Bionatura
Jurnal Ilmu-ilmu Hayati dan Fisik
Journal of Life and Physical Sciences

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Bionatura
Jurnal Ilmu-ilmu Hayati dan Fisik
Journal of Life and Physical Sciences

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E-mail: jurnalbionatura@yahoo.co.id
Website: www.bionatura.unpad.ac.id
(Terbit tiga kali dalam satu tahun : Maret, Juli, dan November)

Terakreditasi B
STUDY ON SEVERAL REPRODUCTIVE ASPECTS OF SOFT CORAL
SINULARIA FLEXIBILIS QUOY & GAIMARD IN BARRANG LOMPO ISLAND,
SPERMONDE ARCHIPELAGO, MAKASSAR CITY

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ABSTRACT

Sinularia flexibilis is one species of soft coral having high economic value due to its pharmacologically active contents. This research was aimed to analyze several reproductive aspects of this soft coral using direct observation in its natural habitat, periodically collecting samples of body tissues, and then preparing and observing its histological preparation. Reproductive aspects found in this research were analyzed descriptively. Results of this research showed that the sexuality type of soft coral S. flexibilis was simultaneous hermaphrodite that reproduced by spawning, its reproductive pattern was hermaphrodite followed by spawning. It spawned from May-September, and the development of its sperm and oocyte was divided into four stages with different characters between one to another, particularly differed in shape, presence of organelles, and organelle colors.

Key words: Sinularia flexibilis, reproduction, Barrang Lompo Island

INTRODUCTION

Sinularia flexibilis is one species of soft corals having a high economical value from coral reef ecosystem. Secondary metabolites contained in this soft coral have been known to have pharmaceutical characters. Compounds produced by this soft coral are sinulariolyde, sinularin, dihydrosinularin (Tursch et al., 1978; Michalek and Bowden, 1997), sandensolide monoacetate, flexibilide (Anjaneyulu et al., 1997), cembranoid diterpenes, sinuflexilide, dihydrosinuflexilide, sinuflexibilin (Duh et al., 1998), diterpenes flexibilide (Aceret et al., 1998), 11-epi-sinulariolide acetate, 11-dehydrosinulariolide, sinulariolide (Hsieh et al., 2003). These compounds have activity as antimicrobe, cytotoxic character, and potential to be used as anticancer compounds.

Beside its significant economical potency to be developed, its population has been threatened worldwide, because most of its habitats i.e. coral reefs have experienced a serious degradation and in terrific condition.
According to Wilkinson (1993) in Tomascik et al. (1997) approximately 10% of global coral reefs have been damaged, and about 30% of these damaged coral reefs will loss within 10-40 years, if level of anthropogenic pressures, population and association of the causal factors are continuously increase, while in Indonesia, according to Suharsono (1999), from 416 locations in 43 areas of Indonesian waters, there were only 6.49% in very good condition, 24.28% in good condition, 28.61% in moderate condition and 40.62% in bad condition.

In order to control high rate of habitat destruction and overexploitation, particularly for utilization as a source of bioactive compounds and production of particular bioactive compounds from technological engineering, it is urgent to provide controlling efforts, especially those related to their culture development. This culture development is focused to produce raw extract and large-scale or industrial-scale of extract fraction and to supply seed for restocking at damaged coral reef areas.

Culture development for production of raw extract and extract fraction is only achieved if reproductive biology aspects of this soft coral have been well understood. Aspects of reproductive biology that should be intensively studied are: sexuality, reproductive style, reproductive pattern, sperm and development stages. The objective of this research was to analyze several reproductive aspects of soft coral *Simularia flexibilis* i.e. sexuality, reproductive style, reproductive pattern, sperm and development stages.

### MATERIALS AND METHODS

Study Location and Time Overall stages in this study were conducted approximately nine months from March to November 2006. Observation and sample collection were done in the vicinity of coral reef of Barrang Lombo Island, Makassar City following lunar cycle. Observation of reproductive behavior was done based on one lunar cycle, while collection of histological samples for determination of sexuality, method and pattern of reproduction, stages of gonad development were done in reproductive season, collection of histological samples was done for five lunar cycles.

