

SPONGE (*Callyspongia* sp., *Callyspongia basilana*, and *Haliclona* sp.) CULTURE WITH DIFFERENT INITIAL EXPLANT SIZES

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ABSTRACT

Sponge global demand for industry and research needs far exceeds supply from the sea. Aquaculture is considered as the only viable method that can supply sufficient and sustainable quantities of sponges. Aquaculture method is also one of efforts to anticipate and avoid the over-harvesting of sponges in nature. However, culture methods need to be determined to provide the platform for commercial success. In this study sponges (*Callyspongia* sp., *Callyspongia basilana* and *Haliclona* sp.) were successfully cultured by transplantation method using polyethylene net with the initial explant sizes of 1 cm, 3 cm, and 5 cm. The result showed that the sponge growth and survival rates were dependent on the species. *Callyspongia* sp. and *Callyspongia basilana* gave the highest growth and survival rates on the treatment 3 cm with the final explants length reached 12.20 ± 2.35 cm and 7.603 ± 0.93 cm and survival rates reached 98.33% and 36.67%. In contrast, *Haliclona* sp. had the highest growth (21.67 ± 0.25) and survival (95%) on treatment 5 cm. Nevertheless, among the three species, treatment using 3 cm and 5 cm of initial explant sizes did not show a significant difference. Therefore for the efficiency of explants use, the best initial explant length for culturing the three species of sponges is 3 cm.

KEYWORDS: sponge, explant size, culture method

INTRODUCTION

Sponge is a member of coral reef ecosystem. Sponge is known as a source of bioactive compounds. For example, *Dysidea herbacea* from West Sumatra produces an antibiotic identified as diphenil eter which is active against *Basillus subtilis* and *Cladosporium cucumerinum* (Handayani *et al.*, 1997). Carletti *et al.* (2000) identified a chemical compound isolated from sponge *Lotrochota purpurae* known as metamone, an oxindole alkaloid containing bromine having an anticancer activity. Study on bioactive compounds of sponge has also been done in the Research Institute for Coastal Aquaculture (RICA) since 1995/1996 for disease prevention on fish culture. The screening on several species of sponges was found that *Auleta* sp., *Halichondria* sp., and *Callyspongia* sp. can be used as a natural bac-

tericide on fish culture (Ahmad *et al.*, 1995; Muliani *et al.*, 1996; Suryati *et al.*, 1997). Muliani *et al.* (1998) reported that sponge extract from *Auleta* sp., *Clathria* sp., and *Thionella cylindrica* were able to inhibit the growth of fungi isolated from diseased shrimps. Sponge can also be used as an antibiofouling source such *Callyspongia* sp., *Clathria* sp., *Auleta* sp., *Clathria reinwardi*, *Halichondria* sp., and *Jaspis* sp. (Suryati *et al.*, 1999). The other investigation on sponge exhibited that *Callyspongia* sp., *Thionella* sp., and *Xestospongia* sp. are also a carotenoid compound source (Rosmiati *et al.*, 2005). The same researcher also identified the presence of oxytetracycline gene in *Sylotella aurantium*, *Acanthella kletra*, *Gelliodes fibulatus*, and *Auleta* sp. and chloramphenicol gene in *Auleta* sp., and *Pericharax* sp. (Rosmiati *et al.*, 2007).

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A good sponge bioactive potency for both industry and research needs has directly caused over-harvesting of sponges from their natural population. A direct harvest from the sea is not sufficient, because global demand has exceeded sponge supply. Several methods for multiplying of sponge bioactive without directly having the sponge harvested from the sea such as chemical synthesis and transfection of gene can be done. However, these efforts are less effective because the chemical synthesis of the target metabolites is technically and economically not feasible due to the compounds' complex chemical structures, which make synthesis too difficult or expensive. Transfection of the genes involved in the production of some bioactive substances to a cultivable microorganism, such as *Escherichia coli*, has also been explored. However, the large number of genes involved in sponges' metabolic pathways greatly complicates this process, and the current results are not conclusive (Caralf *et al.*, 2004).

