Substrate Types on Habitat Breeding Embryonic Development and Larval Morphometric of Sea Urchin *Tripneustes gratilla* (Linnaeus 1758)

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Abstract. Hatchery is the main factor that plays an important role in aquaculture sea urchin *Tripneustes gratilla* (Linnaeus 1758). The quality of sea urchin egg is determined by the absorption of nutrients that carried throughout the gonad development while the quality of nutrients is very likely influenced by the type of food consuming. This research of substrate types on habitat breeding embryonic development and larval morphometric of sea urchin had been done on October - November 2016 at the laboratory of marine, Research Center for Deep Sea, Indonesia Institute of Sciences, Ambon. The Samples taken from three different habitats comprises Hative Besar, Liang and Suli beach. The purpose of this research is to determine the effects of substrate types on habitat breeding embryonic development and larval morphometric of sea urchin. The method that has been used to determine the morphometric was done by measuring the diameter and height of the shell using a digital caliper and total body weight using a digital scale. To observe embryonic development and larval morphometric done by in vitro fertilization through eggs selection then cultured in petri dish at a density of 10 ind/ml. The result of this research is the most dominant quantity of embryonic development (72 hours) indicated by culture vessel from Hative Besar meanwhile the least is indicated by Liang. The greatest larval morphometric is indicated by culture vessel from Hative Besar with length and height of larval body further the larval of culture vessel from Hative Besar shown ossification formation.

1. Introduction

Sea urchin belongs to the Echinoidea class is a thorny sea animal that is found in relatively shallow waters. Sea urchin is one of the valuable commodities exported from the Philippines. Gonads, which are high value fishery commodities, are sold as fresh eggs on the world market, especially Japan, China, Korea, France and Chile. Sea urchin *Tripneustes gratilla* has a high economic value in the international markets, fresh gonad prices on the world market can reach US $ 450/kg wet weight [1]. Sea urchin movement allows the animal to find better eating conditions and improve health through growth and reproduction. Movement also shows population adaptation in heterogeneous environments. Sea urchin is an active herbivorous to find high quality food on the seabed [2]. Uncontrolled and non-selective harvests coupled with other factors led to a decline in fisheries production in 1992,
Tripneustes gratilla is an important economic fishery resource that can increase the incomes of people who employ thousands of families of coastal fishermen [3].

In Indonesia, the potential for sea urchin fisheries has not been optimally developed. However, its population in nature is decreasing due to capture by local people for consumption and commercial purposes without regard to its sustainability aspects. Hatcheries are determinants of production factors that play an important role in the development of marine biota cultivation. The availability of seeds cannot depend on nature alone, because in terms of quality and quantity is not sufficient for cultivation. Efforts to ensure the quality and quantity of seeds will require good candidates with good gonad quality, gonads act as a storage place for nutrients. Types of food, quantity consumed and frequency of eating during the culture phase all influence the process of metamorphosis and the development of sea urchin larvae [4].

In sea urchins, because the process of fertilization and development of larvae occurs externally, inherited nutrient investment (maternal provisioning) in gametes will be completed when spawning. Therefore, the diet consumed and the availability of food for adult has the potential to affect egg quality, larval morphometry and the level of larval development. Furthermore, larvae can develop using food reserves from eggs [5], energy reserves are consumed entirely for development larvae [6] [7]. Larvae are organisms that are between the phases after the embryo and juvenile for both invertebrates and vertebrates. Aquatic invertebrate larvae in their development are supported by the nutrients provided by the egg. At the beginning of the reproductive cycle, the size of the gonads increases due to gonadal nutrient storage. During vitellogenesis, these nutrients are used for development so they enlarge and increase in number. Mature gametes are then stored in the gonads until spawning occurs [8]. Functional differentiation of the digestive tract, enzyme systems and cilia is needed to achieve the feeding phase and metamorphosis. Many echinoderms use egg triglycerides as the main energy in larval development [5]. When the urchin larvae enter the final stage of the planktonic phase, the larvae will find a suitable place to metamorphose into adulthood. The condition of the substrate is closely related to the process of metamorphosis. Sea urchin larvae will choose a hard substrate and are also overgrown with many plants both algae and seagrass. One of the main reasons for choosing a hard substrate is that it is very suitable as a nursery ground that can protect young larvae or sea urchins from currents or predators; this is what causes sea urchins to be found in sandy areas to coral reefs [9].

