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# Embryonic Development of Asiatic Hard Clam, *Meretrix meretrix* (Linnaeus, 1758) in Hatchery Condition

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## ABSTRACT

Asiatic hard clam (*Meretrix meretrix*) is one of Malaysia's highly demanded bivalve species. Studies show that the clam population in some places, particularly in Marudu Bay is experiencing overexploitation. Thus, conservation effort through aquaculture is greatly required. The current study aimed to induce the spawning of *Meretrix meretrix* in hatchery condition using the thermal shock method. Ten specimens of the *M. meretrix* measuring between 5.00 and 7.00cm in shell length were induced to spawn by temperature shock at 5- $6^0$C above the ambient temperature of seawater. Throughout the experiment, the clams were fed a live feed ration of mixed *Isochrysis* sp., *Nannochloropsis* sp., and *Chaetoceros* sp. at the ratio of 1:1:1 with the total concentration of $7.0 \times 10^7$ cells/broodstock twice a day at 07:00 and 17:00. The results demonstrated that the temperature shock induced 70% of the clams to spawn with a high fertilization rate (76.89%) but with a moderate hatching rate (47.39%). It was observed that the egg's first cleavage (2-cell stage) was recorded 20 minutes after fertilization. The trochophore and D-shaped veligers formed 13 and 24 hours after fertilization. Veliger with cilia formed 2 days after fertilization. On the 6$^{th}$ day after fertilization, the veliger was observed to settle on the substrate. On the 10$^{th}$ after fertilization, spat began to develop umbo. Spats with complete umbo were observed 19 days after fertilization and on the 43$^{rd}$ day, the spats reached the juvenile stage. The present study demonstrates that the *Meretrix meretrix* clam can be induced to spawn by temperature shock. It took approximately 43 days for its larvae to fully develop into the juvenile stage. Further studies on improving the hatching rate, survival rate, and growth performance are necessary for economic seed production in the hatchery.

## INTRODUCTION

Aquaculture of mussel (*Perna viridis*) and tropical oyster (*Magallana bilienata*) has been widelyestablished in Malaysia. However, there are many commercially important bivalve species in Malaysia whose productions are dependent on natural sources. This

includes the Asiatic hard clam (*Meretrix meretrix*). *Meretrix* clam belongs to family Veneridae which inhibits the South and Southeast Asian regions **(Liu *et al*. 2006)** and the Sabah on the coastal waters of Malysia **(Tan & Ransangan, 2019)**. Recently, the market demand for the clam has tremendously increased, and it has become one of Southeast Asia's most significant commercial bivalves **(Desrita *et al.*, 2019)**. *Meretrix* also appears to have high market demand in Malaysia **(Hamli *et al*., 2015)**. This has inspired the coastal communities to enhance fishing the clam. However, unregulated exploitation could cause the natural stock of the clam to decline **(Ni *et al*., 2011)**. In fact, the natural stock of the clam in Marudu Bay, Malaysia has already shown signs of overexploitation due to increased fishing activity **(Tan *et al.*, 2017; Tan & Ransangan, 2019; Admodisatro *et al*., 2021)**. Despite overexploitation, attempts to produce the clam through aquaculture have not yet been initiated. Although aquaculture production of this species has been reported as possible in India **(Narasimham *et al*., 1988)**, China **(Tang *et al*., 2006)**, Taiwan **(Huang *et al*., 2016; Chang *et al*., 2020)**, it hasn't yet been initiated in Malaysia.

The findings of recent studies **(Tan & Ransangan, 2019; Admodisastro *et al*, 2021)** justified new management and conservation efforts for this commercially valuable clam to prevent further decline and avoid reaching further points beyond recovery. This can be accomplished through artificial seed production in hatcheries and the transfer of the seeds to natural habitats for stock enhancement and sea ranching. This, however, requires a thorough investigation of the clam's capability to reproduce feasibly in hatchery conditions.

According to **Aji (2011)**, bivalves can be induced to spawn in captivity by a variety of methods including air-drying, the alteration of the temperature of culture water, manipulation of water pH or light intensity, exposure to gamete suspensions, dense phytoplankton concentrations, exposure to reducing agents such as hydrogen peroxide or the combination of these methods. Nevertheless, many studies utilized the heat-shock spawning induction method as it is readily accessible compared to other methods **(Narasimham *et al*., 1998)**. The temperature shock is the most employed spawning induction technique, especially for temperate species. While for tropical species, it does not have such a defined effect due to the general stability of seawater temperature throughout the year **(Nowland *et al*., 2021; Bolasina, 2022**). However, thermal induction has been reported to successfully induce *Meretrix* clam to spawn by raising the water temperature from approximately 4 to $5^0$C above the ambient level **(Utting & Millican, 1997**). The current study aimed to induce spawning in *M. meretrix* study in captivity using thermal shock.

