The effect of concrete tanks for the breeding technique of the sand sea cucumber (*Holothuria scabra*)

D A N Sitoresmi¹ and K T Pursetyo²,³

¹Aquaculture Study Program, Faculty of Fisheries and Marine University of Airlangga, Surabaya 60115 Campus C Airlangga University, Indonesia
²Department of Marine, Faculty of Fisheries and Marine University of Airlangga, Surabaya 60115 Campus C Airlangga University, Indonesia
³Corresponding author: kustiawan@fpk.unair.ac.id

Abstract. Sea cucumber are one of the Echinodermata type that have nutritional value in high protein content and as the sea of commodities with high economical value. The attempts to obtain sea cucumber are increasingly declining. Based on the review above, it is noticed that the cultivation of sea cucumber are needed to fulfill the needs of the market and restore the wild population. Sand sea cucumber breeding begins with the preparation of the broodstock rearing media, the availability preparation of the broodstock, water quality management, the broodstock feeding, the broodstock selection for high quality broodstock, conducting the artificial spawning using the method of temperature shock, egg incubation, hatching egg, and the larval rearing. The larvae were fed using the phytoplankton *Chaetoceros* sp. with a feeding frequency of once per day. The water quality parameters measured include temperature, DO, pH and salinity. The obstacles that were found on the sand sea cucumber breeding is the difficulty of getting the high quality of the broodstock, so the egg produced from spawning process are limited.

1. Introduction

Trepang or sea cucumber is classified into phylum Echinodermata on Holothuridae classes. Sea cucumbers are the one commodity that has a reasonably good prospect and high economic value, both in the domestic and international market [1]. In addition to economic value, the nutritional content is high at 43.1% protein, 2.2% fat, 27.1% moisture content, ash content of 27.6%, calcium, sodium, phosphorus and other minerals from 1.2 to 16, 5% [2]. Sea cucumber type of sand chosen as a target to be cultivated, because this kind of exploitation under pressure. A sand sea cucumbers sea cucumber species hunted to meet the demand of the trade with a relatively expensive price.

Seed supply business has been done, ranging from spawning and hatchery through the hatchery, but success is far from expected. This is partly due to the presence of male and female broodstock mature gonads simultaneously very difficult to obtain, the larvae are very susceptible to death within 2-3 months and only became juveniles and juveniles from the hatchery must adapt to the natural environment. This is further exacerbated by the need for modern technology and costs are very expensive in the implementation of sexual reproduction. Therefore it is necessary preservation and cultivation of sea cucumber effort to reduce overfishing [3].
2. Material and methods
Activities starting from the preparation bathtub seeding aircraft maintenance, supply mains, water quality management, feeding mother, broodstock selection, spawning, egg incubation, hatching eggs, and larval rearing.

2.1. Bak preparation

2.1.1. Bak preparation master maintenance
Bak used for aircraft maintenance sand sea cucumbers in the form of fiber tub with size 250x100x60 cm³. Bak to use cleaned first. Brushed the wall and the primary vessel, with the aim to dispose of pest attached. Cleaning tubs do not require a long time.

Bak is clean filled with sea water which has been filtered using a filter bag. Water quality management is very important, in order to provide the appropriate conditions required in nature. The water used is sea water that has been through a filtration system. Maintenance tub equipped with aeration, water pipes and sewage pipes enter.

Substitution water circulation system is done every day. In addition dilakukan water changes, also do penyiponan every day in the morning to remove excess feed and products of metabolism at the base of the tub. Penyiponan done by sucking dirt and feed residue using 2 cm diameter plastic hose.

2.1.2. Bak preparation larvae maintenance
Larval rearing tubs made of concrete tank with a capacity of 2000 liters. Bak larval rearing groomed and brushed thoroughly and then filled with seawater. The sea water used is filtered with a size of 5 μ purerite cartridge to prevent the protozoa or other microorganisms into the larval rearing tubs. Larval rearing tubs equipped with 24 pieces of aeration to supply oxygen and 4 pieces of heater to regulate the temperature of the water in the tub. The water temperature is set to 28 °C. Giving aeration also intended to keep stirring the food occur evenly.

