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Induced Spawning and Early Development of *Modiolus philippinarum* (Hanley, 1843) (Bivalvia: Mytilidae)

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ABSTRACT

A study was carried out to apply the induced spawning methods for a potential mariculture mussel species *Modiolus philippinarum*. Matured broodstock were collected from Merambong Shoal seagrass bed, Pulau River Estuary Johor and induced to spawn with two induced spawning methods. Mussels were successfully spawned after six hours of normal ambient exposure but failed to spawn when exposed to thermal cycling procedure. A total of 3.735×10^6 eggs were collected with an average of $81.07 \pm 3.42 \mu\text{m}$ in diameter. Early developments of *M. philippinarum* embryos were firstly recorded. First polar body was observed 10 min after fertilisation and early blastula with cilia was recorded 170 min after fertilisation. Embryos developed into late gastrulas after 5 h 30 min and early trochophores seen after 7 h. D-shaped veliger larvae with length averaged $99.51 \pm 9.98 \mu\text{m}$ and height averaged $81.87 \pm 8.14 \mu\text{m}$ ($n = 30$) were recorded after 27 h and only 23% of the eggs reached D-shaped veliger stage. Larval development ceased to progress on the third day into larval stage and failed to reach metamorphosis.

Key words: Induced spawning, larval development, embryonic development, *Modiolus philippinarum*

INTRODUCTION

With long shoreline along the coasts of east and west Malaysia, Malaysia is a country with great potential in mariculture. However, mollusc culture is relatively less established in Malaysian mariculture sector (Anonymous, 2009) with only three types of mollusc cultured in Malaysia for the local food market which are *Perna viridis* (green-lipped mussel), *Anadara granosa* (blood cockle) and *Crassostrea* spp. (edible oysters). Other edible mollusc such as abalone, clams, scallops, several species of gastropods are either being cultured in fully-controlled system invested by foreign company or simply collected from the wild in unsustainable ways. Among mussel species, green-lipped mussel *P. viridis* is still the only species farmed commercially in many Asia countries, other mussel species such as the brown mussel, *Modiolus metcalfei* and *Modiolus philippinarum* which form dense mats on muddy bottoms are simply gathered from the wild (Lovatelli *et al.*, 1990).

Merambong Shoal is a subtidal seagrass bed which only exposed during low spring tide. Located at the Pulau River Estuary, it is the largest seagrass bed in Malaysia with 30 ha exposed area during low spring tide and has the highest seagrass diversity in the country with 10 species

recorded as in Bujang *et al.* (2006). This place has been the collection site for bivalves and gastropods for both self-consume and earning extra income by providing them to the local market vendors. Other than the most targeted gastropod, *Strombus* spp., they also collect *Pinna* spp. and *Atrina* spp. (pen shells), *Macra* spp. and *Meretrix* spp. (clams), *M. philippinarum* (horse mussel), *Placuna ephippium* (saddle oyster) and occasionally *P. viridis* (green-lipped mussel) and *Pinctada fucata* (Japanese pearl oyster). The demands of edible bivalves are high and solely relying on wild harvesting will distress the already disturbed biodiversity in this delicate seagrass ecosystem. *Modiolus philippinarum* has been collected for food in many areas included Malaysia and the Philippines (Poutiers, 1998). Though according to Poutiers (1998) some experiments on the artificial establishment of this species have been carried out in Malaysia, to date the mass culture of this species is yet to develop. The objective of this study was to discover a low cost yet effective induced spawning method of *M. philippinarum* with the intention to explore the possibility of this target species as a new aquaculture product for the local community.

MATERIALS AND METHODS

Mature specimens with estimated length more than 10 cm were collected by August 2011 from Merambong Shoal seagrass ecosystem at Pulau River Estuary, Johor (1°19.55'N, 103°35.57'E) and were brought back to the laboratory in Institute of Bioscience, Universiti Putra Malaysia. After a 48 h conditioning period, specimens were ready for induced spawning.

Two separated induced spawning methods were tested on the mussels, with the first method includes thermal cycling procedure and the second method where specimens were treated with oxygen stress. Ten mussels were randomly selected for each method. They were cleaned externally to remove any encrusting epifauna and thoroughly rinsed by UV-irradiated seawater before being placed into separate spawning tanks. Each tank was installed with mild aeration, a thermometer and covered by black plastic sheet on one side to provide a dark background against which the released gametes can be easily seen.

