

Hatchery Production Performance of the Green Mussel, *Perna viridis* (Linnaeus, 1758)

MOHD SALEH, M.T.

Fisheries Research Institute Pulau Sayak, 08500 Kota Kuala Muda, Kedah

Abstract: The mussel farms in Malaysia are currently dependent on limited spatfall areas and facing inconsistency in the seed supply due to vagaries of nature, pollution, predators, fouling and diseases. As such, the only alternative to overcome the shortage in mussel spat supply is to opt for hatchery production. This project was aimed at producing mussel seeds on a pilot scale so that crucial factors and problems associated with hatchery production of mussel spats in the hatchery could be determined and resolved. Small scale hatchery propagation technique of the green mussel, *Perna viridis* had been developed at Fisheries Research Institute Pulau Sayak, Kedah since 2007. A total of 32 trials on green mussel larviculture were carried out between 2007- 2009 i.e. 8 batches in 2007, 13 batches in 2008 and 11 batches in 2009. From these trials, a total of 43 million eyed larvae were produced with an average survival rate of 6.8% (ranging from 1% to 24%). A total of 569,430 spat ranging from 0.5 to 1.0 cm in size were produced with an average setting rate of 1.6% (ranged from 0.1% to 4.4%). The average density of spat on the rope collectors were 246 spat per meter for low density (ranged from 20 to 527 spat per meter) and 1,067 spat per meter for high density (ranged from 790 to 1,752 spat per meter). Some problems encountered during larviculture were presence of ciliates and uneven spat settlement on the collectors which need to be overcome. The most serious problem was the presence of ciliates which caused heavy mortality of the mussel larvae beginning second week onwards of the culture period.

Keywords: *Perna viridis*, larval rearing, spat production, setting rate, survival rate

Abstrak: Ladang-ladang ternakan siput sudu di Malaysia pada masa ini sangat bergantung kepada sumber benih siput sudu daripada kawasan semulajadi yang begitu terhad dan masih menghadapi masalah bekalan benih siput sudu yang mencukupi secara berterusan kesan daripada faktor-faktor seperti fenomena perubahan alamiah, pencemaran, pemangsa, kekotoran dan penyakit. Oleh itu, alternatif yang ada untuk mengatasi kekurangan bekalan benih siput sudu ini adalah melalui aktiviti pengeluaran benih siput sudu daripada hatceri. Kajian ini dijalankan untuk menghasilkan benih-benih siput sudu di dalam hatceri pada skala percubaan supaya faktor-faktor penting dan permasalahan yang wujud semasa proses penghasilan benih siput sudu tersebut dapat dikenalpasti dan diselesaikan. Pembangunan teknik penghasilan benih siput sudu dalam hatceri telah dijalankan di Fisheries Research Institute Pulau Sayak, Kedah sejak 2007 pada skala kecil. Sebanyak 32 percubaan asuhan larva siput sudu dalam hatceri telah dijalankan di antara 2007-2009 iaitu 8 asuhan pada 2007, 13 asuhan pada 2008 dan 11 asuhan pada 2009. Daripada percubaan-percubaan ini, sejumlah 43 juta larva bermata telah dihasilkan dengan purata kadar hidup sebanyak 6.8% (julat antara 1% hingga 24%). Sejumlah 569,430 benih bersaiz 0.5-1.0 cm berjaya dihasilkan dengan kadar pemendapan larva sebanyak 1.6% (julat antara 0.1% hingga 4.4%). Purata kepadatan benih siput sudu di atas tali pemungut adalah 246 spat per meter tali bagi kepadatan rendah (julat antara 20-527 spat per meter tali) manakala kepadatan tinggi yang dihasilkan adalah 1,067 spat per meter tali (julat antara 790-1,752 spat per meter tali). Antara masalah-masalah yang telah dikenalpasti semasa asuhan larva dan perlu diatasi adalah kehadiran siliat dan ketidaksekataan pemendapan larva di atas tali pemungut. Masalah utama yang wujud adalah kehadiran siliat semasa asuhan larva memasuki tempoh asuhan minggu kedua yang memberi kesan kematian yang amat nyata.

Introduction

Mussel farming is a well established industry in many countries. Although the culture activities deploy intensive labour, it generates good economic returns with relatively little environmental impact. The mussel industry in Malaysia with a production barely exceeding 6,905 metric tons and valued at RM3.86 million in 2006 is sustained by moderate scale cultures in the states of Johor (Selat Tebrau) and Malacca (Sebatu). With the recent increase in the market price for mussels, there is a growing interest among culturists to expand their existing culture areas and explore new areas in other states such as Kedah (Sg.

Merbok), Penang (Penaga), Perak (Sg. Dinding and Kuala Rungkup) and Selangor (Kuala Langat and Kuala Selangor) to farm this species. In addition, there are also a number of opportunities for developing mussel aquaculture activities by exploring and developing new areas including offshore coastal areas.