Aquaculture is considered as the only method that can supply sufficient and sustainable quantities of sponges for supplying a sustainable number of sponges for biopharmaceutical industry and mitigate the environmental effects of over-harvesting. Furthermore, it might also be an economically profitable industry for remote coastal communities. Several species of sponges which were successfully cultured are *Latrunculia wellingtonensis*

and *Polymastia croceus* (Duckworth *et al.*, 2004). RICA has also successfully cultured *Auleta* sp. by a transplantation method using polyethylene net spread on a quadrangle-shaped iron framework 60 cm x 90 cm (l x w) in size with horizontal position and the explant size of 3 cm as an appropriate method (Pong-Masak & Rachmansyah, 2002). The species is now known as *Haliclona* sp.

Based on the information, the research was trying to culture several sponge species, which are potential to be developed, such as *Callyspongia* sp. (Ahmad *et al.*, 1995; Suryati *et al.*, 1999 and Rosmiati *et al.*, 2005) and *Callyspongia basilana* (Rosmiati *et al.*, 2005). *Haliclona* sp. was also cultured as a comparison (Figure 1). The multiplying technique of the three sponges species followed the mentioned above method. This study was aimed to culture *Callyspongia* sp., *C. Basilana* and *Haliclona* sp. and to know the best initial explant size for multiplying these species based on their growth and survival rates. The resulted data will give an information in sponge culture techniques, particularly for *Callyspongia* sp. and *Callyspongia basilana* for the efficient use of sponge explant (natural stock) in relation with the sustainable management of sponge resources. Physical and chemical conditions in Awarange Bay (Culture location) as supporting data on the growth and survival rate of sponges referred to the previous study of Rachmansyah *et al.* (2007).



(a) *Haliclona* sp.



(b) *Callyspongia* sp.



(c) *Callyspongia basilana*

Figure 1. Sponges having a potency as bactericide (a), fungicide (b), and carotenoid source (c)

MATERIALS AND METHODS

Preparing Substrate

Polyethylen net with mesh size of 0.5 inch were spread on a quadrangle-shaped iron framework, approximately 60 cm x 90 cm (l x w) in size and the irons were covered by plastic to prevent corrosion.

Collecting Sponges and Cutting Explants

Three species of sponges (*Callyspongia* sp., *Callyspongia basilana*, and *Haliclona* sp.) were collected from waters of Barrang Lompo Island. To minimize post harvesting impact, up to one third of each sponge was left attached to a rock. The remaining cut sponges of the three species can heal and regrow quickly (Duckworth, 2003 in Duckworth *et al.*, 2004).

The three species of sponges were separated into different oxygen-supplied plastic bags to prevent any antagonistic interactions among the species. All collected sponges were kept in the containers filled with seawater at ambient temperature sourced from the collection site. All specimens brought to a floating net cage in Awarange bay, Barru Regency, South Sulawesi. These sponges were acclimatized one day before cutting process.

The collected sponge tissue was cut into explants with scalpels under flowing seawater with the size of 1 cm, 3 cm, and 5 cm in length as treatments. All explants possessed both side cut.

Experimental Design

For each species, 60 explants (i.e., 20 explants x 3 substrates) were horizontally transplanted on separated three substrates by binding one by one of the explants using nylon

line (No.4) with 15 cm gap among them (Figure 2). These sponge explants, which had been binded on the substrates, were randomly placed at 3 m depth with horizontal position. Study on *Callyspongia* sp. and *Haliclona* sp. was conducted for 75 days from May 28, 2008 to August 10, 2008. Meanwhile, observation on *Callyspongia basilana* was only for 45 days, because all reared-explants of the species were eaten by fish on the 60th day. The experiment was arranged in completely randomized design with three treatments and three replications.

Monitoring Growth

The growth of explants (in terms of length) was monitored every 15 days. However, to simplify the observation, only the final growth and survival rates were statistically analyzed and compared. The measurement of growth of survived explants was conducted by using elastic meter (0.1 cm in scale) to follow the growth direction and the form of sponges length growth. Survival percentage was compared using the formula:

$$\% \text{ Survival} = \left(\frac{\text{The number of final explants}}{\text{The number of initial explants}} \right) \times 100$$

Data Analysis

The growth data (mean of the final total length) and survival rate of each treatment were analyzed by using various analysis (ANOVA), and then continued by Smallest Significant Difference using statistic II programme.

RESULTS AND DISCUSSIONS

Growth of Explants

The increase length of different explant sizes for the three sponge species every 15 days was shown in Figure 3, 4, and 5.



(a) *Haliclona* sp.

(b) *Callyspongia* sp.

