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Use of micron meshed cages for the nursery rearing of hatchery produced green mussel *Perna viridis* spat

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Abstract

The Asian green mussel, *Perna viridis* (Linnaeus,1758) is a major resource of the Indian coast,as they are one of the most preferred edible bivalves. Recent years have witnessed a high demand for mussel seed for farming. Mass production of mussel seed in a technologically sound and cost-effective manner is inevitable for increasing the mussel production. Hatchery production and nursery rearing trials of *P. viridis,* conducted at CMFRI hatchery has rekindled the hope for developing commercially viable mussel seed production technology in India. Mussel hatchery trials revealed that a production rate of more than 0.1-0.5 million spat can be realized from a one ton capacity FRP tank within 30-40 days and 0.1 million spat can be nursery reared to seed size (8-12 mm) in a nursery-cage within 45 days. Larval rearing and use of micron meshed cages for the nursery rearing of hatchery produced *P. viridis* spat; ensuring the

economically feasible large scale production of green mussel seed is discussed.

Keywords Green mussel, hatchery, *Perna viridis*, seed, micron meshed cages

Introduction

Mussel farming is practiced in the estuarine areas during post monsoon months using rack and rope method or on-bottom method along the southwest coast of India (Velayudhan *et al*., 2007). After reaching a peak production of 18400 t in 2009, farmed mussel production has stagnated around 9000-10000 t per year. Large scale collection of seed from the natural mussel beds for farming has, with the increased adoption of green mussel farming in northern Kerala along the west coast of India, led to conflicts between mussel pickers and farmers in the past. Experimental larval rearing and spat production of several bivalves have been successfully carried outin the past (Loosanoff and Davis,1963; Hrs-Brenko,1973; Bayne,1976; Morse *et al*., 1978, Alagarswami *et al*., 1983; Appukuttan *et al*., 1987; Appukuttan, 2001, Narasimham *et al*., 1988; Muthiah *et al*., 1992, 2002; Laxmilatha *et al*., 2011). There is growing demand for mussel spat from mussel farmers from the west coast of India and elsewhere (Biswajit, 2011; Laxmilatha *et al*., 2011). However, the technology of mussel seed production remained restricted to a few experimental trials in the laboratory. Moreover, production volumes are declining

due to the lack of dependable supply of quality seed. The present study was taken up to make the mussel spat production economically viable by standardizing the seed production technology of green mussel, *Perna viridis* and taking the spat at an early stage to sea for nursery rearing reducing the cost of production.

Materials and Methods

Spawning of *P. viridis* and hatchery rearing of larvae

Green mussel specimens were brought from Chennai located along the east coast during the month of January 2015, as it was non breeding season along the west coast. Mussels were kept at 22°C in an air-conditioned room overnight and transferred to seawater at 30°C to induce spawning by thermal stimulation in the hatchery of Vizhinjam Research Centre of Central Marine Fisheries Research Institute. Fertilized eggs were collected using 20 μ sieve and incubated in 200 litre glasstanks at the rate of 40000-60000 eggs litre^{-1} with aeration for hatching and development. On the next day, the D-veligers were filtered through a 40 μ sieve and stocked in one-ton FRP tanks for further rearing. The larval density was maintained at 20 larvae ml^{-1} from D-veliger which was reduced to 10 larvae ml^{-1} when the larvae reached late umbo-stage. Larvae were fed with microalgae species comprising *Isochrysis galbana*, *Pavlova lutheri,* and *Chaetoceros calcitrans* in the proportion 2:1:1 at the rate of 5000-100,000 cells

larvae^{-1} according to the size of larvae. Complete water exchange was given every third day and larvae were shifted to new tank or restocked after thorough cleaning, till the larvae started settling on tank bottom and sides. Bottom was siphoned and the larvae collected in the sieve were washed and restocked on the other days since stocking density was kept high. Once larvae started settling, drained tanks were cleaned by splashing water over the tank sides and bottom and restocking the larvae collected in the sieve during siphoning after washing the larvae.

