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Effect of fragment size on growth and survival rate of soft coral *Sarcophyton* sp.

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ABSTRACT

The *Sarcophyton* sp., are very strong and dominant in many coral reef areas. Sarcophyton species are characterized by a distinct sterile stalk, a broad, flared, smooth, mushroomshaped top, with a wide distribution and dominance in numerous coral reef areas extending from the Red Sea and eastern Africa to the western Pacific Ocean. They are cherished in marine aquariums for their diverse colors and adaptability, but their excessive exploitation has significantly impacted resources and disrupted the balance of the soft coral biome that inhabits coral reefs. The objective of the current study was to assess the impact of fragment size (0.5 × 0.5, 1.0 × 1.0, 1.5 × 1.5, 2.0 × 2.0 and 2.5 × 2.5 cm) on survival rate, growth rate and time of attachment of coral *Sarcophyton* sp. in a closed seawater system. Each treatment involved three replicates with 20 cuttings per replicate and the experimental period was 90 days. The experiment showed that the size of fragment did not influence the growth rate of oral disc diameter, pedal disk diameter and the time attachment of *Sarcophyton* sp. However, the size of fragment effect to survival rate, growth rate of height and weight of *Sarcophyton* sp. colonies. These findings collectively suggest that the initial coral fragment size of 1.5 cm was suitable in laboratory conditions.

Keywords: *Sarcophyton* sp., fragmentation, self-attachment, specific growth rate, survival rate.

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INTRODUCTION

Soft coral as dominant space-occupiers, important structural components of coral reef communities, and contributors to coral reef biomass. They create a source of organic matter, participate in reef formation. However coral reefs in Southeast Asia and throughout the world face unprecedented threat from human activities or natural processes [1]. Numerous coral reef ecosystems worldwide are indeed facing degradation or loss. The aquarium trade, a rapidly growing industry, also contribute to the decline of coral resources in their natural habitats [2]. While this trade can bring economic benefits and raise awareness about marine life, the unsustainable harvesting of corals from the wild can yield adverse effects on biodiversity and coral reef ecosystems. The indiscriminate and excessive collection of corals for the aquarium trade possesses the potential to disrupt the natural equilibrium of coral populations and inflict harm upon the reefs from which they are extracted. Annually, the coral trade generates millions of dollars in global transactions, providing income for countless fishing families worldwide through the trade in marine decorations. Incomplete statistics suggest that approximately 50 million colonies of soft coral are harvested for commercial purposes each year [3]. Coral sources are mainly supplied from Indonesia, Malaysia, Papua New Guinea, the Philippines, the Solomon Islands and Timor-Leste [4]. Currently around 40 countries engage in coral exports for commercial purposes with the volume witnessing an annual increase of over 10% [5].

Promoting the cultivation of corals in controlled environments through aquaculture can effectively alleviate the strain on wild populations. This strategy ensures a sustainable coral supply for the aquarium trade, all while minimizing harm to natural reefs. Corals can be readily reproduced asexually through fragmentation, a widely employed technique that generates subcolonies with notably high survival rates for commercial purposes, rather than resorting to wild harvesting [6]. This method entails dividing the parent coral conglomerates into smaller fragments, which can then attach to new substrates and develop into fresh coral colonies [7, 8]. The resulting subcolonies are fragmented, followed by their attachment to desired substrates through various techniques [7]. The controlled environment propagation of corals is already a well-established practice, streamlining the process and making it cost-effective [9]. Moreover, fragmentation facilitates consistent high growth rates throughout the year, unlike sexual reproduction, which is limited to specific seasons [10]. The recent emergence of economical production methodologies for the replication and cultivation of soft corals in hatcheries marks a significant stride toward satisfying the demand within the ornamental organism market, while also playing a pivotal role in the restoration of degraded coral reefs affected by both natural occurrences and human disturbances [2]. This approach yields the swiftest and most efficient production of new coral colonies [11].

