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Article

Laboratory Cultivation of Sea Urchins for Sustainable Development

J Jumrodah[12*], S Liliasari[1], Yusuf Hilmi Adisendjaja[1], Yayan Sanjaya[1]

[1] Program Studi Pendidikan IPA, Sekolah Pascasarjana, Universitas Pendidikan Indonesia Jl. Dr. Setiabudhi No. 229, Bandung 40154, Indonesia.
[2] Program Studi Tadris Biologi, Institut Agama Islam Negeri Palangka Raya, Indonesia Jl. G. Obos Komplek Islamic Centre Palangka Raya Indonesia.

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a b s t r a c t

Sea urchins are marine biota from phylum echinoderms that have an important role both ecological and economics. The existence of sea urchins in nature has decreased due to over fishing for marketing demand both nationally and internationally. Sustainability cultivation and management of sea urchin is an important way to maintain the marine biological resources. Conservation and cultivation of sea urchins should be done ex situ through education in universities. Sea urchin cultivation in the laboratory consists of four stages: preparation of feed, spawning and fertilization, maintenance of larvae and enlargement of larvae. In the preparation of feed should be pay attention to external factors including: temperature, lighting and chemicals as phytoplankton growing media. In the spawning and fertilization temperature and gonad maturity should not be used chemicals but only using temperature stimulation. The maintenance of larvae should be paid attention to the feed patterns used adjusted to the stages of larvae growth and development, temperature and salinity. The enlargement of larvae should be paid attention in feeding and change of water. The cultivation can provide benefits in the form of conservation or re-stocking in nature and also develop the of students' bio-entrepreneurship skills in the field of marine biology.

1. Introduction

Sea urchins are one of the marine biota that important as a component of the food chain. They have ecological and economic value (Kato and Schroeter, 1985; Darsono, 1999; Yurson, 2009). Sea urchins play an important role in marine ecology, because the biota is determinant of abundance and distribution of shallow marine plants, especially in sea grass ecosystems (Valentine & Heck, 1991; Suskiewicz and Johnson, 2017). Sea urchins are the main species that control seaweed communities and damage to sea grass habitats (Yiu and Feehan, 2017). Juvenile of sea urchins are food for lobsters, crabs and demersal fishs (Scheibling and Hamm 1991; Lumingas *et al*., 1996; Scheibling and Robinson 2008; Fagerli *et al*., 2014; Feehan *et al*., 2014; Yiu and Feehan, 2017). Sea urchins are found on sandy beaches and classified as zero waste products.

Sea urchin has the protein Docosahexaenoic Acid (DHA) (Jose et al., 2014). Also lipids, glycogen, calcium, retinol, vitamin B, riboflavin, cyanocobalamin, nicotinic acid, panthothenic acid, folic acid and carotene, omega-3 and omega-6 found in the gonads (Kato and Schroeter, 1985). Sea urchin gonads are also rich in bioactive compounds such as polyunsaturated fatty acids (PUFAs) and β-carotene (Dincer and Cakli, 2007). PUFAs are important for human nutrition (Lawrence, 2007). β-Carotene and some xanthophils have strong provitamin-A activity and can be used to prevent tumor development and light sensitivity(Britton et al., 2004).

Sea urchins as food ingredient also functions as a development and growth organism model (Ghorani et al., 2012). Davidson and Cameron (2003) used sea urchins as research models in the fields of biology, cell biology, molecular biology of gene regulation, evolutionary biology, biochemistry of metabolite and marine biology, because of several advantages, such as they easy to observe the structure and function of the cis-regulatory system (one-sided regulator), for isolation of transcription factors and for exploration of gene networks.

Sea urchins are also benefits economics, at least twenty-one species of sea urchin gonads are traded in several Asian countries with a total production of around 73,000 metric tons (Castilla-Gavilan et al., 2018). Sea urchins have high economic value in the global market. Fresh, dried and salty sea urchin prices reach USD $ 60 / kg (Sun and Chiang, 2015). Japan is the main market for the sale of sea urchins globally with annual demand of around 50,000 metric tons (Jorge et al., 2018).

