THERMAL INDUCED SPAWNING FOR LARVAL AND SPAT PRODUCTION *PINCTADA MAXIMA* (JAMESON, 1901)*

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Abstract

Species *Pinctada maxima* (Jameson,1901) is one of four major pearl oyster species utilized by the cultured pearl industry for pearl production. They are rich in the banks of Mergui Archipelago in the Andaman Sea. Thermal stimulation is a successful spawning induction in all major commercial pearl oyster species in hatchery. In the present study, the induce spawning method on hatchery of gold-lip oyster and silver-lip oyster *Pinctada maxima* was used for larval and spat oyster culture conduction in Myanmar Pearl Enterprise, Pearl Island, Myeik Archipelago. Different developmental stages of larval oyster were found after the fertilization in the hatchery such as cleavage stage, trochophore stage, D-shape stage, umbo stage, eye-spot stage and spat stage, etc. the time was taken from the selection for mother oysters until the spat collection stage was for 22days. The measurements of larvae were 80-90 µm with the mean size 87.6 µm in gold-lip oyster and 90-110 µm with the mean size 105.2 µm in silver-lip oyster on six days after fertilization. After 20 days, the size of measurement was increased to 170-260 µm with the mean size 234.4 µm whereas 180-280 µm with the mean size 243.0 µm for gold-lip and silver-lip oysters respectively at the optimal environmental condition in hatchery.

Keywords: *Pinctada maxima,* induce spawning, fertilization, optimal environmental condition

Introduction

Mollusca is the most diverse marine phylum on earth. As a fossil record dating back almost 550 million years. They are related to well-known groups such as gastropods (snails), cephalopods (octopus), scaphopods (tusk shells) and other bivalves (clams, edible oysters and mussels) (Southgate and Lucas, 2008). *Pinctada* species (Roding) belongs to family Pteriidae and bivalves of great beauty and age. They occur in almost all the seas of the tropical and subtropical belt (Alagarswami, *et al.*, 1983b). there are 28 species of pearl oysters, the species for producing of pearls with good gem quality and most commercial value are *Pinctada maxima* (Jameson), *P. margaritifera* (Linnaeus) and *P. fucata* (Gould) (Chellam, *et al.*,1991). Pearls are the oldest gems known to man. Pearls and their shells have been used for human adornment since at least 1500BC (Strack, 2006) and the oldest found and documented pearl has been dated back to 5500BC (Charpentier *et al*., 2012).

Plate 1. Shells and Pearls of Silver lip oyster (Introduced from Indonesia, 2017) and Native gold lip oyster (*Pinctada maxima*)

Among these species, a *Pinctada maxima* (Jameson,1901) is one of four major pearl oyster species utilized by the cultured pearl industry for pearl production. Traditionally, pearls were only obtained from nature, but due to scientific advancement, vast majority of pearls today

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are produced from nursing, nucleus inclusion, then breeding a technology dominated by Japan-based companies in South East Asia and northern Australia (Fassler, 1992; Strack, 2006; Septy, *et al* 2018). In Myanmar, *P. maxima* are rich in the banks of Mergui Archipelago in the Andaman Sea. They are considered as the grounds geographically farthest to the west, with the hundreds of islands and coral reefs offering good ecological conditions. The "Mergu Shell", as they were soon known, began to play a role on the world market and were shipped to London and Hamburg via Singapore (Southgate and Lucas,2008).

In nature, the breeding season of *P. maxima* extends from the months of September and October through to the months of April and May. Although there is variability from month to month, the primary spawning occurs from the middle of October to December. A smaller secondary spawning occurs in February and March (Rose *et al.*, 1990; Rose and Baker, 1994). In the present study, the induce spawning method was used for larval and spat oyster culture conduction in Myanmar Pearl Enterprise, Pearl Island, Myeik Archipelago with the following objectives: to study on hatchery of gold-lip oyster and silver-lip oyster *P. maxima* by induce spawning method, to determine the larval development for both gold-lip oyster and silver-lip oyster (Plate 1).

Materials and Methods

Study area and study period

The research was conducted at Myanmar Pearl Enterprise (MPE) in Pearl Island locating 11'16.2' N and 98' 13.8'E, Myeik Archipelago, Tanintharyi Region (Fig. 1). The study period was from July, 2019 to January, 2021.