Hative Besar, Liang and Suli beach in the waters of Ambon Island are the habitats of sea urchin with abundant populations throughout the year [10]. These three habitats are known to have different substrate characteristics, namely the substrate type on the Hative Besar beach is the dominant turf algae attached to the rocks on the beach, the substrate type on the Liang beach is sandy area interspersed with coral and overgrown with macroalgae dominated by Padina sp. and Sargassum sp. which was interspersed with seagrass vegetation while the substrate type on the beach of Suli was a sandy area with a stretch of seagrass fields dominated by Thalassia hemprichii. Different habitat factors in each location with varied food composition need to be studied to determine the effect of the early development of eggs and larvae. This study aims to determine the effect of substrate on brood habitat on embryonic development and larval morphometry of Tripneustes gratilla.

2. Material and Method
Materials used in this study include: buckets, digital caliper (Mitutoyo type CD-8 "C5X, Japan), digital scales (Ohaus type pro adventurers, USA), petridishes, optical microscopes with tools for Dino-Eye Microscope Eye Piece Camera, column counter (Sedgewick rafter counting chambers), hand counter, syringe, 80 ml glass beaker, 1000 ml beaker, micropipette, DO meter, acidity meter, refractometer and
65-80 μm filter construction. While the ingredients used in this study include: sea urchins as many as 5-10 broodstock, macroalgae, 0.5 M KCl and formaldehyde solution. This study was experimental using a completely randomized design (CRD) with three treatments, each consisting of six observation times and three replications. The total consists of 54 experimental units.

The study time was in October-November 2016. The research was conducted at the laboratory of marine aquaculture, Research Center for Deep Sea, Indonesia Institute of Science, Ambon, while sampling was carried out on the Hative Besar beach (the substrate was dominated by turf algae), Liang beach (substrate dominated by macroalgae) and Suli beach (substrate dominated by seagrass) (figure 1).

3. Data Analysis

3.1 Parent Collection Sea urchin Tripneustes gratilla
The parent of Tripneustes gratilla as many as 5-10 individuals were collected from the Hative Besar, Liang and Suli beach that selected based on the size of the cell (diameter>40mm) are then taken to the laboratory to undergo the selection process. The chosen sea urchin parent candidates are healthy, characterized by active biota activity, tube feet that are firmly attached to the substrate, thorns that tighten hard, bright biota colors and most importantly there is no wound on the sea urchin wall.

3.2 Recording of Parent Morphometry Sea urchin Tripneustes gratilla
The selected Tripneustes gratilla as prospective test animals were marked and their morphometric characteristics were major measured, including shell diameter, shell height and total weight. The diameter and height of the shell are measured using a digital caliper (CD-8 type Mitutoyo "C5X, Japan), and the total weight is measured using a digital scale (Ohaustipe adventurer pro, USA).
3.3 Parent Spawning Sea Urchin Tripneustes gratilla
The selected *Tripneustes gratilla* as the prospective parent, after the morphometric characteristics were detected, were then cleaned with sterile sea water. Sterilization is also performed on all equipment and containers used during the spawning process by using autoclave for 15 minutes.

Spawning of sea urchins using KCL 0.5 M as much as 1-3 ml through the peristomal membrane into haemocoel. Individual sea urchins that have been injected with KCl, are then rocked in a ground before placing them on the beaker in an aboral position facing down. Beaker cups are filled with sterile sea water for sea urchins that release eggs and are left empty / dry for sea urchins that release sperm. The collection of gametes is carried out for ± 30 minutes. Besides that, also noted the appearance of color on eggs produced from spawning of sea urchins.