The role and benefits of sea urchin as a food chain in nature are also as source of protein needed by humans. Protein gonads composition in of sea urchins are different varies from each species, *Diadema setosum* per 100 grams, there are 39.18 gr dry or raw conditions (Toha, 2006).

Strongylocentratus nudus 11.70 %, *S. franciscanus* 12.18%, *S. droebhaciensis* 14.96%, *S. purpureus* 14.03% (Hirano et al., 1978; Kramer & Nordin, 1979). *Tripneustes gratilla* in fresh condition has a protein content of 15.43-25.67% (Wiralis et al., 2015). Protein gonad *Tripneustes gratilla* has the most best composition when compared to other species. Gonad *Tripneustes gratilla* can improve health and nutrition especially for children (Wiralis et al., 2015). In the global market the quality of the gonads of *Tripneustes ventricosus* and *Tripneustes gratilla* are the best quality when compared to other species (Jorge et al., 2018).

* Corresponding author.
E-mail address: jum_rodah@student.upi.edu
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Sea urchins have not been utilized optimally in some areas in Indonesia, usually they still become garbage and disturbing fishermen, therefore many fishermen throw them away when caught in fish nets. In 2009 sea urchins in Indonesia have begun to be cultivated, but were in very limited, e.i. in the Tidung Island in the Thousand Islands, until now it has been increased on Panggang Island and Pari Island (Idjo, 2016). The cultivation is only an adult enlargement, while the juvenile have been taken at the sea. Therefore it caused exploitation of sea urchins in nature. However in Indonesia there is a great opportunity for the development of sea urchins cultivation in the laboratory, especially through education in universities.

Exploitation of sea urchins in nature will cause a decline in population. The decreased population of sea urchins in nature will cause an imbalance of ecological functions in their natural habitat, as the destruction of one food chain. In addition to overfishing, the other decline in sea urchins populations caused by very slow growth. At the larvae stage it has a relatively long time between 30 to 40 days (Dworjanyn et al., 2007). The larvae stage until the gonad is ready for harvest or an adult takes about 14 months (Rahman et al., 2016). Sexual maturity of *Tripneustes depressus* occurs when the body size is 4.2 cm at the age of 1 year, but the gonad is not ready to be harvested (Jorge et al., 2018). Sexual maturity will occur at around 3 years of age with a shell size indicator reaching 4 cm (Radjab, 2016).

The sea urchin biodiversity in nature can be maintained, if appropriate actions and strategies are taken, to improve conservation and restocking in nature, also sea urchin development and cultivation can be carried out. The interest in sea urchin cultivation has grown rapidly and has grown well over the past few years (Lawrence dan Agatsuma, 2013; Paredes et al., 2015; Jorge et al., 2018).
Sea urchins, in Indonesia it has begun to be utilized but not optimal yet. The use of sea urchin gonads is still traditional in nature, for example for small busines such as restaurants in the coastal tourism and household consumption of communities coastal (Imrantika, 2017).

Management of marine resources in Indonesia so far is focused on overfishing, therefore it does not consider the negative impacts on the marine ecosystem. This is very contrary to SDGs (Sustainable Development Goals) education point 14 (UNESCO, 2017); about life below water: conserve and sustainably use the oceans, seas and marine resources for sustainable development. The coastal and oceanic regions are the hope of the foundation of human life in fulfilling the needs of the present and the future. Therefore it should be managed and empowered to increase the sustainable economic the community value.

Sustainable development (SD) in Indonesia is stipulated in Peraturan Presiden no. 59 Tahun 2017, one of its aims is to preserve and sustainably utilize marine resources for sustainable development. In order for this goal to be achieved, it must be included in the education curriculum in Indonesia both at the school and university. Based on the results of curriculum studies at several universities in Indonesia, it has not fully provided education that is of a sustainable nature especially in the field of maritim affairs. The curriculum in Indonesia is expected to facilitate learning that leads to sustainable development.

The main goal of sustainability is to improve global living standards by managing and utilizing sustainable marine living resources (Murray et al., 2013). Marine biota is a marine biological source that has the potential to provide economic benefits to humans in a sustainable manner (Pulz and Gross, 2004; Wijffels, 2008;).

