The Behavior of Sea Cucumber *Phyllophorus* sp. during the Period of Adaptation

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ABSTRACT— Study on behavior of sea cucumber *Phylloporus* sp. is the first conducted in Indonesia. The fulfillment of local needs on sea cucumbers Phyllophorus sp were still depends mostly on the catch by local fisherman, so that the Efforts to improve the cultivation of sea cucumbers are important to examine that the cultivation of cucumbers can be started with the adaptation of sea cucumbers in advance. One of the factors influence the success of sea cucumber aquaculture is the success of its adaptation to a new habitat or environment. One of the adaptations required concerns with the depth of the water habitat of sea cucumber. The mud was needed as a substrate to immerse. This study aims to monitor the behavior of sea cucumber rates on varied depth of water and the survival rate of sea cucumbers in each depth. The method used is an experiment with completely randomized design as the experimental design. The treatments use different water depths, the which are 10, 20, 30 and 40 cm, and each treatment repeated 5 times with long maintenance for 30 days .. for how long time period? The behaviors of sea cucumbers during the study were Analyzed using descriptive and statistical analysis. The results Showed that normal behaviors of sea cucumbers occurred in order: fully exposed and fully half-buried body buried. The abnormal behaviors performed by sea cucumbers ranged from secreting intestine sequentially, then secreting the gonads and tentacles, and producing calcareous ring and slimy skin. The results of the survival rate of sea cucumbers show that the average survival period of sea cucumbers is not significantly different at the water treatment depth of 20, 30 and 40 cm.

Keywords— *Phyllophorus* sp., behavior, sea cucumber, survival rate.

1. INTRODUCTION

Local sea cucumbers (*Phyllophorus* sp.), also known as sea ball cucumber, are included in the phylum Echinodermata. These cucumbers are one species of sea cucumbers with high protein (44.39%) and have immunostimulatory potencial against the bacteria *Mycobacterium tuberculosis* [1]. Local sea cucumbers are also among the commodityies from the east coast of Surabaya, which are more widely consumed as snacks, such as chips, traded in Surabaya, Sidoarjo, Lamongan and Gresik. Based on information from multiple collectors, dried local sea cucumber is also a commodity continuously exported to China [2]; [3]. Thus, it suggested that the local sea cucumbers have an important economic value.

Needs for local sea cucumbers *Phylloporus* sp. still depend on provided by natural catches from fisherman. In the long term, sea cucumbers *Phylloporus* sp. may overfishing that can threat the number of local sea cucumbers available in the future. Therefore, the cultivation of local sea cucumbers are needed. Environmental factors will affect the adaptability of sea cucumbers in the cultivation media. High water on the maintenance of sea cucumber provides a number of influences in cultivation. In this study only observed behavior arising from differences in different water depths at the initial stage adaptation of sea cucumbers *Phylloporus* sp.

Inability to adapt will affect sea cucumber physiology. It is shown by some behavior indicating under stress and self-defense either performed mechanically or chemically. Evisceration is one of the signs of stress on sea cucumber [5]. Evisceration is a mechanical attempt of self-defense by sea cucumbers [6]. Evisceration is a spontaneous rejection of internal organs [7] of the sea cucumber that done through the anal and mouth. The self-defense of sea cucumber has varied forms including toxicity, thickened body wall, evisceration, autotomy cryptic behavior, unpalatability, and swimming or other active avoidance from the predator [6].

