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Evaluating the survival and growth rate of *Acropora digitifera* in wild and mesocosm systems

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Abstract

The growing interest in coral culture for restoration and biotechnological applications has prompted researchers to improve their understanding of coral culture, with a focus on *ex-situ* production. This study aimed to understand the performance of common hard coral, *Acropora digitifera* by examining their survival and growth at Pulau Bidong (*in-situ*) and in a mesocosm system (*ex-situ*). First, three branches were tagged from each of eight *A. digitifera* colonies (n = 24). Mortality and linear extension rate were recorded monthly, from July - November 2018. Meanwhile, five branches from each tagged colony were brought back to a mesocosm set up at the hatchery in the Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu (UMT) for the *ex-situ* experiment. All coral nubbins (n=40) were then divided into two coral size groups: small (<5cm) and large (>5cm). After four months, small nubbins showed 100% survivorship, while large nubbins survived for only two months. In contrast, 67% of wild colonies remained alive. However, nubbins in mesocosm extent with almost 2-folds slower (0.091 ± 0.027 mm day^{-1}) than those in the wild (0.166 ± 0.033 mm day^{-1}). Coral nubbins in mesocosm form a basal self-attachment "disc" at the bottom. This suggests that fragmented corals invest more energy in self-stabilization, especially in the early stage of transplantation, which affects their linear growth. This study demonstrates the different demographic traits for corals in both the environments.

Keywords: *Acropora digitifera*, Growth rate, Survival rate, Mesocosm, Pulau Bidong

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Introduction

Coral reef ecosystems are highly valuable with diverse marine flora and fauna underneath the sea, providing biological and ecological benefits to their surroundings. Unfortunately, coral reefs in many parts of the world are declining rapidly (Bruno and Selig, 2007; Burke *et al.* 2011). This degradation resulted from a combination of both natural (Tan et al., 2018) and anthropogenic causes, such as climate change (Munday et al., 2008; Ateweberhan et al., 2013), pollution (Feary et al., 2012; Riegl and Purkis, 2012), sedimentation (Fabricius et al., 2005; Wooldridge, 2009), destructive fishing (Hughes et al., 2007; Caras and Pasternak, 2009), coral mining (Caras and Pasternak, 2009), and exploitation for aquarium trade (Wabnitz et al., 2003; Knittweis et al., 2009). For all those reasons, reefs lose their function and structural complexity, which are crucial for reef growth, fishing habitat, coastal protection, and overall reef biodiversity (Bruckner, 2002; Alvarez-Filip et al., 2009). In Malaysia, coral reef health is continuing to decline from 48.11% live coral cover in 2014 to 40.63% in 2019 (Reef Check, 2019). This has motivated an active intervention of coral restoration (Rinkevich, 2005; Precht, 2006; Shafir et al., 2006; Edwards and Gomez, 2007).

Over the past decade, active restoration to mitigate decline in coral cover has increased worldwide. Coral propagation for restoration is considered an essential component of coral conservation and management plans (Rinkevich, 2005; Precht, 2006; Edwards and Gomez, 2007; Lirman and Schopmeyer, 2016). Coral restoration by transplanting back the corals is seen to produce rapid coral cover. However, considerable research is needed to assess the effectiveness of different methods for coral transplantation as a viable reef restoration tool. One of the most successful ways is the "coral gardening" method, adopted from the silviculture of terrestrial ecosystems (Rinkevich, 1995, 2000). Corals were collected from healthy donor reefs and cultivated in 'nurseries' until they reached a suitable size before being transplanted back onto the targeted reef (Yeemin et al., 2006; Garrison and Ward, 2008; Rinkevich, 2008; Chou et al., 2009; Forrester et al., 2011; Ammar et al., 2013). This approach aims to improve post-transplantation survivorship via the use of either *ex-situ* (aquarium) or *in-situ* (sea-based) nurseries (Rinkevich, 2005).