2. Materials and Methods

2.1 Sample Collection

This research is an experimental laboratory research conducted for three months starting from 29 November 2018 - 25 February 2019 in the LIPI Ambon laboratory. Sea urchins use an average wet weight of 135.05 g and an average diameter of 68.8 cm and as many as 40 prospective adult of *Tripneustes gratilla*.

2.2 Maintenance sample

Taking *Tripneustes gratilla* was carried out at low tide with a depth of 50-70 cm in the morning between 08.00-10.00 WITA on October 26-27 2018 on Liang Beach and Suli Beach in Ambon. Adult sea urchins that have been collected are put into a net with *Padina pavonica* to be taken to the Ambon LIPI cultivation laboratory as a place of research.

3. Result

Marine ecology learning in higher education can be done on a project based by managing and utilizing sustainable marine resource potential, specifically designing sea urchin cultivation in the laboratory.

Stages of *Sea Urchin* Cultivation Method in the Laboratory

Stages of sea urchin cultivation and processing in the laboratory can be done by steps: feed preparation of sea urchin larvae, spawning and fertilization, maintenance of larvae and enlargement of juvenil to adulthood.

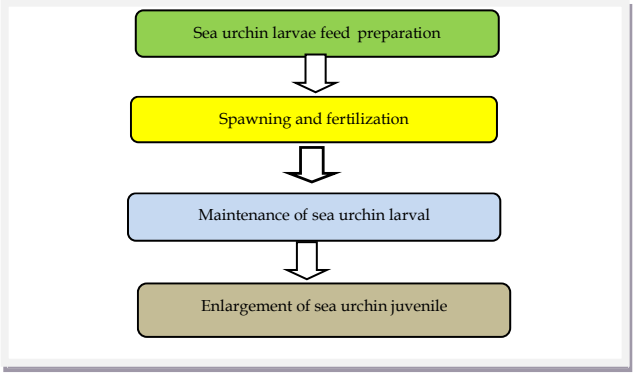

Figure 1. Stages of Sea urchin Cultivation Method in the Laboratory

3.1 Sea Urchin Larval Feed Preparation

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 *Chaetoceros calsitrans* while the larvae at the settle stage then the feed given is *Navicula* sp.

Feed in the form of phytoplankton which is cultivated in a sterile laboratory room with external factors that support for example room temperature ranges from 25-30^{0}C, lighting intensity ranges from 500-10,000 lux. Optimal growth salinity 26-35 $^0/_{00}$.

The stages of making diatome media for the cultivation of *Chaetoceros calsitrans* phytoplankton (Figure 2) and Okinawa media for phytoplankton cultivation of *Navicula* sp. (Figure 3).

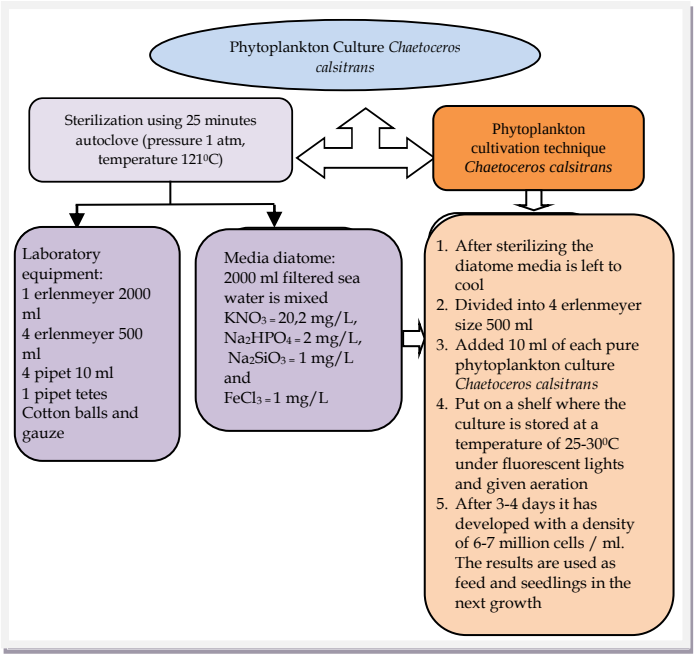

Figure 2. Stages Culture phytoplankton *Chaetoceros calsitrans* in the Laboratory

Phytoplankton cultivation can be carried out both on a laboratory scale and on a mass scale. In other hand it must pay attention to chemical aspects related to nutrient composition and hatchery management aspects related to sterilization. Scheme of phytoplankton cultivation in figure 3.