## MATERIALS AND METHODS

### Sampling of clam

Specimens of the Asiatic hard clam, *Meretrix meretrix* used in this study were sampled from its natural habitat in Marudu Bay, Sabah, Malaysia (Fig. 1) from April to November 2019. Marudu Bay is located about 120 kilometers north of Kota Kinabalu, the state capital of Sabah, and is positioned between latitudes 6°15″ and 6°45″ and longitudes 116° and 117° E. The bay is fringed by mangrove forests rich in fauna and flora **(Zakaria & Rajpar, 2015)**.

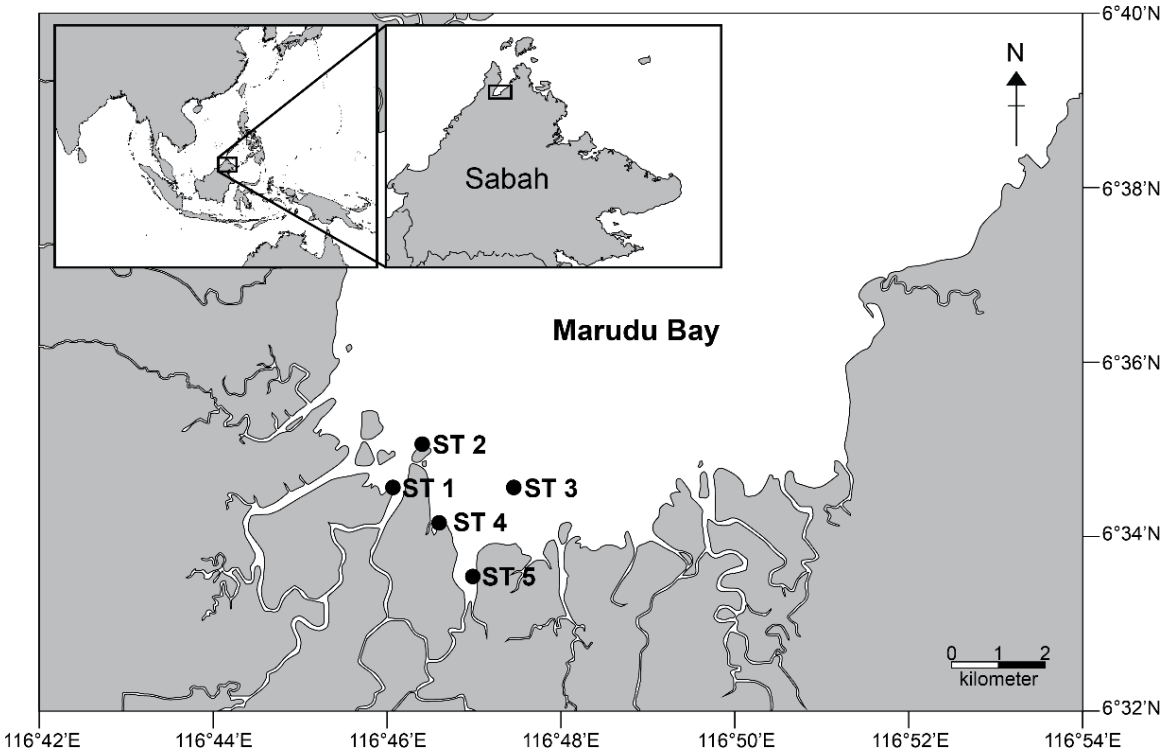


**Fig. 1.** A map showng sampling sites of the *Meretrix meretrix* clam in Marudu Bay

The *Meretrix* clams were sampled using an improvised bivalve fishing gear, locally known as a *kerek* following the method of **Tan and Ransangan (2019)**. The clams buried themselves beneath the sand, hence, the harvesting activity was done during low tide when the sampling areas were dried and exposed. About 120 clam specimens were collected for this study. However, only 10 individuals were used for the spawning experiment.

### Transportation of specimens

The clam specimens were stored in a polystyrene box in wet condition and transported on land back to the Bivalve Mini Hatchery at the Borneo Marine Research Institute, Universiti Malaysia Sabah for acclimation and conditioning before being

induced to spawn. The transportation took about two hours and a half. At the hatchery, the clams were measured, weighed, rinsed with seawater, and kept in a flow-through tank system for acclimatization and conditioning.

**Broodstock conditioning**

The clams were conditioned in two flow-through tank systems with dimensions of 1 x 1 x 0.5m. The bottom part of the tanks was filled with beach sand (100 – 500 µm particle size) of 10cm thickness to function as a substrate for the clams to burry during the conditioning period. Each tank was individually supplied with an aeration tube. The shell length of the clams specimens was measured using a Vernier calliper (Misumi, Malaysia) to the nearest 0.1mm. Clams measured between 5.00 cm to 7.00 cm in shell length were used in this study. The clams were distributed evenly in the tank so that each tank contained 5 clams and were conditioned for 14 days. During the conditioning, the clam specimens were fed on mixed live microalgae (*Isochrysis* sp., *Nannochlorophis* sp., and *Chaetoceros* sp) at a total concentration of $7.0 \times 10^7$ cells/clam with an equal ratio of 1:1:1 twice a day at 07:00 and 17:00.