The expansion of mussel production from its current limited base is expected to bring exponential socio-economic benefits, as it would attract the development of local mussel processing capacity with its associated employment opportunities which will generate income. However, with the increasing areas for culture, the industry is expected to face an acute shortage of mussel seed supply even to sustain the present production. The mussel farms are currently dependent on limited spatfall areas (presently confined to Sebatu, Melaka and Selat Tebrau, Johor) where the problem of inconsistency in supply is further aggravated by vagaries of nature, pollution, predators, fouling and diseases. As such, the only alternative to overcome the shortage in mussel spat supply is to look into ways of producing spat from the hatchery. Jeffs (2003) stated that mussel spat can be produced artificially through breeding mussel and raising them in a shellfish hatchery. There exist the potential to develop the technology for large scale hatchery rearing of mussel spat which will greatly reduce the cost of the spat production. Additionally, the value of hatchery mussel spat can be raised by producing a more valuable product. With the hatchery production of mussel spat, it may also be possible to conduct selective breeding of mussels with more valuable traits, such as enhanced shell colour, faster growth or more nutritious meat. Another area where hatchery spat excels over wild spat is that it is more reliable as there have been several periods of up to a year in Malaysia when wild mussel spat has not been available. A mussel spat hatchery has the potential to supply spat during these periods and attract a commercial premium due to the unavailability of wild spat alternatives. The larval rearing techniques in tropical areas, has been used successfully for the reliable spat production of the green mussel, *Mytilus viridis* (AQUACOP, 1979). A hatchery-nursery program was initiated at the Centre Oceanologique du Pacifique (COP), dealing at first with *Crassostrea gigas*. The technique developed by AQUACOP (1977) was adapted from classical methods described by Loosanoff and Davis (1963), Walne (1966) and Dupuy *et al.* (1977). This technique has been adapted to the tropical conditions and was modified for *Perna viridis* (AQUACOP, 1979; 1980) where about 8.5 million *Perna viridis* spat was produced (Coeroli *et al.*, 1984).

In Malaysia, the hatchery propagation of green mussels, *P. viridis* has been developed at the Fisheries Research Institute (FRI) Pulau Sayak, Kedah on a small scale since 2007 using methods deployed by Tan (1975), Virabhadra Rao *et al.* (1976) and Sivalingam (1977). Preliminary observation indicated that there is great potential for commercial production which would be beneficial in terms of quality and reliability of seed supply. The viability of hatchery production versus wild collection however, will depend on factors such as the cost of production of spat, mussel prices, and the costs of wild spat collection. To date, there is no commercial mussel hatchery operating in Malaysia. As such, this project (at FRI Pulau Sayak) is aimed to record the larvae and spat production performance of green mussel *P. viridis* on a pilot scale which also include the larvae hatching rate, gross observation, survival rate, setting rate and growth.

Materials and Methods

Broodstock and conditioning

A good mussel broodstock batch was procured from mussel culturists and maintained in nearby vicinities (Sg. Dedap, Kedah and Penaga, in Penang) of FRI Pulau Sayak so that they can be readily available for spawning in the hatchery. The gonad conditions of the mussels were monitored from time to time by sacrificing a few adults. Normally ten adults size of 7-8 cm were picked randomly from the cultured site which represents a mark of 10% each during the gonad observation. All the mussel broodstock were opened using oyster knife and each of the gonads were analysed through its shape whether it is full, half full or empty by gross eye observation.

Spawning

Adult mussels were conditioned in holding tanks at ambient temperature and salinity and were fed mixed algal cultures (*Chaetoceros* sp., *Skeletonema* sp. and *Isochrysis* sp.) *ad libitum*. Mature mussels (50 to 100 in number) were selected by plucking them out randomly from the culture ropes. Caution was exercised to retain their byssus to prevent mortality. They were brushed clean and put into a plastic basket

(50 cm x 40 cm x 17 cm). Spawning was induced either by thermal or salinity shocks. For thermal shock, spawning was stimulated by alternately immersing the basket containing mussels in cold water (25°C) for 20 min and subsequently at ambient temperature for another 20 min (29-30°C). As for salinity shock, the basket was immersed alternately in freshwater (0 ppt) first for 20 min and then in seawater (30 ppt) for another 20 min. Usually the males were seen to release the gametes first, followed by the females. The whole spawning process took about an hour. Once spawning was complete, the eggs were collected on a screen (mesh size 15 µm) and subsequently transferred into a bucket for embryo count. After counting, the larvae were transferred into an incubation tank at 20 million/ton. Generally, the fertilised embryo requires about 18 h to attain the straight hinge stage. At this stage, the larvae were harvested and observed under a microscope to confirm that they have successfully developed into the straight hinge or 'D' shaped stage.

Larval growth and survival

At 'D' stage, the larvae from the production trials were stocked into the larval culture tanks of 2 ton capacity at 3-6 million per culture tank. For growth rate monitoring, a total of 100 larvae/day were sampled from each tank. The length and width of the larvae (n=100) were recorded using the micrometer scale on the microscope. As for the survival rate, samples of larvae were taken during complete water change. Larvae were collected in a 20 L bucket and well stirred. Two aliquots of 1 ml samples were taken from each bucket and number of larvae counted. This procedure was repeated for the duration of culture until the larvae attained the pediveliger or setting stage.

