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CULTIVATION STAGES EX-SITU OF *SALMACHIS SPHAEROIDES* ON LARVAL DEVELOPMENT AND JUVENILE THROUGH METAMORPHOSIS FOR SUSTAINABILITY

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Abstract

Sustainability cultivation and management of sea urchin is an important effort to maintain the marine biological resources. Conservation and cultivation should be done ex-situ through education in universities. Purpose of this research is to determine sea urchin stages of cultivation in the laboratory and of embryonic development stages of *Salmachis sphaeroides*. The cultivation method consists of five stages: preparation of feed, taking sea urchin in natural habitats, spawning and fertilization, maintenance and enlargement of larvae. Spawning method is doing by natural stimulating, is raising slowly temperature and sea water adding. *Salmachis sphaeroides* was taken from Senggora, Pangkalanbun and cultured in a laboratory of one of university Palangkaraya. This research has of sea urchin is a good early models organism development, because of spawning can be one naturally and the stages of development the faster and simple and of embryonic development stages it is easy to observe. The success of sea urchin cultivation, because an attention to external factors, such as temperature, salinity, feed and sea water filter. The results of this study can be used as a reference for sea urchin cultivation ex-situ in laboratory and a very helpful for understanding early models of organism.

Keywords: Cultivation, Ex-Situ, Juvenile, Larval development, *Salmachis Sphaeroides*, Sustainability.

1. Introduction

Breeding sea urchins in the laboratory for sustainability is an Ex-situ activity related to real problems and is in line with is one of the SDGs educational goals, it is life under water. The ultimate goal of sustainable development is to raise global living standards by managing and utilizing the resources of the marine in a sustainable manner [1]. *Salmachis sphaeroide*s is one of the sea urchins that is mostly found in shallow waters in sea grass habitats on sandy and muddy sub-litoral zones, besides that it is also commonly found in water that have warm temperature including Senggora, Central Kalimantan as well as in the Johor Strait, between Malaysia and Singapore [2]. Sea urchins play an important role in marine ecology, especially in the food chain, because this marine biota is a determinant of the abundance of shallow marine plants, especially in sea grass ecosystems [3].

Researchers have developed various ways to cultivation of sea urchin in laboratory with the stage a growth of T. gratilla [4-7], of Salmachis spheroids [2], of E. mathei [8]. However, it has not been found systematically the stages of sea urchin cultivation in the laboratory to optimize the growth and development of Salmachis sphaeroides larvae for sustainability. In addition, it will also be presented from cell cleavage to early juvenile, so that it can be used as a model to study the stages of organism development.

Several studies to spawning of sea urchins generally uses chemicals [2], so that after spawning of sea urchins will it die. However, by using a natural way, namely raising the initial temperature, the parent of sea urchins is still alive and can be maintained. The given of aeration at the larval stage with small plastic paddles was stirred constantly by 5 rpm rotating motors was the optimal speed for rearing Salmachis sphaeroides larvae. Meanwhile, other researches employed a speed of 10 rpm and 20 rpm [2, 4].

Some factors that should be considered when we researched materials in sea [9, 10], especially when we tested samples before cultivating of sea urchin. The selection of parents should be having mature gonads (sexual maturity) to carry out fertilization, physical condition and shell size, the individual prospective spawning should be maintained as original habitat conditions, so that when spawning goes according to plan [4-5]. Besides that, of sea urchin larvae feed is an important and crucial factor because of sea urchin larvae have different lifestyles. The feed given to of sea urchin larvae should be adjusted to the condition of the larvae [5].

Therefore, the aim of this research, first to determine of sea urchin stages of cultivation in the laboratory consists of five stages sea urchin, cultivation in the laboratory should be attention to external factors, such as sea water filter used should be clean free of fungi and bacterial contamination. Second, is to determine of embryonic development stages of Salmachis sphaeroides.

2. Methods

In the materials and sample collection, this research method is a laboratory experimental research with treatment parent of sea urchin against predetermined external factors is temperature, salinity, SWF and feed. Then observes and records each phase of its development. Research conducted for two months starting from 30 Oct 2019 until 18 March 2020 in one of university in Palangkaraya, at biology laboratory. Sea urchins used weighing from 50 to 90 gr and body circumference

diameter from 18 to 25 cm and as many as 40 prospective adults of *Salmachis sphaeroides*. Diatome media for the cultivation of *Chaetoceros calsitrans* is phytoplankton and okinawa media for the cultivation of *Navicula* sp. phytoplankton and was employed used to antibiotic penicillin-streptomisin on larvae sea urchin.