### Materials and Tools

Materials and tools in this research were used in the field, laboratory and hatchery Listed in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Materials/Tools</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soft Coral <em>Simularia</em></td>
<td>Tested organism</td>
</tr>
<tr>
<td>2</td>
<td>Fomalal 5-10%</td>
<td>Preservative solution</td>
</tr>
<tr>
<td>3</td>
<td>Sample bottles</td>
<td>Store tissue samples</td>
</tr>
<tr>
<td>4</td>
<td>12 N HCl 10%</td>
<td>Decalcified solution</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol 70%-100%</td>
<td>Preservative solution and dehidration</td>
</tr>
<tr>
<td>6</td>
<td>NaOH 10%</td>
<td>Solution for whitening the skeleton of coral’s larvae</td>
</tr>
<tr>
<td>7</td>
<td>Xylol</td>
<td>Clearing solution</td>
</tr>
<tr>
<td>8</td>
<td>Parafin</td>
<td>Make tissue blocks</td>
</tr>
<tr>
<td>9</td>
<td>Microtom and microtom blade</td>
<td>Incise the tissue (coral polyp)</td>
</tr>
<tr>
<td>10</td>
<td>Object and cover glasses</td>
<td>Stick the histological incision</td>
</tr>
<tr>
<td>11</td>
<td>Hematoxylin and Eosin</td>
<td>Coloring solution for histological preparation</td>
</tr>
<tr>
<td>12</td>
<td>Light microscope</td>
<td>Observe the coral gonads</td>
</tr>
<tr>
<td>13</td>
<td>Photomicroscope and film negative</td>
<td>Take photomicrograph</td>
</tr>
<tr>
<td>14</td>
<td><em>Box glass object</em></td>
<td>Box to store the histological preparation</td>
</tr>
<tr>
<td>15</td>
<td>Scuba diving Set</td>
<td>For diving during observation coral spawning time</td>
</tr>
<tr>
<td>16</td>
<td><em>Water Quality Checker</em></td>
<td>Measure temperature and salinity</td>
</tr>
</tbody>
</table>

### Research Procedure

Observation to know sexuality, reproductive style and strategy and level of gonad development were done based on lunar cycle (Glynn et al. 1994) for two months (two lunar cycles). This observation was done twice for each lunar phase started from the 1st of this phase and 3-4 next days. Sample collection was commenced and ended with new month. The schedule of sample collection was presented in Table 2.

Fragments of sampled branches were fixated by putting them into fixative solution.
(formalin 5-10% in seawater) for a week, then were decalcified with 12N HCl 10% solution (solved in aquadest) for 4-6 hours or more (Wallace 1985, Glynn et al., 1991; 1994). Polyps that had been decalcified were stored in specific container (tissue cassette) and were rinsed at tap water for 24 hours to discharge HCl at the tissue surface. These polyps then were temporarily stored in 70% alcohol solution (Fadallah & Pearse 1982, Glynn et al., 1994) before doing histological preparation. This step followed process of standard tissue technique (Wallace 1985, Kieman 1990, Glynn et al. 1991; 1994).

Table 2. Schedule of field observation and collection of histological samples for observation of sexuality, reproductive style, reproductive pattern, and characteristics of sperm and oocyte development according to the lunar months.

<table>
<thead>
<tr>
<th>Research activity</th>
<th>Monthly week/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moon cycle I</td>
</tr>
<tr>
<td></td>
<td>1  2  3  4</td>
</tr>
</tbody>
</table>

- collection of histological samples for observation of sexuality, reproductive style, and characteristics of sperm and oocyte development
- reproductive pattern

Development of sperm and oocyte was divided into four stages, as follows: I, II, III, and IV stages based on criterion of Glynn et al. (1991, 1994); and Stoddart (1984a, b) in Ward (1992).

Data Analysis

Sexuality, reproductive style, reproductive pattern and characters of each sperm and oocyte development stage of soft coral S. flexibilis were presented as tables and figures to be analyzed descriptively.

RESULTS AND DISCUSSION

Sexuality, Reproductive Style and Pattern

Results of histological observation showed that sexuality of S. flexibilis was simultaneous hermaphrodite, where eggs and sperms developed at the same polyp or at different polyp but in the same colony, and achieved maturity at the same time. This result was supported by observation of spawning both in natural condition and in hatchery where the mature eggs and sperms were spawned simultaneously within the same colony. Based on this fact, it can be inferred that sexuality type of soft coral S. flexibilis is simultaneous hermaphrodite, its reproductive style is spawning, its reproductive pattern is hermaphrodite followed by spawning, and spawned during May-September.

Hermaphrodite sexuality type and spawning reproductive pattern as found at S. flexibilis in Barrang Lombo Island is different from those observed at soft coral Briareum asbestinum (Brazau & Lasker, 1990 in Seo et al., 2008) in Panama, Corallium rubrum (Allemand, 1992 in Seo et al., 2008) in Mediterranean, Pseudopleura porosa (Kapela and Lasker, 1999) in Caribbean, Fannymella spinosa (Orejas et al., 2007) in Antarctic whose sexuality is gonochoristic brooding reproductive style. Similar hermaphrodite sexuality type was also found at stony corals from families Acroporidae, Faviidae, Merulinidae, Mussidae, Pectinidae, and Pocilloporidae as studied by Harrison and Wallace (1990).