Thermal cycling procedure: The tank for thermal cycling induced spawning procedure was filled with UV-irradiated seawater at 32 ppt and maintained at room temperature between 24-25°C. The procedure was started with cold treatment. Temperature of the seawater was first lowered to 16°C with ice packs and maintained for 45 min. After the cold treatment, a heater was used to raise the temperature of the seawater to 28°C and was hold for 45 min before another cooling process begin. The thermal cycling procedure was repeated until the first bivalve start spawning. However, if the mussels do not spawn after in treatment for 4 h, they would be returned to the holding tank.

Oxygen stress (aerial exposure): The method of induced spawning through oxygen stress by air exposure is adapted from Farfan *et al.* (2007). After 6 hours of exposure, 10 selected mussels were placed into a glass tank with UV-irradiated seawater at 32 ppt and 26°C. Temperature were then gradually raised (1°C in every 30 min) and the procedure will be ceased when the mussels do not spawn when the temperature reached 33°C.

Embryonic and larval development: As soon as the mussels started to release gametes, sex of each individuals were determined based on the appearance of the released substances and immediately removed from the spawning tank, rinsed thoroughly with UV-irradiated freshwater and transferred to separate tanks. Gametes were held in UV-irradiated seawater in controlled

condition (32 ppt; 26°C) until the spawning. A small amount of male gametes were then added into the eggs holding tank and stirred gently. Observations were made from time to time until D-hinged shells were observed on larvae. D-hinged larvae were siphoned and retained on a sieve (35 µm) and were rinsed thoroughly with UV irradiated seawater. Larvae were kept in the density of 5 individuals per mL and were fed with *Isochrysis galbana* at a concentration 1.0-1.5×10⁵ cell mL⁻¹. The growth and survival rates were monitored every alternate day by shell-size measurement and survival estimation.

RESULTS

Oxygen stress (aerial exposure): The first male mussel spawned after 45 min and 1°C temperature rise followed by spawning of the first female mussel a minute apart. All specimens released their gametes within the next 20 min. The spawning mussels were removed when the amount of released gametes decreased. The unfertilised eggs (Fig. 1a) are orange in colour, spherical in shape, with diameter ranged from 70-90 µm and averaged 81.07±3.42 µm. A total of 3.735×10⁶ eggs were collected with the average of 6.225×10⁵ eggs per female (n = 6). Holding density during fertilisation was about 120 eggs mL⁻¹.

Embryonic development: First polar body was observed 10 min after the male gametes were added into the eggs holding tank (Table 1). Two-cell was observed 20 min after the fertilisation and reached 4-cell, 6-cell, 8-cell and multicell stages after 45, 55, 65 and 85 min, respectively (Fig. 1b-j). Early blastula with cilia (Fig. 1k) developed 170 min after fertilisation and started spinning slowly with beating cilia. Late gastrula (Fig. 1l) with invagination of the shell field was observed after 5 h 30 min. Early trochophores were observed after 7 h while late trochophore stage with apical tuft and developing shells (Fig. 2a-b) is reached about 18 h after fertilisation with average height 76.24±3.64 µm and width 68.63±3.39 µm (n = 30). The height of central apical tuft averaged 51.64±6.81 µm (n = 5).

Larval development: Early veligers forming velum and without shell were developed after 22 h. Shell formation and degeneration of apical tuft could be observed (Fig. 2c-f) in the following hours and D-shaped larvae were first observed after 27 h. Shell length of D-shaped larvae averaged 99.51±9.98 µm and height averaged 81.87±8.14 µm (n = 30) and approximately 23% eggs survived and reached D-shaped larval stage. At this stage, the free-swimming larvae have straight-hinged

Table 1: Timing of the early embryonic development of *M. philippinarum* at 26°C and 32 ppt

Stage	Time after fertilisation
Egg	0
1st Polar body	10 min
2-celled stage	20 min
4-celled stage	45 min
6-celled stage	55 min
8-celled stage	65 min
Multicelled stage	85 min
Early blastula	170 min
Late gastrula	330 min
Early trochophore	420 min
Late trochophore	18 h