2.2. Procurement parent
Mains quality is one factor that determines the success of the provision of seeds through artificial spawning techniques. Broodstock sand sea cucumbers obtained through the arrest of nature. [4] says that the broodstock sea cucumbers can be obtained from natural and artificial spawning can be done immediately or maintained in a controlled bath. Procurement broodstock that has been done in accordance with the procurement of stem study proposed by [4] that the broodstock may be obtained from natural sea cucumbers. Mains were taken from the catches of fishermen in Pejarakan, Bali. Sea cucumbers are caught in the sand by fishermen not guarantee it can be cultivated to produce eggs.

Broodstock cucumbers are instantly recorded based on place of origin, weight and body length. Sea cucumbers are used as the broodstock is grown to the size range of 250-300 grams. According to [5] used the ideal broodstock is the broodstock who has a weight range of at least 500 grams or 250 grams. The body weight of sea cucumbers will be used as the main candidates in accordance with the declaration [5] that the sea cucumber broodstock minimum weight is 250 grams. The next main candidates cleaned of dirt and parasites attached to his body, and after that prospective parents be mixed together in a bathtub.

Broodstock sand sea cucumbers were fed once a day in the early afternoon to adjust the properties of sea cucumbers actively foraging at night. Feed used for the broodstock in the form of benthos that has been filtered and precipitated first, then squeezed until the water content in the sludge discharged. The feed use in accordance with the statement [4] that the broodstock sea cucumbers were fed in the form of brown algae that has been dried. Broodstock sand sea cucumbers can be seen in Figure 8.
Broodstock cucumbers were fasted beforehand with the aim to remove food debris and dirt in the broodstock body will be cultivated. Before spawning, the selection beforehand with the purpose of obtaining stem that fits the criteria so as to produce a superior seedlings. Broodstock chosen whose body is not deformed or injured weighing not less than 300 grams, or at least 250 grams of body weight. This is in accordance with the statement [5] that the broodstock trepang used must be intact, with no wounds on the skin surface, the skin should be smooth and shiny with a thin layer of mucus and gave the reaction when there is stimulation.

The weight of sea cucumbers stem can be known by weighing. All broodstock cucumbers mixed in a tub with a minimum number of 20 head. The amount is assumed to be obtained 10 breeding sea cucumber breeding male and 10 female sea cucumbers, sea cucumbers because of sex between male and female sea cucumbers morphology can not be known except by surgery can not be recognized also holding a ripe gonads.

2.3. Spawning
Broodstock of sea cucumbers will be cultivated acclimatized beforehand in a tub of fiber volume of 200 liters and equipped with aeration equipment. Broodstock will be done spawning fiber mixed in a tub for holding cucumbers difficult to distinguish gender. All mains were fasted for clean up and remove the dirt that is in the broodstock body of sea cucumbers. Broodstock already finished spawning maintained for the purpose of spawning back.

Sand sea cucumber spawning is done using artificial spawning techniques with methods of thermal shock (temperature shock). Thermal shock method that efforts so that a temperature rise of 3 ° -5 ° C of temperature initially to be able to stimulate the broodstock to spawn. The water temperature is raised by using a heater (heater) to 32 ° C. Thermal shock method used in accordance with one spawning method proposed by [6] that the spawning method using temperature stimuli is to strive for the temperature rose 3 ° -5 ° C of the initial temperature using artificial heating devices. The method gives better results with hatching rate reached 95%.

The influence of the temperature rise causes the stem sea cucumber spawning behavior began to show. Broodstock cucumbers placed into a bath of fiber volume of 200 liters, equipped with an adequate supply of dissolved oxygen aerator.

Broodstock sea cucumber spawning behavior shows squirm or vertically elongate the body and overlap each other at the base of the tub. The male broodstock first white sperm that looks like smoke in the water, and then stimulate the mother to remove the eggs. Spawning lasts ± 30 minutes to an hour. Broodstock who have been spawned marked with water in tub turns white turbid, there are foam on the surface of the water and the water smelled fishy. The fertilized eggs float in water, while unfertilized settles in the bottom of the tub. Sea cucumber spawning process can be seen in Figure 9 and Figure 10.
2.4. Water quality management
Water quality management, is important for seeding, because if the bad water quality, sea cucumber broodstock would be difficult to spawn. Tools used for checking water quality parameters is a thermometer to measure the temperature, DO meter as a measure of the oxygen content, pH pens for checking the pH and salinity refractometer as a measure of water. The water quality measured during sand sea cucumber hatchery activity is 27-30 °C temperature, dissolved oxygen is > 5 mg / L, pH 7.5 to 8.6 and 30-34 ppt salinity. The range of water quality during seeding activity can be seen in Table 1.

