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Study of Gametogenesis Bamboo Coral *Isis hippuris* in Bone Tambung Island, Spermonde Archipelago, Makassar, South Sulawesi

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Abstract

Isis hippuris is a species of coral abundant in Bone Tambung island, Spermonde islands, South Sulawesi. Isis hippuris is known as the bamboo coral, in Indonesia into the spotlight after their retrieval in large quantities while the reproduction has not researched Isis hippuris. Sampling was conducted from February to June 2014 at different stations. Based on observations of coral tissue preparations, Isis hippuris is a Gonokorik coral species that have only ovary or testis in one polyp. The results of observation showed oocytes discovered on February - June 2014. Based on the percentage of its appearance, oocytes are found at least in February, mainly oocytes Stage I. The oocyte diameters have different sizes each month. The average size of the smallest Stage I oocytes contained in May is 39.37 µm while the biggest in May is 68.33 µm. Testes formation begins while the earlier quarter and matures on the full moon or the end quarter early in March, the male gonads are found throughout months of observation, have different diameters. The average diameter of the testes is highest in April in Stage III 222.70 µm range and the lowest for the observation in May at stage I ranges from 54.77 µm

Keywords: Gametogenesis; Males gonads; Females gamet; Histology; Isis hippuris.

1. Introduction

Indonesia is the central distribution of the world's coral reefs. One of the most important reef-building corals is hard corals and soft corals. One of the building reef corals are included in the soft coral is family Isididae, and Isis hippuris.

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Isis hippuris to be spotlight after their retrieval in large quantities are hundreds even thousands of tons were sent abroad for various purposes. Making continuous leads to reduced stock in nature and will lead to the threat of extinction to species Isis hippuris.

Bamboo coral Isis hippuris in the Indonesia waters, scattered mainly in Eastern Indonesia. Hasanuddin University and other researchers noted there were some of the largest colonies in some areas of which Luwuk Banggai, Konawe, Gorontalo, Bitung, Togean, Selayar, and Bone bay. Nevertheless, the presentation of bamboo coral from time to time showed a decrease. It is estimated that due to human activity, either directly or indirectly, such as arrests bamboo coral that are not environmentally friendly, making bamboo coral for accessories and pollution caused by run-off from the mainland. Environmental pressures are expected to affect the reproductive activity of Isis hippuris.

Study on the reproductive characteristics of bamboo coral Isis hippuris types has become an interesting topic and very important because there has been no information about its reproduction. In Indonesia, information and the study of coral reef reproduction are still limited. Sex, gonad composition, fecundity, and models of reproduction and reproductive period a type of coral is very useful in the prediction of recruitment coral populations [1]. Research on aspects of reproduction on soft coral types Isis hippuris concerning gonad development in their natural habitat is an attempt to explore the basic information if you want to do conservation efforts or seeding efforts. The results of this study are expected to provide information about reproduction which is basic information for research further conservation efforts and cultivation later. The purpose of this study was to determine the cycle of gametogenesis and characteristics of Bamboo coral Isis hippuris reproduction in Bone Tambung Island, Spermonde Islands, South Sulawesi. This research is expected to be a reference in the estimation method of reproduction, spawning, and recruitment of bamboo coral Isis hippuris.

2. Methods

Sampling was conducted in February - June 2014, research location in Bone Tambung Island, Spermonde Archipelago, South Sulawesi. Cutting sample preparation and staining performed in the Center for Veteriner, Maros, South Sulawesi, and observations preparations at the Veterinary Laboratory Maros, South Sulawesi. The method used in this research is the descriptive method that aims to give an overview of Isis hippuris gametogenesis. The data collection was done by histology by counting the number of eggs and sperm contained in the sample preparation.

Location Methods

Determining the location of sampling used in this research is the purposive sampling method. This sampling method was selected for determining the location of sampling based on the consideration that each of the sampling stations could represent research areas [2]. Sampling location of this research conducted on Bone Tambung Island, Spermonde Archipelago.

Sampling Method

Sampling was done by cutting the branches of the coral reef Bamboo coral Isis hippuris in the middle colonies 3-5 cm long. Each colony is selected that has entered a period of reproduction (diameter ≥15 cm).