Nursery rearing

On the 42nd day when the spats attained an average size of 2.14 mm antero-posteriorly (APM), 1.36 mm dorso-ventrally (DVM) and with an average weight of 0.001 g; they were harvested (Fig. 9 & 10)from the rearing tanks and transferred to sea for nursery rearing. For nursery rearing trials, micron meshed cages (Fig. 11) with a sieve size of 1x1mm of 37 cm and 93 cm length with corresponding diameters of 9 cm and 10 cm were used. Cages were made of 1mm mesh and had zippers to close them.They had volumes of 7300.5 cm^3 and 9410 cm^3 respectively. The stocking density was 50,000 and 100,000 spats per cage. In the Vizhinjam bay, nursery cages were hung from the raft system. Spats was also stocked in FRP tanks maintained in the hatchery at a stocking density of 2 lakh spat one ton tank^{-1} and fed with mixed culture dominated by *C. calcitrans* at the rate of more than 100000

cells spat^{-1}. Biometric studies were conducted on the spat under two rearing conditions every 15 days.

Hydrology of Vizhinjam Bay

In order to have better understanding of the growth of *P. viridis* spat, water quality parameters of Vizhinjam Bay were recorded during the rearing period of study. Physicochemical and hydrobiological parameters such as temperature, turbidity, TSS, salinity, pH, dissolved oxygen, nitrate, nitrite, phosphate, ammonia and total chlorophyll were measured using standard techniques. Ammonia level was checked using the phenol hypochlorite method (Solorzano, 1969). Nitrate, nitrite and phosphate were checked using nitrate, nitrite and phosphate testing kits (MERCK) and photometric methods (NOVA60, Spectroquent). Temperature (Centigrade thermometer), dissolved oxygen (Winkler, 1888), pH (Compact pH meter, LAQUAtwin), turbidity (Nephelometer), salinity (Master Refractometer, ATAGO) were checked fortnightly. Chlorophyll concentration was analysed with UV Visible Spectrophotometer (Evolution 201) (Strickland & Parsons, 1972).

Results

Larval rearing

Adults were removed after spawning (Fig. 1) from the 200 litre spawning tank (Fig. 2) and the fertilized eggs were filtered through a 20 μ sieve. The collected eggs were incubated in 200 litre glass tanks. Fertilized eggs started diving in 20 minutes and


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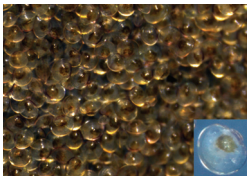
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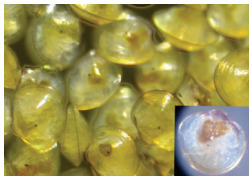
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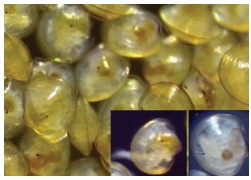
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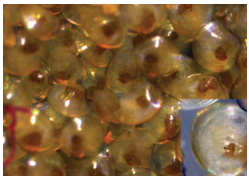
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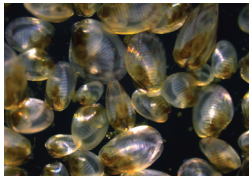
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1: Spawning of *P. viridis*
2: Fertilized egg
3: D-veliger stage
4: Umbo stage
5: Eye-spot stage larvae
6: Pediveliger stage
7:Plantigrade stage
8: Spat (21st day) settlement
9: 42 day old *P. viridis* spat
10: Harvesting of mussel spat
11: Stocking in micron meshed cages
12: Mussel seed - 45th day of nursery rearing

attained trochophore stage in 6 to 8 hours. In 18-20 hours larvae attained D-shape veliger stage (Fig. 3). On day 7, the D-veligers transformed to umbo stage (Fig. 4). Larvae attained eyespot stage (Fig. 5) on days 12-13 with the development of ctenidial edges. Development of foot was observed on 15-17 days indicating the pediveliger stage (Fig. 6) with the formation of gill filaments. During the pediveliger stage, larvae were competent for swimming with velar cilia as well as crawling with the functional foot. Along with the formation of the foot, ciliated velum disappears and the larvae start settling to the bottom with the appearance of gill. Plantigrades (Fig. 7) were seen on the 19th day onwards. Settlement of spat (Fig. 8) was started from the 21st day onwards and continued till 28th day.