| Parameter               | The range       |
|-------------------------|-----------------|
| Temperature (°C)        | 27-30           |
| Dissolved oxygen (mg / L)| > 5            |
| Ph                      | 7.5 to 8.6      |
| Salinity (ppt)          | 30-34           |

There is a difference in the temperature range of the value of water between the water temperature measured during the seeding phase with the statement [7]. [7] said that the condition of the waters for sea cucumber aquaculture preferably at a temperature of 20-25°C, 4-8 ppm dissolved oxygen, water pH 6.5 to 8.5 and 30-33 ppt salinity. The differences are probably due to the different farms and Events fluctuating weather. According to [8] the difference in the value of the water quality is not a problem because cucumbers have the ability to tolerate conditions of temperature and salinity environments.

3. Results and discussion

3.1. Shelter eggs
The fertilized egg, then harvested from spawning tub by using a hose and moved into a plankton net is 60 lm. Plankton net is placed half-submerged in a plastic tub containing sea water so the egg remains in a floating state. Eggs that are in a plankton net was transferred to a plastic container that has been filled with sea water. Plankton net then rinsed with sea water so that no eggs attached. This is in accordance with the statement [4] that the fertilized eggs of sea cucumbers are stored in a container and tub, rinsed with clean sea water to remove excess sperm. The transfer of eggs to be dilakukan carefully so that no egg is wasted. Shelter sea cucumber eggs can be seen in Figure 4.
3.2. Incubation eggs
Incubation of the eggs is done until the eggs hatch the eggs can be transferred to subsequent larval rearing tubs. Eggs that are in plastic containers transferred to the egg incubation tub. Egg incubation bath tub in the form of a transbroodstock glass fiber with a volume of 200 liters. Bak incubation has been first filled with sea water which is filtered by the filter bag as much as 180 liters and fitted with aeration and heater. The incubation temperature in the tub is set to 28 °C. Eggs incubate for 24 hours. The purpose of incubation is to accelerate the process of development of the eggs become larvae stadia auricularia.

3.3. Counting eggs
Eggs produced from spawning process necessary calculation to determine the density of sea cucumber eggs. Measurements of sea cucumber eggs in accordance with the declaration [9]. [9] suggested that measurements of the egg is done by taking a 1 ml sample of 5 different sampling points. Each sample is averaged, it will be obtained an average of 5 samples are then multiplied by the total volume of water in the container. The density of sea cucumber eggs numbered by the method of sampling at five different points then counted under a microscope. Egg is averaged and then multiplied by the total volume.
volume of water of the incubation. Samples of eggs which have been calculated incubation returned to
the tub. Results from sampling measurements of eggs are as follows:

a. \( \Sigma \) eggs from a total sample of 50 ml = 105 items
b. \( \Sigma \) total volume of incubation tub = 180 liters
c. \( X \) eggs = 2.1 grains / ml
   Kepadatan telur = \( X \) telur \( \times \) \( \Sigma \) total volume bak inkubasi
   = 2.1 \times 180000
   = 378.000 butir

3.4. Development and hatching eggs
Eggs that have been fertilized undergo cell division than 2 cell, 4-cell, 8-cell to multi-cell. Time for the
cleavage of about 7 hours after spawning. The next development is followed by a stage of blastula and
gastrula stage. The eggs will hatch approximately ± 32 hours after spawning into auricularia larval
stage. The temperature range for the hatching larvae are 30-32 °C. Auricularia larval stage and then
transferred into a bath of larval rearing. Larvae that will be transferred or stocked in a first sampled
maintenance tub to determine the initial stocking density. The development of sea cucumber eggs can
be seen in Figure 6.