Water exchange

Total water change was carried out on alternate days until the larvae attained the eyed stage. However, at this point, water change was done on a daily basis to transfer the pediveligers (those that retained on 200 µm) to the setting tank while returning the smaller larvae to the culture tank (those that pass through 200 µm). A series of screen sizes ranging from 15 to 250 µm were used during water change to grade the larvae.

Feeding

Feeding of algae commenced from the second day of culture onwards. *Isochrysis* sp. was used during the first 7-10 days and mixtures of *Isochrysis* sp. and *Chaetoceros* sp. thereafter till the eyed larvae stage. Algae densities given ranged from 10-30 x 10³ cells/ml for the first week and then increased to 30-70 x 10³ cells/ml during the second week of culture. Once the larvae had set, mixtures of *Isochrysis* sp., *Chaetoceros* sp. and *Skeletonema* sp. were fed at a density greater than 100 x 10³ cell/ml since the filtering rate of the spat is more efficient.

Water quality monitoring

Physical parameters such as temperature, salinity and pH were recorded daily in the morning between 1100-1200 h using thermometer, refractometer and pH meter, respectively.

Setting and density count on collectors

Pediveligers with a black spot or often termed as the 'eye spot' on the body shell were found to retain on screens with a mesh size of 200 µm. These were transferred into setting tanks with rope collectors suspended in the water column. Generally, all larvae were considered set if no larvae were found retained on the screens during water exchange. From this point onwards, the newly set larvae termed spat were fed on mixed algae (*Isochrysis* sp., *Chaetoceros* sp. and *Skeletonema* sp.). They were fed at a density of more than 100 x 10³ cell/ml as at this stage as the filtering rate of the spat become more efficient. These spat were nursed until they attain a mean length of 5 mm when the density on the cultch could be recorded to determine the setting rate.

Results

Thirty two batches of green mussel larviculture were carried out between 2007-2009 i.e 8 batches in 2007, 13 batches in 2008 and 11 batches in 2009 respectively. Details on the production results are shown in Table 1, Table 2 and Table 3, respectively.

Larval production

A total of 2.9 billion embryos were produced during the spawning processes in the hatchery between 2007-2009 i.e. 422 million eggs in 2007, 1,657 million in 2008 and 819 million in 2009. Out of this number, a total of 860 million developed into D-hinge larvae stage i.e. 167 million larvae in 2007, 429 million larvae in 2008 and 264 millions larvae in 2009. A total of 43.39 million of eyed larvae stage i.e. 6.55 million in 2007, 24.66 million in 2008 and 12.18 million in 2009 were produced (Table 1, 2 and 3). The mean hatching rate of embryo to D-hinge larvae are between 34-51% (51% in 2007, 34% in 2008 and 37% in 2009) (Table 1, 2 and 3). After 14-20 days of culture, the mean larvae survival rate from D-hinge larvae to eyed larvae stage obtained are between 5-9% (5% in 2007, 9% in 2008 and 5% in 2009) (Table 1, 2 and 3). The eyed larvae which retained on the 200 μ m screen and stocked in the attachment or setting tank were allowed for settlement on rope collectors for about one week where the mean average setting rate of the eyed larvae obtained after the process were observed to be very low between 1-2% (1% in 2007, 2% in 2008 and 1% in 2009) (Table 1, 2 and 3).

Spat production

The spat density and production from 2007-2009 are shown in Tables 4. A total of 569,430 spats with a size range of 0.5-1.0 cm were produced from the 32 batches of larviculture between 2007-2009 i.e 76,694 spats in 2007, 447,606 spats in 2008 and 45,130 spats in 2009. The average density of spats on the rope collectors was 246 spats per meter for low density (ranged from 20 to 482 spats per meter) whereas for high density was 1067 spats per meter (ranged from 790 to 1,752 spats per meter) as shown in Table 4.

Table 1: Larvae and spat production performance of green mussel *P. viridis* on a pilot scale in 2007

Mussel Stage	2007 (8 Batches)								Mean /Total
	1	2	3	4	5	6	7	8	
Embryo (million)	13.92	n.a	n.a	n.a	374.40	n.a	n.a	47.5	422
Larvae (million)	5.20	14.59	0.65	012.03	84.70	5.25	27.20	37.50	167
Hatching Rate (Embryo to D hinge larvae) (%)	37%				23%			79%	51%
Eyed larvae (million)	Dead D11	Dead D11	Dead D7	Dead D13	2.08	Dead D15	1.97	2.50	6.55
Larvae survival rate (D-hinge larvae to eyed larvae) (%)					2%		7	7%	5.3%
Setting rate (%)					1.5%		0.2%	1.6%	1.1%

Table 2: Larvae and spat production performance of green mussel *P. viridis* on a pilot scale in 2008