Soft coral S. flexibilis in Barrang Lombo Island spawned from May-September, while stony corals Porites lobata, Acropora squamosa, and Acropora hyacinthus spawned in June and July (Seo et al., 2008).
and Montipora foveola in Central Pacific spawned during July (Richmond and Hunter, 1990), Goniastrea palauensis, Goniastrea retiformis, and Platygyra lamellina in Great Barrier Reef spawned during October-August (Babcock et al., 1986 in Richmond et al., 1990), Pavona varians and Pavona maldivensis in Hawaii spawned during June (Kolinski and Cox, 2003), and Madracis senaria, M. mirabilis M. decactis and M. pharensis in Curacao spawned from April-December (Vermeij et al. 2003).

**Characteristics of Sperm Development**

Characteristics of each sperm development stage of soft coral *S. flexibilis* could be seen at Table 4, whereas, characters of each stage could be viewed from photomicrograph of histological preparation at Figure 1.

From the results of histological observation to the colony of the soft coral *S. heflexibilis*, its sperm type could be determined as well. Soft coral *S. flexibilis* has sperm type pier-like or ovoid at head and middle parts. Its center shape was ovoid or bullet-like, and its upper end had a zone with less compact material. At the midpiece of sperm, there was mitochondrial sheet or group of single mitochondria, lipid body and complex centriol structure, and cytoplasm at neck of front sperm. This type was similar to that introduced by (Harrison 1985 in Harrison and Wallace 1990) explaining that sperm type like this was commonly found at scleractinian corals having hermaphrodite sexuality type. Further, Benayahu (1991); Gutiérrez-Rodríguez and Lasker (2004) described that at soft corals, sperm was developed within gastrodermal mesentery, particularly in ventral and lateral parts, and attached to the mesentery during maturation process.

Sperm diameter of *S. flexibilis* at all stages (I-IV) was varied in size ranges and mean size. Sperm had size ranges and mean size 25-100 μm and 66.531 μm (Stage I), 62.5-200 μm and 118.218 μm (Stage II), 150-350 μm and 197.290 μm (Stage III), and 225-500 μm and 327.437 μm (Stage IV) (Table 4). Sperm diameter differences between soft coral *S. flexibilis* and species *A. dimorpha* was caused by difference in polyp size and difference in size of gastrovascular cavity at mesentery between these two soft corals.

**Characteristics of Oocyt Development**

Based on modification of Glynn et al. (1994), characteristics of oocyt development of soft coral *S. flexibilis* was divided into four oocyt development stages as presented at Table 5 with structure as shown at Figure 2.

Oocyt character I of soft coral *S. flexibilis* found during this research (Table 4) was relatively different from oocyt characters I, II, III, and IV of coral *Caulastrea furcata* and *Lobophyllia corymbosa* as studied by Kawaroe *et al.* (2007). According to Kawaroe *et al.* (2007), oocyt characteristics I of coral *C. furcata* and *L. corymbosa* had relatively large nucleus and the oocyt tend to occur singly.
Table 3. Characteristics of each sperm development stage of soft coral *S. flexibilis* based on Photomicrograph of Histological preparation

<table>
<thead>
<tr>
<th>Sperm Maturation Stage</th>
<th>Characteristics of <em>S. flexibilis</em> Sperm</th>
<th>Sperm Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>S.I</td>
<td>Like a dumbbell, small and clumped, filled by interstitial cells in mesoglea, dark bluish red color</td>
<td>25 - 100</td>
</tr>
<tr>
<td></td>
<td>Its shape started to prolong and spherical, showed a distinct sperm border, extended to mesoglea, bluish violet color</td>
<td>62.5 - 200</td>
</tr>
<tr>
<td>S.II</td>
<td>Prolonged, there was distinct lumen and tail, more solid spermatozoid nuclei with dark color (blue/red)</td>
<td>150 - 350</td>
</tr>
<tr>
<td></td>
<td>Prolonged (spherical oval), there was solid lumen and decreased cytoplasm volume and had distinct tail, dark blue/red color</td>
<td>225 - 500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± (SE)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66.531 ± 1.626</td>
</tr>
<tr>
<td></td>
<td></td>
<td>118.218 ± 2.616</td>
</tr>
<tr>
<td></td>
<td></td>
<td>197.290 ± 5.061</td>
</tr>
<tr>
<td></td>
<td></td>
<td>327.437 ± 8.583</td>
</tr>
</tbody>
</table>

Table 4. Characteristics of Oocyt Development at soft Coral *S. flexibilis* Based on Results of Histological Observation