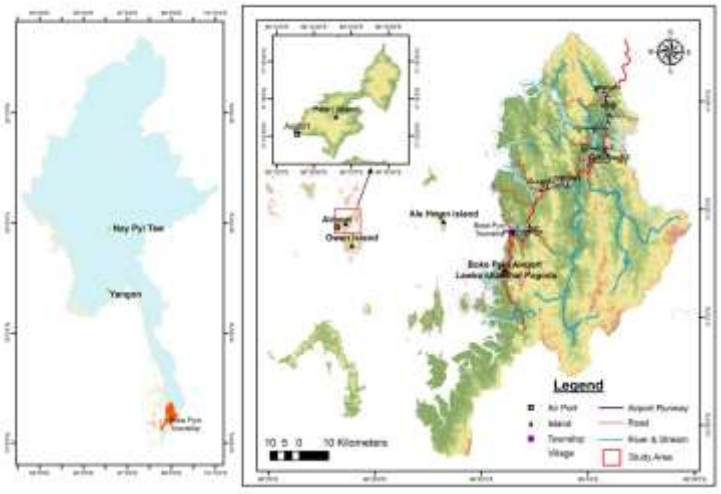

Figure 1. Location map of Pearl Island, Myanmar Pearl Enterprise, Pearl Island, Myeik Archipelago, Tanintharyi Region

Selection of Oysters Breeders

The pearl oysters were collected from selective brood stock in pearl farm. The fouling organisms from the collected oyster were removed by using a knife and clean. The cleaned oysters were put by row in the rectangular water tanks and the gonad condition were examined with naked eyes for each individual by using a shell opener which putting between two shells. If an individual which was at the suitable gonadal development, it was kept separately as the sex group for the culture. And then these selected oysters were cleaned with a brush and marking sign for each individual. After cleaning, these selected oysters were put 15 individuals per plastic

basket by separate sexes such as Male oysters, Female oyster and transferred into a 500L water tank (Plate 2).

Spawning and rearing in hatchery

A total of 21 individuals males and six females of Gold-lip oysters whereas two individuals of male and two individuals of female of Silver-lip oysters were taken for the spawning in the hatchery. These mother oysters in the blue tank were spawned by induce spawning method. This process was done by putting heater 2000 Watt for heat. Water temperature was taken to raise between 32°C to 35°C. The water temperature was checked and recorded the time of recording gonads for gold-lip oysters (Table 1)and silver-lip oyster(Table 2). Firstly, male oysters were spawned and released sperm and later the female oysters were releasing the egg. When this condition, the both sexes were kept separately into the new tank again and pouring sperm water in female spawning tank. The water sample from the spawning tank was collected using a pipette and examined the fertile larvae under the compound microscope (Plate 3).

After fertilization, the 2 days oyster larvae were moved from a larval culture tank through a various size of sieve and cleaning with seawater. After cleaning the oyster larvae were transferred to a beaker and replaced back into another clean culture tank. The seawater was exchanged in larval rearing tanks and reared for 40 days (Table 3). The stocking densities were checked for 500 L tank capacity (Plate 4). For the feeding, the five species cultured micro-algae planktons in the hatchery as the species *Chaetoceros simplex, C.calcitrans, C. gracilis, C. ceratosporum, Isochrysis galbana* fed to the cultured oyster larvae. The feeding rate was determined from six days to 20 days. In this study, the growth and developmental of oyster larvae were examined from first polar body to spat stages in the hatchery. The larvae were fed on chloramphenicol (Antibiotic) from day four to the stage before the collector hanging.

Results

There were different developmental stages of larval oyster after the fertilization in the hatchery such as cleavage stage, trochophore stage, D-shape stage, umbo stage, eye-spot stage and spat stage, etc. From fertilized eggs to D-shape stage were become within ~20 hours. The early umbo stage was within six to seven days. The umbo stages were become for 10-12 days after. The eye-sport stage was become 16-18 days and then become to the spat stage (Plate 5).

The larval development for both gold-lip and silver-lip oysters was shown in Table 4. The measurements of larvae were 80-90 µm with the mean size 87.6 µm in gold-lip oyster and 90-110 µm with the mean size 105.2 µm in silver-lip oyster on six days after fertilization. After 20 days, the size of measurement was increased to 170-260 µm with the mean size 234.4 µm whereas 180-280 µm with the mean size 243.0 µm for gold-lip and silver-lip oysters, respectively. The amount of feeding rate was 1~2% on that six and eight days of culture and then the rate became 2% on 10 days, 2~3% on 12-14 days, 3% on 16 days, and 3~4% on 18-20 days during the larval development. The measurements of larvae were 80-90 µm with the mean size 87.6 µm in gold-lip oyster and 90-110 µm with the mean size 105.2 µm in silver-lip oyster on six days after fertilization. After 20 days, the size of measurement was increased to 170-260 µm with the mean size 234.4 µm whereas 180-280 µm with the mean size 243.0 µm for gold-lip and silver-lip oysters, respectively.