Soft coral communities and Sarcophyton coral are becoming the dominant organism on many reefs. Soft coral, specially *Sarcophyton* sp. were loved in marine aquariums due to its diverse colors and adaptable, so they have been exploited in large quantities, affecting the resources and balancing the coral reef ecosystem. There have been several studies on the *Sacorphyton* coral fragments such as the effects of temperature [12], light intensity [13, 14] and there is a lack of studies on the effect of the size of fragments to time of self-attachment, survival rate and specific growth rate. This study was carried out on *Sarcophyton* sp. corals to establish the scientific basis for cultivating *Sarcophyton* sp. corals in the circulating seawater system on the basis of assessment of the above indicators.

MATERIALS AND METHODS

Materials

Twenty colonies of *Sarcophyton* sp. with a mouth diameter of 10–15 cm were collected by SCUBA from Nha Trang Bay. At the Institute of Oceanography, the colonies were cleaned of all epibiotic organisms and placed

in a flow-through seawater system, use aeration 24/7. These colonies were then kept in aquaria for a period of 7 days under controlled conditions. The aquaria provided natural light, and no supplementary feeding was given to the corals during this time. To ensure optimal conditions for the corals, the water temperature was maintained at 26–28^{o}C using a water cooler, and the salinity was set between 29–30. The purpose of this controlled environment was to remove mucus from the corals and closely monitor their health during the observation period.

Defragmentation method

Only healthy parent colonies with no sign of stress (contraction of the polyp, bleaching or apparent disease infection) were used for the preparation of the cuttings [15, 16]. The perimeter of the polypary was removed using a scalpel (Figure 1a) and then thoroughly rinsed with filtered seawater for the removal of mucus. Then, five size: 0.5 × 0.5, 1.0 × 1.0, 1.5 × 1.5, 2.0× 2.0 and 2.5×2.5 cm cuttings were made using a sharp sterilized stainless scissors and immediately placed into porous plastic baskets with filtered seawater and gently aerated for approximately 24 hours before attachment to the substrata (Figure 1b). A total of 300 cuttings were made for the experimental setup. After cutting, the parent colonies were also maintained in 500 liters capacity tanks with a flow-through ambient seawater system for recording the healing time of the injury in the cutting areas of the parent colonies [12]. Fragmentations and the parent colonies were all soaked for 10 minutes in 5% Lugol solution, then the cuttings were stored in baskets with aeration for 24 hours before being fixed with wire to dead coral substrates, each cut piece and substrate are weighed separately before being fixed to each other, and these pieces of wire are removed after the coral attacks to the substrate by themselves, so it does not affect the mass of the coral at the next weighing. The parent colonies after cutting continued nourishment and monitoring of wound healing, recovery status and survival along with the subcolonies of the experiment. After 24 hours, the cut pieces are fixed on the substrate (death coral pieces) and immersed 2nd in 5% Lugon solution (5 minutes) for antiseptic (Figure 1c). The parent colonies after being fragmented are still kept and monitored regularly along with the experimental process.