**Water quality monitoring**

The seawater used in the current study was pumped from the sea nearby the Bivalve Mini Hatchery. The seawater was passed through a sand filtration system (Waterco, Malaysia) before use. Monitoring of water quality parameters (water temperature, salinity, dissolved oxygen, and pH) was done three times daily i.e., before the cleaning routine in the morning (7.00 to 7.30 am), at noon (12 to 12.30 pm), and in the evening (5.00 to 5.30 pm). The seawater was allowed to flow through the tank system throughout the conditioning period at the rate of 1000 ml/h. Throughout the study period, the seawater recorded the following measurements: temperature ranged from 24.0 to $31.5^0$C, salinity ranged from 29 ppt to 34 ppt, dissolved oxygen ranged from 3.74-4.71 mg/l and pH ranged from 6.1 to 8.3, respectively.

**Spawning induction**

A total of 10 clam specimens with size between 5.00 and 7.00 cm in shell length as suggested by **Duisan *et al*. (2021)** were induced to spawn. Briefly, every clam was first put in a 2-L aerated induction tank and exposed to a gradually increasing temperature of 5°C - 6°C higher than the ambient seawater temperature using an electrical heater for two hours. Afterward, the clams were transferred to another 2-L spawning tank containing ambient temperature filtered and sterilized seawater and monitored for any spawning activity up to three hours following the induction. The total number of eggs released by the clam was counted using the Sedgewick-rafter under a light microscope (Leica ICC50 HD). The eggs of the *M. meretrix* were observed either pear-shaped or ball-shaped. According to **Chen *et al*. (2020),** pear-shaped eggs are immature whereas ball-shaped are the ones matured and ready for fertilization. The sperm

of *Meretrix meretrix* is considered primitive or termed ectaquasperm and consists of three parts: a head, a midpiece, and a flagellum (**Dong *et al*., 2010; Chen *et al*., 2020**). Under the light microscope, the sperms of *Meretrix meretrix* appeared to have bright yellow reflections of a round shape (the head) and move very rapidly.

**Embryonic development**

As soon as the clams spawned following temperature shock, 250ml of male gametes was collected and mixed with 250ml of female gametes in an incubation tank containing 2.5L of filtered and UV-treated seawater. Then, every significant embryonic developmental stage was recorded and observed every minute using an inverted compound microscope (Olympus, IX73) for up to 24 hours. The fertilization rate was estimated using the following formula.

$$Fertilization\ rate\ (\%/ml) = \frac{Total\ fertilized\ eggs\ (1ml)}{Total\ eggs\ (1\ ml)} \times 100$$

Fertilized eggs were identified based on the number of eggs undergoing cell division (mitosis) over the total number of eggs in a 1 ml sample. Likewise, the hatching rate was estimated based on the number of fertilized eggs successfully developed into the D-stage veliger over the total number of fertilized eggs in the 1 ml sample.

$$Hatching\ rate\ (\%/ml) = \frac{Total\ D-shaped\ larvae\ (1ml)}{Total\ eggs\ (1\ ml)} \times 100$$

Subsequently, 1000ml of the water containing D-shaped veligers was transferred into a 7-L plastic transparent tank for further observation. The density of the D-stage veligers in the tank was adjusted to 90 larvae/ml, and 1 ml of the larvae was taken using a sterile pipette for observation every day up to the tenth ($10^{th}$) day and continued observation on the nineteenth ($19^{th}$) day and on the forty-third ($43^{rd}$) day respectively. During observation, the larvae were photographed and preserved in 10% buffered formalin for later analyses. Throughout the monitoring of the embryonic development, the larvae were fed with mixed live microalgae consisting of *Isochrysis* sp., *Nannochlorophis* sp., and *Chaetoceros* sp at the total concentration of 3.5 x $10^5$ cells/larvae, with an equal ratio of 1:1:1 twice a day at 0700 and 1700.

## RESULTS AND DISCUSSION

In the present study, the *M. meretrix* clams measuring 5.0 to 7.0 cm in shell length were ready to be induced to spawn by the temperature shock technique. Previous studies showed that mature bivalves could successfully be induced to spawn by several methods as summarized in Table (1).