Table 1. Thermal Induced Spawning for Gold lip oysters

Sr. No.	Steps	Time (26.11.19)	Duration (mins)	Remarks
1	Transfer the selected mother oysters in blue water tank	6:25 AM	5	Males-21 & Female- 6
2	Heating (32°-35°-32°C)	6:30 – 6:45 AM	15	
3	Leaving in the water tank	6:45 - 7:00 AM	15	
4	Heating (up to 35°C)	7:00 – 7:20 AM	20	Giving food-10 liters
5	Leaving in the water tank	7:20 – 7:50 AM	30	
6	Heating (up to 34°C)	7:50 – 8:10 AM	20	Giving food-15 liters
7	Leaving in the water tank	8:10 – 8:25 AM	15	
8	Heating (up to 34°C)	8:25 – 8:35 AM	10	
9	Leaving in the water tank	8:35 – 9:00 AM	25	
10	Running water started	9:00 AM		
11	Gonads leave start and pouring sperm water for fertilization	9:15 AM	15 mins after running water	
12	Finished all eggs fertilization	9:40 AM	25	

Table 2. Thermal Induced Spawning for Silver lip oysters

Sr. No.	Steps	Time (17.12.19)	Duration (mins)	Remarks
1	Transfer the selected mother oysters in blue water tank	6:15 AM	5	Males-2 & Female- 2
2	Heating (up to 32°C)	6:30 – 7:10 AM	40	Giving food- 15 liters
3	Leaving in the water tank	7:10- 7:25 AM	15	
4	Heating (up to 35°C)	7:25– 7:55 AM	20	Giving food- 15 liters
5	Leaving in the water tank	7:55 – 8:25AM	30	
6	Running water	8:25 – 8:55 AM	30	
7	Heating (up to 32°C)	8:55 – 9:20 AM	25	Giving food- 20 liters
8	Leaving in the water tank	9:20 – 9:35 AM	15	
9	Heating (up to 35°C)	9:35– 10:00 AM	25	Giving food- 20 liters
10	Leaving in the water tank	10:00 – 10:30 AM	30	
11	Running water start	10: 30 AM		
12	Gonads leave start and pouring sperm water for fertilization	10: 45 AM	15 mins after running water	
13	Finished all eggs fertilization	11 :20 AM	35 mins	

Table 3. The used of different sieve's size for cleaning oysters

Sr. No.	Gold-Lip Oyster		Silver-Lip Oyster	
	Day	Used of sieve's size (μm)	Day	Used of sieve's size (μm)
1	2	65, 40, 20	2	65, 40, 20
2	4	85, 58, 40	4	85, 58, 40
3	7	118, 85, 75	7	118, 95, 85
4	10	132, 95, 85	10	132, 95, 85
5	13	180, 118, 100, 95	13	180, 132, 118, 100,
6	16	212, 150, 132, 118	16	212,150, 132
7	20*	400, 180, 160	19	400, 160, 150, 132
8	23	400, 180, 160	22*	400, 180, 160
9	27	400, 180, 160	25	180
10	30**	180	28	180
11	33	180	31**	180
12	37	180	34	180
13	40		37	180

Remarks: * Hanging collectors start, ** Running water start

The amount of feeding rate was 1~2% on that six and eight days of culture and then the rate became 2% on 10 days, 2~3% on 12-14 days, 3% on 16 days, and 3~4% on 18-20 days during the larval development. Three individuals of abnormal larvae were recorded on 12 days in gold-lip oysters whiles non abnormal larvae in silver-lip oysters. Eight nos. of eye spot larvae were found on 18days in gold-lip oysters as compare as three nos. and 15 nos. of eye spot larvae were observed on 16 days and 20 days, respectively. The density (%) was 3.7 % for gold-lip oysters whereas 4.8% of silver-lip oysters in 500L culture blue tank on six days. But it was become 1% on 20 days for both gold-lip oyster and silver-lip oyster. During the study period as the environmental condition the air temperature was minimum 19.8°C and maximum 27°C. The water temperature was minimum 25°C and maximum 28°C. The salinity of water was 30-31 ppt and humidity 46-60%.

