different tank systems without daily cell counts.

For instance, according to Riisgård et al. (2011) for *Rhodomonas salina* Chla (µg L^{-1}) = 1.251×10^{-3} × Cell

The calibration of the AquaFluor® Turner raw fluorescence = 1.3502 * Cell - 5.6928

Thus, the raw fluorescence was aimed at values between 30-50 corresponding to cell count of 40-60 cells μl^{-1}. The use of a flow cytometer to recognize the volume of each microalgae species and the effect of grazing on the ratio could also be used, as well as continuous monitoring with Chl *a* sensor to ensure that food is not limited.

3.1.2. Transfer (1 day)

Once 50% of the larvae presented foot activity (pediveliger stage with black eye), the conical tanks were emptied using a pipe and filtered onto an 80 µm sieve and all the pediveligers were transferred to downwelling, 50L, 140 µm sieve tanks for settlement.

3.1.3. Larvae counts

Larvae concentration was initially recorded at 8-10 mL^{-1}. Aliquot samples of less than 10 mL were found not sufficient to count the larvae. Samples of 15 mL sieved through 50 µL mesh allowed a more accurate count and inspection of the larvae. However, sampling should not occur every day to reduce mechanical stress and larger volumes are recommended.

3.2. Settlement (1-2 weeks)

3.2.1. Preparation of settlement substrate

Substrates for larvae settlement were prepared during the larvae rearing phase, since all settlement material need to be soaked in freshwater for three days to remove industrial chemicals. After this process, the settling material was rinsed in seawater and deployed in the sieve tanks. The settling material was placed in the sieve tanks 24-hours before settling. The settling material were hanged vertically in the settling tanks.

3.2.2. Settlement

The downwelling 50L, 140 µm-sieve tanks were set up prior to receival of competent larvae. Larvae are introduced to 20°C, 1 µm, UV filtered seawater in a flow-through system (Figure 5), where aeration was delivered through perforated pipes attached to the bottom of the tank.

The number of larvae used for settlement were 8.4 larvae ml^{-1}, or 420 000 larvae sieve $tank^{-1}$. Larvae were collected from the conical tanks and suspended in 5L buckets and counted to obtain a final pre-settlement density and length measure. Within a couple days settled larvae were visibly attached to the collectors.

A continuous feeding system can be used to feed the mussels. The system is made of a peristaltic pump connected to a feeding bucket with a daily ration of 5L of microalgae mix per sieve tanks at a food ratio as described in Table 1. Feeding tanks were cleaned every two days with freshwater to ensure high food quality.

4. Larvae to micro-nursery for large-scale hatchery production

The water quality in the large-scale hatchery production was 20 µm, UV filtered seawater.

4.1. Larvae rearing (3-4 weeks)

The fertilized eggs at an approximate density of 10 fertilised eggs mL^{-1} were placed in 400L tanks at 20°C with no airflow (Figure 6). There was no food for 24-hours and no water-flow for 36-hours. After 48-hours post fertilisation, the feeding of the larvae began.

Six times a week, during a period of 3h there was a water exchange at a flow rate of 60 L h^{-1} to avoid potential mechanical damage to the larvae. A 30 µm filter was attached to the outflow pipe to prevent loss of larvae. There was no aeration until the settling material was added where aeration was initiated to allow mixing and oxygenation of the 400L volume.

The various steps from larvae rearing to micronursery are summarized in figure 6.

4.1.1. Microalgal Feeding of the larvae

Feeding occurred once a day after water changes to avoid clogging the filters during water changes with 400-800 mL added per day. A mixed feed consisting of three microalgae species were used throughout larval rearing and spat settlement. One diatom species, *C. muelleri*, and two marine flagellates, *T. lutea* and *P. gyrans* are fed in the ratio detailed in Table 1 according to the time after fertilisation. The raw fluorescence measured by AquaFluor® Turner is aimed at values between 30-50 corresponding to cell count of 40-60 cells $µl^{-1}$ (see section 3.1.1).

4.2. Settlement (3-4 weeks)

Once 50% of the larvae become eyed settlement substrate were added to the tank. Larvae were not transferred.