**Table 1.** Bivalve species successfully induced to spawn using different induction techniques

| Induction method | Bivalve species | Procedure | Reference |
|---|---|---|---|
| Temperature shock | *Meretrix meretrix* | Clam broodstock (5.0-7.0 cm shell length) exposed to seawater that was adjusted 50C to $6^0$C above ambient temperature for two hours | This study |
| | *Amusium balloti* | Scallops were exposed for 2 hours to seawater that was gradually heated up to 24°C. Ceased if the sample is unresponsive. | **(Cropp, 1993)** |
| | *Pinctada margaritifera* | Oysters kept in fresh seawater were heated up to 30 to $32^0$C for 2 hours period. | **(Southgate & Beer, 1997)** |
| | *Pinctada maxima* | Oysters kept in fresh seawater were heated $1^0$C to $2^0$C above the ambient seawater temperature for 2 hours period. | **(Rose & Baker, 1994)** |
| | *Amusium pleuronectes*<br>*Perna viridis* | Exposed to seawater that has 10°C higher than ambient temperature. | **(Belda & Norte, 1988)** |
| | *Modiolus philippinarum* | Subjected to low temperature (16℃) for 45 minutes and then exposed to 28℃ for another 45 minutes | **(Wong & Arshad, 2013)** |
| | *Perna viridis*<br>*Crassostrea madrasensis*<br>*Anadara granosa*<br>*Meretrix casta* | Maintained in hot seawater (35℃) for 45 minutes and then returned to cold seawater (25℃) for 30-45 minutes. | **(Rao *et al.*, 2018)** |
| | *Crassostrea virginica* | Exposed to cold seawater (10℃) for 30 minutes and then at 30℃ for 30 minutes | **(Magaña Carrasco *et al.*,2018)** |
| | *Asaphis violascens* | Exposed to increasing temperature (28℃ to 32℃) for 30 minutes | **(Bolasina, 2022)** |
| Salinity shock | *Saccostrea cucullata*<br>*Saccostrea commercialis*<br>*Perna viridis*<br>*Perna indica* | Maintained in 34 ppt seawater for 2 months before returning to 26 ppt | **(Stephen & Shetty, 1981)** |
| | *Striostrea prismatica* | Exposed to 96 PSU for 30 | **(Argüello-Guevara *et*** |

| | | | |
|---|---|---|---|
| | | minutes and then returned to 32 PSU for 30 minutes. | ***al.*, 2013)** |
| | *Perna viridis*<br>*Crassostrea madrasensis*<br>*Anadara granosa*<br>*Meretrix casta* | Exposed to seawater with decreasing salinity level (30 ppt to 24 ppt) | **(Rao *et al.*, 2018)** |
| | *Crassostrea virginica* | Exposed to 10 PSU for 30 minutes and then returned to 33 PSU for another 30 minutes | **(Magaña Carrasco *et al.*,2018)** |
| | *Striostrea prismatica* | Subjected to a 10℃ lower than the ambient temperature for 1 hour before returning to ambient temperature for another 1 hour. Exposed gain to 10℃ lower ambient temperature for another hour. Subsequently, exposed to 30℃ water for 30 minutes before reducing the temperature to 22℃. | **(Argüello-Guevara *et al.*, 2013)** |
| Air-dry method | *Meretrix lyrata* | Broodstocks were taken out from the conditioning tank for 5 hours before returning them to the conditioning tank. | **(Chu & Kumar, 2008)** |
| | *Asaphis violascens* | Air-dried the clams for 2 hours before placing them into the conditioning tank. | **(Bolasina, 2022)** |
| Serotonin injection | *Spisula solidissma*<br>*Argopecten irradians*<br>*Crassostrea virginica* | Inject 0.4 ml of 2mM serotonin solution directly into the gonad. | **(Gibbons & Castagna, 1984)** |
| | *Geukensia demissa*<br>*Artica islandica*<br>*Mercenaria mercenaria* | Inject 0.4 ml, 2mM serotonin solution through the adductor muscle. | **(Gibbons & Castagna, 1984)** |
| | *Mactra chinensis* | Inject 0.4 ml, 2mM serotonin solution into the foot. | **(Fong *et al.*, 1996)** |
| | *Tridacna* sp. | Injection of serotonin through mantle below the excurrent siphon to avoid heart or injection into the gonad through the muscle covering the byssal opening. | **(Braley, 1985)** |
| | *Saccostrea echinata* | Exposed to 30 g/l $MgCl_2$ solutions for 3 hours and then injected with 0.2 ml serotonin solution at the adductor | **(Nowland *et al.*, 2021)** |

| | | | |
|---|---|---|---|
| | | muscle. | |
| Sex steroid injection | *Placopecten magellanicus* | Injection of 200 µl of steroid solution containing testosterone, estradiol, and progesterone into the gonad. | **(Wang & Croll, 2006)** |
| Hydrogen peroxide | *Tridacna gigas*<br>*Tridacna squamos*<br>*Tridacna derasa* | Injection of 200 µl of steroid solution containing testosterone, estradiol, and progesterone into the gonad. | **(Beckvar, 1981)** |
| Neuropeptides | *Saccostrea echinata* | Ejection with 5 µL of both APGWamide and buccalin or 10 µL of either APGWamide or buccalin or 10 µL of saline solution | **(Nowland *et al.*, 2021)** |
| Sperm addition | *Striostrea prismatica* | 100 ml sperm (extracted from ripe *Striostrea prismatica males* were introduced to the spawning tank every 30 minutes for 2 hours. | **(Argüello-Guevara *et al.*, 2013)** |
| Air-dry shock, thermal shock, and exposure to the sperm fluid | *Ruditapes philippinarum* | Exposed to air for one hour and then kept in a 200 mL beaker with a sufficient amount of phytoplankton and then subjected to temperature shock (24℃ to 29℃) for 40 minutes. Exposed to sperm fluid at 14-17 days intervals. | **(Chung *et al.*, 2001)** |
| | *Meretrix lusoria* | The clams were exposed to the air for one hour and then transferred to a 200 mL beaker with a sufficient amount of phytoplankton. The water temperature was increased to 29℃ from the initial 25℃ for 40 minutes. Exposed to sperm fluid at intervals of 14-18 days. | **(Chung *et al.*, 2005)** |
| Air-dry and thermal shock | *Modiolus philippinarum* | Exposed to air for 6 hours before returning to UV-irradiated seawater (32 ppt and 26℃). Then gradually raised the temperature (1℃ every 30 min). Stop the treatment when no spawning at 33℃. | **(Wong and Arshad, 2013)** |
| | *Striostrea prismatica* | Exposed to air for 30 minutes and then soaked for 1 hour in seawater that has 5℃ higher than the ambient seawater. | **(Argüello-Guevara *et al.*, 2013)** |