2. MATERIAL AND METHOD

2.1 Materials

The experiment was conducted at the Laboratory of the Faculty of Fisheries and Marine Education, The University of Airlangga from April to May 2014. The primary equipment used in the study is the aquarium (30 cm x and other equipment needed for sea cucumber aquaculture. Sea cucumbers collected from the east coast of Surabaya and the species used was the local sea cucumber (Phyllophorus sp.). Sea cucumbers are collected by fishermen with iron comb tools as fishing gear. Collecting sea cucumbers carried out at low tide with water depth range between 10-50 cm. Sea cucumber is then used as the animal test. Sea cucumbers are grouped with an average weight of 25 grams without distinction of male and female. This study was Carried out as an experimental study with CRD (completely randomized design) consisting of 4 treatments (10, 20, 30 and 40 cm) and 5 replications. Initial adaptation of sea cucumbers collected performed on aquarium tank with a volume of 20 m2 with a stocking density of each aquarium is 4 individual/L. In the aquarium substrate depth of 10 cm mud. Salinity and temperature maintenance with attempted temperature and salinity at the making of the sea that is 29oC and salinity of 29 ppt. Maintenance on the research carried out for 30 days.

2.2 Maintenance procedures Method

The adaptation processes have done in a semi-outdoor space. Sea cucumbers were placed in an aquarium containing of seawater and fitted to aeration. Aeration system using Hi-blow Resune® Air pump LP-40 which will channel the air through a small pipe has given solver air to form tiny air bubbles. Maintenance system was performed by using a recirculation pump Aquaman® AM-1600 liquid filter 25 Watt without filtering. The seawater has taken from the sea cucumber habitat. Seawater was done physical filtration and sterilization with chlorine of 100 ppm for 24 hours. A substrate of 10 cm thick layer of mud was lay at the bottom of the aquarium. Mud taken from the habitat of sea cucumbers, sterilization has done with the drying under the sun for two days. *Phylloporus* sp. is a type of burrowing sea cucumber, so they requirements of substrates. Chlorella sp. was given up as a natural food with the density to 20 x 10^3 cells / ml were given two times a day at 15:00 pm, 20:00 pm and 1:00 am in a day. Behavioral observations and the survival of sea cucumbers during the study has examined every hour a day. Behaviors observed were fully exposed body, half-buried and fully buried a body, secreting of tentacles, secreting of intestines, secreting of gonads, the occurrence of a calcareous ring and slimy skin. The observation of sea cucumber survival has conducted by observing the posterior movement of sea cucumbers or touching the posterior of sea cucumbers. Observations were made on the morning and afternoon. Recording all behaviors have done manually with a direct view of sea cucumbers in the aquarium maintenance every hour. Water quality parameters are dissolved oxygen (DO), salinity, temperature and brightness. DO was measured by DO meter, and hand Refractometer was used measure salinity. Temperature was measured by thermometer, than sechidisk was used measure on brightness.

2.3 Statistical Analysis

Data from the observation on sea cucumber will be Analyzed behaviors both descriptively and statistically using SPSS Version 21. Besides, the water qualities have used as supporting data is to support the data from sea cucumber behaviors. The data have presented in the form of tables and figures.

3 RESULTS AND DISCUSSION

3.1 Results

a. Sea Cucumber Behavior

The results show that in all treatments, sea cucumbers perform self-embedding behaviors, which are fully exposed, fully buried and half-buried body. Changes in self-embedding behavior occur consecutively, first is fully exposed, then half-buried and finally fully buried body. In addition, abnormal behaviors (evisceration and production of slimy skin) performed in conjunction with self-embedding behavior. On the first to the fourth day, sea cucumber performed abnormal behaviors in the treatment with the water depth level of 20 cm. The abnormal behavior performed was secreting intestine. At the water depth level of 30 cm, the visible abnormal behavior was producing slimy skin. Compared with other treatments, at the10 cm water depth level, sea cucumbers showed several abnormal behaviors like secreting intestine, producing gonads, calcareous ring and slimy skin.