Mussel Stage	2008 (13 Batches)													Mean /Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Embryo (million)	142.00	121.00	210.48	156.30	119.00	36.69	74.43	248.00	66.97	15.94	5.17	95.43	384.00	1675
Larvae (million)	24.33	61.58	40.61	39.20	27.37	26.83	23.50	31.40	12.91	7.50	2.92	45.55	85.00	429
Hatching Rate (Embryo to D hinge larvae) (%)	17%	51%	19%	25%	23%	73%	32%	13%	19%	47%	56%	48%	22%	34%
Eyed larvae (million)	1.08	1.17	Dead D13	Dead D13	3.99	1.67	5.09	4.59	2.67	0.27	Dead D12	1.00	3.13	24.66
Larvae survival rate (D-hinge larvae to eyed larvae) (%)	4%	2%			15%	6%	24%	13%	21%	4%		2%	4%	9.5%
Setting rate (%)	3.0%	2.6%			3.8%	4.4%	1.0%	1.8%	0.4%				0.5%	2.2%

Table 3: Larvae and spat production performance of green mussel *P. viridis* on a pilot scale in 2009

Mussel Stage	2009 (11 Batches)											Mean /Total
	1	2	3	4	5	6	7	8	9	10	11	
Embryo (million)	36.88	172.54	12.00	17.65	62.48	36.77	26.62	30.00	105.00	34.45	285.00	819
Larvae (million)	18.16	34.31	8.90	3.57	12.99	8.84	13.15	10.87	27.65	17.00	108.57	264
Hatching Rate (Embryo to D hinge larvae) (%)	49%	20%	74%	20%	21%	24%	49%	36%	26%	49%	38%	37%
Eyed larvae (million)	1.50	1.30	0.20	0.15	0.55	0.23	2.30	0.60	0.40	0.35	4.60	12.18
Larvae survival rate (D-hinge larvae to eyed larvae) (%)	7%	3%	2%	4%	4%	3%	17%	5%	1%	2%	4%	4.7%
Setting rate (%)	1.4%	-	-	-	-	0.6%	0.9%	0.1%	-	-	0.001%	0.6%

Table 4: Spat production performance of green mussel *P. viridis* on a pilot scale in 2007 to 2009

Density/Year	2007 (3 Batches)			2008 (8 Batches)								2009 (4 Batches)				Mean /Total
	5	7	8	1	2	5	6	7	8	9	13	1	6	7	8	
No. of Collector	270		85	100	100	310	120	100	130	67	120	50	40	100	27	1619
Spat Density : Spat/rope	-			853	790	1752	1108	801	1096	-	-	-	-	-	-	1067
High No. of rope	-			17	24	79	39	30	31	-	-	-	-	-	-	220
Spat Density : Spat/rope	116		477	213	149	53	382	399	482	169	128	527	71	154	20	239
Low No. of rope	270		85	83	76	231	81	70	99	67	120	50	40	100	27	1399
Total spat produced	31,400	4,749	40,545	32,180	30,284	150,651	74,154	51,960	81,694	11,323	15,360	26,350	2840	15,400	540	56,9430

Note: Spat settled on the wall of the culture tank

Larval growth

The growth of the green mussel larvae from 8 trials in 2007-2009 are shown in Fig. 1. Results showed that the D-hinge larvae (length and width size of $83.0 \pm 5.0 \mu\text{m}$ and $61.0 \pm 5.0 \mu\text{m}$, respectively) attained the eyed larvae stage mostly at day 14 with length and width size of $274.0 \pm 25.0 \mu\text{m}$ and $249.0 \pm 27.0 \mu\text{m}$, respectively.

Larval survival

The survival rate of the green mussel larvae from 25 trials between 2007-2009 are shown in Fig. 2. Survival rate was around 57% at day 7 and dropped drastically thereafter from day 9 until the end of the larviculture i.e. at day 15 attaining mean survival rate of 7%.

Water quality

The physical water quality parameters i.e. temperature, salinity and pH are shown in Fig. 3. These parameters monitored did not exhibit any major fluctuations during the culture periods and were observed to be within the optimal values for larval rearing. The temperature was in the range of $28.5 - 30.0^\circ\text{C}$, salinity 30-31 ppt and pH ranged from 7.4-8.0 throughout the culture period.