![Figure 6. Development of eggs.](image)

3.5. Handling larva

3.5.1. Early counting larvae density
The eggs were incubated to hatch or reach stadia auricularia. Auricularia stage eggs were immediately
moved into a tub maintenance. Flyblow at the same time is used to determine the value of hatching or
Hatching Rate (HR). Hatching Rate (HR) is calculated by dividing the number of eggs fertilized by the
total number of eggs. Hatching Rate Calculation in accordance with the statement [9] that the
estimated level of fertilization is to divide the number of eggs fertilized by counting the total number
of eggs. Results of calculation Hatching Rate (HR) are as follows:

a. \( \Sigma \) larvae of the total sample of 40 ml = 55 individuals
b. \( \Sigma \) larvae incubation total of 180 liter tub = 247 500 individuals

\[
\text{Hatching Rate (HR)} = \frac{\Sigma \text{telur fertil}}{\Sigma \text{total telur}} \times 100\% \\
= \frac{247.500}{378.000} \times 100\%
\]

= 65.47%  

3.5.2. Larval development
Sea cucumber eggs develop into larvae have hatched. The development of sea cucumber larvae of
sand divided into several stages, namely:
a. Stadia *auricularia*
Auricularia stage is the initial stage larvae after eggs hatch. This stage has the characteristics of a transbroodstock body, the shape of the mouth like a funnel and take food by means of filtration using vibrating bristles help on the surface of the mouth. Auricularia stage consists of three stages: early auricularia, auricularia auricularia middle and end of which has an average size of 563 μ - 1.1 mm. Auricularia larval life stage as planktonis. Stadia auricularia larval form can be seen in Figure 7.

![Figure 7. Larva stadia auricularia.](image)

b. Stadia doliolaria
Stadia doliolaria thrive on day 13 to day 14. Stadia doliolaria shaped like a tube and can be contrasted with previous stadia views of the movement, which is moving rapidly toward the front. Feed taken by filtration with the aid of tentacles that extended out of the mouth. Stadia doliolaria known to consist of three phases, namely the transition doliolaria, doliolaria doliolaria middle and end that have a size of between 460-620 μ. The larvae live as planktonis doliolaria stadia. Doliolaria stadia larval form can be seen in Figure 8.

![Figure 8. Larva stadia doliolaria.](image)

c. Stadia pentactula
Entering the day 17 until day 18, the stadium will be turned into stadia doliolaria pentactula. The larvae stadia pentactula has the size range of 600-700 μ. Stadia pentactula with a tubular-shaped with five tentacles at the anterior part of the open cloaca, tentacles more clearly and making up the skin is already visible. Stadia are benthic pentactula. Pentactula stadia larval form can be seen in Figure 9.

![Figure 9. Larva stadia pentactula.](image)

3.6. Larvae maintenance
When the larvae reach the stadia doliolaria the end, be the installation of shelters of plastic fiber wrapped in a cloth into a tub maintenance. Mounting position vertically shelter is aimed at expanding the area of larval settlement and feed. This is in accordance with the statement [5] that the sea cucumber larvae stadia doliolaria seek substrate to settle and metamorphose into larvae pentactula. Suitable substrates include plastic sheets or sheet of fiberglass. Larval stage is a very critical stage, due to the low survival of larvae to the juvenile stage. Critical phase usually takes place at the stadium doliolaria end until early pentactula. Maintenance of sea cucumber larvae can be seen in Figure 10.

3.7. Feeding larvae
Auricularia larval stage until early pentactula stadia given natural feed in the form of phytoplankton of the type of *Chaetoceros* sp. *Chaetoceros* sp. derived from activities performed in a laboratory culture of sea cucumber hatchery[10, 11, 12, 13, 14]. This is in accordance with the statement [4] that if facilities for culture are limited, the use of plankton plankton *Chaetoceros* sp. is a good choice, because in addition to being one of the plankton used as sea cucumber larvae feed, *Chaetoceros* sp. able to live at high temperature tolerance and fairly easy to culture. The density of phytoplankton were given a minimum of 20,000 cells / ml, according to the statement [4] that on the second day of the larvae after hatching, used for up to four types of plankton as feed at a density of at least 20,000 cells / ml and increased to 40,000 cells / ml after 14 days. Phytoplankton density was calculated using the tool haemacytometer [15, 16, 17].

Frequency of feeding on larvae done once per day. Phytoplankton given after bath larvae undergo water exchange system. Before the natural food given in stages, the water is removed with a hose and filter for a total volume of plankton will be given. The volume of water sought to remain stable, so that no larvae were carried out.