Ex-situ nurseries, which are based on land, provide a "preliminary foster period" for only a short period of time (Epstein et al., 2003). Then, after all the coral fragments have achieved a favourable size, they are relocated to *in-situ* nurseries or directly to the actual targeted transplant area. Parameters indicative of coral health such as survivorship, growth, self-attachment times and bleaching rates, which are commonly monitored in *in-situ* nurseries (Becker & Mueller, 2001; Shaish et al., 2008; Guest et al., 2011) are very useful and pertinent when managing *ex-situ* nurseries. However, such data is scarce and typically anecdotal, and lacks scientific scrutiny for *ex-situ* coral rearing (Arvedlund et al., 2003; Olivotto et al., 2011). This is because most knowledge on coral culture practices and husbandry is present in grey literature in the aquarium hobbyist's forum (Leal et al., 2016). Expanding the interaction between marine aquarium hobbyists and coral aquaculture scientists may contribute to improving and validating the current knowledge on coral aquaculture (Leal et al., 2016; van Os *et al.,* 2012). Moreover, many coral species are yet to be investigated for culture optimisation, and new combination of culture parameters still need to be verified (Arvedlund *et al.* 2003).

Coral growth rate was suggested as a standard ecological tool to determine the growth tolerances of reef-building organisms (Shinn, 1966). Quantitative studies on coral growth are scarce in Malaysia, particularly on the common *Acropora* species. Among the various species, acroporid corals are keystone species (Carpenter et al., 2008), which makes them appealing for restoration efforts. On the other hand, branching *Acropora* species are known as the hardiest types to grow in mesocosm tanks as they are sensitive to changes (Jimenez et al., 2001 and Mc Clanahan et al., 2001). Hence, this study aimed to evaluate the performance in terms of survival and growth rate of *Acropora digitifera* nubbins transplanted in *ex-situ,* with donor colonies in the wild. In addition, the size variation between coral nubbins (small; <5cm and large; >5cm length) was also examined. Large fragments might have higher survival (Bowden-Kerby, 2001; Okubo et al., 2005). Small coral fragments, however, require less initial source material (i.e., less damage to donor colonies) and ,therefore, are more desirable, providing the fragments survive well. Thus, by carrying out this pilot study of branching *Acropora* coral growth in a mesocosm system, a better understanding of their growth dynamics is likely to be achieved.

Materials and Methods

Description of the study site

This study was conducted at Pantai Pasir Cina, Pulau Bidong, Terengganu. Pulau Bidong is located about 18 nautical miles from the mainland, to the northwest of Kuala Terengganu, East Coast Peninsular Malaysia (Figure 1). It is an uninhabited island, with three sandy beaches (Pantai Pasir Cina, Pantai Pasir Pengkalan and Pantai Pasir Tenggara). Coral reefs at Pantai Pasir Cina were dominated by fast growing corymbose, *Acropora digitifera* (Dana, 1846) ranging from 2m to 6m depth.