In the instrumentation and characterization, autoclave was used to sterilize diatome media and okinawa media also glassware to cultured *Chaetoceros calsitrans* and *Navicula sp*. phytoplankton. Microscope equipped with an optilab viewer and image raster 3 was used to observation of larval development and measuring it. Laboratory room equipped with cooler, and a shelf equipped with fluorescent lighting intensity ranges from 500-10,000 lux was used for phytoplankton culture. Sedgewick Rafter Counting Cell 1801-G20 was used to observation of larval development. Aquarium size (50x30x40) cm was used to maintenance larvae. Small plastic paddles were stirred constantly by 5 rpm rotating motors was used to aeration also to making sea water movement.

In the maintenance sample, taking of *S. sphaeroides* was carried out at low tide with a depth of 50-100 cm in the morning between 08.00-10.00 WIB on 20 Nov 2019 and 17-21 Jan 2020 on Senggora Beach in Pangkalanbun. Adult sea urchins that have been collected are put into a tank with *E. acoroides* and SWF to be taken to the cultivation laboratory as a place of research.

3. Results and Discussion

Stages of sea urchin cultivation in laboratory can be done by steps: feed preparation of sea urchin larvae, taking adult sea urchin in natural habitats, spawning and fertilization, maintenance of larvae and enlargement of early juvenile.

In the feed preparation of sea urchin larvae, sea urchins' larvae feed is an important and crucial factor that should be prepared before conducting sea urchin cultivation. Feed given to sea urchins larvae should be adjusted to larvae condition. At the floating stage the feed given is *C. calsitrans*, it is at the stage of pluteus larvae of 4 arms. The larva at settle stage then the feed given is *Navicula sp*, it is of larvae regression tissue of the pre-metamorphic larvae with ambulacral feet. While the young juvenile until adults they are given lamun or algae feed. The movements or colours are physical characteristics of feed for fish and non-fish cultivation [8]. The difference in the cultivation of *C. calsitrans* phytoplankton with *Navicula sp*. is of *C. calsitrans* should be given aeration. Whereas *Navicula sp*. not given aeration during cultivation in the laboratory.

In the taking adult sea urchin in natural habitat, the gloves should be being used when picking up the adult of urchin so as not to get pricked by the thorns it and the sea should be at low tide. External factors such as temperature, pH and salinity should be checked when taking of sea urchin. The goal is that the storage tank for the adult of sea urchin was adapted to it has the natural habitat, so can adapt to new habitats. Sea urchins have a low tolerance for changes in salinity, it this can cause pigment change, the spines fall out, the animal becomes inactive and does not want to eat, so can cause death [11].

In the spawning and fertilization, gonad maturity is an important factor that should be considered when conducting cultivation, because the gonad maturity will affect the survival of larvae. Healthy and ready to fertilize eggs cell for *S. sphaeroides* an average size of 100-120 µm. Following the methods are:

(1) Spawning method: Spawning of this study was carried out with slowly raising the water temperature (2-4 ºC was raised the early temperature of in the tank). It can occur spawning caused due to a response to high temperatures. The sea water is put into the aquarium until it reaches 5 L and salinity 3-7 0/00. The adult sea urchins are placed evenly in the bottom of the aquarium without aeration. For 30 minutes one of the parents takes out sperm cells. With the indicator of the liquid released is milky white. After 5 minutes later one of the adult sea urchins took out the egg cell, which is the yellow liquid. But to ensure the fluid is an egg or sperm cell observed under a microscope with a magnification of 10 times. Then measure the diameter. Based on observations when spawning is one parent secretes a white liquid, then the other parent is yellowish, this is because the scent of sperm cells stimulates the other parent. At the time of spawning, the male parent generally releases the sperm cells and then the female parent ejects the egg cell [5].

(2) Fertilization: After spawning is left for 60 minutes sperm cells and egg cells in the aquarium to fertilize. Observations were made by using a microscope to determine changes in egg cells, with indicators of cell nucleus appearance or cell cleavage. Then filtering is done with multilevel sieves (nylon mesh 40 µm, 60 µm, 100 µm and 140 µm). Before the egg cell is moved into fiberglass and aquarium, the calculation is done first, so that it can adjust the area, volume of water and feed. Comparison between the number of sperm cells and eggs should be balanced therefore that decay does not occur [5]. Spawning with raising the temperature and providing fresh sea water makes spawning easier, effective and efficient. Besides that also did not commit killing on sea urchin adult and can be maintained for further spawning. This method to be done, does not use chemicals and according to reproductive behaviour in nature, so that gamete cells that come out of the parent mature sexually and increase the natural stock of sea urchin aquaculture [12].

