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The Study of Sea Cucumber (Phyllophorus sp.) Gonads Histology: Thermal Shock to the Spawning Process

Sapto Andriyono[1] Endang Dewi Masithah[1] Dwi Winarni[2]
1.Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga, Kampus C UNAIR Jl. Mulyorejo, Surabaya, 601145, Indonesia
2.Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR Jl. Mulyorejo, Surabaya, 601145, Indonesia

Abstract
Sea *cucumbers Phylloporus* sp. obtained from the east coastal waters of Surabaya. This sea cucumber has potential to develop as a source of the active ingredient. Exploitation of sea cucumbers is very high today. Local sea cucumber hatchery activities *Phyloporus* sp. is important so that the availability of sea cucumbers need not depend on the nature and this program also has benefit to increasing quality of the resulting sea cucumbers.
This study aims to determine the effect of heat shock on the seeding process by looking at the histology preparation. By knowing the profile of the resulting influence gonad development it is expected to be applied in the local sea cucumber hatchery activities. The results have shown that the temperature shock treatment did not affect the increase in mass of the gonads and gonad maturity level. However, the provision of heat shock capable of inducing spawning of sea cucumber with a marked presence of sperm and egg is released. The resulting larvae have survival is still very low, this is possible because of the quality of eggs and sperm are also forced spawning by temperature shock do.
Keywords: *Phyllophorus* sp., gonad, thermal shock, spawning

1. Introduction

Sea cucumbers are also known as sea cucumber (sea cucumber) . In Surabaya and surrounding areas , sea cucumbers found in the form of a regular snack food , which is marketed in the form of fried sea cucumber. In certain markets, found in its raw forms, usually used as a Chinese cuisine which is believed to have certain properties.

Sea cucumber habitat is widespread throughout the world, ranging from tidal zones to the deep sea. Samad (2000) states that 10 % of the number of species that exist in the world of sea cucumbers in the waters of Indonesia. Please also note that about 25 species of sea cucumbers were identified commercial potential originating from Indonesian waters. International trade of sea cucumbers, 80 % goes to Hong Kong, with major suppliers from Indonesia and the Philippines. Hong Kong itself and then export back to China, Singapore and Taiwan.

Search results researchers showed that the rate of export of dried sea cucumber from Indonesia is so big, it is not offset by the many useful products are made from sea cucumber from Indonesia. Food supplement products made from sea cucumber mentioned has benefits for health and healing disease (Farouk *et al*, 2007), is imported , and no one has mentioned that the material is essentially a sea cucumber living in Indonesian waters.

The largest sea cucumber producing regions are the beaches in Eastern Indonesia (Samad, 2000). The observation of researchers, more than 10 years ago, sea cucumber populations in Surabaya East coast is much larger than current population. Similarly, reported by Darsono (2005) occurs in other places in Indonesia. It is not possible over the next 10 years in the Indonesian state of East suffered the same fate as the East coast of Surabaya, if the rate of exploitation is not balanced with the preservation and cultivation efforts.

Species of sea cucumbers collected at study sites in the East Coast Surabaya are 7 species, namely: *Phyllophorus* sp.*, Paracaudina australis, Colochirus quadrangularis, sanctori Holothuria, Holothuria* sp.*, Holothuria forskali,* and *Holothuria turriscelsa*. No species abundance is very high, with only one species abundance and distribution of high-level, while others range from low to moderate. Preferred habitat is generally sandy. During these commonly consumed is *Phyllophorus* sp. and *P. australis*. Both are used for snacks or soup mix. Some collectors claim that *Phyllophorus* dry has been exported to Taiwan and Hong Kong. Even so, has never been scientifically reported efficiency of sea cucumber.

2. Study location

The study was conducted through several stages: (1) Phase collection and selection to get the best broodstock (2) The provision of feed enrichment and thermal shock, and (3) Histology of the gonads of the sea cucumber samples had been shocked by given temperature and normal sea cucumbers.