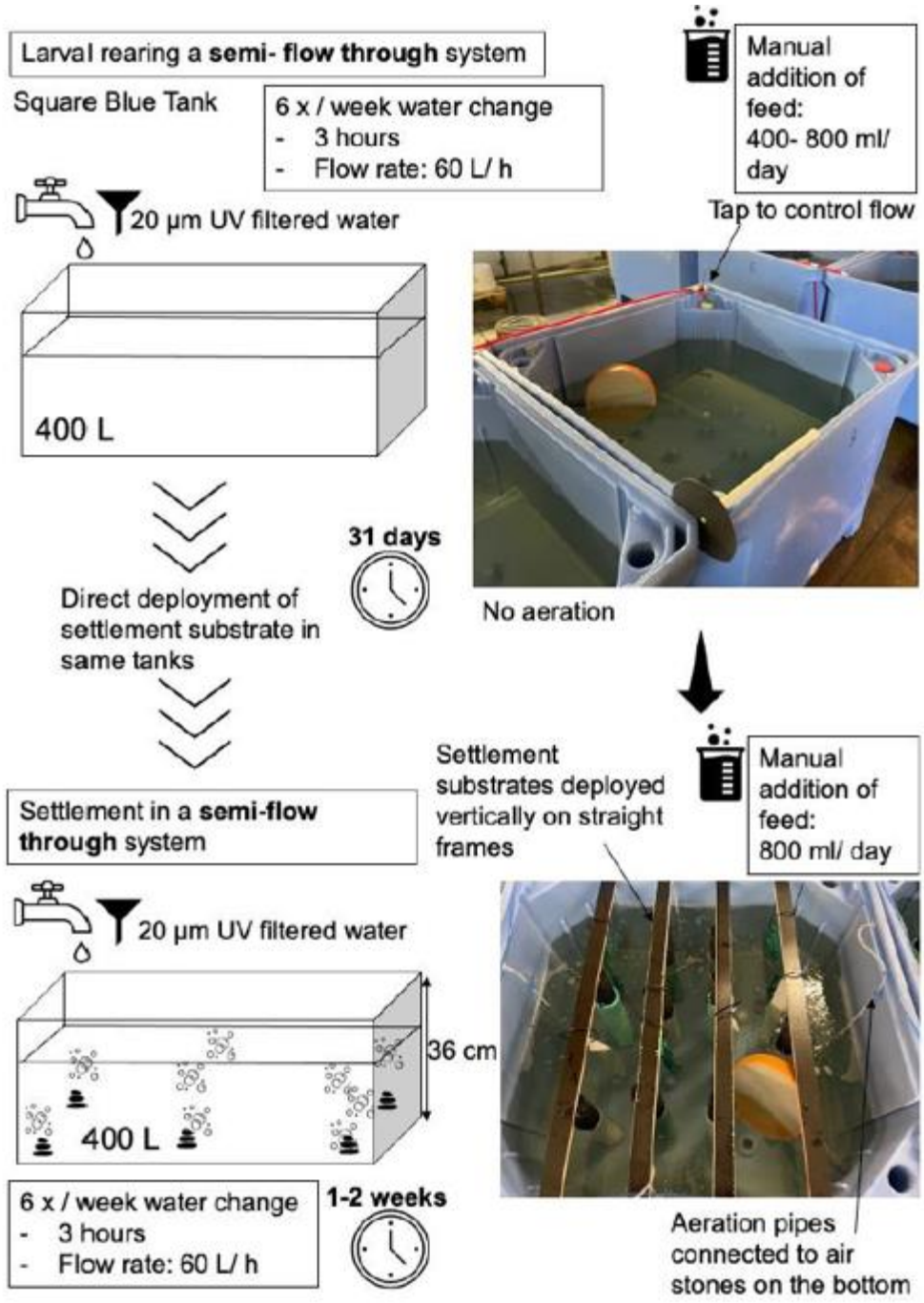


Figure 6. Graphical overview of protocol from larval rearing to micronursery of the large-scale production protocol. Fertilized eggs are added to 400 L square tanks without aeration. Settlement substrates are directly added to the same tanks together with some aeration. During the entire process 20 µm UV filtered seawater is used. Design: Clarie Ng

4.2.1. Preparation of settlement substrate

Substrate for larvae settlement was prepared during the larvae rearing phase, since all settlement material needed to be soaked in freshwater for three days to remove industrial chemicals. After this process, the settling material was rinsed in seawater and deployed in the 400L tanks.

4.2.2. Settlement (1-2 weeks)

Aeration was added to the bottom of the tank at the same time as the settlement material.

4.2.3. Microalgal Feeding of the spats

Feeding occurred once a day after water changes to avoid clogging the filters during water exchange. Around 800 mL of food was added daily. Food was not limited in the tank and concentration was adjusted to the concentration of 60 cells ml^{-1} or the equivalent raw fluorescence.

5. Micronursery to grow-out

5.1. Micronursery

Acclimation to grow-out temperature conditions was made by reducing the temperature slowly (e.g. 1°C per day) until it reached the ambient temperature.

5.2. Grow-out

The time of deployment of the spat depends on the substratum used (e.g. lines rolled around coils, spat collectors, nets), and the local environmental conditions. This must be tested for each location to time optimal deployment for grow-out to avoid biofouling of the hatchery produced seed.

6. Conclusion

The knowledge transfer of mussel seed production in the hatchery from Cartron Point Shellfish to DTU Aqua was successful. DTU Aqua adapted and tested the protocol of Cartron Point Shellfish to its own hatchery technology. More, DTU Aqua consequently optimised the protocol to upscale production and simplify it, by reducing the numbers of steps. Several protocols for mussels have been developed in Europe and elsewhere (e.g., Galley et al., 2010, Kamermans et al., 2013; Pronker et al., 2008) and were consulted while testing the Cartron Point Shellfish protocol. Previous research has focused on broodstock conditioning, spawning, fertilization, larvae rearing, water conditions, larvae concentration, food quality, food ratio and microalgae species, settlement systems and the possibility of triploidy and tetrapoidy inductions. Less work has been done on nursery rearing of mussel spat up to seed and transfer to grow-out system (see review in Kamermans et al. 2013).