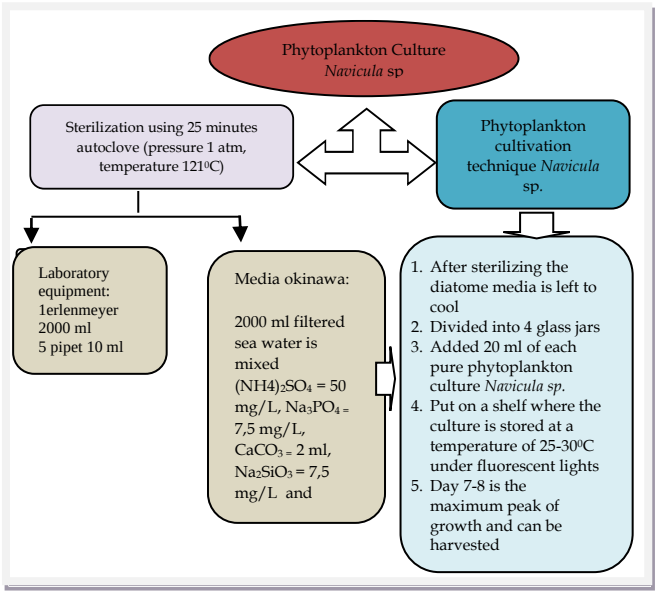

Figure 3. Stages Culture Phytoplankton *Navicula* sp. in the Laboratory

3.2 Spawning and Fertilization

Some things that should be considered before conducting sea urchins cultivation are the selection of the gonads (sexual maturity) (egg cells and sperm cells) to 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 fertilized eggs cell for *T. gratilla* sea urchins with an average size of 40-60 µm.

Spawning of adults sea urchins does not use chemicals but uses temperature stimulation (raising the initial temperature). Before stimulating sea urchins spawning, adults are weighed and measured next then cleaned the surface of the shell using sea water.

Spawning Method: Spawning at the time of the study was carried out with slowly raising the water temperature (the early temperature of 29⁰ C is increased to 34⁰ C). The sea water is put into the tank until it reaches 50 L and salinity 35-39 ⁰/₀₀. The adult sea urchins are placed evenly in the bottom of the aquarium without aeration. Indicators of spawning are usually seen as the parents' behavior tends to rise to the surface of the water through the aquarium wall. For 45 minutes one of the parents takes out sperm cells. With the indicator of the liquid released is milky white. After 7 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 x. Then measure the diameter and make sure healthy eggs cell are ready for fertilization. At the time of spawning, the male parent generally releases the sperm cells and then the female parent ejects the egg cell (Radjab, 2001; Levitan 2005). About 120 minutes after the spawning process, the adults sea urchins are removed from the aquarium and moved into the tank to be re-maintained.

Fertilizatition: 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 to do larval maintenance, the calculation is done first, so that it can adjust the area, volume of water and feed for sea urchin cultivation in the laboratory. Comparison between the number of sperm cells and eggs should be balanced therefore that decay does not occur (Radjab, 2001).

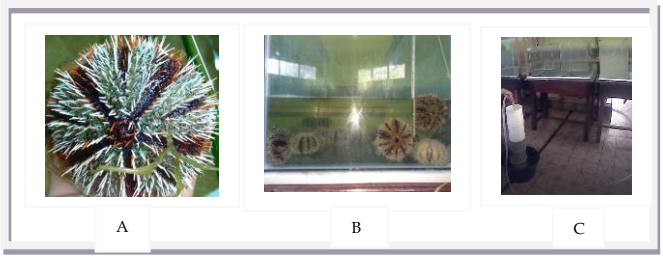

Figure 4. Stages Spawning and Fertilization of Sea urchin in the Laboratory *Tripneustes gratilla* (A) Temperature spawning process (the early temperature of 29⁰C is increased to 34⁰C); (B) The process of filtering fertilized egg cell using multilevel sieves (nylon mesh 40 µm, 60 µm, 100 µm and 140 µm).