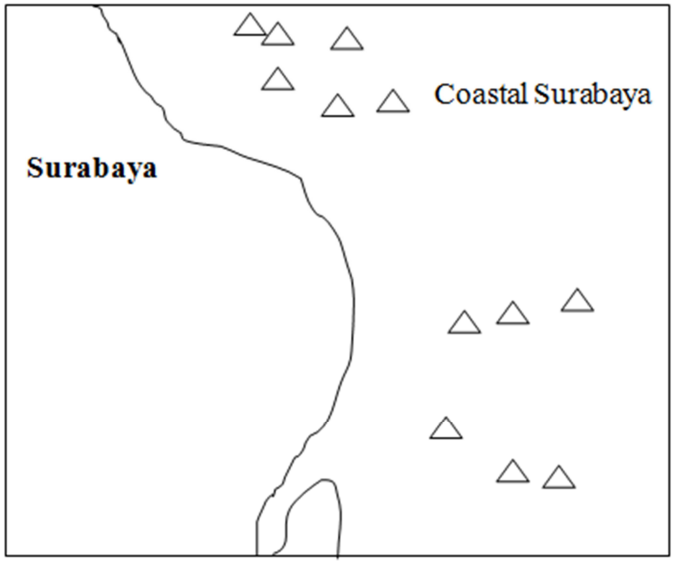


Figure1. Sampling Distribution Point (triangle sign) in Surabaya East Coastal triangle denoted with an asterisk**.**

2.1 Collection and Broodstocks Selection

Parent Selection Stage Collection and performed at the east coast of Surabaya with location based distribution division which had been previously marked point coordinates to facilitate a survey conducted in accordance with Figure 1.

In cultivation, first broodstocks selection obtained from the results of a survey conducted in Surabaya East Coastal region. The next stage will be done will be done spawning process is done by manipulation of the environment based on thermal shock by Hendri et al., 2009. In addition, the selected broodstocks also given feed enriched with Vitamin E prior to gonad development and improve the quality of the resulting larvae. Most sea cucumbers gonad from nature will be taken for histological examination for the level of gonad development and general overview.

2.2 Enrichment of Natural Feeding and Heat Shock

Enrichment of feed made with natural food immersion method at a dose of 500 IU of vitamin E for five minutes. Furthermore, the entire median feed and given to the broodstocks in a tub maintenance. Natural feeding is expected to provide an increase in the maturity of gonads of sea cucumbers.

Temperature shock was given to the media by moving of sea cucumbers in different medium have been given the thermostat to the temperature difference of 3 and 5 degrees above the normal temperature. Normal condition for sea cucumber habitat is 26^{o}C and 32^{o}C maintenance. Laxminarayana (2005) mentions the change in temperature of 3-5 degrees on container maintenance can stimulate spawning in the sea cucumber. Sea cucumbers male will release sperm first and then will stimulate the female will release the eggs of sea cucumbers. Giving shock performed on 10 animals each container of sea cucumbers. Comparison of males and females was not done because of sea cucumbers can be distinguished not only by anatomical sex outside. Mass spawning is done with the hope that there is enough sperm to fertilize the eggs produced by a sea cucumber or otherwise provided enough eggs to produce the number of larvae that much. This activity is carried out at 18.00 according to the natural spawning occurs in the sea cucumber. Observations were made after 48 hours of spawning with filter media using a plankton net size of 100µm. Of the filtered sample is then observed whether the auricularia larvae found. Observations were made using a binocular microscope with a magnification of 100 times.

2.3 Analysis of Sea Cucumber Eco-habitat *Phyllophorus* sp.

In the first year of this study is to get an optimal environmental conditions and information about the reproductive cycle of sea cucumbers. Optimal conditions of cultivation are done by comparison of natural habitats such as water quality and appropriate substrates and the right feed for the parent prior to the manipulation in the hatchery in year 2. In the information gathering activities of the reproductive cycle of sea cucumbers will be conducted by analysis of gonad indices and histology of gonads in sea cucumbers in nature within an interval of about 4 months. So in summary in the first indicators to be achieved are:

Tabel 1. Water Quality Data on *Phyllophorus* sp.Habitat.

Month	Salinity (ppt)	Temperature (^oC)	pH	Brightness (cm)	Weather condition
Mei 2013	26-27	24-26	6.5-7.0	60	cerah
June 2013	26-28	24-26	6.5-7.5	70	cerah
July 2013	27-28	25-27	6.0-7.5	65	cerah

Histology results were obtained in the gonads of sea cucumbers, then analyzed with regard to sex and gonadal stadia. Gonads were obtained generally found in all stadia, although many do not spawning, but found stadia which is post-spawning gonads. While the male sea cucumbers, gonadal histology showed most of the gonads of sea cucumbers in its infancy and advanced growth although only a small portion of sea cucumbers have sperm mature and ready for spawning.