The results from the protocol testing and development are promising and present an optimistic outlook for a readily adoptable commercial hatchery protocol for *M. edulis*. However, results also reveal that these cost savings come at the expense of larval growth, where a high-technological conical tank system optimised for larval rearing is better suited for accelerating larval development. Nonetheless, this could also mean that the low technological protocol could be further optimised by adopting some features of the high-technological protocol such as aeration to stimulate circulation. These two protocols would gain in more testing and optimisation at DTU Aqua and other hatcheries, in relation to the upscaling and grow-out using local substrate material to optimize, reduce the costs and become more attractive to the industry.

Another step to adapt this protocol to the industry is to test the easy to use already made mix of microalgae mixed in liquid solutions for bivalve culture, which is currently available in the market from various distributors in Europe. This would allow the industry to be independent of microalgae hatchery and produce spats close to the production sites.

References

Alfaro, A. C., McArdle B., Jeffs A. G. (2010). Temporal Patterns of Arrival of Beachcast Green-Lipped Mussel (*Perna Canaliculus*) Spat Harvested for Aquaculture in New Zealand and Its Relationship with Hydrodynamic and Meteorological Conditions. *Aquaculture* 302 (3–4): 208–18. https://doi.org/10.1016/j.aquaculture.2010.02.028.

Avdelas, L., Avdic-Mravlje E., Borges Marques A. C., Cano S., Capelle J.J., Carvalho N., Cozzolino M., Dennis J., Ellis T., Fernández Polanco J.M., Guillen J. (2021). The Decline of Mussel Aquaculture in the European Union: Causes, Economic Impacts and Opportunities. *Reviews in Aquaculture* 13 (1): 91–118. https://doi.org/10.1111/raq.12465.

Beaumont, A.R., Turner G., Wood, A.R., Skibinski, D.O.F (2004). Hybridisations between *Mytilus edulis* and *Mytilus galloprovincialis* and performance of pure species and hybrid veliger larvae at different temperatures. *J Exp Mar Biol Ecol 302,* 177–188. hpp://doi.org/:10.1016/j.jembe.2003.10.009

Costello, C., Cao, L., Gelcich, S., Cisneros-Mata, M.Á., Free, C.M., Froehlich, H.E., Golden, C.D., Ishimura, G., Maier, J., Macadam-Somer, I., Mangin, T. (2020). The future of food from the sea. *Nature*, 588(7836), 95-100.

Dias, P.J., Dordor A., Tulett D., Piertney S., Davies I. M., Snow M. (2009). Survey of Mussel (*Mytilus*) Species at Scottish Shellfish Farms. *Aquaculture Research* 40 (15): 1715–22. https://doi.org/10.1111/j.1365-2109.2009.02274.x.

Galley, T.H, Batista, F.M., Braithwaite, R., King, J., Beaumont, A.R. (2009). Optimisation of larval culture of the mussel *Mytilus edulis* (L.). *Aquaculture International, 18*, 315-325. hpp://doi.org/:10.1007/s10499-009-9245-7

Kamermans, P., Galley, T., Boudry, P., Fuentes, J., McCombie, H., Batista, F., Blanco, A., Dominguez, L., Cornette, F., Pincot, L. (2013). Blue mussel hatchery technology in Europe. In Advances in aquaculture hatchery technology. Elsevier, New York, pp 339–373

Mero F.F.C., Pedroso F.L., Apines-Amar M.J.S., Cadangin J.F., Rendaje D.C., Verde C.S., Maquirang J.R.H., Piñosa L.A.G. (2019) Influence of water management, photoperiod and aeration on growth, survival, and early spat settlement of the hatchery-reared green mussel, *Perna viridis*. *Int Aquat Res* 11:159–172.

Parodi, A., Leip, A., De Boer, I.J.M., Slegers, P.M., Ziegler, F., Temme, E.H.M., Herrero, M., Tuomisto, H., Valin, H., Van Middelaar, C.E. (2018). The potential of future foods for sustainable and healthy diets. *Nat Sustain*, 1, 782-789.

Penney, R. W., Hart, M. J., Templeman, N. D. (2007). Shell strength and appearance in cultured blue mussels *Mytilus edulis*, *M. trossulus*, and *M. edulis* × *M. trossulus* hybrids. *North American Journal of Aquaculture*, 69(3), 281–295. https://doi.org/10.1577/a06-044.1.

Pronker, A.E., Nevejan, N.M., Peene, F., Geijsen, P., Sorgeloos, P. (2008). Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus 1758). Part I. Impact of different micro-algae mixtures on broodstock performance. *Aquac. Int., 16,* 297-307.

Riisgård, H. U., Egede, P. P., Saavedra, I. B. (2011). Feeding behaviour of mussels, *Mytilus edulis*: new observations, with a mini-review of current knowledge. *Journal of Marine Biology*, *2011*, Article ID 312459.