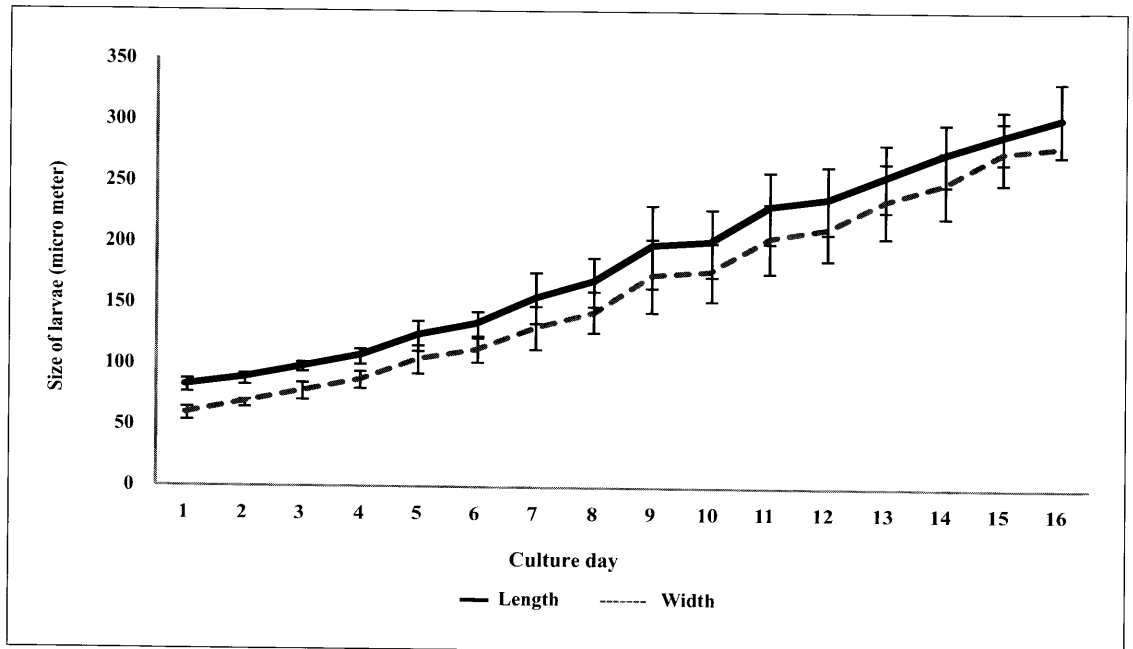


Figure 1: Growth (length and width) of the green mussel larvae from D-hinge larvae to eye larvae stage in culture tank

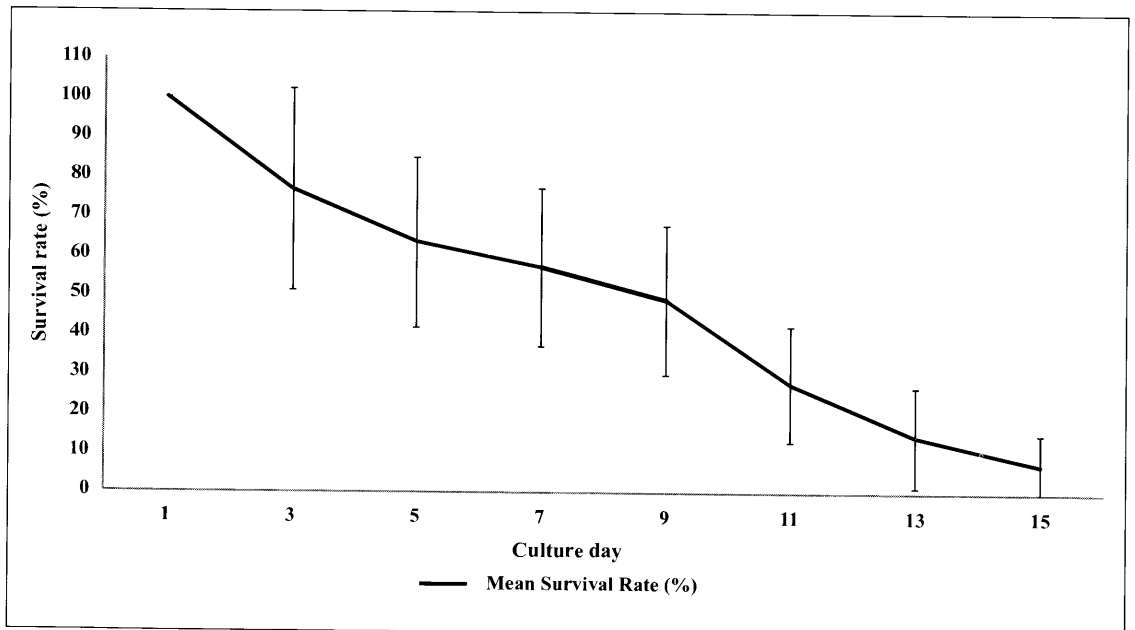


Figure 2: Mean survival rate (%) of the green mussel larvae from D-hinge larvae to eyed larvae stage in culture tank

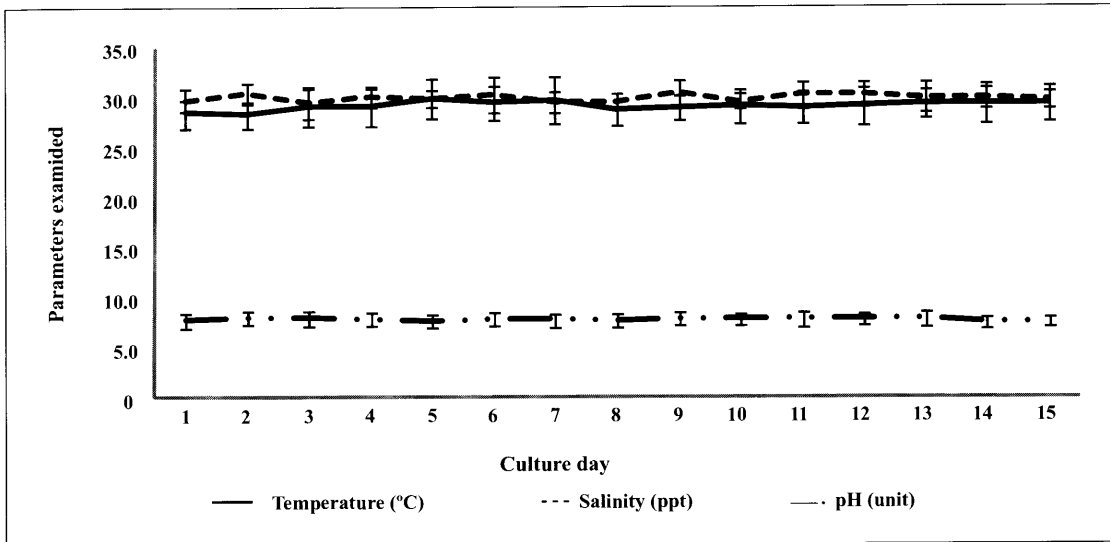


Figure 3: Water quality parameter of the green mussel larviculture trials from 2007-2009