3.3 Maintenance of *Sea Urchin* Larval

Larvae maintenance from fertilization to juvenile is a critical point of growth and development of sea urchin larvae. The factors that should be considered are population density and area, feeding, sterilization of seawater used, aeration, salinity, water temperature, water pH and turnover time of seawater. The time needed for the critical point of larval growth from 3 days after fertilization to 40 days. Larvae are maintained at 29,5⁰ C – 30⁰ C and salinity 37,5-40 ⁰/₀₀. *T. gratilla* species has a relatively long larval growth stage of 30-40 µm (Dworjanyn et al., 2007).

Larvae maintenance was carried out using 2 liters of filtered seawater with a size of 0.5 µm 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 (Dworjanyn and Pirozzy, 2008). Larvae deaths often occur due to density in maintenance (Rahman et al., 2012).

Giving aeration at the maintenance of larvae is carried out 30 hours after fertilization or embryo development at the stage of larval prism. Giving aeration can be done if can swim freely at the larval stage (Patrick, 1986; Rahman et al., 2012).

Feeding on larvae begins on the third day after fertilization, namely embryo development at stage 4 arms pluteus larvae. Larvae are fed 36 hours after fertilization, when functional intestines appear (Jorge et al., 2018). Feed pattern given to pluteus larvae 4 arm, 6 arm and 8 arm each 5000, 10000 and 15000 cells/ml per day (Rahman et al., 2012). Feed given should be in accordance with the number of larvae, because good larvae maintenance should be fed according to the stages and there are 1 to 2 algal cells in the stomach of the larvae (Gregory et al., 2004). At the floating stage given phytoplankton feed *Chaetoceros calcitran*, namely at the stage of pluteus larvae 4 arm to competent larvae with complete

rudiment growth. Whereas at the sedentary stage, feed giving phytoplankton *Navicula* sp. namely at the stage of larvae regression tissue of the pre-metamorphic larvae with ambulacral feet to juvenile. While in the juvenile stage until adults are given feed *Ulva* sp.

Based on the results of observations after spawning the morphologically healthy form of eggs *T. gratilla* are full round with a size between 40-60 µm and is able to divide and develop into juvenile (Figure 5A). Egg cell division occurs the first 60 minutes after fertilization, namely 2 cells. First meridional. The first meridional cleavage occurs in the formation of two cells of the same size (Fig. 5B) and 4 cells ((Fig. 5C). 2 hours after fertilization the egg cells undergoes cleavage 8 cells. At the eight-cell stage a small cavity appears between the blastomeres. As cleavage continues this space will enlarge to form the blastocoel. The fluid inside this space is largely sea water (Conway et al., 1984) (Fig. 5D) and 16 cells (Fig. 5E). 32 cell cleavage of the cell occurs 200 minutes after fertilization (Fig. 5F). 4 hours after fertilization the egg cell undergoes cell cleavage 64 (Fig. 5G). The morula stage occurs 5 hours after fertilization (Fig. 5H). The next stage is 8 hours after fertilization the egg undergoes cleavage of the blastula (Fig. 5I dan 5J). At this stage, the cells form an empty ball that surrounds the central blastocoel. The most important morphological characteristic at this stage is formation of cilia around cell mass and embryonic mobility (Ghorani et al., 2012). 18 hours after fertilization the egg cell undergoes embryonic development in the gastrula stage (5K). Latter-point of grastula stage, through invagination at the vegetal pole (5L). The invaginated region is called the archenteron that eventually made contact with the blastocoel wall and formed the mouth. hen continuous digestive tube was formed by connecting the mouth to the archenteron. Red-pigmented cells originate on the vegetal pole and migrate through the ectoderm to the apical plate while the archenteron elongation is continuous (Rahman et al., 2012).