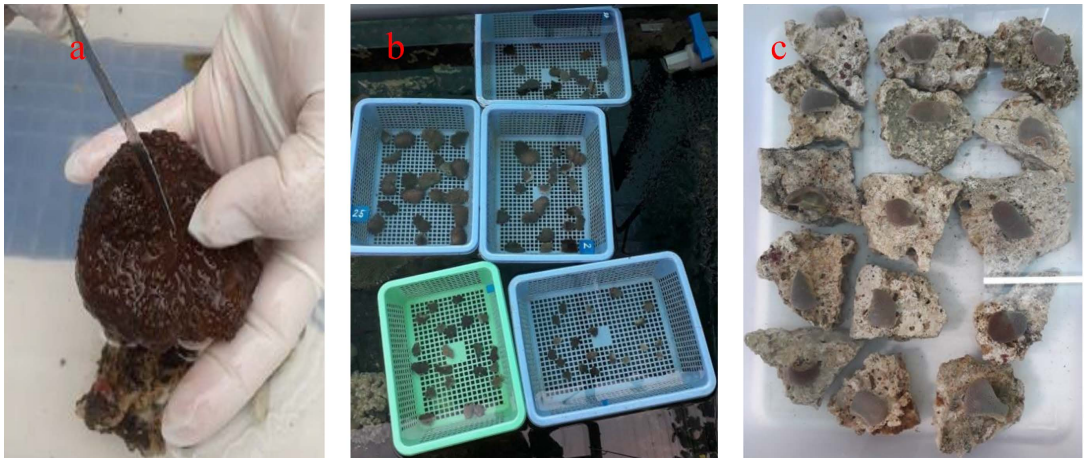

Figure 1. Method of fragmentation and fixation on the substrate: fragmentation (a), coral sizes (b) and fix on death coral pieces (c)

Experimental set-up

3 tank systems (length × width × height: 2.2 m × 0.6 m × 0.4 m) sharing the same circulating filtration system (filter volume 0.5 ton). The bottom of the tank consists of a layer of coral sand and gravel (size 0.3–1.5 cm) 8 cm thick as the substrate.

Experimental corals were placed outdoors with a tole to get light and cover the orchid net. Using natural seawater, natural light with intense of 400–500 µmol $m^{-2}s^{-1}$.

Sarcophyton sp. fragments pieces were fixed by wire on dead coral pieces and arranged completely randomly in a recirculation system with 5 different treatments of coral fragment sizes of 0.5 × 0.5 cm (T1), 1.0 × 1.0 cm (T2), 1.5 × 1.5 cm (T3), 2.0 × 2.0 cm (T4) and 2.5 × 2.5 cm (T5), each treatment was repeated 3 times, each experimental unit was 20 cuttings (total 300 pieces) and the experimental period was 3 months.

Care and monitoring

The entire experimental facility was with natural light. In this study, a recirculating seawater system (RSS) was used for growing out cuttings. Each experimental system had a completely randomized design with three replicate per treatment. Water exchanges of 10% of the volume of each system/day in the first week to remove all the mucus of corals secreted by the initial damage. The experiment was arranged in the condition of not adding food for corals, so the water was change 20%/time/week after that. The indicators of survival rate, time of attachment were observed and recorded daily. Oral disc diameter (ODD), peduncle disc diameter (PDD) and height of coral sub-colonies was determined once/month.

Data collection

Environmental figures: the temperature is maintained by water coolers, salinity: measured with a refractometer, light is measured in the water at the coral placement site using the MQ-200 (serial *4735).

Time of self-attachment: The self-attachment time was determined as the time needed for the cuttings that were permanently fixed over the substrata and they did not fall down from the substrata when flippedupside down. Coral self-attachment was observed the recovery of all 300 cutting pieces, daily monitoring of foot signs of each cut piece.

Survival rate was defined as the presence of the cutting completely attached to the substrata after the experiment had been started and the loss of a cutting that was detached from the substrata or partly decaying was defined as dead. The final survival was calculated at the end of the experiment.

$$SR(\%) = N_t / N_0 \times 100$$

in which: N_t is the number of colonies alive at the end of the experiment; N_0 is the number of initial cut corporations.

Oral disc diameter (ODD), pedal disc dimeter (PDD) and height of coral sub-colonies over time were determined with a caliper (1mm subdivision), measured directly in the water. The mass of corals was determined using an electronic balance (accuracy of 0.001 g) when taking them out of the water and drying the substrate in the same time. One day before weighing, the coral colonies were cleaned to remove all algae on the substrates and corals. The initially height of the coral colony was determined after 30 days because initially the surface of the fragments were fixed directly on the substrate, they did not appear (after about 18 days, the “peduncle” is formed and lengthened, raise the “disc” upwards and measured on days 30, 60, 90 days of the experiment. The growth rate in volume and height is determined: once per month. determined according to [17] (Figure 2).