Sample Processing Method

Samples have been taken and then preserved in 10% formalin in seawater. Samples decalcified in 10% formalin and 10% acetic acid, then dehydrated in graded alcohol solution with different concentrations. Then Xylene-paraffin is put in a solution of xylene and embedding use paraffin. Samples that have been in embedding processed for histological preparations were made according to the procedure at the Center of Veteriner, Maros, South Sulawesi. Sampling was conducted from February - June 2014.

Data analysis

The diameter of gonad was calculated using the geometric mean method by calculating average its geometric, which is the root of the product of the longest diameter and shortest diameter of the male gonads (testis) and female gametes (oocytes) [3]

$$\text{Geometrik Mean} = \sqrt{(\text{The longest diameter x The shortest diameter}} \; 00$$

The percentage of the gonad is calculated by noting the appearance of each stage of maturity that is found in the entire colony, either male, female, or hermaphrodite at each sampling date. It aims to see whether the coral colonies Isis hippuris produce male and female gonads throughout the year.

The calculation percentage of gonad with the following formula [4]:

$$\text{The percentage of male gonads} = \frac{\text{The number occurences the male gonads phase-n}}{\text{Total appearance of the male gonads}} \text{ x } 100\%$$

$$\text{The percentage of female gametes} = \frac{\text{The number of occurrences female gamete phase-n}}{\text{Total appearance of female gametes}} \text{ x } 100\%$$

Phase-n = Phase I, II, III, and IV

3. Result

Based on observations of coral tissue preparation, known as Bamboo coral Isis hippuris is a species of Gonokorik coral that have only ovary or testis in one polyp. Gamete development includes male gametes (spermatogenesis) and female gametes (oogenesis) have 4 stadia to reach maturity gonad, each stage has different development.

Oogenesis

The results showed oocytes began to be discovered on February 18th - June 25th, 2014. Based on the percentage of its appearance, the oocytes are found at least in February, mainly oocytes Stage I. Oocytes through four distinct stages of development to reach maturity gonad. Stage I oocyte is characterized by a gonad diameter of about 55 µm, with the irregular shape of the egg and the egg is still in the mesoglea layer (Figure 2a). Stage II oocytes showed the increased size of the nucleus and nucleolus, oval egg shape, seen the addition of yolk and the average diameter of the eggs reach 82 µm (Figure 2b). Stage III oocyte diameter size of eggs grew about 91 µm, yolk began to appear speckled with more volume (Figure 2c). Oocyte stage IV is the final stage of the egg to mature, at this stage of the germinal vesicle is not visible then the yolk increasingly mottled and increasingly moves to the edge of the egg, the oocyte diameter sizes ranging from 238 µm (Figure 2d).

The Frequency of Occurrence of Female Gametes

Results obtained from oocytes observations throughout the year show the differences in the percentage of each stage (Figure 3). Oocytes stage I is lowest found on the February 5% and the highest at 24.19% in June. Stage II the lowest was found in February t is 8.33% and the highest at 40.32% in June. For stage III was found the percentage of the lowest found in February at 0% and the highest in May amounted to 56.52%. Stage IV is the lowest emergence there in May amounted to 0% and the highest in February amounted to 86.67%.

Diameter of Oocytes per Month Based on the Maturity Stadium

Diameter oocytes have different sizes each month. The average size of the smallest Stage I oocytes contained on May 2nd is 39.37 µm while the biggest is on May 31 is 68.33 µm. Stage II the average size of the smallest oocytes contained in May amounted to 76.71 µm, while the greatest in February was 102.58 µm. Stage III oocytes average size of the smallest found in February at 0 µm or not found stage III while the biggest in May 2nd at 164.05 µm. In May the average size of Stage IV is not found (0 µm) and the biggest was found on May 2nd is 223.46 µm (Figure 4).

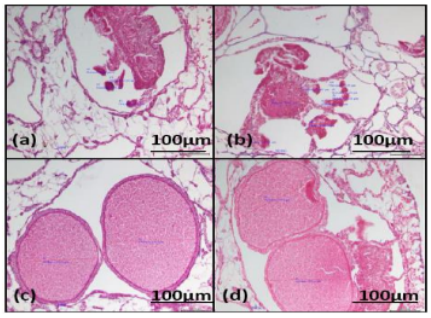

Figure 2: Stages of oogenesis Coral Isis hippuris at Bone Tambung Island, Spermonde Archipelago, South Sulawesi: (a) Stage I oocytes; (B) Stage II oocytes; (C) Stage III oocytes; (D) Stage IV oocytes.