| | | | |
|---|---|---|---|
| Thermal and salinity shock | *Saccostrea echinata* | Maintained for 30 minutes in seawater that was heated up to 7℃ above ambient seawater. Subsequently, treat with reducing salinity (36 ppt to 22 ppt) for 2 hours. | **(Nowland *et al.*, 2021)** |
| Salinity shock and sperm addition | *Saccostrea echinata* | Maintained in reduced salinity seawater (36 ppt to 22 ppt) and then added with 2 ml sperm suspension. | **(Nowland *et al.*, 2021)** |

It was speculated that the increase in temperature during the temperature shock creates stress thereby causing the high energy expenditure cells, especially gametes to consume a large amount of oxygen **(Zhuang & Liu, 2006; Chávez-Villalba *et al.*, 2013)**. This creates a burden on the bivalve. Consequently, the bivalve needs to release its gametes to reserve energy for other basic metabolic processes and continue to survive. **Hassan *et al.* (2017)** noted that the production of gametes is a high-energy demanding process. They also found that egg formation (oogenesis) consumes higher energy than sperm formation (spermatogenesis). However, **Utting and Millican (1997)** warned that changing water temperature alone does not guarantee a spawning response in clams as gonad maturity is also crucial. Thus, to induce spawning successfully, the clams must be fully matured with ripe gonads or at least have a high gonad index **(Duisan *et al.*, 2021)**.

In the present study, it was found that the eggs acquired through the temperature shock seemed normal (224 eggs/ml) and had satisfactory quality as the gametes exhibited a high fertilization rate (76.89%) and acceptable hatching rate (47.39%). According to **Lahnsteiner and Mansour (2012)**, temperature affects sperm motility. The high temperature makes the sperm more active and collides with eggs more frequently. This enhances the fertilization rate **(Byrne *et al.* 2010)**. However, accumulation of waste parallel to an increase in temperature during the induction reduced water pH and affected sperm motility in oysters, *Crassostrea gigas* **(Barros *et al.*, 2013)**.

In addition, many other factors affect the fertilization rate in bivalves, including the age of the male bivalve, sperm concentration per egg, and water turbulence **(Barros *et al.*, 2013)**. In external fertilizing species such as bivalves, the sperms are not deposited within the female hence the probability of sperm-egg encounter can be highly variable and depends on a variety of factors ranging from attributes to those populations, individuals and gametes **(Levitan, 2018)**. An experimental study recorded a decline in the percentage of fertilized eggs with increasing water flow **(Pennington, 1985; Levitan *et al.*, 1992; Petersen *et al.*, 1992)**. If gametes are sufficient, fertilization may be inhibited by water movement. Laboratory experiments demonstrated that the shear forces experienced by gametes in a highly exposed environment could disrupt sperm-egg interactions, resulting in decreased fertilization success **(Mead & Denny, 1995)**. The

depth of water in which the gametes are mixed can also influence fertilization success. As the volume of water decreases, the concentration of gametes increases, hence the collision among gametes increases, and the chance of fertilization increases.

Gamete concentration and the probability of sperm-egg encounters are controlled by the rate of diffusion and the rate and timing of the gamete's release. At the population level, a factor influencing the local concentration of gametes is the distance between individuals and the abundance of individuals. Experimental data showed that the fertilization rate is reduced to 20% when the female is about 1m from the male spawner and further to 1% when the distance between spawners is about 10m **(Levitan & Young, 1995)**. However, this could be less significant in an artificial spawning setting, where gametes are mixed in a small tank or females, and males are placed within the same tank.

The variation also influences the fertilization rate. This variation has been noted to occur among and within species **(Benzie & Dixon, 1994)**. It is linked to gametes attributes which include egg size, the receptiveness of the egg surface to sperm, sperm velocity, and sperm longevity **(Denny & Shibata, 1989)**. Egg traits that have been demonstrated to vary among taxa are: buoyancy, size, the proportion of sperm-egg collision that results in fertilizations, the size and the presence of jelly coats or other structures that can capture sperms, as well as the presence of sperm chemoattractant. For sperms, variations are detected in velocity, longevity, buoyancy and behavior **(Levitan, 2000)**. Greater sperm swimming velocity increases fertilization rate **(Levitan *et al.*, 1991)**.