Gross and microscopic observation

Gross observations were recorded during the larviculture activity as shown in Photo 1 - 4. Photo 1 shows observation on colour changes of larvae within the 14 days of culture period. The colour changed from orange at day 1 (embryo or egg) to purplish from day 1-2 (D-hinge larvae), brownish from day 3-9 (early to mid umbo), greyish brown from day 10-13 (late umbo) and finally to black from day 14 (eyed stage) to pediveliger stage. Photo 2 shows the microscopic shape of the various larval stages according to colour changes as mentioned above. During the eyed larvae stage, lots of whitish streaks of byssal thread were seen in the bucket filled with mussel larvae (Photo 3) harvested from the culture tank, indicating that it is time to transfer the larvae into the setting tank.

Low survival rate experienced throughout the culture trial from 2007-2009 showed that the main cause of mass mortality in almost every culture batch was due to the mass presence of ciliates in the culture tank. Ciliates are living protozoans and are known to multiply rapidly by consuming dead organisms in which in this case is dead mussel larvae. Once they are dominant in the culture tanks, they can be seen in every empty shell of the dead larvae (Photo 4).



Photo 1: Gross observation of mussel larva on harvest screen

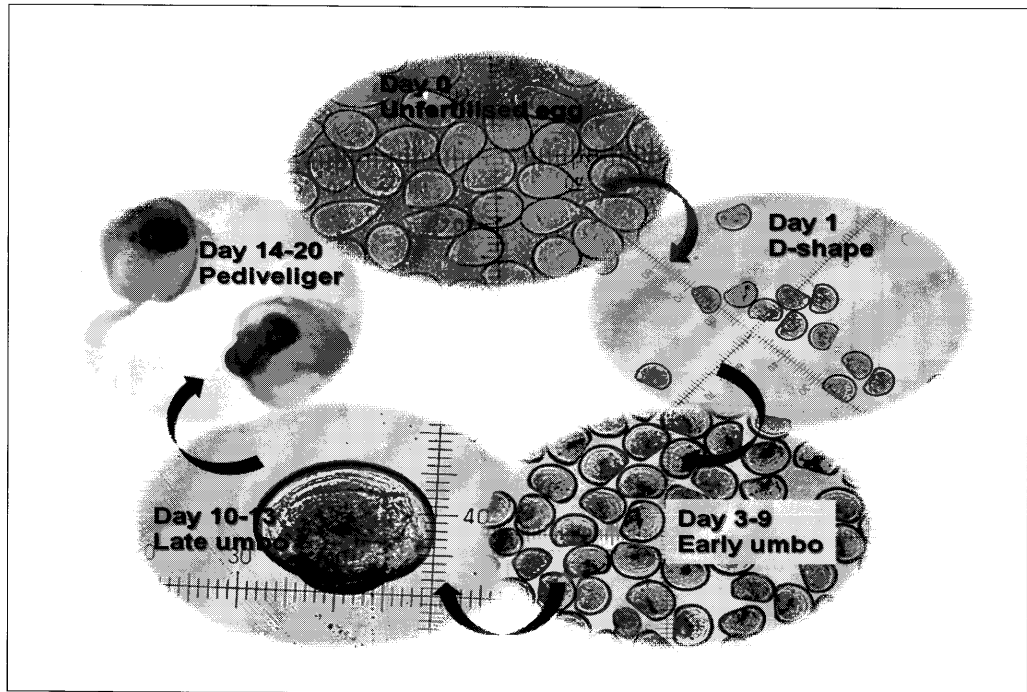


Photo 2: Microscopic observation of mussel larva under microscope

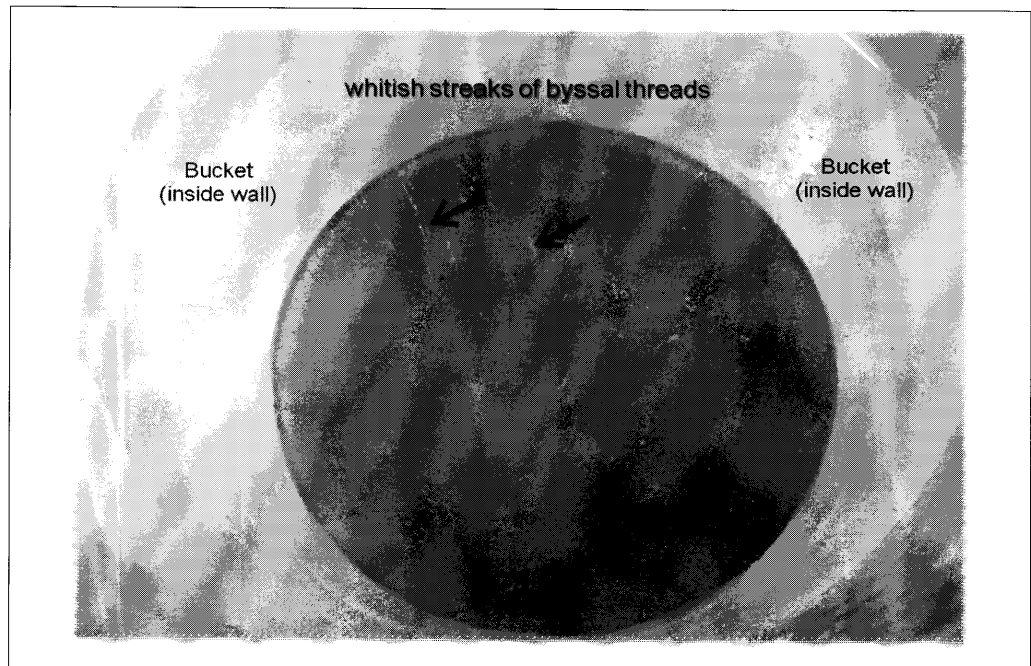


Photo 3: Gross indication of larvae setting (arrows - whitish streaks of byssal threads)

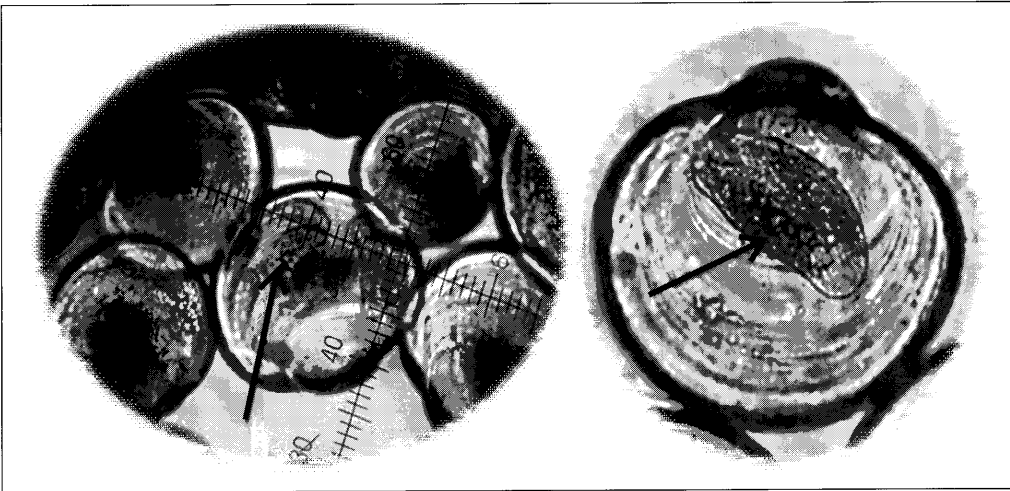


Photo 4: Presence of ciliates in mussel larvae shell at pediveliger stage (arrows)

Discussion