3.4 Selection and Measurement of Sea Urchin Tripneustes gratilla
The eggs used in this study are eggs that have a size between 65-82 μm. Eggs of this size are obtained by passing eggs through a 80 μm pore membrane filter on the top and a 65 μm pore membrane filter at the bottom. The eggs that are trapped in between, are then transferred into a measuring cup to count (gr/ml) with the help of the column counters (sedgewick rafter chambers) under an optical microscope at 4x10 magnification. Then, using the Dino-Eye Digital Microscope EyePiece Camera and software (Dino Capture 2.0) the integrated egg is measured in the egg diameter (figure 2).

![Figure 2. Measurement of sea urchin *Tripneustes gratilla* eggs using DINO-EYE Digital Eye Piece Camera Microscope and Dino Capture 2.0 software.](image)

3.5 Fertilization in vitro
The fertilization process is carried out by mixing filtered eggs in 1000 ml of sterile sea water with 1 ml of concentrated sperm. One hour after fertilization and more than 99% of eggs have been fertilized (embryonic phase), then filtered and cleaned of sperm remnants. The embryos that have been cleaned are then stored in a 1000 ml beaker glass containing sterile sea water. Embryo density was calculated using the count column that was observed using an optical microscope at 4x10 magnification.

3.6 Embryo Culture
Cultures were made with a density of 10individuals/ml with three replications for each sub culture treatment in a petri dish with a volume of 25 ml containing sterile sea water. During its development the embryo is not fed. This aims to find out the quality of the investment that is passed down by the parent to the eggs and eggs to the larvae. Sampling to count the number of embryos and morphometry of larvae was carried out as many as five replications for each treatment by placing 1 ml of sample in the counters pool (sedgewick rafter counting chambers) and observed with an enlargement of 100x under an optical microscope according to the specified time. The sampling time for routine embryo counting after fertilization is 1 hour, 6 hours, 12 hours, 24 hours, 48 hours and 72 hours.
3.7 Evaluation of Embryonic Development and Larval Morphometry of Tripneustes gratilla

Evaluation of embryonic development and larval morphometry was carried out by measuring several parameters in the form of embryo development and larval morphometry at the end of 72 hours of maintenance.

Data analysis was carried out descriptively for data related to morphometric characteristics, egg quality, embryonic development and water quality. The data obtained from the results of a completely randomized design (CRD) in the form of larval morphometry were analyzed using One Way ANOVA by Duncan's test on 5% error.

4. Result

4.1 Morphometry Recording of Parent Tripneustes gratilla

Morphometric recording including shell diameter, shell height and total body weight are shown in table 1.

| Location and Substrate type | Morphometry  |
|----------------------------|--------------|
|                            | D (mm)       | H (mm)     | W (g)      |
| Hative Besar: the massive sand and rock substrate is dominated turf algae | 71.60±10.40  | 43.14±10.59 | 142.25±10.47 |
| Liang: the substrate of dead coral and sand is dominated macroalgae, Padina sp. and Sargassum sp. | 59.19±10.81  | 41.92±30.13 | 85.04±50.61  |
| Suli: the sand substrate is muddy and overgrown with dominant seagrass T. hemprichii | 52.05±70.52  | 38.30±30.50 | 61.24±15.60  |

*Description: D (shell diameter), H (shell height), and W (body weight)*

The results of morphometric recording of sea urchin Tripneustes gratilla from three locations showed that sea urchin from the Hative Besar beach had a larger morphometry with a shell diameter of 71.60 mm, a shell height of 43.41 mm and a total body weight of 142.25 g compared to the parent sea urchins from Liang beach with shell diameter of 59.19 mm, shell height of 41.92 mm and total body weight of 85.04 g. While the morphometry of sea urchins from Suli beach is the mother with the smallest morphometry where the shell diameter is 52.05, the shell height is 38.30 mm and the total body weight is 61.24 g.