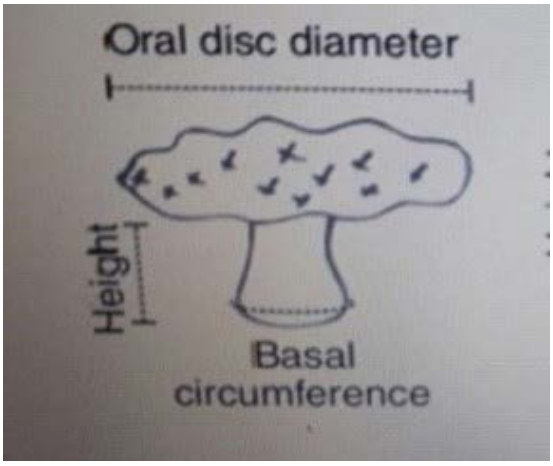

Figure 2. Determination of the size of *Sarcophyton* sp.

Coral fragments specific growth rate [16]:

$$SGR(\%/day) = 100 * \left(\ln\left(w_{t_i}\right) - \ln\left(w_{t_0}\right)\right) \Big/ \Delta t$$

in which: W_t and W_i are the mass or size (ODD/PDD) of the colony of baby reefs at time t_0 and t_i is the experimental time.

Data processing

Using one-way ANOVA analysis method on SPSS 18.0 software to compare significant differences in growth, survival, self-attachment time between species with 95% confidence. The metric is represented as an average ± standard error (SE).

RESULT AND DISCUSSION

Environment factors

The experiment was arranged in a covered system, using a water cooling system during the experiment. Environmental factors remained quite stable throughout the culture period (Table 1).

Table 1. Some environmental factors

Factor	Range
Temperature (^oC)	26–27
Salinity (psu)	32–33
Light (µmol $m^{-2}s^{-1}$)	400–500

Time of self-attachment of *Sarcophyton* sp.

During the experiments, the miniature cuttings of *Sarcophyton* sp. underwent regeneration and developed the morphology of naturally growing juvenile colonies, exhibiting a mushroom-shaped structure. The cuttings had already healed the wound area with pigmentation and new tissue had started to cover the wounded area within 3–5 days of the experiment. Approximately 3–4 days the cuttings started to attach to the substrate and the newly detached coral colonies began to form “stalks”. All the cuttings were completely covered with new polyps inside the wounded area and the permanent attachment of the cuttings to the artificial substrata occurred by days 9–11. There was no significant difference in the average time of self-attachment among the *Sarcophyton* sp. cuttings using the five different fragment sizes ($p > 0.05$). The average time of self-attachment was 10.02 ± 0.22 days in treatment 1, while it ranged from 9.84 to 9.59 days in treatments 2 to 4. Treatment 5 exhibited the fastest attachment time, with an average of 9.38 ± 0.13 days (Figure 3). The time of self-attachment in this experiment is similar to the 2021 results of the Marine Biology Technology Department when considering the same type of substrate as dead coral (9.1 ± 0.24 days, fastest is 8 days and slowest is 11 days). The average time of self-attachment of *Sarcophyton* sp. in these treatments were different from previously published results. Specifically, the time of attachment for *Sarcophyton glaucum* was reported to be 5-18 days [12]. Then, the average time of self-attachment of the cuttings using the impaling method (6.1 ± 0.1 days), the natural attachment method (7.6 ± 0.1 days) and the containing methods (7.2 ± 0.1 days), was significantly shorter than that of the tethering method (8.8 ± 0.1 days) and the adhering method (10.1 ± 0.1 days) [15].