Discussion

Thermal stimulation has been reported as a successful means of spawning induction in all major commercial pearl oyster species (Alagarswami *et al,* 1983a, b; Alagarswami *et al,* 1989; Chellam *et al*., 1991; Rose and Baker, 1994; Southgate and Beer, 1997). In the present study,

thermal induce spawning method used for pearl oysters in hatchery. The growth of pearl oyster seeds starts from zygote, followed by larvae, then spat (Gervis and Sims,1992).

Table 4. Larval development of Gold-lip oyster and Silver-lip oysters (n=25)

No	Day	Measurement (µm)		Average size (µm)		Amount of eat (%)		No. of Abnormal larvae		No. of Eye spot larvae		Density (%) in 500L culture blue tank	
		Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip
1	6	80-90	90-110	87.6	105.2	1~2	1~2					3.70	4.80
2	8	90-110	90-110	103	107.4	1~2	1~2					2.80	2.80
3	10	100-130	110-150	116.2	130	2	2					3	3
4	12	100-160	120-190	130	154	2~3	2~3	3				2.40	2.00
5	14	110-200	120-220	169.4	183	2~3	2~3					2.20	1.60
6	16	130-210	140-240	187.2	207	3	3				3	2.10	1.40
7	18	160-250	160-270	219.8	233	3~4	3~4			8		1	1
8	20	170-260	180-280	234.4	243	3~4	3~4				15	1	1