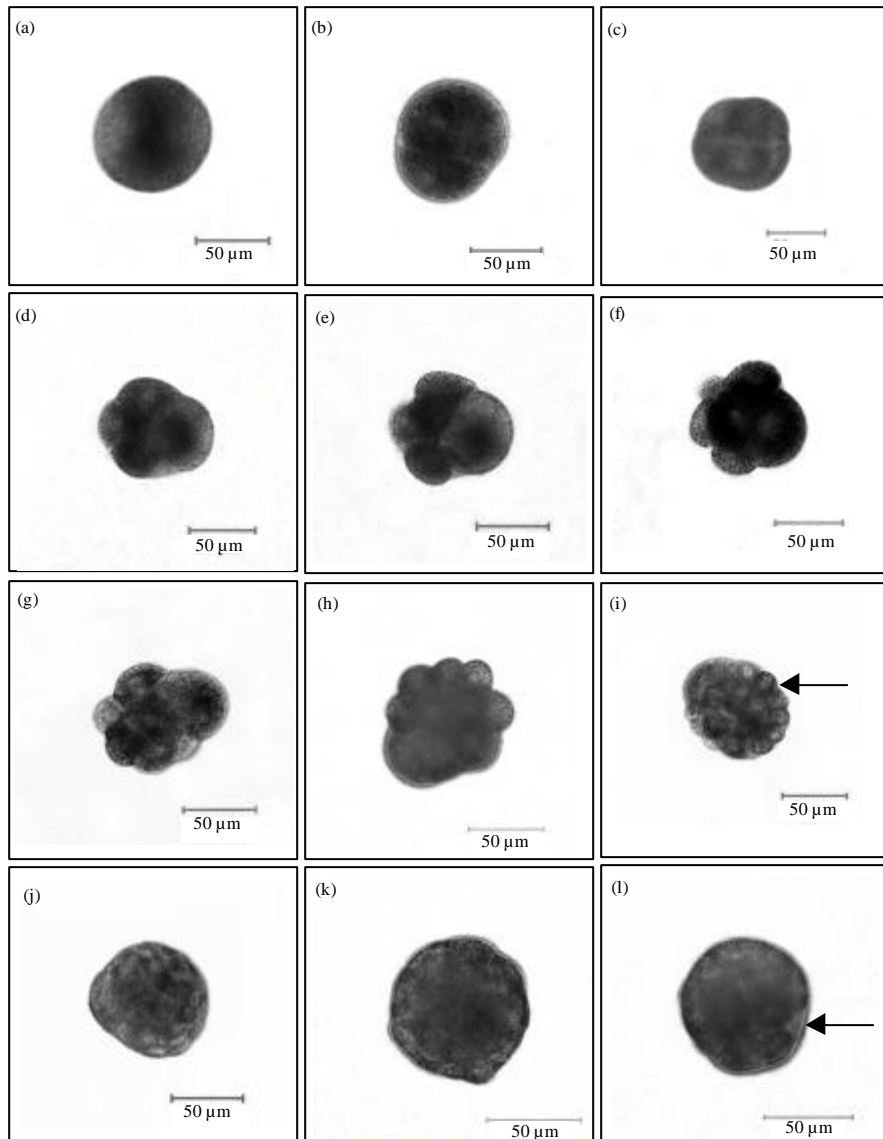


Fig. 1(a-l): Early development of *Modiolus philippinarum*, (a) Unfertilised egg, (b) 2-celled stage, (c-j) Developments from 4-cell, 6-cell, 8-cell to multicellular stage, (k) Early blastula and (l) Late gastrula stage with imaginations of the shell field (arrow)

shell and visible velum and started feeding on microalgae. Despite having successfully reached D-shaped larval stage, the larvae only survived for less than three days and little survivals were left on the fourth day.

DISCUSSION

Through this study, the induced spawning procedure for *M. philippinarum* has been determined. The procedure only involved the use of basic tools and equipment excluding the use of any chemical. Aerial exposure of six hours prior to water temperature increase produces sufficient stress in *M. philippinarum* where the specimens responded swiftly to seawater