Utting S.D., Spencer B.E. (1991). The hatchery culture of bivalve mollusc larvae and juveniles. Lab Leafl, vol 68. MAFF Fish Res, Lowestoft, p 31.

Annex I – Hatchery protocol Ireland

Hatchery Production of Mussels (*Mytilus edulis*) at Cartron Point Shellfish Ltd. New Quay, Burrin, Co. Clare, IRELAND

Authors: Iarfhlaith Connellan

Location: The hatchery lies at the entrance to Aughanish Bay on the south shore of Galway Bay.

Introduction

The hatchery building and ancillary facilities e.g. pumps and algal tanks at Cartron Point Shellfish Ltd. are designed specifically for the production of shellfish and more specifically for the production of bivalves. To date the hatchery has produced spat of oysters, clams, scallops and mussels

Regardless of the species being cultivated, the hatchery is used sequentially in a series of steps to match the facilities with the lifecycle stage and the environmental requirements of the bivalve under cultivation. Only one species is cultivated at any one time

To operate this methodology the hatchery facilities must provide for:

1. Broodstock
2. Spawning
3. Fertilisation
4. Larval rearing
5. Settlement (Metamorphosis)
6. Nursery for spat

The species, whether clams oysters scallops or mussels have their own individual specific requirements of temperature and salinity as well as water flow and dietary requirements and so it is not just convenient, it is also very important from an economic point of view that shellfish requiring raised temperature seawater are cultivated when ambient sea temperatures are highest and equally that cold water species are cultivated in colder periods of the year to thus reduce heating bills. For these reasons as well as the necessity to avoid cross contamination of larval and spat stocks only one species at any one time is cultivated in the hatchery. It is not possible to obtain stocks of shellfish from the hatchery containing other shellfish species as there is both a separation of time and space in the cultivation regimes used. To prevent extraneous material from the wild entering the hatchery, filtration to 1-micron for algal cultivation and 10-micron filtration for larviculture is utilised in the hatchery.

Hatchery mussel production

This programme consisted of

1. Provision of suitable mussel conditioners and a conditioning regime.
2. Provision of suitable microalgal diet.
3. Supply of equipment necessary to control the conditioning regime and spawning events of *Mytilus edulis*
4. Provision of sufficient larval rearing capacity and conditions as well as the microalgal ration to ensure successful metamorphosis and settlement of larvae.

Conditioning

A conditioning regime for various batches of mussels is set up in the hatchery. This conditioning or the control of the events of gametogenesis takes place in a dedicated mussel conditioner. Mussels in various developmental stages are placed in the conditioner at successive intervals.

Methods

The conditioners consisted of 1500 litre air lifted recirculated and biologically filtered seawater, or a filtered recirculation system with 5 holding trays each with a capacity of 5 kgs. of mussels. The biological filtered system had a capacity of 15-18 kgs of *Mytilus edulis* broodstock which are held in mid water holding trays.
A pulse pumped feeding regime provides a mixed diet of *Skeletonema, Isochrysis* and *Chaetoceros* with some additions of *Thalassiosira*. Throughout the conditioning period a cell concentration of food organisms at approx. 60 cells per microlitre is maintained.

Seawater flow in the biologically filtered system is held at 30 litres per minute through the mid water trays. These trays (3 or 4 in number) are repositioned vertically once per week throughout the conditioning period to obviate food access advantage in the uppermost tray. In the individual tray conditioner, a recirculated seawater supply of 5 litres per minute is provided through a spray-bar to each tray. The entire seawater contents of this type of conditioner has to be changed twice a week with 10 micron filtered seawater.

Conditioning times vary and are always a function of the gonad condition of the mussel broodstock on arrival at the hatchery. However, using either regime of conditioning it seldom exceeded 6 weeks to produce ripe gonads in *M. edulis*.

Conditioner showing 5 mussel trays and 1500 l. recirculation reservoir. Photo: I. Connellan

Spawning

Induction of spawning is achieved using thermal stimulants.
When *Mytilus edulis* adults are conditioned sufficiently then the preferred method for spawning is using thermal shock. A minimum of 10 degrees difference from the ambient holding seawater temperature is required for spawning to be effective This is the only method used in Cartron Point Shellfish's hatchery.