Other studies highlighted that the concentration of sperm is the most important factor determining fertilization success **(Powell *et al.*, 2001; Kupriyanova, 2006; Liu *et al.*, 2008)**. Nevertheless, increasing the concentration of sperms could create a polyspermy condition that may decrease viable embryos **(Narvate & Pascual, 2003)**. In the case of clams, high concentrations of sperm affect the success of fertilization. For instance, concentrations higher than 10 million spermatozoids/ml are required for successful fertilization **(Powell *et al.*, 2001)**. It was speculated that oocyte quality is also crucial in determining fertilization success. However, up to date, there are still no predictable egg traits that can be used to quantify the quality of eggs **(Migaud *et al.*, 2013)**. The fecundity levels among bivalves are usually high, and fertilization success is also high. For example, an individual of *Tridacna gigas* released $5 \times 10^8$ eggs in a single spawning event **(Crawford *et al.*, 1986)**; whereas, *Anadara antiquata* produced an average of $1.6 \times 10^6$ eggs per female **(Mzighani, 2005)**.

**Embryonic development**

The embryonic development of *Meretrix meretrix* recorded in hatchery condition is presented in Fig. (2). It was noted that the development of fertilized eggs of *Meretrix meretrix* into D-shape veliger occurred within a short period. Ten minutes after

fertilization, a polar body was formed, and the fertilized eggs began to cleave into a 2-celled stage in about 20 minutes (Fig. 2c). The 16-celled (Fig. 2d) and multi-celled (morula) (Figs. 2e, f) stages emerged in 2 hours and 7 hours after fertilization, respectively. The early blastula (Fig. 2g) and the late gastrula (Fig. 2h) stages were observed 8 and 9 hours after fertilization, respectively. It was also noted that the early blastula started to spin slowly. After the late blastula stage was reached, it began to spin rapidly, and the cilia were seen to develop. The trochophore stage (Fig. 2i) was observed 13 hours after fertilization. At this stage, the trochophore with fully developed cilia was swimming randomly and rapidly. At the veliger stage, the apical tuff from the trochophore stage started to degenerate. At this stage, the shell began to form at about 20 hours (Figs. 2j, k) after fertilization. In the following hours, the D-shaped larvae with developed velum were first observed after 24 hours of fertilization. Besides, the larvae possessed a straight-hinged shell and began to feed on microalgae. On day 6, the larvae began to transform into spat (Fig. 2l). At this time, most of the veligers settled on the bottom of the tank and exhibited very limited movement. Moreover, the veligers had fully developed internal organs. On day 10 (Fig. 2m), the early spat stage with visible umbo was observed to utilize its foot to find substrate. On day 19, the spats had a fully developed umbo (Fig. 2n). Then, on day 43, the spats were fully developed with thicker umbo (Fig. 2o). At this stage, the average shell length of the spat was about 729µm, and the shape already resembled the adult. The developmental stages of the *Meretrix meretrix* and other venerid clams are given in Table (2).