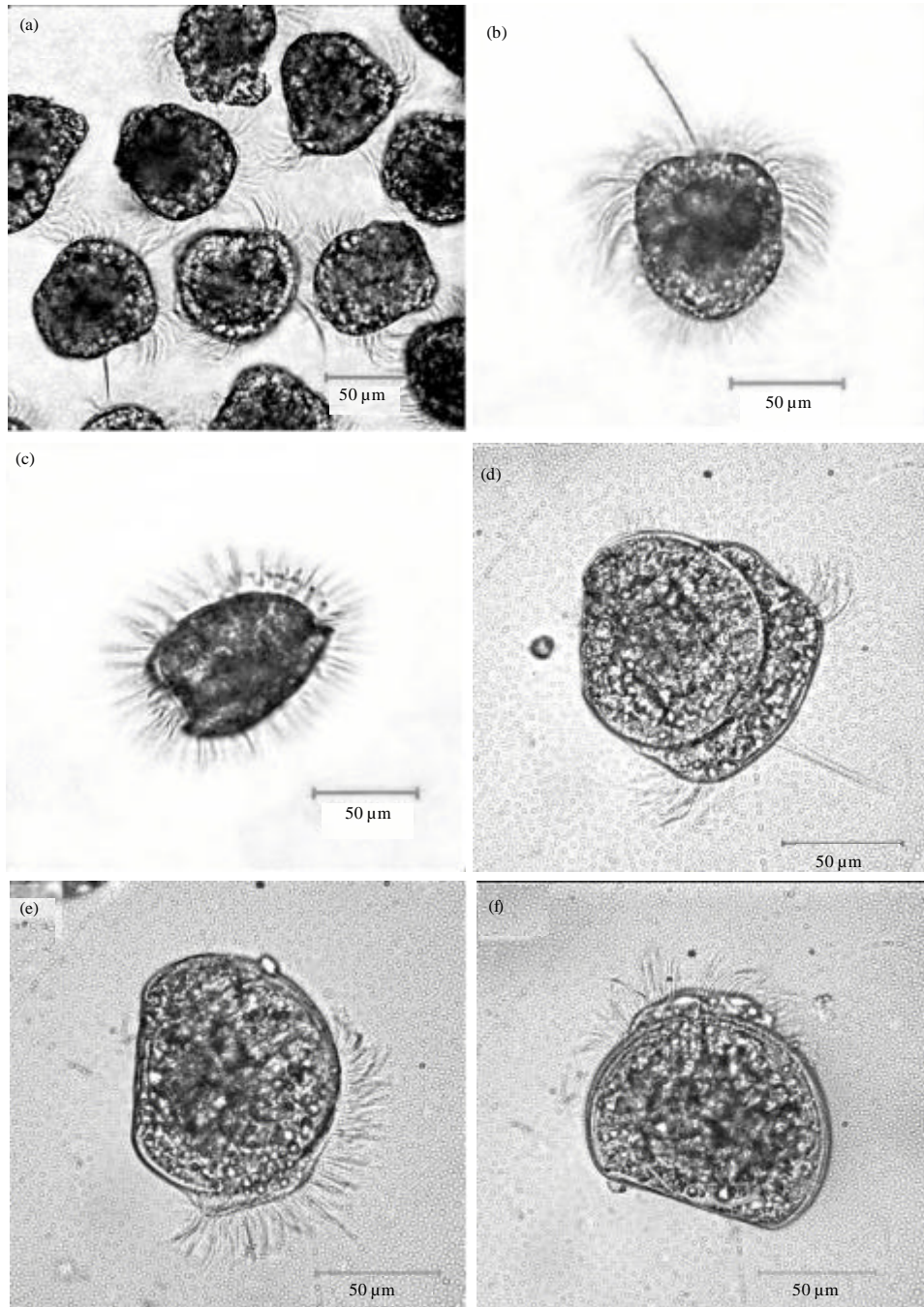


Fig. 2(a-f): *Modiolus philippinarum* (a-b) Trochophore stage with apical tuft, (c-d) Early veliger stage forming shells but apical tuft still visible and (e-f) D-shaped larval stage losing apical tuft

temperature raise stimulation and started to spawn in just 45 min into the procedure. On the other hand, thermal cycling procedure with cold and heat shocks as described in Helm *et al.* (2004) failed to stimulate the spawning of *M. philippinarum*. While the fertilisation and embryonic development

of this species have been observed and monitored up until the shelled larval stage, the larvae failed to survive beyond the third day.

Various induced spawning methods have been used on different mussel species in order to produce seeds in hatchery. Induced spawning, larval rearing and spat production of several mussel species such as *P. viridis* (Sivalingam, 1977; Stephen and Shetty, 1981; Rajagopal *et al.*, 1998; Manoj Nair and Appukuttan, 2003; Laxmilatha *et al.*, 2011), *Perna perna* (Stephen and Shetty, 1981), *M. capax* (Farfan *et al.*, 2007), *Mytilus edulis* (Galley *et al.*, 2010), *Mytilus galloprovincialis* (Satuito *et al.*, 2005; Ruiz *et al.*, 2008) have been successfully produced in laboratories and hatcheries, but little attention was paid in the production of mussel spat in large scale hatchery and the mussel industry still relied on the supply of seed from the wild (Alfaro *et al.*, 2010; Laxmilatha *et al.*, 2011).