In Tahiti, where no species of mussels are found in the natural environment, large scale culture operations had to rely on the hatchery spat production. Since 1978, induced spawning was routinely achieved throughout the year using imported broodstock, and larval rearing method proved to be reliable (AQUACOP, 1979). Among those who had put into effort on developing the hatchery spat production is Rao *et al.* (1976) who described spawning, fertilization and larval development of the green mussel *P. viridis* from the Goa Coast in India and Appukuttan *et al.* (1987) who detailed larval rearing and spat production of the brown mussel *Perna indica*. To determine the viability of green mussel seed production in the hatchery, the survival and setting rate should be an important indicator besides the growth (length and width). In this study, the survival rate obtained was 57% at day 7, 30-50% at day 10 and further dropped below 10% at day 15 (Fig. 2). These survival rates are generally low as compared to the results achieved by French Polynesia (Table 6). AQUACOP (1980) reported that only 5-20% of larvae attained settlement stage in many bivalve species, whereas these rates recorded about 50% for *P. viridis*. As for the survival rate between the D-stage and marketable sized spat obtained was only 10% (AQUACOP, 1979). Efforts are being geared towards increasing the survival rate from Day 7 onwards through the target lines as shown in Fig. 8 from 10% to 30-40%. This could be done by controlling the feeding and stocking densities in the culture tank. The use of tropical algal strains *Isochrysis* sp. and *Chaetoceros gracilis* improved the quality of diet. The use of a mixture of two tropical strains *Isochrysis* sp.-*Chaetoceros gracilis* enhanced the growth rate and resulted in better survival rates at day 10. Another suggestion was to deploy bigger tanks in the second week of culture where eight 800 L tanks were compared to a 10,000 L tanks for an equivalent production which consumed less water. The use of such rearing tanks is cheaper and easier to build besides increasing the efficiency of the design as well (AQUACOP, 1979).