Table1. Results behavior of the sea cucumber

Behaviors	Water depth			
	10 cm	20 cm	30 cm	40 cm
Fully exposed	61,80±19,61[a]	5,20±3,49[b]	6,60±6,50[b]	8,40±7,27[b]
Fully buried	28,20±10,83[b]	77,20±4,76[a]	76,40±5,18[a]	79,80±4,44[a]
Half buried	37,80±8,53[a]	17,40±18,12[b]	9,60±5,86[b]	11,60±7,30[b]
Expend of tentacle	13,60±9,24[a]	1,20±1,64[b]	1,60±2,30[b]	2,60±4,34[b]
Expend of intestine	13,40±9,61[a]	2,20±3,03[b]	0,80±1,79[b]	0,60±1,34[b]
Expend of gonads	2,60±5,81[a]	0[a]	1,20±2,68[a]	0[a]
Expend of calcareous ring	11,80±12,03[a]	0[b]	0[b]	0[b]
Slimy skin	14,20±3,56[a]	0[b]	1,60±2,30[b]	0,60±1,34[b]

Remarks: Different superscript in one line showed no significant differences ($p<0,05$).

On the fifth to the eighth day of sea cucumbers placed at the treatment of water depths of 20, 30 and 40 cm, the stabilized behavior shown fully buried although at the treatment of water depths of 20 and 30 cm sea cucumbers also showed half-buried behavior. At a water depth of 10 cm, sea cucumbers performed fully exposed, fully buried and half-buried behaviors. Along with self-embedding behavior that occurring at a water depth of 30 cm treatments, the behaviors shown were secreting tentacle and intestines where as at 40 cm water depth, the behavior performed was only the secreting of intestine. At a depth of 10 cm of water treatment, more behaviors shown: secreting tentacles and intestines, and producing calcareous ring and slimy skin. Unlike day one to four and five to eight, on day 9-12, sea cucumbers at a water depth of 10 cm treatment died. The behavior of fully buried in the water treatment of 20, 30 and 40 cm deep was quite stable, although the behavior of fully exposed to the water depth of 20 and 40 cm was more visible and the behavior of half buried was clearly seen at the water treatment of 20, 30 and 40 cm deep. In a water treatment depth of 20 cm, the abnormal behavior that was seen was the secreting of intestine and gonads.

On the 13rd-16th days, a fully buried behavior at the depth of the water treatment of 20, 30 and 40 cm looked more stable than the previous days although the abnormal behavior of producing slimy skin still found at the water treatment depth of 20 and 40 cm. At the depth of 20 cm water treatment, sea cucumbers also produced a calcareous ring. A stable fully buried behavior was also seen on day 17th-20th and 21st-23rd though the sea cucumbers placed at the 30 cm water depth was found dead on day 21st-23rd.On day 17th-20th and 21st-23rd, the sea cucumbers placed at the treatment of water depth of 40 cm were found to secrete their tentacles.

Fully exposed behavior of sea cucumber has characterize by the position of the whole body is on top of the substrate and the position of the analyses facing the water surface (Figure 1A). Other behavior performed in conjunction with a fully exposed behavior is the secreting of tentacles from the mouth (Figure 1B) and the secreting of gonads (Figure 1C) and intestine from the anal of sea cucumbers (Figure 1D). Tentacles secreted from anterior body are more protrude than those by posterior side. Moreover, gonads and intestines secreted have oval shape and secreted when the anal position is facing the water surface. Fully buried behavior is characterized by the whole body of sea cucumbers, except the anal hole, is buried (Figure 2A). The position of the anal hole is facing the water surface.