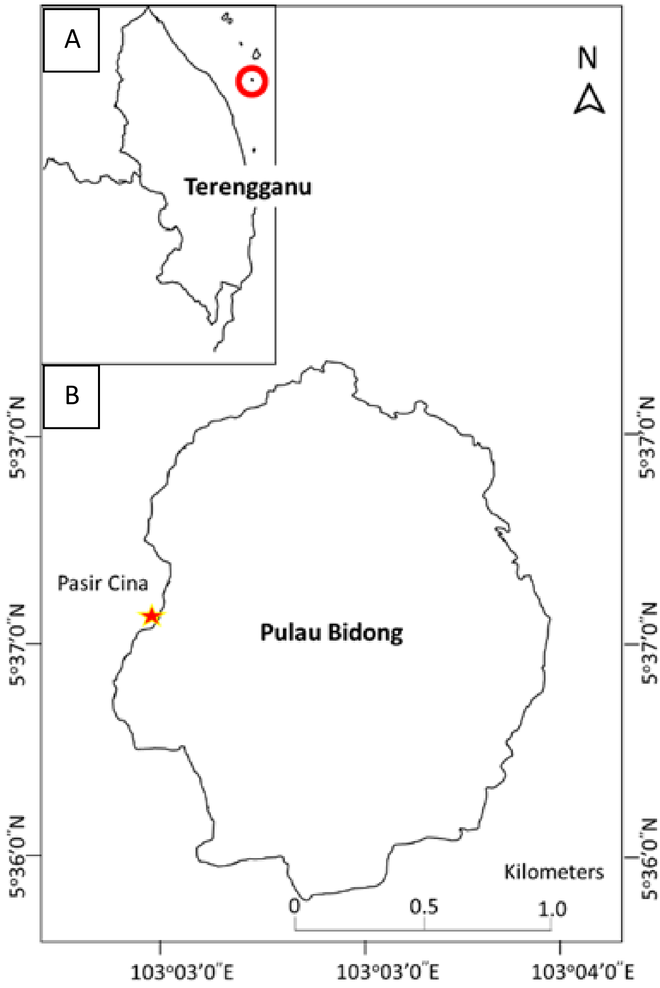


Figure 1. (A) Location of Pulau Bidong Terengganu, Malaysia (in red circle). **(B)** Location of Pantai Pasir Cina.

In-situ sampling

To examine the growth, eight healthy appearing (without nany lesion) *A. digitifera* colonies were randomly selected as donor colonies and tagged using Allflex Lazatag (Figure 2A). Then, three branches from each selected colony were tied with different colours (blue, red, and yellow) cable ties (Figure 2B) to serve as a baseline to measure the coral linear growth rate (Lirman et al., 2010b). To measure growth, pictures of the cable tied branches (total n = 24) were taken using an Olympus TG4 camera with underwater casing. A ruler was placed next to the branches as a scale bar when the picture was taken. Monthly sampling was conducted from July to November.

Ex-situ data collection

From each of the tagged *A. digitifera* colonies, five coral nubbins (n=40) were detached and brought to the hatchery at the Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu, for *ex-situ* experiment. The coral nubbins were sampled according to two size groups (small: <5cm and large: >5cm length) to explore the size variation in linear extension growth rate. Coral nubbins were carefully fragmented with a screwdriver and placed in individual zip-lock bags filled with sea water. Then, these coral nubbins were transported to the AKUATROP hatchery in a water-filled ice chest container with an ice pack. Upon arrival, all coral nubbins were attached to cement base frag plug (Figure 2C).