Fertilisation

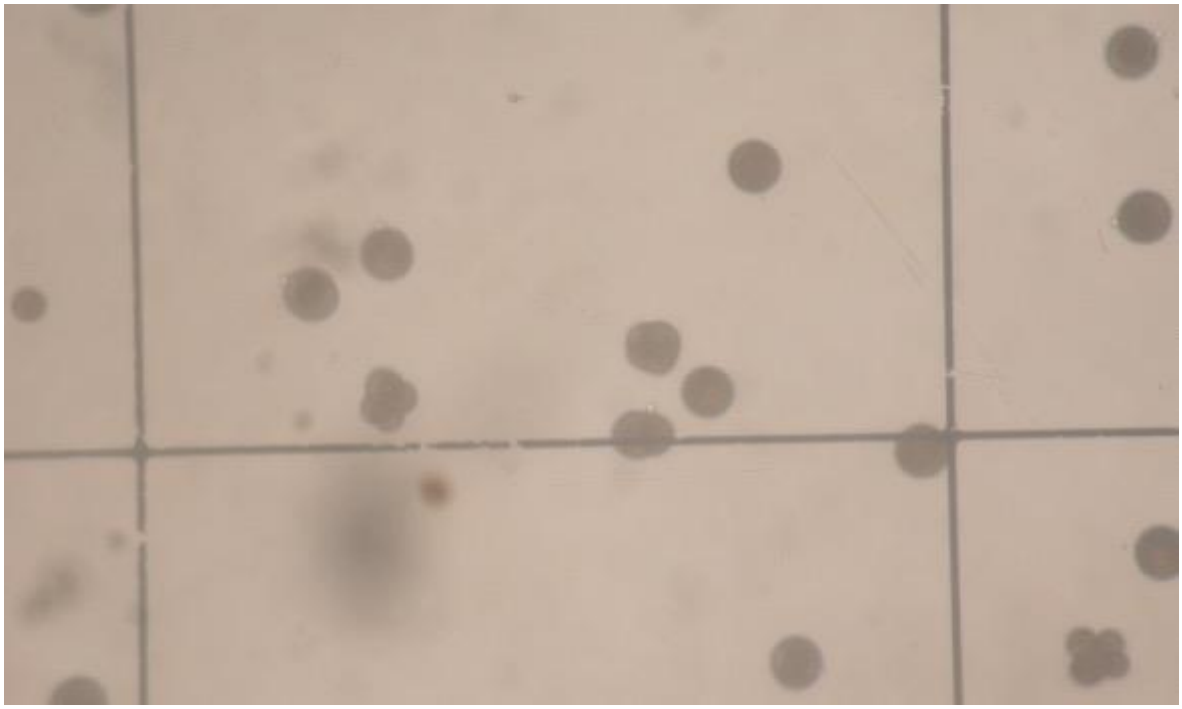
Two fertilisation regimes are used. The first method which is common in hatchery rearing of other bivalves is to permit the free release of both gametes into 20L tanks or vessels. This then involves an assessment of the degree of fertilisation and when sufficient is achieved either by the addition of more sperm or further eggs the fertilised eggs are placed into larval rearing bins along with the accompanying sperm.

Fertilisation method 2

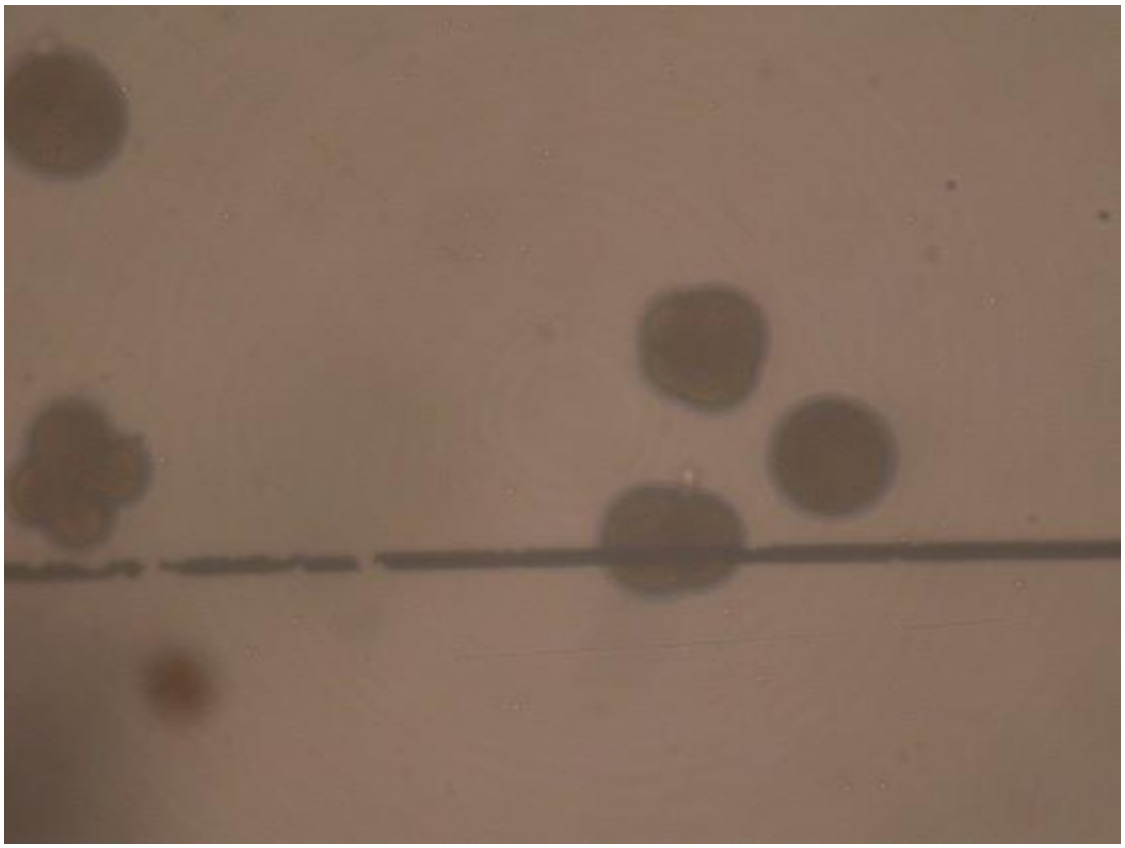
The second method involves the separation of the adult by gender. This is achieved by inducing spawning in the adult mussels and constant vigilance to immediately identify the gender of the gametes. The individual adult are then immediately removed and placed in a separate vessel. Here they continue to release all the mature gametes.
When sufficient eggs have been released, and this is a function of the capacity of a rearing facility as well as the food algal ration available, the sperm from a number of individuals is counted using a haemocytometer and diluted to match the egg numbers in a ratio of 5-6 sperm to each egg.
Microscopic examination of the gametes is used to measure the success of fertilisation.