Almost 30 hours after fertilization, embryos development entered to prism stage and formed the skeletal elements (5M). 32 hours after fertilization, embryos development entered to pluteus larvae stage and will formed the 2-armed pluteus larvae (5N). 3 days after fertilization, embryos development forming to 2- armed pluteus larvae and 4 - armed pluteus larvae, showing first pairs of arms (1st). Scala bar = 163, 32 µm (5O) and and second (2nd) scala bar = 60,28 µm (5P). 14 days after fertilization, embryos development forming to 6-armed pluteus larvae. The condition of the larvae is healthy and the movement backwards. (5Q). The 17 days after fertilization, embryos development forming to a competent 8-armed pluteus larvae with juvenile rudiment occupied most of the body and displaced the larval gut (5R). At the 21 days after fertilization, embryos development entered to pre-metamorphic larvae. This stage has many armed, therefore they cannot calculated and measured (5S). In 24 days after fertilization, embryos development entered to pre-metamorphic larvae with pedicels. This stage at the armed tip forms a pedicel and a considerable amount will later develop into ambulacral feet (5T). More than 24 days fertilization, embryos development forming to larval tissue regression of the pre-metamorphic larvae with ambulacral feet (settle) (5U). In the 29 days after fertilization, embryos development forming to competent larvae, with complete rudiment growth (5V). This stage a continued degeneration of larval tissue and arms accompanied by the emergence of the adult spines and tube feet may be seen slightly below the left corner of the larval.

Almost 43 days after fertilization, embryos development entered to aboral view of a recently metamorphosed sea urchin showing podia and spines (juvenile) (5W). In this stage, well-formed spines and extended tubefeet were evident. Metamorphosis occurred when larvae attached firmly to the bottom with the protruding tubefeet and the larval tissues began to regress and accumulate on the aboral surface of the rudiment. During this process, larval spicules became exposed and broke off and the larval tissues accumulated on the aboral surface of the rudiment forming a globoid structure. Early postlarval juveniles had no skeleton on the aboral surface, except for the remnants of larval rods. The gut was not yet formed and neither mouth nor anus was present. During the resorption of larval tissues, the rudiments of Aristotle's lantern and teeth were visible in the oral region under polarized lights (Rahman et al., 2012).

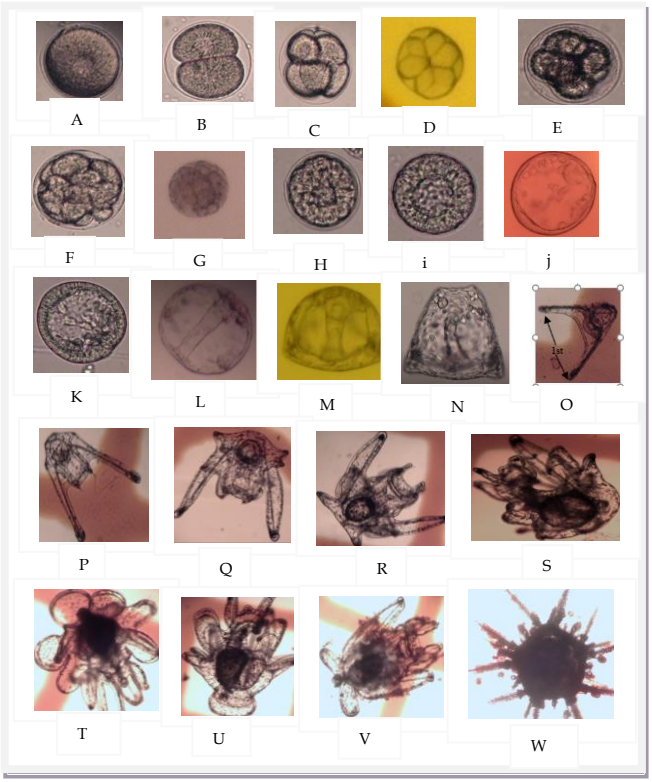

Figure 5. The early Development of *T. gratilla* in the Laboratory
Ovum after fertilization (A); 2-cell stage (B) and 4-cell stage (C); 8-cell stage (D) and 16-cell satge (E); 32-cell stage (F); 64-cell stage (G); Morulla stage (H); Blastophore prior to heatching stage (I); Latter-point of blastula stage (J); Grastula stage (K); Latter-point of grastula stage (L); Prism stage (M); Pluteus larvae stage (N); 2- armed pluteus larvae (O); 4- armed pluteus larvae stage (P); 6 - armed pluteus larvae (Q); 8 - armed pluteus larvae (R); Pre-metamorphic larvae (S); Pre-metamorphic larvae with pedicels (T); Larvae tissue regression of the pre-metamorphic larvae with ambulacral feet (settle) (U); competent larvae with complete rudiment growth (V); aboral view of a recently metamorphosed sea urchin showing podia and spines (juvenile) (W).