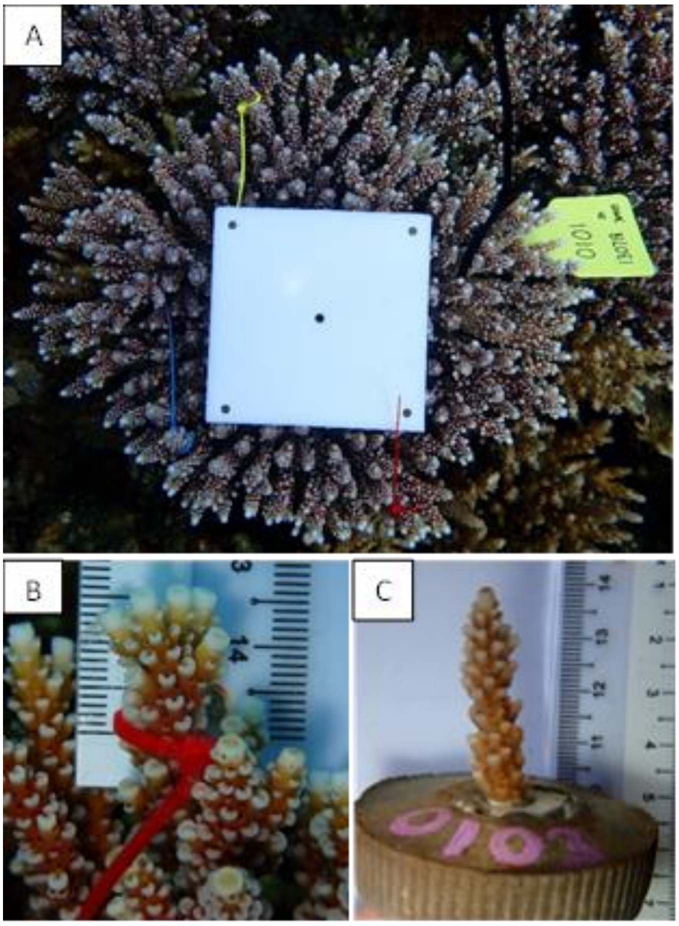


Figure 2. (A). Mature donor coral colony in Pulau Bidong tagged with Allflex Lazatag for monthly monitoring. **(B).** Close-up of coral branches with a red cable tie. **(C).** *Ex-situ* coral nubbin is attached to cement base plug.

The coral nubbins were first placed in a quarantine tank for two weeks for acclimatization. Transfer of wild-caught corals to aquaria can lead to an initial decline in coral health, often expressed as excess mucus secretion, the onset of bacterial infection, and light sensitivity (Calfo, 2001). Generally, any unhealthy looking corals are to be removed prior to the experiment (Sabater and Yap, 2004). However, in this study, none of the coral fragments showed any signs of stress during the acclimatization period. After the acclimatization period, the coral nubbins (n=40) were divided equally into two size groups (small and large) and transferred to the 1ft x 2ft experimental tank. Similar to the *in-situ* experiment at Pulau Bidong, monthly pictures of the coral nubbins were taken using an Olympus TG4 camera with underwater casing, and a ruler was placed next to the branches as scale bar when the picture was recorded.

Experimental tank water quality maintenance

In the experimental tank, nine water parameters were monitored weekly using Salifert Profi Test to ensure that the water conditions were stable (Table 1). The water parameters were maintained within these ranges according to Arvedlund et al. (2003). Temperature and salinity in the tanks were maintained at an averaged 27-28°C and 32-33 ppt, respectively. The photoperiod was set up with 12-hour light: 12-hour dark (i.e., 7 a.m. to 7 p.m.) using AI Hydra light with an average of 350-370 µmol $m^{-2}s^{-1}$. Coral nubbins were fed twice a week with newly hatched artemia (~1g) and Reef Roid coral food. Partial water renewal (10% of total water volume) using filtered seawater were performed weekly.

Table 1. Water parameters in the *in-situ* and *ex-situ* nursery tanks.

Parameter	**Unit**	**Average value**	
		***In-situ* (wild)**	***Ex-situ***
pH		8.0	8.4
Alkalinity	dKH	6.7-7.0	9
Calcium (Cal)	mg L^{-1}	400-420	440
Magnesium (Mg)	mg L^{-1}	1150-1200	1330
Ammonia (NH_3)	mg L^{-1}	0	0 – 0.05
Nitrate (NO_3)	mg L^{-1}	0	0 – 0.05
Phosphate (PO_4)	mg L^{-1}	0	0 – 0.05
Salinity	ppt	34-35	32-33
Temperature	°C	28-30	27-28

Coral growth measurement

Back into the laboratory, the photo captured length of coral nubbins (from wild and *ex-situ*) was analysed using ImageJ Image Analysis Software version 1.52a (https://imagej.nih.gov/ij/download.html). For *in-situ* coral nubbins the length was measured from the cable tie up to the axial polyp (Okubo et al., 2005; Johnson et al., 2011). For *ex-situ* nubbins, measurement started from the bottom encrusted part up to the axial polyp of the branch (Ferse and Kunzmann, 2009; Gomez et al., 2014). Along with this experiment, a survival rate was also recorded. Corals that showed 80-90% tissue loss were considered dead and excluded from the experiment.