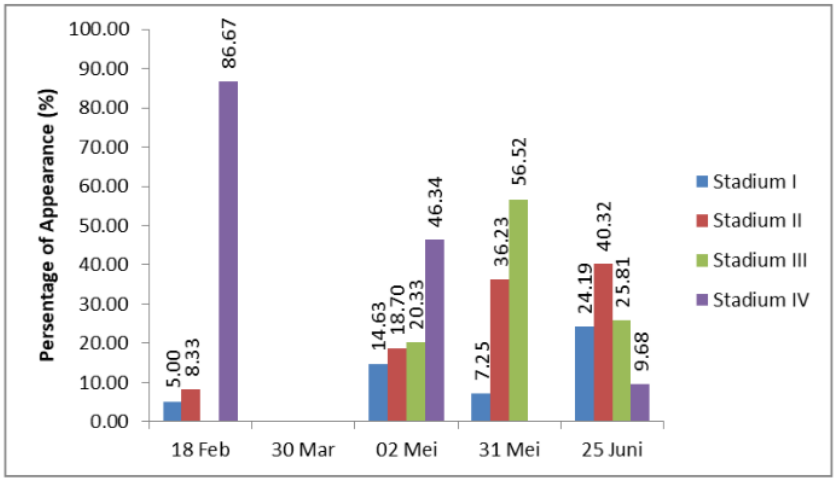

Figure 3: Percentage of appearance female gametes (%) Bamboo Coral Isis hippuris on Bone Tambung Island, Spermonde Archipelago, South Sulawesi.

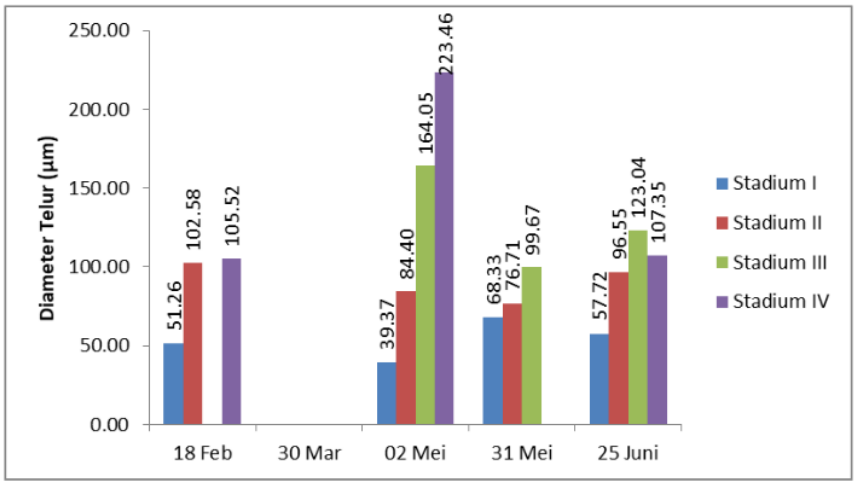

Figure 4: Diameter female gametes (µm) Bamboo Coral Isis hippuris on Bone Tambung Island, Spermonde islands, South Sulawesi.

Spermatogenesis

Morphology spermatogenesis

Spermatogenesis started in February 2014. The male gonads or testes are attached to the trunk mesenteric in the gastrodermis cavity. Old youth of testis is seen from the thick and thin walls, testicular size as well as

morphology. It has been found four stadia maturity testicular cells that stage one to four (Figure 5d).

Stage I or the early stages of spermatogenesis characterized by the emergence of the bag of thick-walled small testes containing cells spermatocytes, average size diameter at stage one is 24µm (Figure 5a). Stage II is characterized by images of spermatocytes shaped spots and began to fill the bag of the Testis, the average size of the bag can reach 59µm (Figure 5b). Stage three is characterized by walls of sperm that are still thick, bags testis that has begun to dilate and diameter average 72 µm (Figure 5c) forming testis, stage IV characterized in wall pockets testicular getting thinner and gonad contains spermatozoa, bag size Testis greater with the average diameter of 125µm.