In the maintenance of larva, larvae maintenance from cell cleavage to metamorphosed showing podia and spines (settle toward early juvenile) is a critical point of growth and development of sea urchin. The factors that should be considered are population density and area, feeding, SWF used, aeration, salinity, temperature and turnover time of SWF. The time needed for the critical point of larval growth from 1 day after fertilization to 25 days. Larvae are maintained at 28 ^{o}C - 31 ºC and salinity 3-7 $^{0}/_{00}$.

Cell cleavage periods until of grastula was carried out using 2 litres of SWF inserted in the suspension of 2 ml eggs cell. Larval maintenance in the laboratory with a ratio of sea water to larvae of 10:1 [6]. Larvae deaths often occur due to density in maintenance [2]. 10 larvae that have metamorphosis be reared in 100 ml of sea water, after 21 days one larva/3 ml and usually in 6-8 weeks, to one larva/l0 ml [4]. Feeding of larvae begins on the second day after fertilization, it is 4 arms pluteus stage and after having functional intestines. Larvae are fed 36 h after fertilization, when functional intestines appear [13]. Feeding the of pluteus larvae in this study followed protocol [2] is of 4 arms, 6 arms and 8 arms, each 5000, 10000 and 15000 cells/ml per day. Feed given should be in accordance with the number of larvae.

The change of water should be done every 2-3 days, therefore that the sea water using is kept clean and sterile. To avoid contaminants of bacteria and fungi can be

used antibiotics, with a ratio of 2 litre SWF: 2 drops of antibiotics. The aeration given on blastula stages it is 1 day after fertilization. The tool use is small plastic paddles was stirred constantly by 5 rpm rotating motors. The larvae attained free swimming blastula then given movement on 10 rpm rotating motors [2]. The embryo develops into a blastula to maintenance its viability stirred by a plastic paddle attached to a plexiglass rod operated by a 20-rpm electric clock motor [4].

In the enlargement of early juvenile to adulthood, enlargement of aboral view of a recently metamorphosed sea urchin showing podia and spines (settle toward early juvenile) carried out in laboratory from 25 days after fertilization until 43 days. The early juvenile is maintained at the water temperature 28-31^{0}C and salinity 3-7 $^0/_{00}$. Every 2 days the SWF using for of juvenile enlargement should be is replace. Enlargement of the early juvenile for 25 and 43 days, it has body size 375,12 µm and 451,17 µm. Oral and aboral views juvenile at laboratory for 92 days has body size 6,35 mm. To reach the adult size and gonad ready to harvest sea urchins, it takes 2 years [4, 6].The size of the body diameter to reach 1 cm takes 152 days, this stage is said to be the young sea urchin [6].

Larval development stages until juvenile

The stages of larval development in *Salmachis sphaeroides* from cell division to juvenile can be seen in Table 1.

Table 1. Development of Salmachis sphaeroides from fertilization to juvenile. Time is 1 h after fertilization

Stages	Description	Time
Embryo	Fertilized eggs with complete fertilization	5-10 m
	Fist division (2cell stage)	15 m
	Second division (4-cell stage)	20 m
	Third division (8-cell stage)	33 m
	Fourth division (16- cell stage)	68 m
	Fifth division (32- cell stage)	1.33 h
	Multicell (morula stage)	2 h
	Blastula	4.33 h
	Grastula	8.36 h
Larvae	Prism	13.51 h
	2-arm pluteus	24.19 h
	4-arm pluteus	26.07 h
	6-arm pluteus	4 d
	8-arm pluteus	7 d
	Competent larva with complete rudiment growth	17 d
	Larvae tissue regression of the pre-metamorphic larvae with ambulacral feet	19 d
Juvenile	aboral view of a recently metamorphosed sea urchin showing podia and spines (settle) (early juvenile)	25 d

(i) Cleavages and embryonic

Based on Fig. 1, the results of observations the morphologically healthy form of eggs *S. sphaeroides* are full round with a size between average 100-120 µm and is

able to divide and develop into juvenile. The egg cell has a thin 'jelly layer' membrane protect them, Fig. 1(a). Egg cell division occurs the first 15 minutes after fertilization, it is 2 cells first meridional, Fig. 1(b). The first meridional cleavage occurs in the formation of two cells of the same size. Then of egg cell will it divide into 4 cells, 8 cells, 16 cells and 32 cells at different times. The stages of larval development in *Salmachis sphaeroides* from cell division to juvenile can be seen in Figs. 1(a)-1(j). The next stages are 4.33 h minute the egg undergoes cleavage of the blastula. The most important morphological characteristic at this stage is formation of cilia around cell mass and embryonic mobility [7]. Then 8.36 h shaped of gastrula stage, Fig. 1(c). Grastula stage results in the internalization of the blastula vegetation area, which consists of mesoderm and endoderm. At this stage the blastopore becomes the anus, while the mouth forms as a secondary opening between the ectoderm and archenteron, it also has cell movements, it is ingression, epithelial invagination, and oriented cell rearrangement [7].