(c) *Callyspongia basilana*

Figure 2. Explant position on substrate

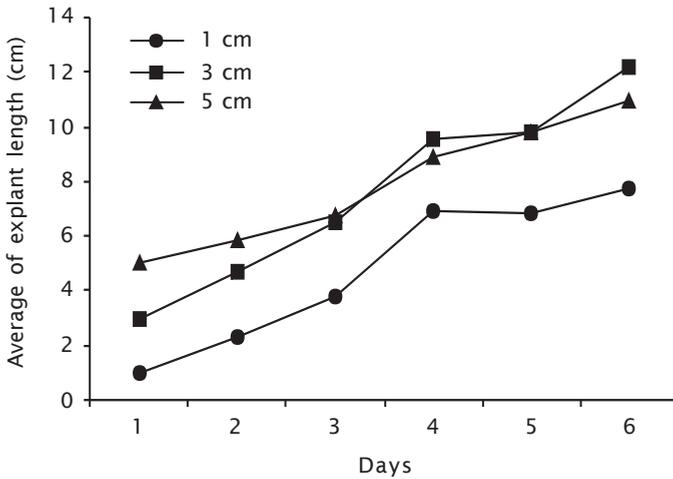


Figure 3. Growth of *Calispongia* sp. with different explant sizes

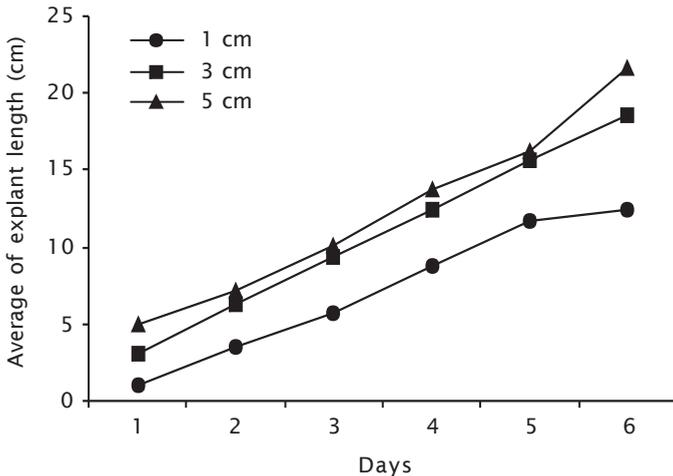


Figure 4. Growth of *Haliclona* sp. cultured with different explant sizes

Explants with the size of 1 cm, 3 cm, and 5 cm in length of the three sponges species grew well during this study. The initial size of the transplanted explants gave valuable information in culture technique of the three sponges species. The highest average explant length was found in *Callyspongia* sp. (12.20 cm) and *Callyspongia basilana* (7.60 cm) with each initial explant size of 3 cm. Meanwhile for *Haliclona* sp. was 21.67 cm transplanted using 5 cm explant size. The different final explant lengths at the size of 3 and 5 cm compared to 1 cm were affected by two factors. First, small size decreases the ratio of cutting surface to the explant increased volume, which likely will

increase stress. Second, small explants have smaller reserved tissues and comparatively invest more energy into regeneration process and diverting energy away from somatic growth.

The high sponges growth, furthermore, was supported by a good environmental condition in Awarange Bay (culture location). As reported by Rachmansyah *et al.* (2007), the physical condition such as water temperature in May to August was 29°C–30°C. It caused the increase of plankton abundance reaching 580 ind./L, that can be used by sponges as their food. There were also found high Total Organic