Statistical analysis

The normality of distribution was confirmed by using Shapiro-Wilk test, and followed by Lavene`s homogeneity test. Due to survival rates affecting the sample sizes, the growth rate data was unequal (unequal variance) until the end of the study. Since the data violates the assumptions of homogeneity of variances, Welch’s ANOVA test was done to compare two mean groups. The significance of differences was defined at $p < 0.005$. Statistical analyses were performed using IBM SPSS Statistic software version 20 and data were reported as mean±standard error of the mean (SE).

Results

Survival rate

Overall, coral survival in the mesocosm system was higher than tagged coral colonies in the wild. Mortality recorded for wild colonies was slightly low throughout the months with 67% surviving by the end of this study (Figure 3). The dead specimens of tagged corals were observed either missing from the study site due to strong currents or found to have with algae growing on them. In comparison, survivorship for coral nubbins in mesocosm varied between sizes. At the end of the experiment all small (<5cm) coral nubbins survived, while large (>5cm) nubbins recorded 100% mortality during the third month (Figure 3). Large *ex-situ* coral nubbins showed a drop in survivorship from 85% in the second month to zero in the third month. These nubbins had tissue lesions and showed signs of bleaching before they died.

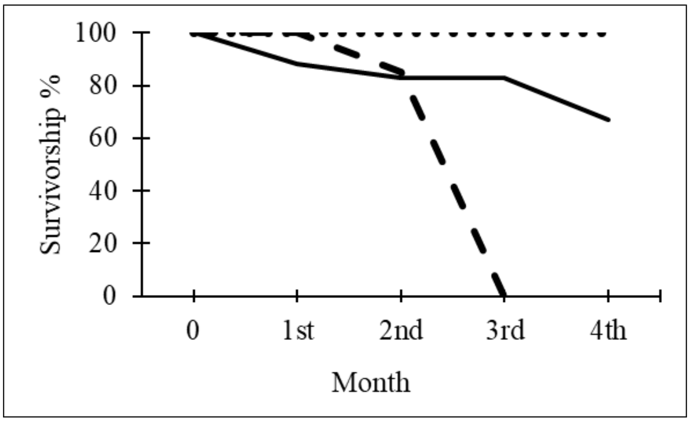


Figure 3. Survivorship of *A. digitifera* nubbins over four months. Solid line represents *in-situ* branches (n=24), dotted line represents small *ex-situ* nubbins (n=20), and dashed line represents large *ex-situ* nubbins (n=20).

Growth variation

On the other hand, while having higher survivorship in the mesocosm system, coral nubbins turn out to have growth of almost 2-times slower than those in the wild. The average linear extension rate of *A. digitifera* nubbins measured in mesocosm was 0.091 ± 0.027 mm day^{-1}. The growth rate varied throughout the study (Figure 5B) and was not reaching its full potential when compared to the wild. Then, the growth rate dropped significantly on the fourth sampling interval with a negative reading recorded (-0.020 ± 0.011mm day^{-1}, mean + SEM). This data was assumed to be negative due to no growth or very minimal growth. In addition, this study also recorded the extra tissue developing around the base of coral nubbins in the mesocosm system (Figure 4).

For the coral nubbins at Pulau Bidong, the average extension rate was 0.166 ± 0.033 mm day^{-1} (mean + SEM) in all sampling intervals. But in the 2nd sampling interval, the coral grew highest (0.239 ± 0.036 mm day^{-1}). Overall, their growth was consistent throughout the study period (Figure 5A).

According to the Shapiro-Wilk test, all the data were normally distributed for *in-situ* (W (68) = 0.985, p = 0.565) and *ex-situ* (W (65) = 0.981, p = 0.411). However, according to Lavene`s test of homogeneity, the variance between the data was unequal (p=0.01) due to survivorship of coral nubbins. Thus, from Welch's ANOVA test, there were statistically significant differences between both *in-situ* and *ex-situ* (p=0.000). Negative values of more than -0.05 mm day^{-1} were excluded from the sample pool to obtain statistical accuracy.