The same situation was also experienced with the setting rate i.e. very low setting rate (mean setting rate of 1.6%) obtained in this study as compared to 30-60% setting rate achieved by French Polynesia as shown in Table 5. The settlement rate was estimated at 45% where this rate takes into consideration the spat settled on the collectors and on the walls of the 10 m³ tank. Improvement in the settlement rate could be obtained by hanging up more nylon net collectors in the rearing tanks to maximise surface areas for settlement. The density before settlement had to be adjusted to 2,000 larvae/L, as it was shown that higher density at that stage induced a delay in settlement (AQUACOP, 1979).

Results from these propagation trials appear promising i.e. achieved 57% survival at day 7 while more research work is needed to reduce mass mortality during the second week of the culture period.

However, several other problems related to production and survival rates also need to be overcome. Some of the problems include the presence of ciliates, poor water quality condition due to silt, solving inconsistent supply of matured broodstock due to unpredictable weather conditions and innovations to solve uneven density of spat settlement on collectors. AQUACOP (1979) had reported that chemicals such as sulfadimerazine can be used to control ciliates. It was observed that bacteria control using this chemical gave good results when culture water was treated with 14 mg per liter of sulfadimerazine every 2 days (Coeroli *et al.*, 1984). However, Utting and Spencer (1991) were of the opinion that successful hatchery production of larvae and spat is more related to the skill and experience of the staff rather than the facilities and equipment. Among future focus of studies are on effects of water flow and aeration bubbles on spat settlement, using bigger capacity tanks i.e. more than 10 tons of water during the second week of culture, using other types of collectors and controlled feeding by changing the feeding rate from number of cells per ml of water to number of cells per individual larvae.

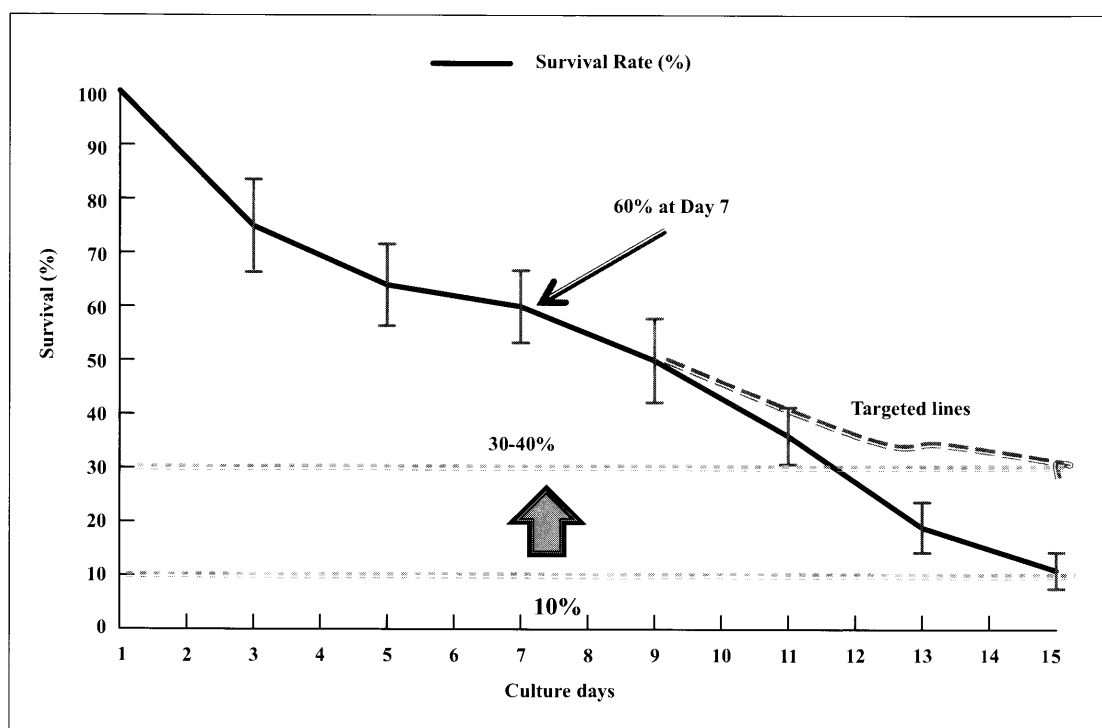


Figure 5: Target for survival rate improvement plan of the mussel larviculture in the hatchery

Table 5: Comparative results of mussel larviculture between the present study (Malaysia) and other countries

Country	Present study	French Polynesia	India
Fertilisation rate	34-51%	90%	85-90%
Larval survival rate (Day 10)	30-50%	70-90%	n.a
Setting rate	1-2%	30-60%	2.5-23.5%

Acknowledgments

The author wishes to gratefully acknowledge Ms. Devakie Nair (Senior Research Officer) for sharing her ideas and experiences on this project and also for reviewing the manuscript. Thanks are also due to Mr. Mohd. Amer Halib (Research Assistant) for the hard work during hatchery operation and to Mr. Mohd. Khairul Anwar Zakaria (Contract Worker) for helping out with the ground work.

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