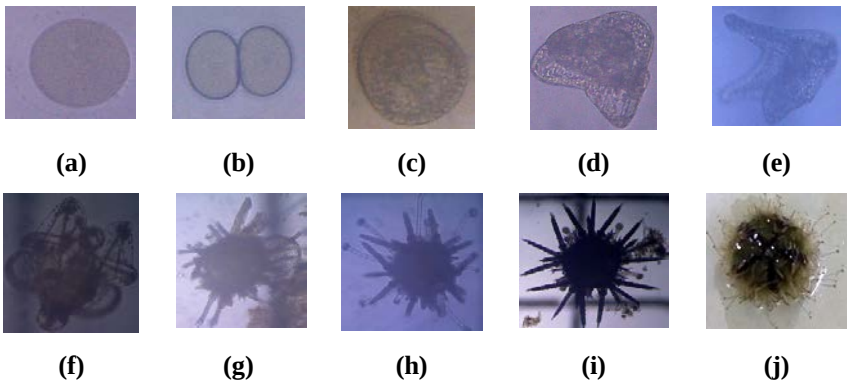

Fig. 1. Embryonic and larval development stages until juvenile of *S. sphaeroides*: (a) egg cell complete fertilization; (b) 2-cell stage; (c) Grastula; (d) Prism; (e) 2-arm larva; (f) Complete rudiment growth; (g) pre-metamor phic ambulacral feet; (h) Showing podia and spines (early juvenile 25 days); (i) showing podia and spines (early juvenile 43 days); (j) Oral and aboral views juvenile (young sea urchin)

(ii) Larval development

This report study of *S. sphaeroides* cultivated in Kalimantan Tengah had shorter time developments when compared to other or its similar of sea urchin species it is 13.51 h form a prism, Fig. 1(d). The prism formation took 22.25 h of S. sphaeroides [2] and 48 h of T. depresses [4] and 20 h of E. mathei [7]. This difference is probably caused by external factors, especially the temperature. Larvae development was relatively short at 29^0C when the temperature compared of 26 ºC [7]. 24,19 h, embryos development entered to of pluteus larvae stage and will it form the 2-armed, Fig. 1(e). Then of pluteus larvae will it divide into 4 arms, 6 arms, 8 arms at different times. 17 days, embryos development forming to the competent larva with complete rudiment growth, Fig. 1(f). Pedicellariae not seen on the body surface [2]. 19 days, the larvae tissue regression of the pre-metamorphic larvae with ambulacral feet, Fig. 1(g). This stage at the armed it will tip forms a pedicel and develop into ambulacral feet, which will be used for settle [4].This indicated that larvae were ready for the settlement and metamorphosis. The

next stages metamorphosed showing podia and spines (settle) will into develop early juvenile. This stage is formed 25 days with size with 375.12 µm, Fig. 1(h). In this stage, well-formed spines and extended tube feet were evident. Then 43 days the body shape looks round and the body spines are sharper and pointed has a size of 451.17 µm, Fig. 1(i). The condition of the post larval is healthy using tube feet and pedicellaria to move the body in a circular motion.

4. Conclusion

This research is an initial laboratory scale research that has succeeded in cultivating *Salmachis sphaeroides* in the laboratory. Sea urchin is a good early models organism development, because of spawning can be one naturally and the stages of development the faster and simple and the growth of process it is easy to observe.

The success of sea urchin cultivation is because it is supported using with small plastic paddles was stirred constantly by 5 rpm rotating motors was the optimal speed for rearing *Salmachis sphaeroides* larvae, so that the survival rate of the larvae was greater when compared to using 8 rpm and 10 rpm.

The larval stage of *Salmachis sphaeroides* on metamorphosed showing podia and spines (settle toward early juvenile) faster when compared to of *Salmachis sphaeroides* cultivated in different places. *Salmachis sphaeroides* of the larvae to reach aboral view of a recently metamorphosed sea urchin showing podia and spines (settle) (early juvenile) its 25 days after fertilization.

Further researches are expected to can cultivate other marine biota that have economic and ecological value in a wider scope not only in the laboratory.

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