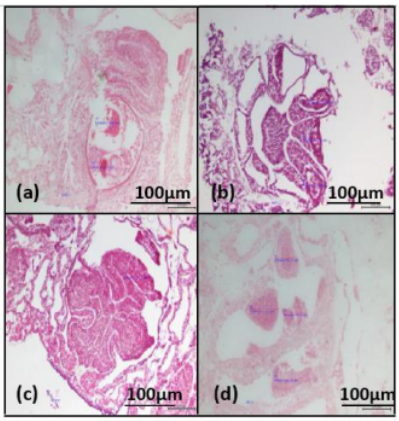

Figure 5: Spermatogenesis of coral Isis hippuris on Bone Tambung Island, Spermonde Archipelago, South Sulawesi: (a) Testis Stage I; (B) Testis Stage II; (C) Testis Stage III; (D) Testis Stage IV.

The Frequency of Occurrence Stadium Year-round Observations of Male Gonads

Almost every month observation male gonads are found, except in May. In February, the fourth stage appears, while in other months there are only two stages that appear, in March amounted to 81.82% stage II, and stage III amounted to 18.18%. On May 2nd, the percentage of testes of stage I is 80% and stage III is 20%. Whereas in the last month's observation is in June, the percentage of testes for stage I is 42.86% and stage II amounted to 57.14% (Figure 6).

Testicular Diameter Size per Month Based on the Stage of Maturity

Male gonads are found throughout the months of observation, have different diameters. The average diameter of the testes is highest in May 2nd in Stage III 222.70 µm range and the lowest is in May 2nd at the first stage ranges from 54.77 µm.

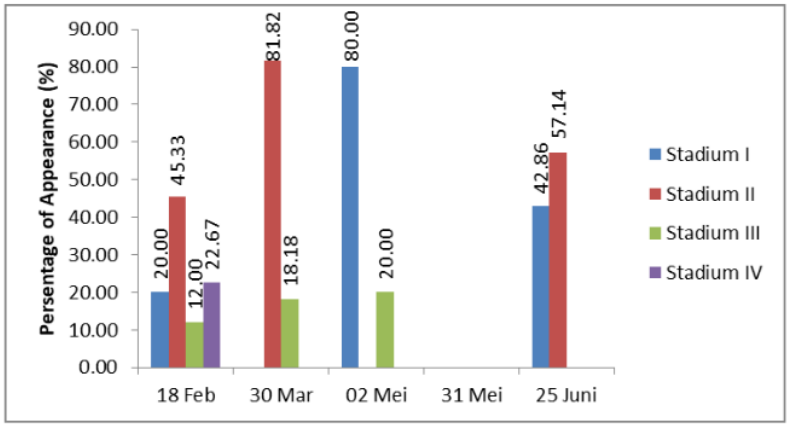

Figure 6: Percentage of Appearance Testis (%) Bamboo Coral Isis hippuris on Bone Tambung Island, Spermonde islands, South Sulawesi.

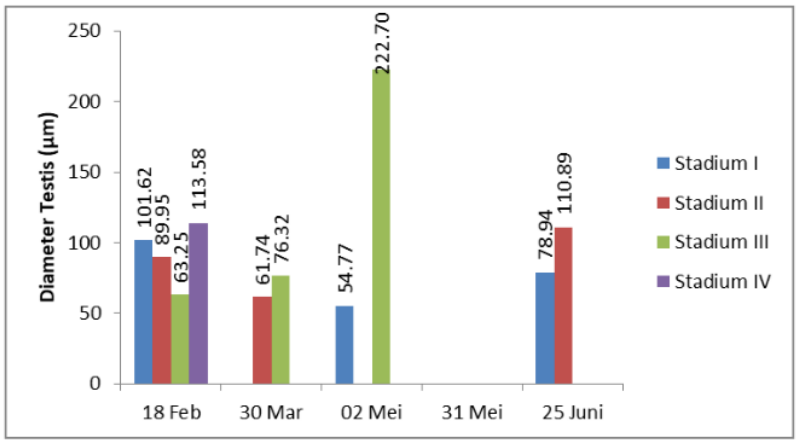

Figure 7: Diameter of testis Bamboo coral Isis hippuris on Bone Tambung Island, Spermonde islands, South Sulawesi.