Results of the biometric studies conducted on the spat under two rearing conditions (nursery cages and FRP tanks) are given in Table 1. After one month interval the spat reared in micron meshed cages show an increase in growth by an average APM of 8.42 mm, an average DVM of 4.24 mm and an average weight of 0.063 g. On the other hand, spat reared in hatchery showed only limited growth.

At the end of 45 days, spat reared in nursery-cages showed significant increase in size (average of 9 mmAPM, 4.48 mm DVMand 0.1g in weight) with a survival rate of more than 95% (Fig. 12). The similar sized spat reared in one ton FRP tanks didn't show any notable increase in the growth at the end of 45 days rearing (average of 2.7mm APM, 1.62 mm DVM and 0.009 g in weight). Seed grown in the nursery cages can be used for seeding

nursery ropes for further growth. After 45-60 days of rearing in micron meshed cages, the spat can be transferred to ropes.

Table 1.Growth of *P. viridis* spat in the two rearing systems.

	In micron mesh cage			In hatchery tank		
	APM & SD (mm)	DVM & SD (mm)	Wt (g)	APM & SD (mm)	DVM & SD (mm)	Wt (g)
Initial average	2.14± 1.09	1.36± 0.53	0.001	2.14± 1.09	1.36± 0.53	0.001
Final average (45th day)	9.02± 1.22	4.48± 0.96	0.1	2.7± 0.86	1.62± 0.72	0.009

Table 2. Hydrographical parameters during nursery rearing

No.	Parameter	Vizhinjam Bay Surface	Mid	Bottom
1	Depth (m)			6
2	Temperature	26.6±1.08	26.6±1.08	26.6±1.08
3	DO (mg/l)	6 ±0.37	6±0.37	5.4±0.36
4	pH	8±0.05	8.1± 0.06	8.1±0.06
5	Salinity (ppt)	34±1.31	34.9±1.33	34.6±1.3
6	Turbidity (NTU)	0.32±0.05	0.28±0.03	0.24±0.02
7	TSS (mg/l)		0.008±0.0007	
8	Nitrate (mg/l)		0.336±0.006	
9	Nitrite (mg/l)		0.008±0.0007	
10	Total N (mg/l)		0.373±0.006	
11	Ammoniacal N (mg/l)		0.069±0.003	
12	Reactive P (mg/l)		0.008±0.0007	
13	Total P (mg/l)		0.050±0.0006	
14	Total Chlorophyll (mg /m^3)		2.946±0.01	

Hydrological parameters during nursery rearing

Hydrological parameters of the Vizhinjam Bay (surface, mid and bottom) recorded during the nursery rearing of *P. viridis* larvae are given in Table 2. During the nursery rearing trial, hydrological parameters did not show any significant variation from the normal coastal seawater conditions of southwest coast of India.

Fouling and predation

Every fourth day cleaning/scrubbing of the cages was done to prevent silt accumulation and clogging by sponges and other epi-fauna growing on the cage, facilitating the free flow of water and algae through the mesh. Since tiny crabs entering the cage can cause considerable mortality, cages were inspected for small crabs and removed. Bigger crabs were found tearing the cages containing seed sized mussels of more than 10 mm. Subsequently all the cages were covered by old fish net of 15-20 mm mesh size to prevent crab attacks.