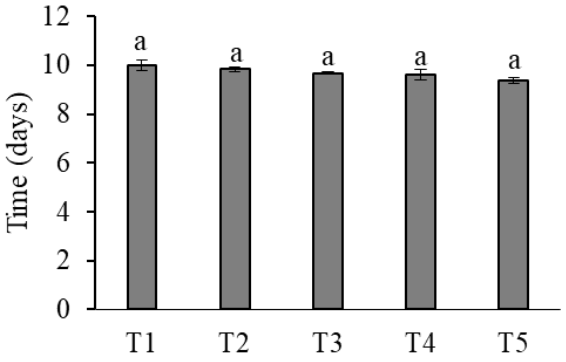

Figure 3. Time of self-attachment of *Sarcophyton* sp.

The rectangular cuttings gradually transformed into rounded shapes as natural attachment occurred. By days 30–37 the colonies had fully developed and exhibited the typical characteristics of *Sarcophyton* sp. The shape and orginal size of parent colonies were restored after approximately 90 days (Figure 4).

Furthermore, the monitoring resulte also indicated that the contact position of the cutting pieces with the substrate did not affect their attachment time.

Figure 4. Colony after cut (a) and recover after 90 days (b)

Survival rate

The experiment also recorded that the mortality of young coral colonies occurred only in 7–10 days and then remained stable throughout the experiment period. A coral transplant operation is considered successful if the total number of viable transplanted corals is more than 50% [18]. After 90 days of culture, the survival rate of corals in T1 and T2 was 80% and 78.33 ± 7.26%, while both T3 and T4 reached a survival rate of 96.67 ± 3.33%. Similarly, T5 also achieved a survival rate of 96.67 ± 3.33% (Figure 5). The results indicated that there was a statistically significant difference in survival between the small cut pieces (0.5–1 cm) and the larger cut pieces (ranging from 1.5 cm to 2.5 cm) ($p < 0.05$).

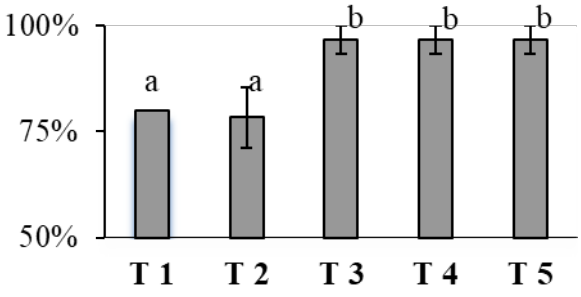

Figure 5. Survival rate *Sarcophyton* sp.

This result is also consistent with previous publications. For instance, the survival rate of *Sarcophyton glaucum* coral with a 36 mm^2 cut fragment separated from the broodstock and glued to porcelain substrate with the glue after 60 days was 88% ($p > 0.05$) [12]. It can even reach 100% [19]. However, when cut with a size of 0.5 × 0.5 cm, the survival rate can vary widely depending on different methods of fixation to the substrate, ranging from 26.6% to 93.3% [15]. On the other hand, the survival rate can reach 93–100% when considering the effects of different media (as reported by the Division of Aquaculture Technique in VNIO, 2021). Satisfactory results of 100% survival rate were also achieved after 3 months when the cuttings were 3 inches (7.62 cm) in size for *Sarcophyton* sp. [20].

Growth rate

There were no significant health status differences between methods. no significant difference in growth rate of oral disc diameter (ODD) and growth rate of pedal disc dimeter (PDD) of corals among the different treatments of cut size ($p > 0.05$) over the 90-day culture period. However, during the initial 30 days, the corals exhibited a faster growth rate in ODD compared to the growth rates at 60 days and

90 days. Overall, the size of the ODD in all treatments increased proportionally with the culture time. Regarding the growth rate in height, a statistical difference was observed between the coral treated with a size of 2.5 cm (Treatment 5) and the other treatments ($p < 0.05$). The height tended to be proportional to the fragment size, meaning larger cut sizes generally resulted in taller coral colonies (Table 2).