4.2 Egg quality of Tripneustes gratilla

The indicator used to determine egg quality in this study is the diameter, number and color of eggs. The size of the egg diameter used in this study is 65-80 μm. The mean diameter and range of Tripneustes gratilla eggs from three locations is shown in table 2.
Table 2. Average egg diameter and egg diameter range

| Location and Substrate type | Average egg diameter (µm) | Range egg diameter (µm) |
|-----------------------------|---------------------------|-------------------------|
| Hative Besar: Massive sand and rock substrate dominated turf algae | 73.87±20.52 | 68.35-80.13 |
| Liang: Massive sand and rock substrate dominated macroalgae, Padina sp. and Sargassum sp. | 73.66±30.37 | 65.81-81.91 |
| Suli: The sand substrate is muddy and overgrown with dominant seagrass T. hemprichii | 74.93±30.17 | 65.21-81.60 |

While the number of eggs and colors in eggs produced by female sea urchin from three locations are shown in table 3.

Table 3. The number of eggs and eggs color spawned by female parent T. gratilla.

| Location and Substrate type | Individual (Treatment) | Number of eggs | Color Egg |
|-----------------------------|------------------------|----------------|-----------|
| Hative Besar: Massive sand and rock substrate is dominated turf algae | 1 | 16.69 x 10⁶ | Orange |
|                            | 2 | 19.70 x 10⁵ | Orange |
|                            | 3 | 13.10 x 10⁵ | Orange |
| Liang: The substrate of dead coral and sand is dominated macroalgae, Padina sp. and Sargassum sp. | 1 | 47.7 x 10⁴ | Orange |
|                            | 2 | 23.96 x 10⁵ | Orange |
|                            | 3 | 35.41 x 10⁵ | Yellow |
| Suli: The sand substrate is muddy and overgrown with dominant seagrass T. hemprichii | 1 | 16 x 10⁵ | Yellow |
|                            | 2 | 16.8 x 10⁵ | Yellow |
|                            | 3 | 2 x 10⁶ | Orange |

The color of the eggs affected by carotenoids is absorbed through the feed consumed by sea urchins in nature and accumulates in the gonads. The color of the gonads varies from dark brown, greenish, orange, dark yellow, light yellow and transparent (clear). This color variation is related to gender and gametogenesis level [12].

4.3 Embryonic Development of Tripneustes gratilla
Embryonic development was calculated based on the observation time of 1 hour, 6 hours, 12 hours, 24 hours, 48 hours and the final 72 hours of maintenance on each culture container. The results of the One-Way ANOVA test for embryonic development are shown in figure 3.
A. Histogram culture embryo Hative Besar beach

Description: Different colors indicate the time of observation
- 1 hour
- 6 hours
- 12 hours
- 24 hours
- 48 hours
- 72 hours

Development phase
1. 2, 4, 16, > 32 cells
2. (b) blastula beginning, (e) blastula end
3. (g) gastrula beginning, (m) gastrula middle
4. (e) gastrula end, (p) prism beginning, (e) prism end
5. 2 arms, 4 arms and 8bs blastula

B. Histogram culture embryo Liang beach

Description: Different colors indicate the time of observation
- 1 hour
- 2 hours
- 12 hours
- 24 hours
- 48 hours
- 72 hours

Development phase
1. 2, 4, 16, > 32 cells
2. (b) first blastula, (e) end blastula
3. (g) first gastrula, (m) midle gastrula
4. (g) end gastrula, (p) first prism, (e) end prism
5. 2 arms, 4 arms and 8bs blastula
Figure 3. Histogram of embryo culture from three locations with different substrates.

The embryonic development histology of sea urchins *Tripneustes gratilla* showed that in the culture container of the Hative Besar beach, the quantity was greater than the other two culture containers, namely from the Liang beach and Suli beach.

The embryos that were cultured from the Hative Besar beach, at 1 hour observation enters the 16-cell division phase and many embryos go into the cleavage phase > 32 cells, yet some are still at the two-cell division stage. At 6 hours of observation, the embryo in the cleavage phase > 32 cells have developed to form the early blastula and at 12 hours of observation there was the formation of the late blastula. At 24 hours of observation, the gastrulation was formed. But its development is not uniform because of the presence of embryos still in the early gastrula stages, the middle gastrula, and the late gastrula stages that developed form a prism and for 2 arms at 48 hours of observation and at the end of 72 hours observation were already formed 4 arms. But also found dominance in the blastula phase was imperfect.