3.8. Disease
Pests and diseases is one of the risks facing the sea cucumber hatchery sand. Infection diseases found are ulcer disease or injury occurs on the surface of the skin of sea cucumbers. Wound or ulcer disease is caused by bacteria. This type of bacteria causing the disease is unknown. [18] explained that Vibrio harveyi marked by exfoliating the skin surface until lightly injured in the sea cucumber. Control is done on the body of the diseased sea cucumber is using a disinfectant albaju. Albaju a drug for ornamental fish, but can be used for fish that do not have scales like cucumbers. Albaju form of a powder containing an active ingredient Nifurstyrenat-Sodium effective against bacteria Columnaris and Aeromonas. The bacteria are able to cause infection in the body of the fish or sea cucumbers injury occurs. The use of disinfectants albaju is by applying albaju the sea cucumber body injured and left briefly before being put back into the tub. Sea cucumbers are attacked by the disease can be seen in Figure 11.
4. Conclusion
Based sand sea cucumber hatchery activities that have been carried out for 30 days at the Center for Research and Development of Marine Aquaculture Gondol, Bali, it can be concluded that: 1) sand sea cucumber hatchery activities undertaken include the preparation of aircraft maintenance tub, supply mains, water quality management, feeding mother, broodstock selection, spawning, egg handling, development and hatching eggs, handling larvae, larval rearing and feeding larvae. Broodstock spawning sand sea cucumbers using the thermal shock method that efforts so that a temperature rise of 3-5 °C from the initial temperature to be able to stimulate the broodstock to spawn and 2) barriers that occur on the sand sea cucumber hatchery activity is difficulty in obtaining quality maternal and limited production of natural food.

5. References
[1] Darsono P 2011 J. Oceanol. Limnol. in Indonesia 37, 57-70
[2] Tuwo A and Conand C 1992 Inform. Bull. 4, 2-3
[3] Sukmiwati M, Salmah S, Ibrahim, S D and Handayani P P 2012 J. Natur Indonesia 14, 131-137
[4] Battaglene S C 1999 The ICLARM Quarterly 4, 1-8
[5] Agudo N 2006 Int. Agric. Res., 1-40
[6] Seeding T 2001 White Sea cucumbers (Holothuria scabra) Office of the Deputy Utilization and Correctional Science and Technology )wwwarintekristekgoid accessed on October 31, 2015
[7] Rustam 2006 Marine Aquaculture Training Coremap Phase II (Selayar District) pp 3-4
[8] Pitt R 2001 Inform. Bull. 14, 14-21
[9] Al Rashdi K M, Eeckhaut I and Michele R C 2012 A Manual on Hatchery of Sea Cucumber Holothuria scabra in the Sultanate of Oman (Muscat: Ministry of Agriculture and Fisheries Wealth, Aquaculture Center) pp 1-20
[10] Setyawati F, Satyantini W H, Arief M, Kismiyati and Pujiantuti 2018 J. Aqua. Fish Health 7, 50-56
[11] Daughter ADA and Tjahjaningsih W 2018 J. Aqua. Fish Health 7, 111-117
[12] Mubarak A S, Sulmartiwi L and Tias D T R 2009 J. Ilmiah Perikanan dan Kelautan 1, 67-72
[13] Arsad S, Stavrakakis C, Turpin V, Rossa P, Risjani Y, Sari L A, Prasetiya F S and Mouget J L 2019 IOP Conf. Series: Earth Environ. Sci. 236, 012 044
[14] Sari L A, Masithah E D and Alamsjah M A 2018 J. Fish. Mar. Res. 2, 9-14
[15] Sari L A, Satyantini W H, Manan A, Purseyto K T and Goddess N N 2018 IOP Conf. Series: Earth Environ. Sci. 137, 012 029
[16] Sari L A, Purseyto K T, Arsad S, Masithah E D, Setiawan E and Affandi M 2019 Poll. Res. 38, S27-S32
[17] Sari L A, Sari P D W, Nindarwi D D, Arsad S and Affandi M 2019 Eco. Env. Cons. 25, S26-S31
[18] Becker P, Gillan D, Lanterbecq D, Jangoux M, Rasolofonirina R, Rakotovao J and Eeckhaut I 2003 *Aquaculture* **242**, 13-20

**Acknowledgement**
The authors gratefully acknowledge the financial support from the Annual Budget of the Faculty of Fisheries as well as the instrument support.