Each individual mussel can produce many millions of eggs and thus it is clear that few adult individuals are required to provide for the entire larval requirements within the hatchery.
In practice (see plates) it is both prudent to ensure a broader genetic spread and more economical use of the hatchery facilities to ensure that at least 10 or 12 individuals are used in any one production run.

Gamete separation prior to fertilisation. Photo: I. Connellan



Mytilus edulis fertilization and the beginning of cell division. Photo: I. Connellan



Close up of M. edulis fertilized eggs showing polar body extrusion and cell division. Photo: I. Connellan.

Algal Culture

Phytoplankton for both conditioning and larval rearing are production in microalgal form from the continuous algal culture system. All seawater used in this system must be pasteurised. This ration is sometimes augmented when food demand is heaviest with a batch culture system. The seawater used for batch cultivation is sterilised using UV radiation. The algal food organisms used are divided into two groups:
The flagellate and Diatom mix chosen were selected for their broad nutritional spectrum.

1. *Isochrysis galbana*
2. *Monochrysis lutherii*

1. Thalassioira pseudonana
2. *Chaetoceros muelleri*
3. *Skeletonema costatum*

Algae from both systems, collectively as well as individually were fed to mussels in cultivation. There is a known nutritional difference between the same species cultured under the different regimes (continuous as opposed to batch culture). Both systems are used to feed adults and larvae of *M. edulis,* and no nutritional deficiencies never became apparent.

Stock Culture Holding System.

Stock algal culture isolates are maintained in the hatchery in a stock culture room. The stocks themselves are maintained in Erdschreiber medium (this is maintenance medium for both

flagellates and diatoms). Generally, the algal stocks are stored in 200 ml. Erlenmeyer flasks with cotton wool stoppers and aluminum foil covers.

Batch microalgal stocks and growing vessels. Photo: I. Connellan

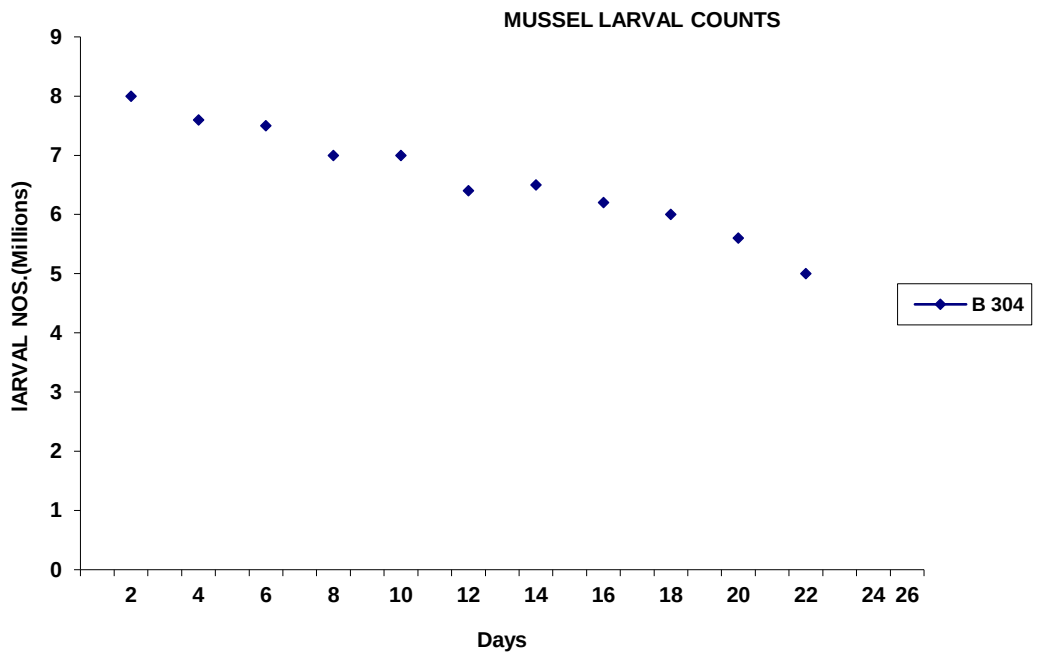
Continuous microalgal culture bags (600 l.). Photo: I. Connellan.