The embryos that were cultured from Liang beach showed a significant difference in terms of quantity and stages of development. At 1 hour observation, a huge number of embryos developed from 2 cells, 4 cells and reached the cleavage stage > 32 cells. At 6 hours of observation the embryos developes forming the blastula, until the 12 hours of observation forms the late blastula. At 24 hours of observation, gastrulation occurred until the 48 hours of observation, the final prism formed, but the number decreased at the end of 72 hours of maintenance where the number of larvae with 4 arms was only slightly compared to the number of larvae in the culture container of the Hative Besar and Suli beach. This is indicated to be possible because the embryo at the morula phase is unable to continue development and die.

The embryos that were cultured from the coast of Suli showed different variations where at 1 hour of observation there was still a cleavage phase of 1 cell to 4 cells. At 6 hours of observation develop into the phase > 32 cells. That number increases during 12 hours of observation when the number of embryos that develop forms a larger late blastula phase. Then at 24 hours of observation gastrulation occurred but the amount decreased. This is indicated to be possible because of the
The measurement of the larvae at the end of the maintenance of each container of the culture shows that the larval morphology of sea urchin from the Hative Besar beach is greater than the larval morphology of sea urchin from the Suli and Liang beach. According to table 4, it can be seen that the larval morphology of sea urchin on a culture container originating from Hative Besar beach has a postoral arm length (PA) of 154.08 μm, body width (BW) of 143.41 μm, body length (BL) of 60.34 μm and overall body length (OBL) of 307.74 μm. Larval morphology of sea urchin on culture containers from the Suli beach has a postoral arm length (PA) of 142.36 μm, body width (BW) of 130.81 μm, body length (BL) of 51.41 μm and overall body length (OBL) of 268.21 μm. While the larval morphology of sea urchin in culture containers from Liang beach are the smallest average size remains of postoral arm length (PL) is 93.40 μm, body diameter (BD) is 117.99 μm, body length (BL) is 44.41 μm and overall body length (OBL) of 201.84 μm. After further tests of Duncan on the measuring of the larva (table 4) were obtained the following results:

a. The length of the postoral larval arm on the culture container of the Hative Besar beach and the Suli beach is the same and different from the length of the postoral larva arm on the culture container from the Liang beach.

b. The larval body width in the culture container from the Hative Besar beach is different from the larval body width in the culture container from the Liang beach and both differ significantly from the larval body width in the culture container from the Suli beach.

c. The larval body length in the culture container from the Hative Besar beach is different from the larval body length in the culture container from the Liang beach and both are significantly different from the larval body length in the culture container from the Suli beach.
The entire length of the larval body in the culture container of the Hative Besar beach and the Suli beach is the same and different from the length of the larval body in the culture container of the Liang beach. In the final 72 hours of maintenance, in each culture container showed differences in morphology of the larvae, as formed in figure 4.

From the picture it can be seen that the larvae in the culture container of the Hative Besar beach have prominent differences with the length of the larval arm that is longer and also has reinforcement compared to larvae in the culture container of the Suli and Liang beach which have shorter arms and none reinforcement. The average measurement of water quality in culture containers from three locations indicates that DO (Dissolved oxygen) is in the range of 4-5 mg/l. The salinity value in the culture container is in the range 35-360 / 00. This acidity value is in the range 9.8-9.9. The temperature is in the range of 30°C. From the measurement results, the value of water quality in the culture container is in optimum condition.

![Figure 4](image)

**Figure 4.** The morphological difference between sea urchin larvae at the end of the 72 hours of culture maintenance from all three locations.