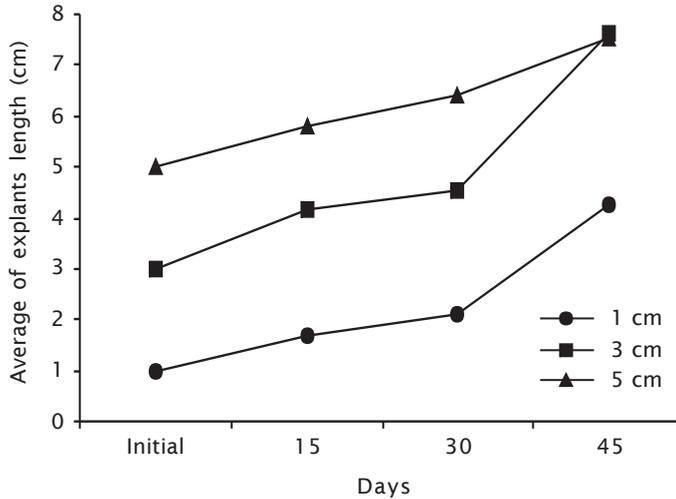


Figure 5. Growth of *Callyspongia basilana* with different explant sizes

Matter (TOM) (11.21—16.00 mg/L), nitrate (0.0982—0.5869 (0.2663 ± 0.1709) mg/L) and phosphate (0.005—0.0460 mg/L) concentrations. This high TOM concentration can also be used by sponges as food source by filtering it in the water. Meanwhile, The high concentration of nitrate and phosphate was suspected to be able to increase symbiotic microbes in sponge tissues and cells. According to Wilkinson *et al.* (1999), symbiotic microbe in sponge tissue can reach 60% of its volume. The increasing number of symbiotic microbes will directly increase biomass in tissue and cell.

The average of explant length of the three sponge species will increase correspondently with rearing time. The Smallest Significant Difference analysis of the three sponge species showed that explant length with the initial explant sizes of 3 cm and 5 cm did not show a

significant effect on *Callyspongia* sp. and *Haliclona* sp. In contrast, explant length on the 3 cm treatments showed a significant difference compared to the 1 cm treatments on *Callyspongia* sp. Meanwhile for *Haliclona* sp., the explant final length with the initial size of 5 cm showed a significant difference with explant final length with the initial size of 1 cm (Table 1). On the other hand, the explant size difference found in *Callyspongia basilana* gave a similar result (Table 1).

Survival

Survival analysis of transplanted sponges (Figure 6 and 7) exhibited that the highest survival rate for *Callyspongia* sp. and *Callyspongia basilana* were found on explants with the initial length size of 3 cm with the value of 98.33% and 36.67% respectively. Meanwhile, for

Table 1. Average of the total length of sponge (*Callyspongia* sp., *Haliclona* sp. and *Callyspongia basilana*) after cultured

Type of sponge	Cultured	Explants length (cm) at the initial explants size		
		1 cm	3 cm	5 cm
<i>Callyspongia</i> sp.	75 days	7.763±0.47 ^b	12.20±2.35 ^a	10.93±2.52 ^{ab}
<i>Haliclona</i> sp.	75 days	12.50±1.66 ^b	18.59±1.68 ^{ab}	21.67±0.25 ^a
<i>Callyspongia basilana</i>	45 days	2.829±1.07 ^a	7.603±0.93 ^a	7.511±0.85 ^a

Note: Values at the same row followed by the same letter are not significantly difference

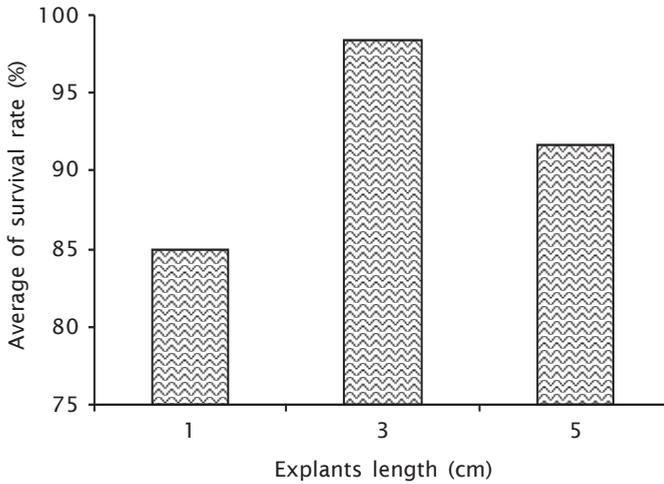


Figure 6. Survival rate of *Callyspongia* sp. after 75 days

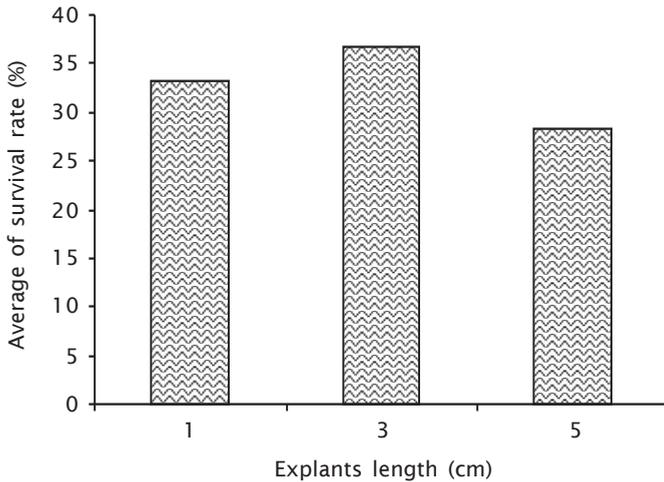


Figure 7. Survival rate of *Callyspongia basilana* after 45 days

Haliclona sp., the survival rate was 95% on the explants with initial size of 5 cm (Figure 8).