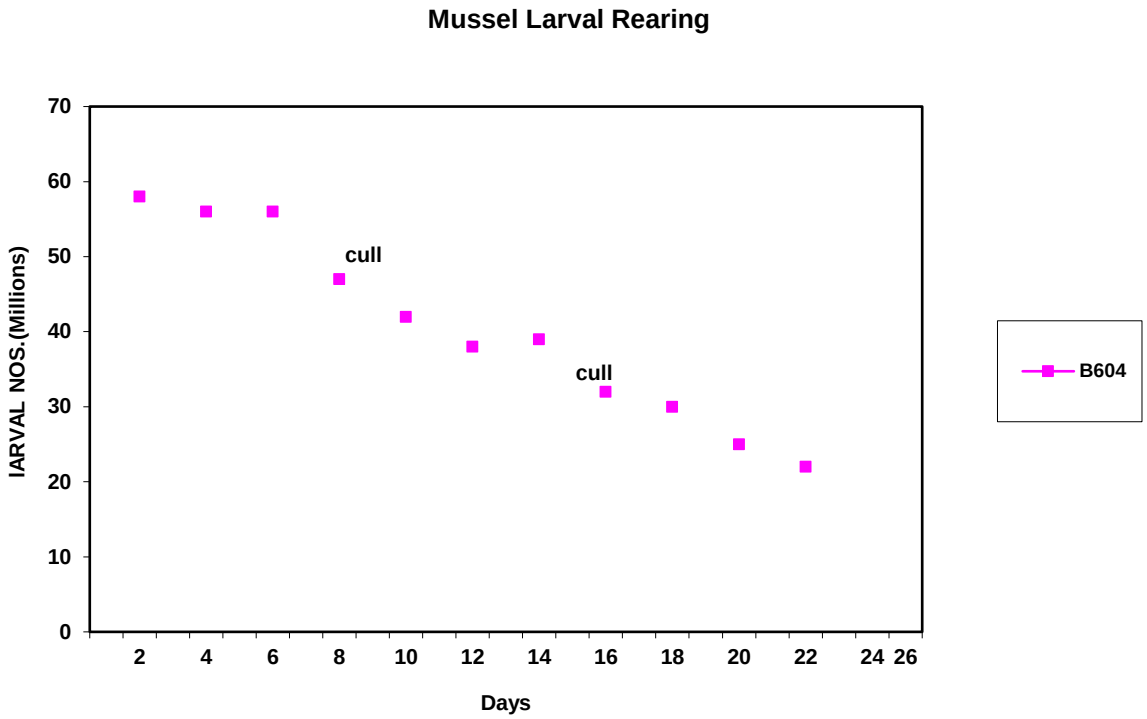
Larval rearing

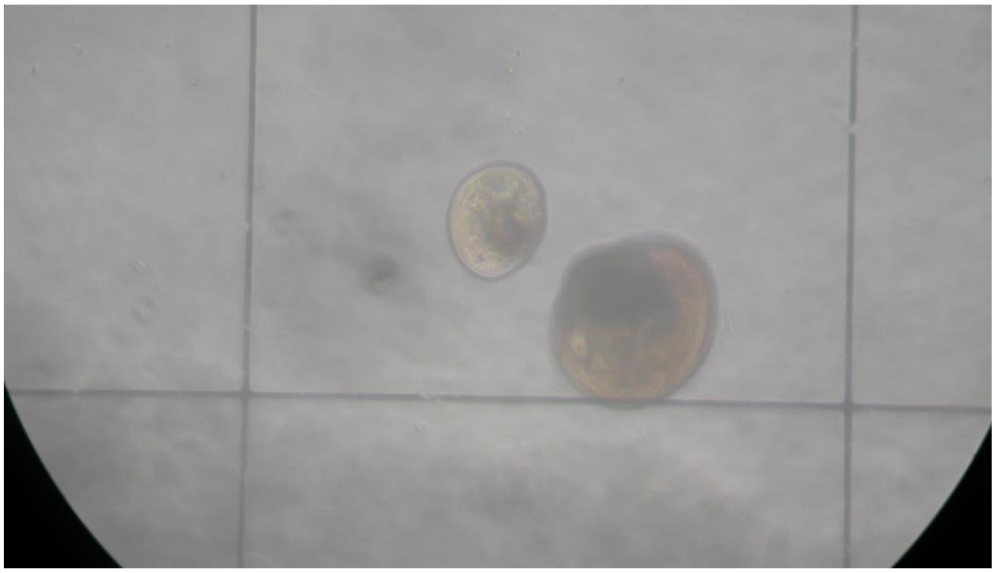
One thousand and 5000l larval bins were used for larval rearing.