### 4.5 Water Quality

The results of water quality measurements in culture containers (petri dishes) are shown in table 5.

| Hydrological Parameters | Location and Substrate type |
|-------------------------|-----------------------------|
| Dissolved Oxygen (Mg/l) | Hative Besar. The massive sand and rock substrate is dominated *turf algae* 5.25 |
| Salinity (%/00)         | 35.75 9.94 30.47 |
| Acidity                 | Liang. The substrate of dead coral and sand is dominated *macroalgae, Padina sp.* and 4.45 36.25 9.93 30.05 |
| Temperature (°C)        | 30.05 |
| Location and Substrate type | Hydrological Parameters |
|-----------------------------|------------------------|
|                            | Dissolved oxygen (Mg/l) | Salinity (‰) | Acidity | Temperature (°C) |
| *Sargassum* sp.             |                        |              |         |                 |
| Suli                        | 5.05                   | 36.25        | 9.80    | 30.10           |
| The sand substrate is muddy |                        |              |         |                 |
| and overgrown with dominant seagrass *T. hemprichii* | | | | |

5. Discussion

The composition of sea urchin size used in this study includes shell diameter, shell height and total body weight. The results showed differences in parent morphometry from three locations with different substrates. In addition to habitat, density, shell size, another important thing about sea urchins is the type of food. According to [13], sea urchins feed on seagrasses, detrital material is also micro and epiphytic and epibenthic macroalgae. The process of food ingestion is an important aspect of nutrition. The amount and frequency consumed by sea urchins are influenced by the physical and chemical characteristics of the food. Therefore, differences in the types of feed found at each location are indicated to have an effect on larval growth and development.

During the sea urchin larval phase, *Tripneustes gratilla* still use maternal food to fuel the development of larvae contained in yolk [5]. Maternal food is invested by the mother into eggs from food obtained from nature. The food consumed contains carotenoids which function as color pigments in the gonads and also as reserves of facultative food during embryonic development when food sources from nature are not available. The waters of the Hative Besar beach with massive sand and rock or substrate types are areas that are dominated by turf algae from *Rhodophyta* or red algae. The size of the algae is very small, which is less than 2 cm, making it easier for herbivorous organisms in the process of swallowing food.

Liang beach with substrate types in the form of dead coral fragments, sand and also dominated by macroalgae *Padina* sp. and *Sargassum* sp. The content and composition of pigments in *Padina* sp., namely violaxantin (5.99%), β-carotene (4.70%) and chlorophyll (4.93%). The content and composition of pigments of *Sargassum* sp., namely chlorophyll a (52.82%); fucoxanthin (20.95%); chlorophyll a derivative (14.88%); total xanthophyll (8.46%); β-carotene (1.49%); chlorophyll c (1.05%); and chlorophyll c derivatives (0.35%) [14]. Based on the composition and percentage of the content, it is seen that the pigment in the crude extract of *Sargassum* sp. the most abundant is chlorophyll a while the most carotenoids are xanthophyll, especially fucoxanthin.

Suli beach waters with muddy sand substrate types dominated by seagrass vegetation *Thalassia hemprichii*. Phytochemical content of these seagrasses is steroids or triterpenoids, flavonoids, saponins and tannins. Several studies have shown that carotenoids are very important for the development and survival of larvae [15]. Carotenoids, β-carotene and β-echinenone, xanthophyll and fucoxanthin play an important role in the biological defense of sea urchins. Xanthophils, such as cantaxanthin and astaxanthin, may increase fertility, reduce mortality during larval development and increase the ability of larvae to tolerate harsh environmental conditions such as light effects. [16] found that β-carotene increased gonadal growth, egg content, energy and larval development rate in *Strongylocentrotus droebachiensis*. found that fucoxanthin, β-carotene and β-echinenone increase the biological reaction of defense and increase egg production [17] [18].
This is what allows the quantity and quality of embryos and morphometry of sea urchin *Tripneustes gratilla* from the Hative Besar beach to be cultured better than the Liang and Suli beach. The results of laboratory studies show that sea urchin living on abundant turf algae shows a high rate of growth and reproduction due to the high quality of food [2]. Furthermore it is said that the quality and quantity of food has a significant effect on gonadal growth and larval development [19].

6. References

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