Since the bloom of mollusc aquaculture, researchers have been exploring induced spawning procedures and trying to determine the most efficient protocol for each bivalve species. Procedures such as temperature and salinities stimulation, aerial exposure and chemical stimulations have been tested for several mussel species. Sivalingam (1977) reported that induced spawning by plain temperature jumps, pH or salinity variations were ineffective. However, four temperature cycles of 20 min duration between 25 and 35°C with 0.2% NH₄OH in seawater medium had successfully induced the spawning of *P. viridis*. Stephen and Shetty (1981) reported that three experimental stimulations, e.g., rapid salinity change, rapid salinity change with addition of gametes and stable salinity with addition of gametes triggered the spawning of ripe *P. viridis* and *P. perna*.

Though chemical spawning inductions are common practice among researcher, such as Fluvoxamine used on *Mytilopsis leucophaeata* (Verween *et al.*, 2007), induced spawning methods which involve stimulation of physical factors (e.g., temperature and salinity) are still preferred especially for small scale fisheries farmers. The result of this study was intended to be duplicated by the local fish farmers; therefore, induced spawning procedures with simple tools were selected and tested on selected mussel, *M. philippinarum*.

Modiolus philippinarum developed into D-shaped veliger 27 h after fertilisation. However, early development progress might be subjected to change in different geographical environments as reported in green-lipped mussel *P. viridis*. *Perna viridis* embryos developed into veliger at 16 h in Sivalingam (1977) but took 20-22 h to reach D-shaped veliger stage in Laxmilatha *et al.* (2011). Aside from different localities, larvae of *P. viridis* are temperature sensitive where in some study the optimum larval development occurred at 31°C while higher temperature (33 and 35°C) caused total mortality and at lower temperature (24°C) larvae took longer to settle (Manoj Nair and Appukuttan, 2003).

There are many factors influencing the survival rate of bivalve early development, egg density is one of the most debatable factor. During fertilisation, *M. philippinarum* eggs were kept at 120 eggs mL⁻¹ and only approximately 23% survived and reached D-shaped larval stage. Galley *et al.* (2010) commented that high egg density beyond a certain concentration during embryonic development in *M. edulis* will reduce the percentage of embryos that develop into normal larvae. The theory is proven in *Pinctada maxima* and *P. margaritifera* as in Southgate *et al.* (1998) where highest survival was shown at 10 eggs mL⁻¹ and lowest at 100 eggs mL⁻¹. As *M. edulis* eggs settle to the bottom after released, the surface area of the holding tank and the concentrations of eggs were critical factors in early development before the embryos become mobile (Galley *et al.*, 2010). However, the optimum egg concentration per surface area is still debatable. While low surface area density (20 eggs cm⁻²) was suggested by Sprung and Bayne (1984) for *M. edulis*,

results by Galley *et al.* (2010) suggest that reliable concentration is at 200 eggs cm⁻², while densities as high as 1,000 eggs cm⁻² are advocated by Helm *et al.* (2004). Thus, Galley *et al.* (2010) concluded that though egg density does influence the proportion of normally developed larvae, it cannot be assured solely by manipulating the egg densities.

The bivalve culture industry in Malaysia is still in its infancy while the actual consumption and trade rate are still not clear. Annual Fisheries Statistics in 2008, it is reported that the sum value of squid, cuttlefish, octopus and other mollusc, live, fresh, chilled or frozen only accounted for 12.1% of the total export in fisheries products while canned crustaceans and molluscs was 0.8% of the total export (Anonymous, 2008). All these products contributed in more than RM435 million in export value. However, the true export value of mollusc is unknown. On the other hand, similar products were import with the total import value RM114 million in 2008 and again, the true import value of mollusc is not specified. Bivalves such as *Anadara* spp., *Macra* spp., *Spinulosa* spp., *Pholas* spp. and many other species are commonly seen on Malaysians' dining table, relying solely on wild harvesting will only bring negative environmental impact and brings temporary benefits to the harvesters. Therefore, more study and research should be carried out to explore potential marine bivalve species for aquaculture; for the benefit of the natural environment and to expand the potential of marine aquaculture industry.

CONCLUSION

The result of this study is encouraging as this can be successfully induced to spawn in a low cost yet effective way. Though more works need to be done to establish a complete larval rearing protocol, this study shed positive lights on introducing new species to the local mariculture industry, empowering the local with simple yet effective bivalve culture technique and enable species restocking in the seagrass ecosystem when necessary in future.

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