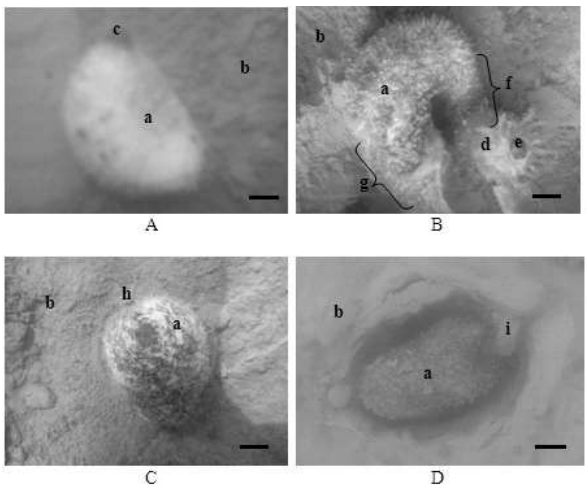

Figure 1. Behaviors of sea cucumber *Phylloporus* sp. in different condition: fully exposed (A), fully exposed with secreting tentacles (B), the producing of gonad organ on fully exposed (C), and the secreting of intestine organ on fully exposed. sea cucumber (a), substrate (b), sea cucumber anal (c), tentacles (d), mouth (e), anterior (f), posterior (g), gonad (h) and intestines (i) scale bar: 1 cm

Fully buried behavior is performed in conjunction with other behaviors, i.e secreting tentacles from the mouth (Figure 2B) and secreting intestines from the anal of sea cucumbers (Figure 2C). The whole body of sea cucumbers when secreting tentacles and intestines immersed in the substrate except the mouth, tentacles and intestines. When the tentacles are coming out, tentacle insertion into the mouth characterized by curving tentacles toward the mouth of the branch as well as the inclusion of tentacles into the mouth. Perhaps include a video in the supplementary material

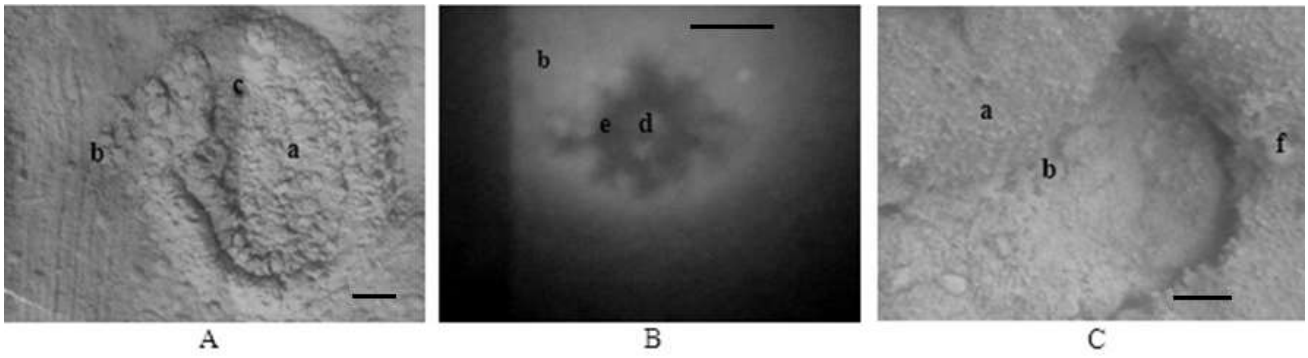

Figure 2. *Phyllophorus* sp. Behavior of fully-buried (A), fully exposed buried with secreting tentacles (B), fully exposed buried with secreting intestines (C). sea cucumber (a), substrate (b), anal of sea cucumber (c), mouth (d), tentacles (e) and intestine (f). scale bar: 1 cm

Half-buried behavior has indicated by half body of sea cucumbers in the substrate and the position of the anus is facing the water surface (Figure 3A). Half-buried behavior performed in conjunction with secreting gonads (Figure 3B). The gonads secreted from the sea cucumbers body.