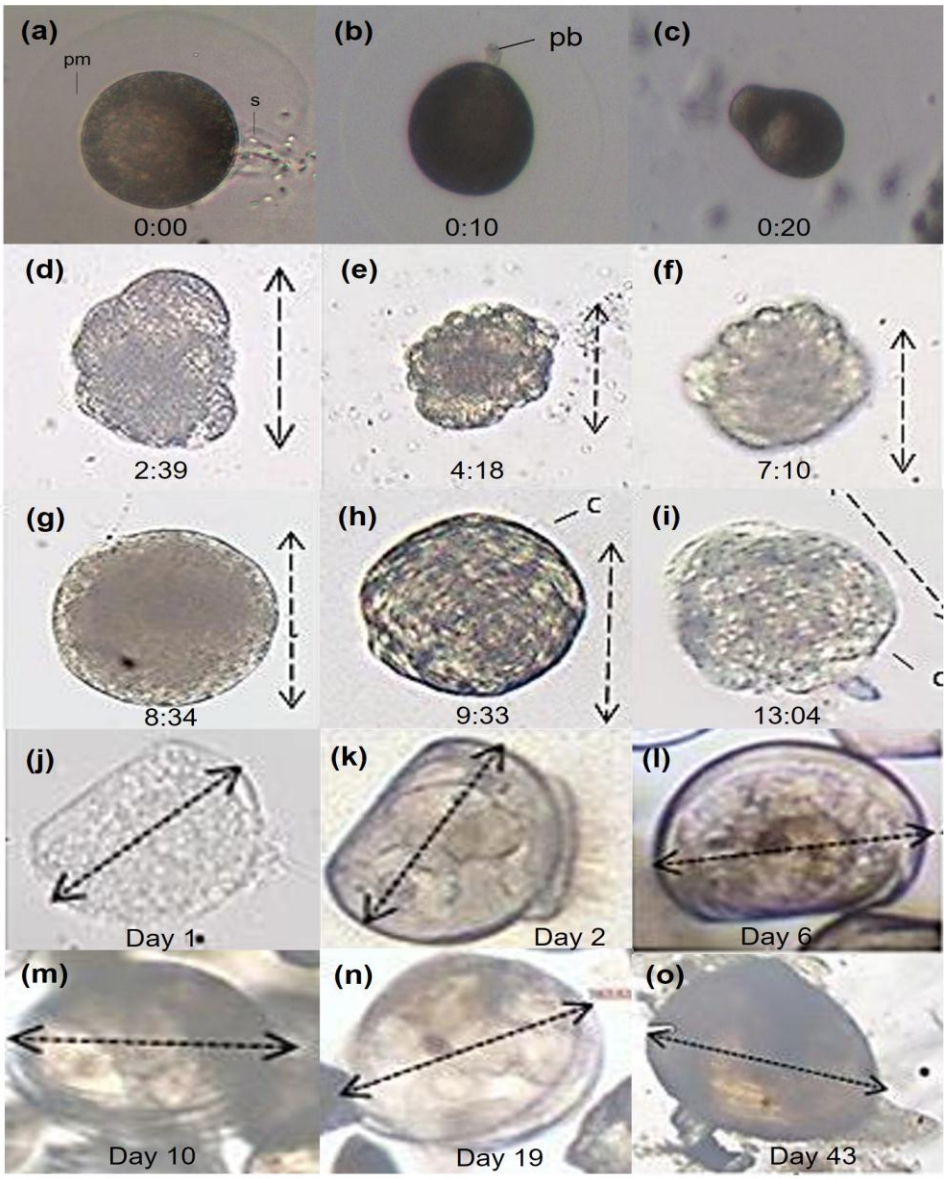


**Fig. 2.** Timeline (hour: minutes) of the embryonic development of Asiatic hard clam (*Meretrix meretrix*) in hatchery condition

Note: (a) Egg fertilized by sperms; (b) Egg with polar body (c) 2-cells division; (d) 16-cells division; (e)-(f) Continued cell division; (g) Blastula; (h) Gastrula; (i) Trocophore; (j) D-shape veliger; (k) Veliger with cilia; (l) Veliger ready to settle at the bottom; (m) Spat with umbo begin to form; (n) Spat with developed umbo; (o) Spat reached the juvenile stage with a fully developed umbo. pm: Perivetilline membrane; pb: Polar body; c: Cilia; s: Sperm.

**Table 2**: Comparison of timing for important embryonic and larval development stages in Veneridae species

| **Veneridae species** | **Developmental Stage** | | | | | | **References** |
|---|---|---|---|---|---|---|---|
| | 1st Cleavage | Trocophore | D-stage | Umbonate | Pediveliger | Metamorphosis | |
| *Meretrix meretrix* | 20 min | 13 hAF | 24 hAF | Nd | 6 dAF | 10 dAF | This study |
| *M. meretrix* | 10-15 min | 9 hAF | 20 hAF | 4 dAF | 6 dAF | 7 dAF | **Narasimham *et al*. (1988)** |
| *Anomalocardia brasiliana* | 30 min | 9 hAF | 24 hAF | 5 dAF | 8-10 dAF | 11 dAF | **Mouëza *et al.* (1999)** |
| *Marcia opima* | 30 min | 6 hAF | 14 hAF | 5 dAF | 6 dAF | 9 dAF | **Muthiah *et al.* (2002)** |
| *Cyclina sinensis* | 55 min | 10 hAF | 24 hAF | 7-16 dAF | 17 dAF | nd | **Hur *et al*. (2005)** |
| *Mactra veneriformis or M. quadrangularis* | 55 min | 6 hAF | 18 hAF | 6-13 dAF | 14 dAF | nd | **Hur *et al*. (2005)** |
| *Meretrix lusoria* | 25 min | 9 hAF | 17 hAF | 3-6 dAF | 7 dAF | nd | **Hur *et al*. (2005)** |
| *Ruditapes philippinarum* | 1 hour and 10 min | 10 hAF | 20 hAF | 6-12 dAF | 13 dAF | nd | **Hur *et al*. (2005)** |
| *Ruditapes decussatus* | 20 min | 13 hAF | 26 hAF | 7 dAF | 15 dAF | 27 dAF | **Aranda-Burgos *et al.* (2014)** |
| *Chionista fluctifraga* | nd | nd | 20 hAF | 4-6 dAF | 8 dAF | 9-13 dAF | **Castillo-Durán *et al.* (2016)** |
| *Chione cortezi* | 15 min | nd | 24 hAF | 5 dAF | 9 dAF | 11 dAF | **Olivares-Banuëlos *et al.* (2017)** |
| *Tawera elliptica* | 15 min | 56 hAF | 87-107 hAF | Nd | nd | Nd | **Barria *et al.* (2021)** |

According to **Liu *et al.* (2006)**, apart from nourishment, stocking density is also an important factor influencing the survival of clam larvae. It was noted that the lower the stocking density, the higher the survival rate. The authors also demonstrated that larger space shortens the larval swimming period and reduces clam larvae' risk of mortality.