A. Uncleaning oysters in panels

B. Cleaning oysters with a knife

C. Oysters put in tanks

D. Examination on Gonad's developmental stages

E. Cleaning oysters with a brush and marking on the shell

F. Leaves the selected oysters breeder in water tank

Plate.2 Selection of oyster breeders

A. Heating Oysters in blue tank

B. Feeding on oysters

C. Checking the water temperature

D. Record the time of releasing gonads

E. Keeping of both sexes separately into the new tank again

F. Pouring sperm water in female spawning tank

G. Taking the water sample from fertilized tank with a pipette

H. Examining samples under a compound microscope

Plate. 3 Spawning of oysters in the hatchery

A. Water from a larval culture tank through a sieve

B. Larvae cleaning with seawater

C. Water through various size of sieve

D. Oyster larvae in a sieve

E. After cleaning the larvae transfer into a beaker

F. Replaced back into another clean culture tank

Plate. 4 Cleaning and changing of water tank during the stage of D-shape oysters

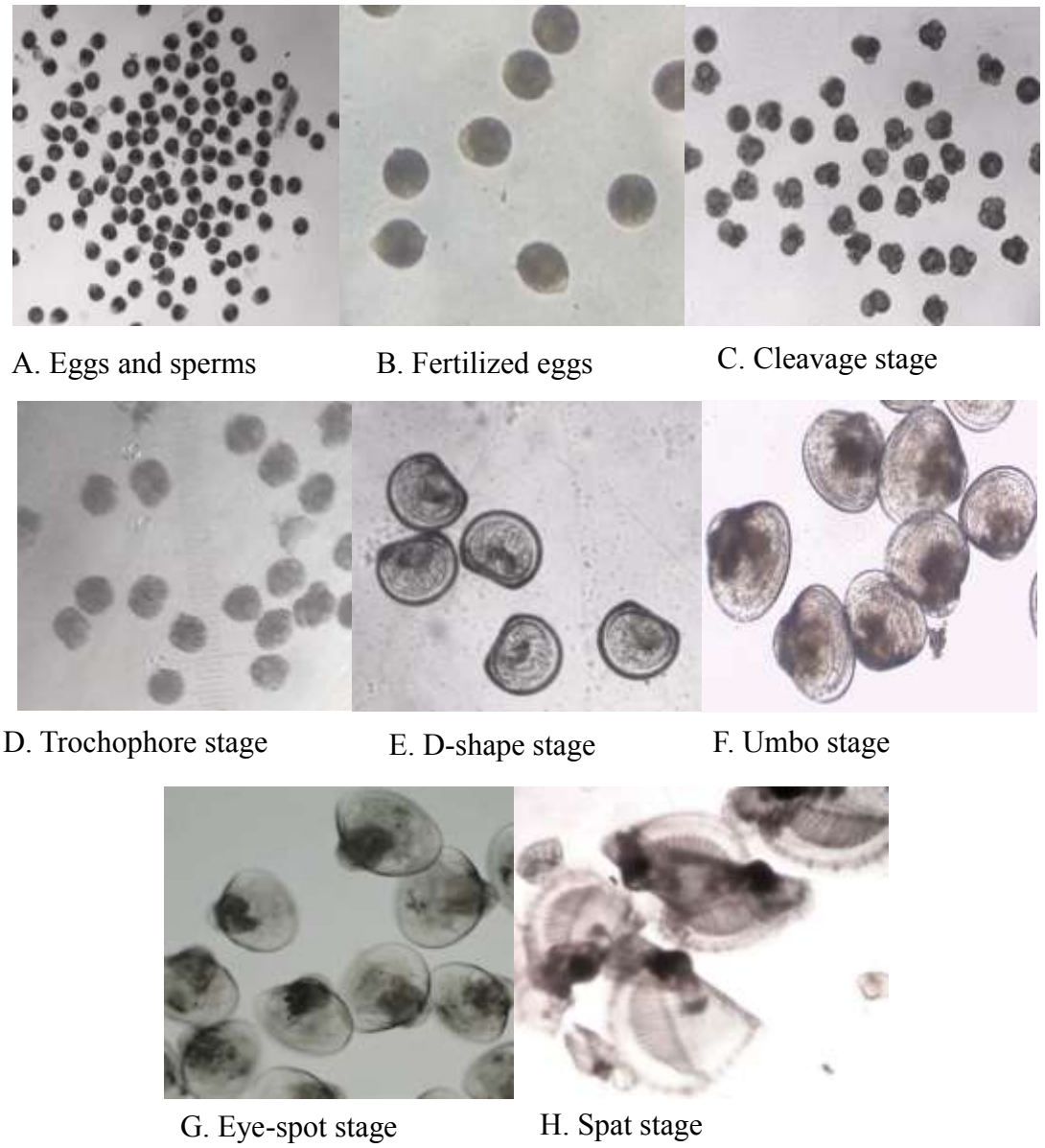

Plate. 5 Developmental stages of oyster larvae

Rose (1990) recommended that the egg density should not exceed 30/mL for Pinctada maxima. Initial larval stocking density should be no more than 5/mL and this should be reduced to 2/mL by day 10 and to 1/mL by day 14. Larval stocking density should be adjusted at water change. In the present hatchery the egg densities were 21/ml of gold lip oysters and 10/ml of silver lip oysters. Larval stocking density were adjusted at water change as the reduction of density from 3.7 % for gold-lip oysters whereas 4.8% of silver-lip oysters on six days to become the similar density 1% on 20 days for both oysters. All fertilized eggs transformed into the D-shape stage within ~20 hours. The early umbo stage was within six to seven days. The umbo stages were become for 10-12 days after. The eye-spot stage was become 16-18 days and then become to the spat stage. Thus, the duration between the day of selection for breeder oysters until the spat collection stage was prolonged for 22days.

Tranter (1958) described that spawning results from muscular contractions and oocytes are activated immediately prior to spawning in the follicle. In developmental stages of larval oysters, the extrusion of the first and second polar body occurred within 5minutes and 15-

20minutes of insemination, respectively, in Akoya pearl oysters (Wada *et al.,* 1989) whereas Doroudi and Southgate (2003) stated that first polar extrusion in *Pinctada margaritifera* was recorded after 24minutes. In the present study recorded that six different developmental stages of larval oyster after the fertilization in the hatchery namely cleavage stage, trochophore stage, D-shape stage, umbo stage, eye-spot stage and spat stage. The first and second polar body occurred within 5minutes and 15-20 minutes after fertilization take place in both gold-lip oysters and silver-lip oysters. Trochophore stage appear within 5 hours to 8 hours and measuring around 75µm. Thus, the data indicated that around the same size as compare to Saucedo and Southgate (2008) who stated that trochophore stage measure around 75µm *Pinctada maxima* and 70µm in *Pinctada margaritifera*.

According to the previous literatures stated that the growth rate of pearl oyster larvae is influenced by their surrounding environment, in particular food availability (Doroudi *et al.,*1999a; Doroudi and Southgate, 2002), food quality (Martinez – Fernandez *et al*.2006, water quality (Doroudi *et al.,* 1999b). Mills (2000) reported that growth of *Pinctada maxima* spat was optimal at a temperature of 26-29ºC and at an algal concentration of 54 cells/µL. Also, Doroudi *et al*., (1999a) reported maximum survival of 6-day-old *Pinctada margaritifera* larvae to occur within a salinity range of 26.5-33.5% and within a water temperature range of 22.5-26.5ºC. In the present hatchery, the ambient temperature 27.8ºC and an increase from 32-35ºC and the salinity of water was 30-31 ppt and humidity 46-60%. Therefore, the environmental condition was in the optimal condition for the growth of spat *Pinctada maxima* in the hatchery of the study area.

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