<table>
<thead>
<tr>
<th>Oocyt Maturation Stage</th>
<th>Characteristics of <em>S. flexibilis</em> Oocyt</th>
<th>Oocyt Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Oocyt I</td>
<td>Originated from extension of interstitial cells, rounded and small, red and pale in color, unclear wall and nucleus</td>
<td>25 - 87.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.6 ± 1.4</td>
</tr>
<tr>
<td>Oocyt II</td>
<td>Rounded (oval), larger, red, unclear nucleus, thick wall had been formed</td>
<td>37.5 - 150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84.0 ± 3.5</td>
</tr>
<tr>
<td>Oocyt III</td>
<td>Rounded (tend to ellipse), there were more solid fatty granules, distinct nucleus and wall, bluish red in color More oval in shape, larger and more solid size, its position moved to the edge, dark red in color, there were sub-nucleus within several nucleus</td>
<td>100 - 275</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150.3 ± 6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 - 62.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.6 ± 10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Oocyt IV</td>
<td></td>
<td>187.5 - 625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>277.0 ± 12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37.5 - 150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.1 ± 8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.3</td>
</tr>
</tbody>
</table>

Cortical wall was still very thin, and contain less cytoplasm and fatty granules. Oocyt character II had increased cytoplasm volume and egg size. Sub nucleus has been differentiated from the nucleus. Vitellogenesis has occurred, however, numbers of fatty granules was still limited with relatively large diameter. Oocyt character III had high cytoplasm volume and high content of fatty granules; however, cortical membrane has not well developed yet. Oocyt character IV was mature oocyt. Cortical membrane has compressed and clearly observed compact and solid fatty granules.

Oocyt development of soft coral was also developed at mesentery at polyp autozoid calyx, similar to sperm development. After maturation, oocyt moved from mesentery to gastrovascular cavity. Immature eggs hanged at mesentery,
and gradually mature eggs detached from the mesentery (Kawaroe et al., 2007; Seo et al., 2008). Autozoid or fertile polyps had separate gonads (male and female). Gonad was located at each mesentery. Reproductive processes included egg and sperm releasing by each polyp into water column. External fertilization occurred outside the coral body. Larvae formed had cilia, and then freely swim to look for suitable hard substrates for attachment before forming polyp or new colony (Manuputty, 2002).

Oocyt diameter of *S. flexibilis* at various stages (I-IV) had different size ranges and mean size. Oocyt stage I had size range between 25-87.5 μm and mean size was 38.6 μm. Oocyt stage II had size range between 37.5-150 μm and mean size was 84.0 μm. Oocyt stage III had size range between 100-275 μm and mean size was 150.3 μm, and Oocyt stage IV had size range between 187.5-625 μm and mean size was 277.0 μm (Table 5). Besides difference in size range and mean diameter, oocyt of *S. flexibilis* had also difference in size range and mean size of nucleus, especially at oocyt stage III and IV, while oocyt I and II have no clear nucleus.

As a comparison, oocyt diameter of soft coral *A. dimorpha* as studied by Seo et al. (2008) had relatively different size ranges and mean size compared to *S. flexibilis* at all stages of oocyt development. Seo et al. (2008) found oocyt I of coral *A. dimorpha* had size <51 μm and mean diameter of 43 μm. Oocyt II had diameter range of 51-175 μm and mean diameter 114 μm. Oocyt III had diameter range between 176-275 μm and mean diameter was 211 μm. Oocyt IV had diameter range between >276 μm and mean diameter was 359 μm, while Kawaroe et al. (2007) studied oocyt development of coral *Caulastrea furcata* and found mean diameter of oocyt I by 0.275 μm, oocyt II by 0.533 μm, oocyt III by 1.508 μm, oocyt IV by 2.424 μm, and at species *Lobophyllia corymbosea*, they found mean diameter of oocyt I, II, III and IV

![Oocyt I](image1)

![Oocyt II](image2)

![Oocyt III](image3)

![Oocyt IV](image4)

Figure 2. Oocyt structure (I-IV) of soft coral *S. flexibilis* obtained from photomicrograph of histological preparation.
as much as 0.185 μm, 0.456 μm, 1.092 μm, and 1.558 μm, respectively.

CONCLUSION

Sexuality type of soft coral *S. flexibilis* was simultaneous hermaphrodite reproducing spawning, and its reproductive pattern was hermaphrodite followed by spawning, while sperm and oocyst maturity was divided into four stages each, where each stage has different characters compared to others, particularly in shape and presence of organelles.

AKNOWLEDGEMENT

This work was funded by Hibah Bersaing DP2M DIKTI. I wish to thank M. Rajab and Nasruddin for their help to carry out the field and laboratory work.

REFERENCES