Table 2. The growth rate of *Sarcophyton* sp.

Factor	Days	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
ODD (%day^{-1})	30 days	0.252 ± 0.129^{a}	0.454 ± 0.155^{a}	0.571 ± 0.117^{a}	0.794 ± 0.321^{a}	0.875 ± 0.416^{a}
	60 days	0.360 ± 0.042^{a}	0.399 ± 0.210^{a}	0.359 ± 0.127^{a}	0.309 ± 0.082^{a}	0.520 ± 0.267^{a}
	90 days	0.398 ± 0.087^{a}	0.399 ± 0.106^{a}	0.405 ± 0.136^{a}	0.418 ± 0.149^{a}	0.521 ± 0.015^{a}
PDD (%day^{-1})	60 days	0.095 ± 0.028^{a}	0.149 ± 0.039^{a}	0.100 ± 0.027^{a}	0.186 ± 0.042^{a}	0.174 ± 0.010^{a}
	90 days	0.108 ± 0.025^{a}	0.126 ± 0.020^{a}	0.105 ± 0.019^{a}	0.178 ± 0.039^{a}	0.093 ± 0.029^{a}
Height (%day^{-1})	60 days	0.316 ± 0.003^{a}	0.320 ± 0.002^{a}	0.318 ± 0.004^{a}	0.326 ± 0.143^{a}	0.609 ± 0.002^{b}
	90 days	0.277 ± 0.118^{ab}	0.299 ± 0.006^{a}	0.295 ± 0.002^{a}	0.302 ± 0.006^{a}	0.397 ± 0.003^{b}
Weight (%day^{-1})	30 days	0.040 ± 0.004^{a}	0.025 ± 0.002^{b}	0.036 ± 0.004^{ab}	0.034 ± 0.001^{ab}	0.034 ± 0.001^{ab}
	60 days	0.016 ± 0.002^{ab}	0.019 ± 0.003^{a}	0.012 ± 0.002^{ab}	0.011 ± 0.001^{b}	0.010 ± 0.001^{b}
	90 days	0.010 ± 0.001^{a}	0.008 ± 0.002^{a}	0.007 ± 0.001^{a}	0.009 ± 0.001^{a}	0.007 ± 0.000^{a}

Note: the metric is presented as an average ±SE. Different exponential symbols in the same row indicate a statistically significant difference ($p < 0.05$).

The highest growth rate in weight was recorded in the group with the smallest initial fragment size (Treatments 1 and 2) followed by the coral group at Treatment 3 and lower at Treatments 4 and 5. Preliminarily it can be determined that the smaller the size, the faster their growth in weight. After 30 days of culture, growth rate in weight reaches 0.025–0.04% per day, at 60 days reaches 0.01–0.019% per day and after 90 days reaches 0.007–0.01% per day (Table 3). This result is quite low compared to previous published records, such as [13], which showed that the growth rate of *Sarcophyton glaucum* reached 0.040 ± 0.010% per day; 0.038 ± 0.007% per day and 0.035 ± 0.009% per day ($p > 0.05$). Meanwhile, other studies reported higher growth rates, ranging from 0.210 to 0.380% per day [21], 0.027–0.028% per day [22], 0.11–0.39% per day [19], or 0.055–0.380% per day [23]. Furthermore, *S. glaucum* was reported to achieve a growth rate of 0.025–1.828% per day [16].

CONCLUSIONS

The size of the initial fragment did not affect the time of attachment or the growth rate of PDD of *Sarcophyton* sp. under experimental conditions. However, it did have an impact on growth rate of height, survival rate, and mass growth of juvenile corals. The experimental results also indicate that the growth rate of corals slows down over time. This study indicated a fragment size of 1.5 cm suitable for *Sarcophyton* sp. in asexual reproduction by fragmentation at experimental conditions.

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Disclaimer: We hereby declare that these are the results obtained from the research conducted by the authors.

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