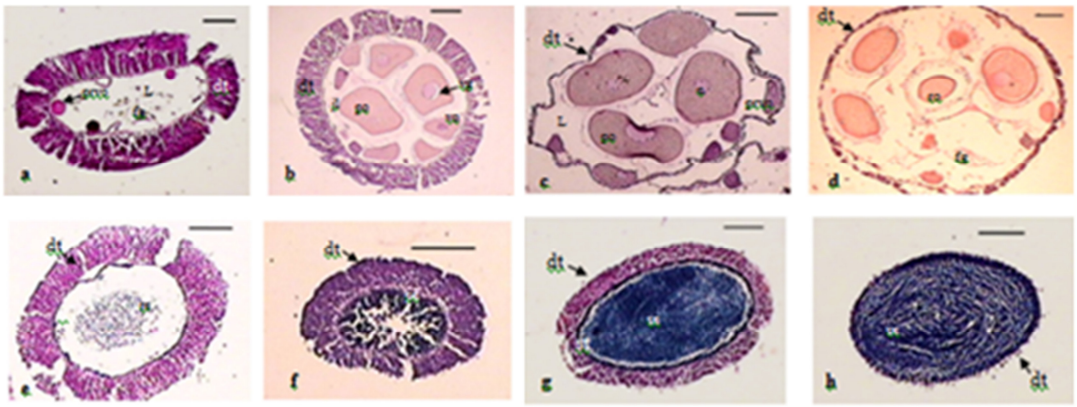
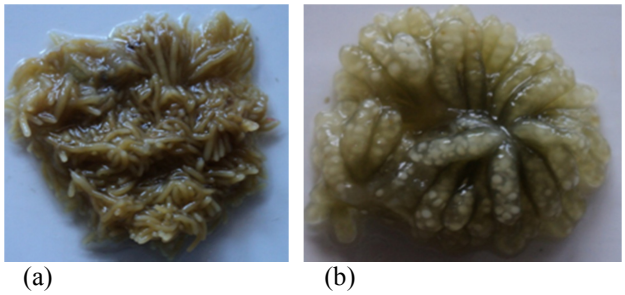


Figure 2. Histological cross-section of female gonads (ad) and male gonads (er) *Phyllophorus* sp. dt: the tubular wall; fg: phagocytic cells; jl: jelly layer; l: lumen, n: nucleus; prvo: previtelogenik oocytes; po: postvitelogenik oocytes; pz: proliveration zone; ro: relict oocytes; rs: relict spermatozoa; st: spermatocytes; SZ: spermatozoa; vg: germinal vesicle; vo: vitelogenik oocyte (scale: 200µm)

In natural feeding and temperature shock on media maintenance is performed after spawning is done, the sea cucumber is still alive then analyzed gonad organs to perform surgery. Surgery is performed by opening the ventral part with the aid of a scalpel and surgical scissors. The gonads are preserved in 70% formalin immediately prior to making preparations histology. Method for making histology with paraffin method was performed in the Department of Biology, Faculty of Science and Technology, Airlangga University.

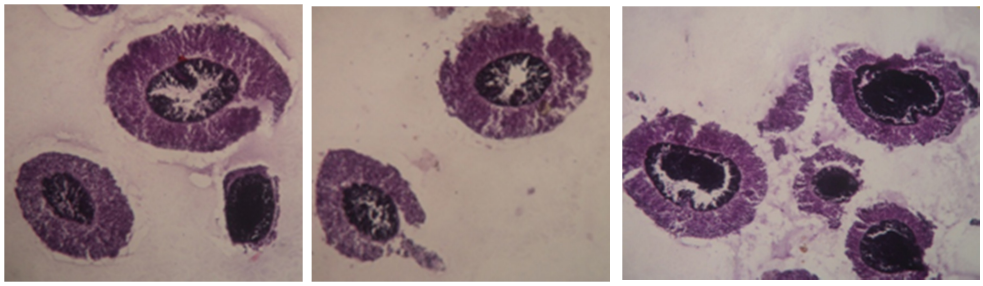
On treatment with shock temperature 27^{o}C (control), there are five male sea cucumbers are still alive and then do surgery to take gonads. Of the five sea cucumbers, the tails are male and the other female. While at 30^{o}C and 33^{o}C treatment and the remaining 5 and 2 tailed sea cucumber living. At 30^{o}C treatment, sex of sea cucumbers found 4 females and 1 male. From the observation gonads organ by histology showed three females on growth stadia and one female gonad after spawning in the stadia, while the male gonads were found in condition after spawning. At the highest temperature of 33^{o}C treatment caused mostly dead of sea cucumbers. Sea cucumbers are only the tail remaining two males who were in the development of the gonads after spawning stadia.