3.4 Enlargement of Sea Urchin Juvenile

Enlargement of larval until juvenil takes 6 months. Treatment at the time of enlargement is not as intensive as during maintenance of larvae, because what needs to be considered is the pattern of feed, aeration and length of time of change of water.

Enlargement of juvenile sea urchin carried out in the laboratory from 43 days after fertilization until 92 days. Feed given during enlargement of the juvenile stage, in addition to Navicula as well as Ulva sp. Juvenile urchin sea is maintained at water temperature 29.5-30^0 C and salinity 38-39 $^{0/00}$. Every 2 weeks the water used for juvenile enlargement is replaced, to clean food that is not eaten and the feces and dirt that are attached to the wall of the jar. Aeration is given with moderate strength. At night there is no direct lighting, because it adapts to natural habitat conditions.

Enlargement of the juvenile Sea urchin for 92 days conducted at the Ambon LIPI cultivation laboratory has body size 20 mm. To reach the adult size and gonad ready to harvest sea urchins, it takes 2 years and during cultivation the feed pattern, temperature, salinity and sea water turnover time sould be considered. Feed pattern is very important to determine the quality of gonads (Dworjanyn and Pirozzi, 2008; Jorge et al., 2018).

4. Discussion

4.1 Sea Urchin Larval Feed Preparation

The availability of feed should be adequate both in terms of quantity, quality and sustainable before conducting fish and non-fish cultivation. Until now in Indonesia, natural food cultivation (phytoplankton and zooplankton) is still a limiting factor in the development of marine aquaculture especially in certain biota's (Isnansetyo and Kurniastuty, 1995).

Phytoplankton as natural food in urchin sea larvae cannot be replaced by artificial feed, because the size of feed should be adjusted to the mouth openings

of the sea urchin larvae. The size of feed that is suitable with the mouth opening will optimize the activity and amount of biomass that is eaten, besides that it also does not act as a host for a pathogenic or parasitic organism and does not produce toxins in its life cycle (Isnansetyo and Kurniastuty, 1995).

The difference in the cultivation of *Chaetoceros calsitrans* phytoplankton with *Navicula* sp. in the laboratory is the treatment of aeration. *C. calsitrans* should be given aeration in order to provide the oxygen *C. calsitrans* needed for growth. The optimal aeration rate of 3 cms^{-1} can produce a specific growth rate of 7,41 × 10^{-2} $hour^{-1}$ with a maximum cell concentration of 8,88 × 106 sel mL^{-1} cells for *C. calsitrans* cultivation (Krichnavaruk et al., 2005). Whereas *Navicula* sp. not given aeration during cultivation in the laboratory. This is adjusted to the natural habitat of *Navicula* sp. which is settle and originates from deep sea waters (Nurachman et al., 2012).

4.2 Spawning and Fertilization

Spawning with raising the temperature of sea water is considered more effective and efficient because it does not use complex equipment, only by heated sea water under the sun. The advantage of this method is that the adult sea urchin used for spawning is still alive and can be maintained for further spawning. Spawning with raising the temperature and providing fresh sea water makes spawning easier, saving costs and energy (Radjab, 2016). Besides that also did not commit killing on sea urchin adult. This spawning method was first carried out by Kikutani & Patris (1991) called "noncirculated stimulation". This method continues to be done because it naturally does not use chemicals and according to reproductive behavior in nature, so that gamete cells that come out of the parent mature sexually. Spawning with raising temperatures is also done in Japan to increase seed production and increase the natural stock of sea urchin aquaculture (Walker et al., 2007).