Discussion

The present study revealed that *P. viridis* larvae can be reared at a high density of 20 larvae ml^{-1}during D-shape and 10 larve ml^{-1} from late umbo stage. As in any larval rearing trial, water quality and feed play important roles in addition to stocking density. According to Kamermans *et al*. (2013) stocking densities of approximately 10 larvae ml^{-1} appear to be optimal for most bivalves, allowing good survival rates, although larvae can be

cultured at higher concentrations using flow-through or recirculating systems requiring complicated rearing systems. Current hatchery phase trials proved that larvae can be stocked upto 20 larvae $ml^{-1}$and reared successfully in one ton flat rectangular FRP tanks. Mussel larvae are temperature sensitive and show better growth rate at higher temperatures providing a rapid growth rate at tropical temperatures; this is evident from the faster growth of mussel larvae in the present study. Diet is the major component affecting the growth and survival of *P. viridis* larvae. A mixed diet is recommended (Marshall *et al*., 2010) to be added to rearing vessels at the required density. In the larval phase, mussel larvae are fed with mixed microalgal feed viz; *I. galbana*, *P. lutherii*, *Dicretaria* spp., till spat settlement and after that they were provided with mass cultured diatom species dominated by *C. calcitrans* as well as *Thalassiossira* spp. Since the size and nutritional quality of each algae varies, mixed diet is proved to be better than monodiet for *P. viridis* larvae in every stage. By providing the appropriate diet, maintaining cleanliness and with an appropriate stocking density; production of *P. viridis* spat can be enhanced.

According to Kamermans *et al*. (2013), mussel seed are ready for transfer to on growing site at 5 to 10 mm size but he opines that early transfer to field is associated with greater loss of mussels. In Europe and US various upwelling and down-welling rearing techniques are used to rear oyster, scallop and clam spat to required size for growing on farm. Upwelling/down-welling

systems use a series of cylindrical containers in which spat are stocked for nursery rearing and water is circulated with pump or air lift and fed with algal culture in fresh or preserved form. In some cases, larvae are allowed to settle on ropes kept in the rearing systems and the ropes are taken out for further rearing. In this case a good proportion spat attached to the rope will be lost due to detachment. In Europe, the blue seed project was initiated in 2005, to standardise seed production of *M. edulis* and *M. galloprovincialis* on commercial scale (http://www.blueseedproject.com) and found mussel seed production uneconomical (in places where large scale seed collection from the wild is possible), unless some improvement is made in the quality of seed such as triploid production. Floating upweller System (FLUPSY), Solar flupsy, flowthrough systems and recirculation system are being developed for nursery rearing of bivalves in USA, Canada and Europe (http://www.reproseed.com andhttps://viudeepbay.com). Faster growth rate reported from tropical region and cost effective rearing methods can make the mussel seed production economically viable in tropical countries such as India. Larval/juvenile crabs entering the cages must be meticulously removed without which there can be considerable mortality. Attacks by larger crabs are more evident in cages stocked with larger mussel seed with disastrous consequences. This can be effectively prevented by covering the micron-meshed cage with old netting of 15-20 mm mesh size. In the present study, micron meshed cages used were simple and effective for spat rearing with good

growth performance and survival rate of more than 90% if cleaned properly on every 3rd or 4thday to wash away the silt and fouler clogging the mesh.

Conclusion

There is a high demand for mussel seed for farming. Mass production of mussel seed in a cost-effective method is inevitable for increasing the mussel production. However, the technology of mussel seed production remained restricted to experimental trials in the laboratory. Moreover, production volumes are declining due to the lack of dependable supply of quality seed. The present study was taken up as a step to make the mussel spat production economically viable by standardizing the seed production technology of green mussel *P. viridis* and taking the spat at an early stage to sea for nursery rearing reducing the cost of production. Present hatchery trials proved that a production rate of more than 0.1-0.5 million spat can be realized from a 1 ton capacity FRP tank within 30-40 days and 0.1 million spat can be nursery reared to seed size (8-12 mm) in anursery-cage within 45 days.

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