(a) (b)

Figure3. The gonads of sea cucumbers *Phyllophorus* sp. males (a) and females (b)

Observations on the gonads of sea cucumbers made on tubules I, II and III. So we get the repeat observations on histological preparations of each sea cucumber. In the male gonad tubule wall thickness seen in the lumen appears the growth of spermatogenic cells moving towards the lumen, the area is called the proliferation of growth zone. From the analysis of histological preparations showed that the gonads of sea cucumbers *Phylloporus* sp. in the growth conditions.

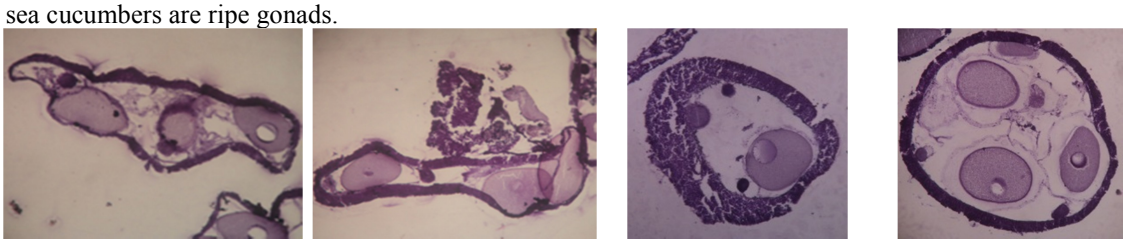


(a) (b) (c)

Figure4. Histology of males sea cucumbers *Phyllophorus* sp. gonads in the tubules I, II and III.

While the gonads of sea cucumbers *Phyllophorus* sp. females only one who showed stadia gonads on growth of advanced, while the others are still in the growth stage gonads. This is indicated by the thick walls of the tubules in the gonads. Based on the results of the histology of the gonads of sea cucumbers *Phyllophorus* sp. who has done that several stadia gonad becomes a parameter in spawning. Conan (1981) in Kesharvars *et al*. (2012) mentioned that there are at least five stadia in gonad developments. The development of the gonads can be seen from the length of tubules, diameter, and branch of the tubules, saccules presence, color and presence of gametes.

Further Kesharvars *et al*. (2012) suggested that the broodstock collection for sea cucumber hatchery should be done at the time of maturity and spawning time relatively close. However, with limited information about the sea cucumber *Phyllophorus* sp. cannot be done. To answer this problem will require highly accurate monthly information related to the reproductive season so that when the broodstocks collection conducted found that sea cucumbers are ripe gonads.



(a) (b) (c) (d)

Figure 5. Histology of females sea cucumbers *Phyllophorus* sp. gonads in the tubules II and IV (Fig. a and b) on the stadia advanced growth, and on the other organisms in the II and I in stadia growth (images c and d).

Sea cucumbers are currently obtained as a broodstocks is also less than perfect in the arrest. Catching up was done by fishing gear in the form of a large comb drawn by boat for a while and then removed. Sea cucumbers are raised mostly injured embedded in the ends of the comb, so no doubt cause stress for catching sea cucumbers eject most of the internal organs out through its oral side. The existence of a complete lack of organs makes this survival rate of sea cucumber *Phyllophorus* sp. very low. Within a relatively short maintenance time is a maximum of 7 days can survive in container of culture. Spawning may occur also possible because of stress due to changes in environmental parameters such as temperature. Given temperature changes are known as the factors that trigger spawning. Laxminarayana (2005) lowering the temperature of the sand sea cucumber maintenance 3-5 degrees below the normal temperature of the sea water by adding ice cubes. Immersion was carried out at a temperature for 5 minutes and then returned to maintenance medium that has a normal temperature (3-5°C higher) than the previous cultivation temperature on the container . The value of 3-5^{o}C increase in temperature is sufficient to trigger the spawning. Individual males will first spawn by releasing sperm and about 30 minutes later will be followed individual females who will release their eggs.

5. Conclusion

From the results of some tests in this study it can be concluded that the provision of feed enrichment (enrichment) on the local sea cucumber broodstock *Phyllophorus* sp. not directly affect on gonad maturation. Nutrition through a feeding may directly affect growth. Consideration should be given additional hormones that feed the media through the hormones directly affecting gonad embankment. Giving temperature shock on the local sea cucumber spawning *Phyllophorus* sp. not produce good spawning. This is due to the limited information on the development of sea cucumber gonads *Phyllophorus* sp.

From the result of performance in this study, we can advise further research on the use of pro-biotic in feed combined with the addition of hormones that stimulate gonad development can be applied to the local sea cucumber aquaculture *Phyllophorus* sp. In addition, the collection of information about the development of the gonads of sea cucumbers are local in nature will be a portrait of the reproductive cycle of the local sea cucumber is better to be a very important information .

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