Adult sea urchins after spawning moved into the tank to be re-maintained. Maintenance of adult sea urchin in nature does not require special handling, but it is different when doing maintenance in the laboratory. Parent should be considered physiologically therefor as not to be easily stressed and experience death. Feed patterns should be considered, aeration and change of water and external factors such as water pH, water temperature and salinity. An important factor in maintaining sea urchin adult in the laboratory is the number of animals per unit volume of sea water and oxygen needed for maintenance should be adequate (Leahy, 1986). External factors should be adapted to natural habitat conditions.

During the fertilization process takes place the observation of egg cells is done randomly, based on the observation that the development of the embryo is not the same as the splitting. For example, some have 8 cell division, but some only have 2 cell division and cleavage for other stages. This shows that the maturity of the egg in one individual is different and the ability of sperm cells to fertilize the egg.

4.3 Maintenance of *Sea Urchin* Larval

Larvae maintenance in the laboratory will be different in its natural habitat, the change of water must be done every 3-4 days therefore that the sea water used is kept clean and sterile. If there are many dead sea urchin larvae found in observations, water changes should be done even though it is not yet time. The purpose is to change the water because it does not provide an opportunity for bacteria, fungi and predators to grow in the aquarium where larval are kept. Nother important component of the successful maintenance of sea urchin larvae is free from fungal, bacterial, protozoa and ciliate contamination which can cause 100% larval death (Gregory et al., 2004).

Larvae maintenance should be attention to external and internal factors, one of which is on feed. Feeding should be adjust to the stage of development of the larvae. *Chaetoceros calsitrans* is given to sea urchin larvae when it is planktonic, because *Chaetoceros calsitrans* has buoyancy, which can float on the surface of the water, making it easier for larvae to catch feed. Settled larvae of sea urchins are fed with *Navicula* sp. because golden yellow can be easily seen by larvae. Attractive movements or colors are physical characteristics of feed that should be fulfilled during the selection of feed for fish and non-fish cultivation (Isnansetyo and Kurniastuty, 1995).

4.4 Enlargement of Sea Urchin Juvenile

Larvae can be moved in the tank enlarging the prospective parent or released in nature as conservation of sea urchins and re-stockings, if the size of the body diameter reaches 4 cm. The size of the body diameter to reach 4 cm takes 188 days, this stage is said to be the juvenil stage (Jorge et al., 2018). The body size reaches 4.2 cm therefore it can be said to reach adulthood (prospective parent), although the quality of the gonad is not ready to be harvested but spawning can be done (Jorge et al., 2018).

The weaknesses found during the study were the survival of 2- arm pluteus larvae to competent larvae with complete rudiment growth very low. This is because at the time of observation it was found that many bacteria and predators eat carcasses of larvae. In addition, feeding *Ulva* sp not sterilized. Therefore that suspected bacterial contamination of cultivation is the cause of mass death in larvae. To ascertain whether bacterial contamination is correct, further research is needed. The algae leaves that will be given as larvae should be sterilized to reduce the abundance of bacteria (Dworjanyn and Pirozzi, 2008).

This research is an initial laboratory scale study that has succeeded in cultivating *T. gratilla* from spawning to enlargement of tillers at LIPI Ambon. This research needs to be carried out further so that *T. gratilla* cultivation can be carried out not only on a laboratory scale but more broadly.

5. Conclusion

Sea urchin cultivation in the laboratory has 4 steps that should be done, namely preparation of feed, spawning (stimulation) and fertilization, maintenance and enlargement of larvae. Of the four stages there are 3 stages that require accuracy and intensification in the work, because they should be sterile and done in the laboratory. Even for the maintenance of larvae there is a critical point that larval should be pass through to be able to live. In stage 4 (enlargement of larval) it is not as intensive as in the previous three stages and the work can be done outside the laboratory.

Sea urchin cultivation in the laboratory can be done from spawning until adolescent enlargement takes 3 months. While from adolescents to adults, it takes quite a long time, which is around 12 months. During the cultivation of sea urchins in the laboratory feed patterns and external factors should be adjusted to their natural habitat.

Sea urchin cultivation can provide benefits both economic and ecological in the form of conservation or re-stocking in nature and can develop the skills of bio-entrepreneurship students in the field of marine. Sea urchin cultivation material can be given at the school and universities by incorporating sustainable development learning programs.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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