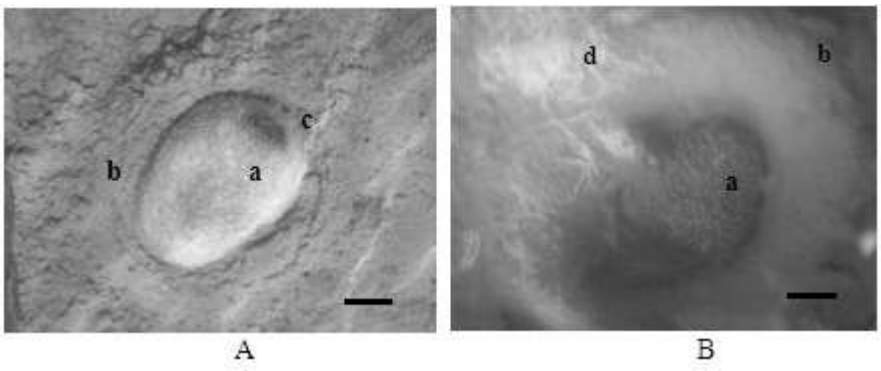

Figure 3. *Phyllophorus* sp. half-buried behaviors (A), expend of gonad with half buried (B). Sea cucumber (a), substrate (b), anal (c) and gonads (d). scale bar: 1 cm

The production of slimy skin as an abnormal behavior of sea cucumbers can see in Figure 4A. The body of sea cucumbers in this condition is oval with their whole body is filled with mucus. The shape of the sea cucumber, when producing calcareous ring from the mouth shown in Figure 4B.

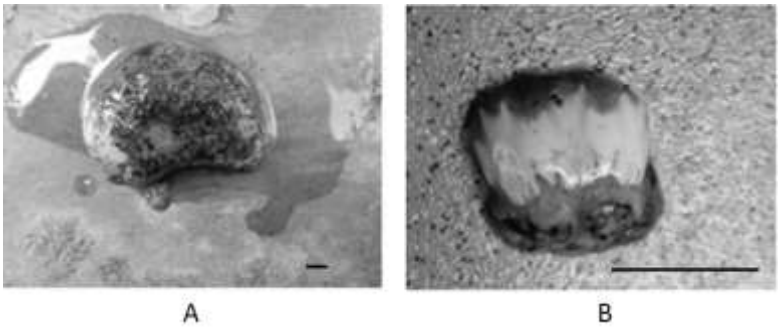

Figure 4.The production of the slimy skin of sea cucumbers *Phyllophorus* sp. (A) and calcareous ring (B) scale bar: 1 cm

Based on the results of behavioral observations on sampling sea cucumbers, it is found that the fully-buried behavior will appear on sea cucumbers until 100% from 5 am to 6 pm when the sea water is at the lowest tide. The low water level caused sea cucumbers rarely come to the surface [4]. It shows that sea cucumbers are negative phototoxic [8].

Based on the observations, on the behavior of sea cucumbers during the study, that placed at the water treatments of 20, 30 and 40 cm deep showed normal behavior. The normal behavior meant here is self-embedding behavior which is performed in a sequence, starting from the fully exposed, then the last half-buried and finally fully buried. This is consistent with the behavior of the sea cucumber *Holothuria scabra* species whose self-immersion activity starts from fully exposed, half-buried and last, fully buried in normal conditions [9].

Abnormal behaviors on sea cucumbers occur in the forms of secreting intestines, removing gonads, producing tentacles, calcareous ring, and slimy skin. Those occur at a water treatment depth of 10 cm. The sea cucumber genus *Thyone* also performs abnormal behavior [10] similar to the behavior of *Phyllophorus* sp. Most abnormal behaviors shown by this genus performed after evisceration; they are the secreting intestine, then the gonads, and the last is the production of calcareous ring of tentacles. Not like other behaviors, the production of slimy skin carried out before evisceration. Sometimes, the secreting of gonads and the production of calcareous ring after intestines have secreted does not occur. This suspected to cause severe stress to sea cucumbers. It shows that without secreting gonads and producing calcareous ring, sea cucumber may die. The death of sea cucumber typically has caused by severe stress [10].