However, in the present study, the D-shape veliger of the *Meretrix meretrix* experienced a high mortality rate. Similarly, previous studies on *Ruditapes decussatus* and *Pinna nobilis* **(Trigos *et al.*, 2018; Sami *et al.*, 2021)** recorded higher mortality at this stage. **Sami *et al.* (2021)** attributed the high mortality of D-shape veliger to the probability of bacterial infections. Studies have shown that broodstock is the most crucial bacterial source in the larval culture facility **(Schulze *et al.*, 2006; Sandaa *et al.*, 2008)**. Vertical transmission of bacteria from broodstock to larvae occurred when the bacteria contamination in broodstock's gonads and intestinal tracts passed to off-spring **(Beninger *et al.*, 2003; Prado *et al.*, 2013)**. In this context, **Fontanez and Cavanaugh (2014)** highlighted that infection could occur horizontally by the bacteria escaping the filtration system or other broodstocks. Microbiota such as *Vibrio* sp has been reported as the common pathogen in bivalve larval culture facilities worldwide **(Sainz-Hernandez & Maeda-Martínez, 2005; Gómez-León *et al.*, 2008; Prado *et al.*, 2013)**. The pathogen causes necrosis to soft tissue and ciliary structures of bivalve larvae, disrupting the larvae's filtration and feeding ability **(Sugumar *et al.*, 1998; Neo *et al.*, 2011)**. According to **Trigos *et al.* (2018)**, *Vibrio* sp. also disrupts the vellum of larvae during the veliger phase and is responsible for 80% of larvae mortalities during the first 2- 9 days. The authors also noted that weak larvae are particularly vulnerable to Vibrio infection due to insufficient feeding. However, excess feeding was also observed to enhance bacterial proliferation. Unfortunately, the high mortality of the D-shape veliger of the *Meretrix meretrix* in the present study cannot be ascertained because no bacterial study was conducted.

A study on larval rearing of *Pinna nobilis* recorded more than 80% mortality when the larvae were exposed to darkness. Some bivalve veliger larvae tend to have a negative phototaxis that vertically migrates to deeper waters during daylight and returns to the surface at night **(Manuel and O'Dor, 1997; Gosling, 2003; Weinstock *et al.*, 2018)**. However, **Bricelj *et al.* (2017)** demonstrated that the veliger of hard clam *Mercenaria mercenaria* appeared phototactic as it had broader vertical distribution, and no maximum densities of larvae were observed near the bottom upon exposure to darkness. On this note, further investigation is needed to ascertain the effect of photoperiod on *Meretrix meretrix* larvae.

Besides reproductive cycles **(Hamli *et al.*, 2015),** population dynamics **(Admodisastro *et al.*, 2021)**, and gonadal dan condition indices **(Duisan *et al.*, 2021)**, the present study was the first investigation involving embryonic development of *Meretrix meretrix* under hatchery condition in Malaysia. This certainly contributes to the essential understanding of the biology of this species in the Malaysian context. The embryonic

development of this species in Malaysia was not significantly different from that observed in other bivalve species (Table 2). **Narasimham *et al.* (1988)** reported that, the first cleavage of the egg of the *M. meretrix* species in India began 10-15 minutes after fertilization, which is 5 minutes earlier than what was recorded in the present study. For the morula stage, it was noted that the current study recorded 3 hours delay than what was reported in the study of **Narasiham *et al.* (1988)**. The trochophore stage was observed 9 hours after fertilization with a size of 101 x 81um, which is slightly earlier than the finding of **Narasiham *et al*. (1988)**. The straight hinge stage with a well-developed ciliated velum was observed 20 hours after fertilization with the length and height of the D-shaped larvae recorded at 116.4 µm and 91.3 µm, respectively **(Narasimham *et al*., 1988)**. The duration required by the larvae to reach the metamorphosis stage is similar to what was reported in India. Interestingly, the eyed larval stage was not observed in the current study. Comparably, the absence of the eyed larval stage has also been observed in several other bivalve species including *Rangia cuneata* **(Chanley, 1965**), *M. lateris, Pitar morrhuana*, *Laevicardium mortoni* (**Loosanoff *et al*., 1966)** and *Marcia opima* **(Muthiah *et al*., 2002)**.

## CONCLUSION

The current study revealed that the *Meretrix meretrix* which have already attained shell length between 5.00 to 7.00 cm could successfully be induced to spawn using the temperature-shock method. It was observed that egg fertilization of *M. meretrix* occurred within 20 minutes after being released into the water column. The D-stage larvae was formed approximately 24 hours after fertilization. The larvae began to consume exogenous feed once they developed velum. On the 6$^{th}$ day after fertilization, most veligers of the *M. meretrix* larvae had already settled on the substrate and exhibited limited movement. On the 43$^{rd}$ day after fertilization, the veliger had a fully developed and thicker umbo, and the shape resembled an adult clam. The current study also demonstrated that the seed procurement of *M. meretrix* in hatchery conditions is possible which warrants sustainable production of the bivalve in the future.

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