4. **Discussion**

Based on observations of the composition of the gonads and the discovery of oocytes and Tests in one polyp in which oocytes were earlier found from Testis, then Isis hippuris at Bone Tambung Island is a coral Gonokorik type. But in a colony of bamboo coral, Isis hippuris is a type of hermaphrodite, which generally reefs have the hermaphrodite type of model, reproduction, and fecundity of different [5]. It is also reported on a coral A. Aspera in Panjang Island also has a type of reproduction hermaphroditic type is hermaphroditic- spawner. The formation of female gonads is expected to begin in February 2014 until the final quarter of the new moon, it can be seen the biggest stage I oocytes appearance on May 2nd, 2014, and the final quarter of the new moon. Appearance oocytes of stage I which also appears on the full moon in February (2014) (5%) and the first quarter

at May 2nd (14.63%), May 31th (7.25%), June 25th (24.19%) it means that the early stages of male gonads are also available in the period around the full moon and earlier quarter. The stadium that often appears in-stadia IV. Almost every month stadia II there is arise. Stadia III was found in May and June. The high appearance of Stage II oocytes almost every month of the year means that this stage requires the longest development time. It can also be seen from the large variations in size which is between (mean 90.06) µm. The range is from 24.06 to 224.8 µm. Stage II can be found around the new moon-the final quarter where the highest production is on the full moon. Stage IV can be found at the final quarter to the beginning of the quarter and the highest production in the earlier quarter approaches the full moon in March. In Oocyte stage I, II, III number of eggs in February is 8, at May 2nd is 66, on May 31st is 69, at June 25th is 56, decreasing the appearance in March, while the appearance of oocytes at stage IV the number of eggs at February is 52, at May 2nd 57, at June 25th is 6 the greater this is mean that the oocytes of stage I, II, III has been manufactured into oocytes of stage IV in March. Permata et, al., (2000) suggest that small-sized oocytes were observed throughout the monthly cycle are likely to mature in the next reproductive cycle. In the full moon in March estimated female gametes have been released into the waters. This was expected since the beginning of March quarter the size of stage IV oocytes reach a maximum up to of 238,34µm and gastrodermis wall thinning as well as information from local residents mentions the mass spawning on the full moon in March.

Male gonads are found only in the initial quarter in March. The development of male gonads stage I to stage IV of Isis hippuris is fast, suspected formation stage I have been taking place in February because of the appearance of stage IV is very high as well as the appearance stage I which is relatively low, it is suspected production stage III and IV are from stage I in January or February, the male gonad development at Isis hippuris faster than female gametes. On the reef species, oocyte development in one period takes about 9 months, and Testis is around for more than 10 weeks in the annual gametogenesis cycle [6,7].

Male gonads are estimated to spawn at the beginning of the full moon in March and May. It is suspected due to Stage IV which was found in the early February quarter has average size is very large (113,58µm), the range (47.44 - 245.4)µm, spermatid cells contained in the bag Testicles have been fused and wall spermatogonial to be thin indicating spermatozoa ready to be released.

The appearance of Stage IV Male and female gonads was highest in the early quarter of February. A similar thing by Richmont and Hunter says that coral spawning usually occurs once a year [8]. There are coral of Acropora from other types namely Acropora nubilis in Lombok, West Nusatenggara also spawn approached in March that at the end of February [9], while in the Great Barrier Reef, Australia, coral of Acropora spawning in the summer October-November [10,11]

In conclusion, the results of this study that (i) Bamboo Sea Isis hippuris is a species of coral Gonokorik that only have male gamete (testes) or only females (oocytes) in one polyp where production of female gonads earlier than the male gonads. (ii). Bamboo coral Isis hippuris has 4 maturity stages (stage I, stage II, stage III, and stage IV) that were discovered during observations. (iii).The results of the development of the reproductive gametes bamboo coral Isis hippuris spawning tendency for two months.

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