The secreting of tentacles on sea cucumbers, have seen in all depths of water treatments. Sea cucumbers at a depth of 10 cm of water treatment secreted tentacles along with the behavior of the fully exposed and the anterior body is more prominent than the posterior one. On the other hand, at the water treatment of depth of 20, 30 and 40 cm, the secreting of tentacles performed in conjunction with a fully buried body and tentacle insertion into the mouth. The activity of secreting tentacles at a depth of 10 cm of water treatment is similar to the behavior of the sea cucumber genus *Thyone* when experiencing severe stress [10]. This is due to the lack capability of sea cucumbers to adjust with its surroundings. The secreting of tentacles at the depth of 20 cm, 30 cm and 40 cm, have shown by the species *Cucumaria frondosa* sea cucumber during a feeding activity [11].

b. Survival of Sea Cucumbers

The life span and survival data of sea cucumbers used to determine the success of the adjustment process of sea cucumbers. Based on statistical analysis test results in Table 2, the life span of sea cucumbers is not significantly different at the water depth of 20, 30 and 40 cm. Meanwhile, at a water depth of 10 cm, the result shows significant difference with the result on other water depths. This data also supported by the results of the average survival rate of sea cucumbers. The results indicate that the survival rate of sea cucumbers at the water treatment depth of 20, 30 and 40 cm is not significantly different from one to others. On the other hand, the results of the treatment at the water depth of 10 cm show significantly different results from other depths. The results of the ANOVA test the life span of sea cucumbers can see in Table 2. Water quality parameters for the study presented in Table 3.

Table2. Results of ANOVA survival of sea cucumbers during the study

Parameters	Water depth			
	10 cm	20 cm	30 cm	40 cm
Living time (hour)	53,40±3,13[b]	513,80±3,40[a]	454,20±10,42[a]	522,60±3,89[a]
Survival rates (%)	0[b]	86,80±1,81[a]	80,00±2,99[a]	93,40±[a]

Remarks: Different superscript in one line showed no significant differences (p<0,05).

Table3. The range of water quality data on the sea cucumbers adaptation medium during the study

Water depth (cm)	Salinity (ppt)	DO (mg/l)	Temperature (^oC)	Brightness (%)
10	29	8,3	29	100
20	29	8	29	100
30	29	8,1	29	100
40	29	8	29	100

The life period of sea cucumbers at the water depth of 40 cm is the longest among others. This indicates that sea cucumbers are able to perform the adaptation. The figure for this depth is equal to 93.40%. This means that 93.40% of sea cucumbers is alive while 6.6% other died.

Water quality in the adaptation medium is also measured. The parameters are salinity, DO, temperature and water transparency in the entire basin. The adaptation success indicates that the water quality is similar to the natural habitat of sea cucumbers. According to the study on *Phyllophorus* sp. habitat on the east coast of Surabaya, it is found that the habitat has natural condition like 28-33 ppt salinity, DO from 6.5 to 7.9 mg/l, temperature of 29,5-32^oC, and water transparency from 25-100%. The water condition is still acceptable for the life of sea cucumbers [13].

4 CONCLUSION

The water depth influences the behavior and the life span of sea cucumbers during the adaptation period. Water depth of 40 cm is the most appropriate medium for sea cucumber adaptation, although that no statistical difference have observed among 20 and 30 cm of depth. that no statistical difference was observed among 20, 30 and 40 cm of depths. Based on the research results, it is also advisable to use a water depth of 40 cm in the adaptation process of *Phyllophorus* sp. Further research is required to determine the effect of salinity, temperature, light intensity, DO on behavior, survival, growth of *Phyllophorus* sp. during adaptation. It is also necessary to examine to know how sea cucumber responds to stressors physiologically.

5 ACKNOWLEDGEMENT

The authors would like to convey their gratefulness to Universitas Airlangga Research Program year 2014 that supported this research and made this research realized. We would also like to acknowledge the cooperation of all research members, the LPPM Universitas Airlangga, and the Faculty of Fisheries and Marine that provide assistance in the development of research in Universitas Airlangga. We would like to particularly thank Dr. Dwi Winarni who provided meaningful support in conducting this research.

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