The smallest significant difference analysis of sponges survival can be seen in Table 2.

The difference analysis on the survival rates of *Callyspongia* sp., *Callyspongia basilana* and *Haliclona* sp. cultured with different explant sizes showed that the explants of 3 cm and 5 cm gave a similar result of survival rates during rearing time and both treatments resulted a better response than the explant with initial size of 1 cm. Previous study also stated that the same method for culturing

of *Haliclona* sp. gave the survival level of 92.86% on the treatments with initial size of 3 cm and 94.45% on the treatments with initial size of 5 cm (Pong-Masak & Rachmansyah, 2002). These investigations revealed that the survival rate of explants with initial length of 5 cm was higher than that of the explants with initial length of 3 cm.

According to Pong-Masak & Rachmansyah, (2002), the ability to adapt to a new environment and biota symbiosis around them are required for the survival of sponge species. The high survival rate is resulted from sponge ability to use nutrient sources around the culture

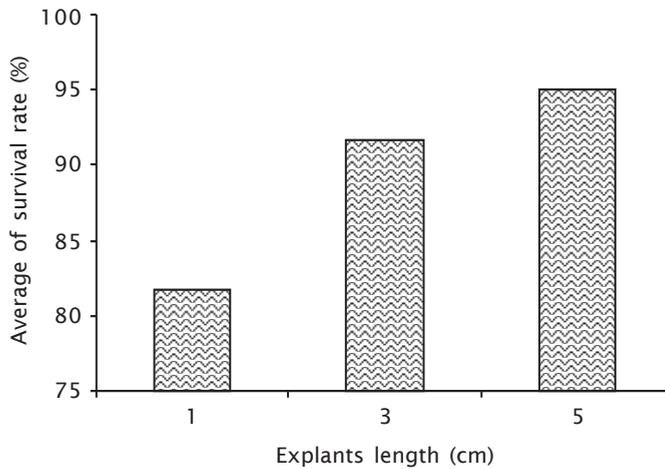


Figure 8. Survival rate of *Haliclona* sp. after 75 days

Table 2. Average of survival percentage of the different explant lengths in the final study

Explant length (cm)	Survival rate (%)
<i>Callyspongia</i> sp.	
1	85.00b
3	98.33a
5	91.67ab
<i>Callyspongia basilana</i>	
1	33.33a
3	36.67a
5	28.33a
<i>Haliclona</i> sp.	
1	81.67a
3	91.67a
5	95.00a

environment such as bacteria, fungi and zooxantella as well as nutrient inside the sponge (Scheuer, 1978). The ability to use micro symbiosis elements was proven on isolation of *Pseudomonas* sp. and *Aspergillus niger* from *Callyspongia* sp. (Suryati *et al.*, 2000). Sponge survival depends not only on species, nutrient, explants size but also on culture method. In our study the use of horizontal transplantation method in sponge culture obtained a high survival rate because explants position were horizontally bound on substrate. This position causing sponge did not need to

spend more energy for current restraining. Previous study also showed that the use of Polyethylene net as a substrate was the best material for doing sponge transplantation in open waters (Rani & Haris, 2005).

CONCLUSION

Callyspongia sp., *Callyspongia basilana*, and *Haliclona* sp. can be cultured by transplantation method using polyethylene net with the best size for transplanted explants of 3 cm or medium size, because it will guarantee

good growth and high survival rate, which in turn will produce more harvested explants using less stock of sponge biomass.

ACKNOWLEDGEMENT

We thank Ridwan and Ulla for their help in collecting sponges specimen and Sri Redjeki Mulyaningrum and Rifka Pasande for helping us with field work. This project was part of Biotechnology and seaweed, shrimp and sponge culture research, which received funding from Ministry of Marine and Fishery.

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