Batches of mussel larvae in excess of 10 million are reared in 5000 litre larval bins in a regime of 48 hour water changes and a feeding programme of a mixed diet of diatoms and flagellates fed twice per day to maintain a cell count of 40 to 60 cells per microlitre in the larval bins.

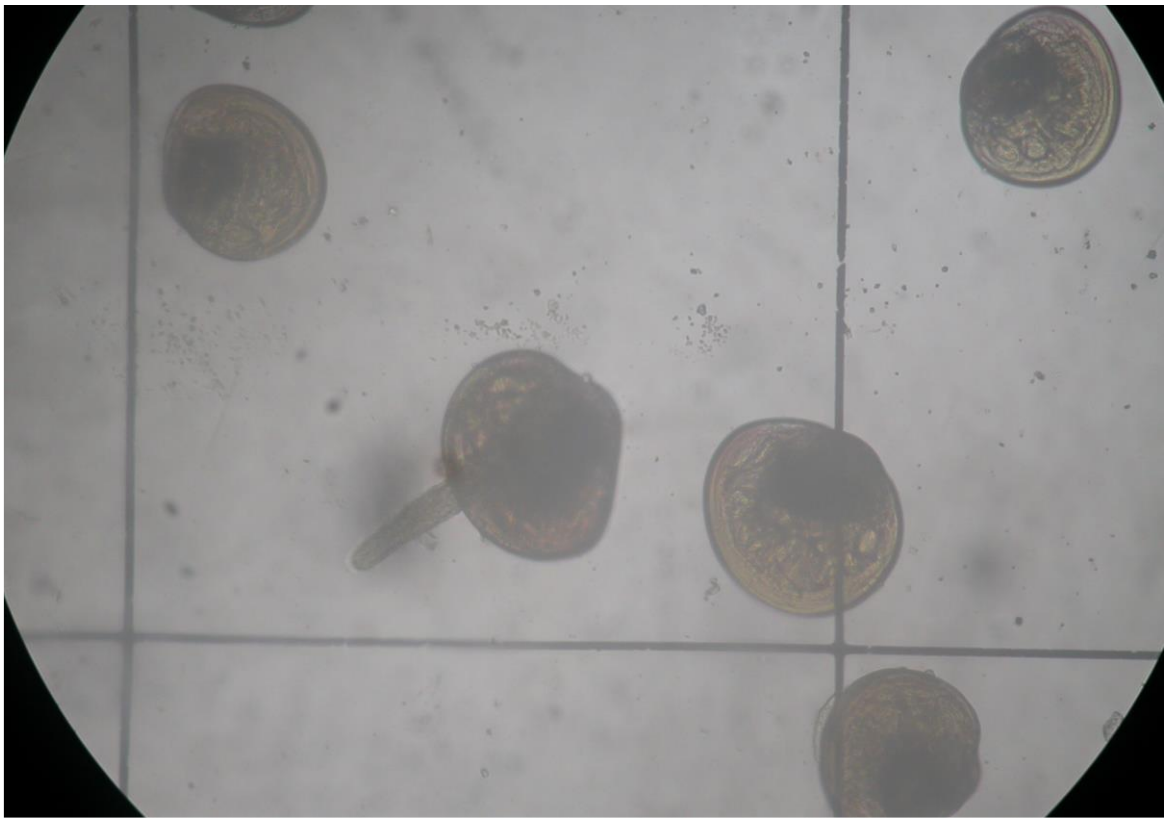
The larvae develop with alacrity at temperature of 20 -21 degrees C reaching the pediveliger stage within 20 days (see plates) the stage of metamorphosis (immediately after the crawling pediveliger stage is attained when the larvae are sitting on a 120-micron sieve.
Larval rearing of *Mytilus edulis* does however present some interesting problems:
There is a greater spread of developmental stages over time in mussel cultivation than is ever the case with oysters or clams. This may result from the fact that all broodstock has been obtained from the wild and thus no selection for rate of development has ever taken place. However, it does mean that in larval rearing in the hatchery an early intervention to cull slow growers is a very effective device to improve the quality and uniformity of the final spat settlement.







18 day old larvae of Mytilus edulis (note contrast in development at same age). Photo: I. Connellan



M. edulis larvae (pediveliger stage) note foot activity. Photo: I. Connellan

Mytilus edulis spat on base of downweller (spat size 2mm). Photo: I. Connellan

Settlement

At day 21, when most mussel larvae are exhibiting foot activity, they are placed in downwelling columns. These are PVC columns with 120-micron base meshes. The settling larvae are fed a constant supply of airlift seawater in the nursery system at a recirculating rate of 16 litres per minute. The water change regime for dowelling sieves is a complete change every 48 hours. Water changes are affected using a bank of 4 cartridge filters achieving a filtration efficiency of 10 microns. The algal ration is fed to maintain algal food density above 30 cells per microlitre. Mussel spats once settled remain within the downwelling system until they leave the hatchery either for sale or for ongrowing. No other species is cultivated in these systems, which are dedicated to mussel culture.

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