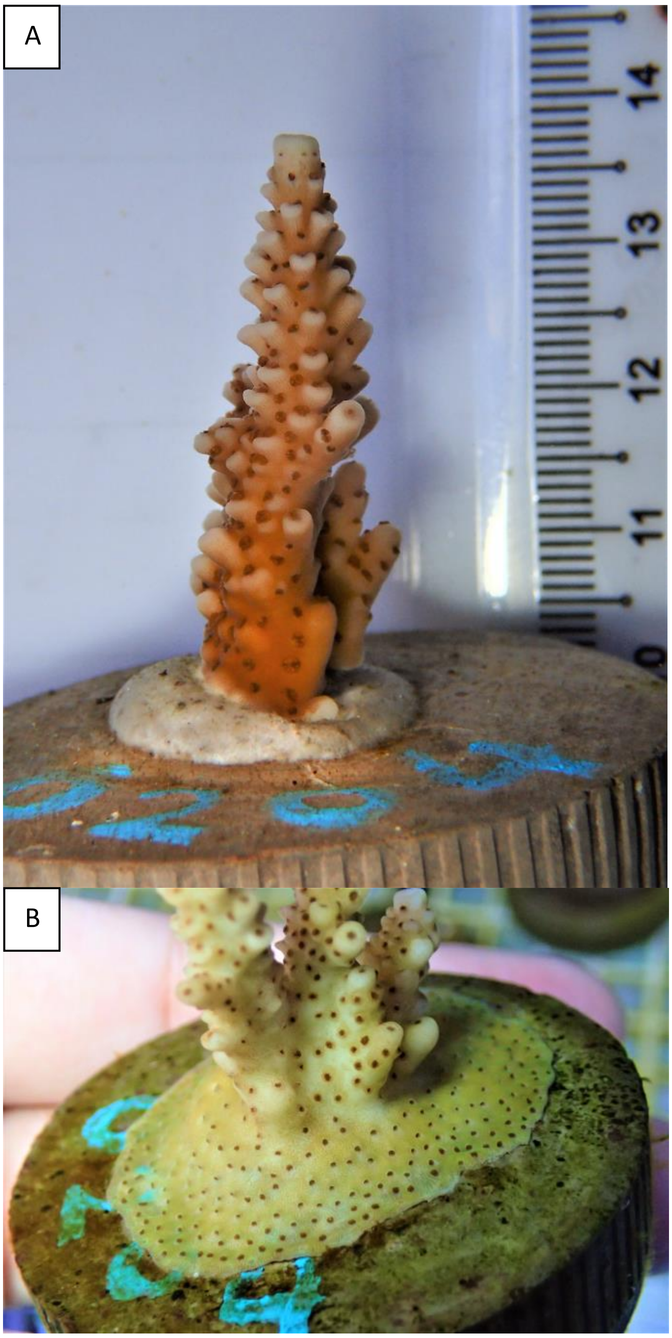
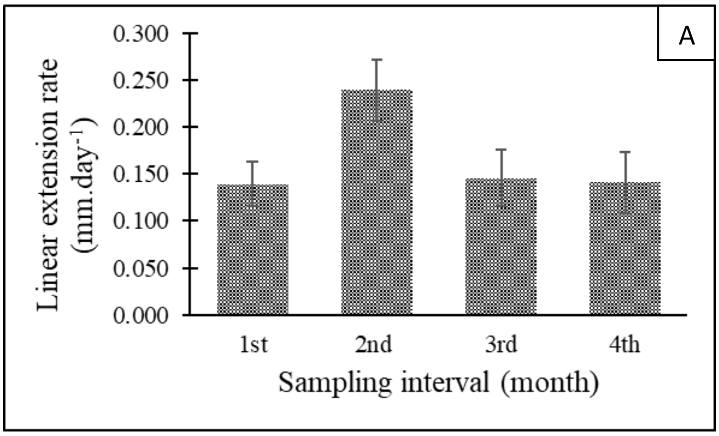


Figure 4. Coral nubbins in *ex-situ* tanks in **(A)** the first month and **(B)** after the fourth month.



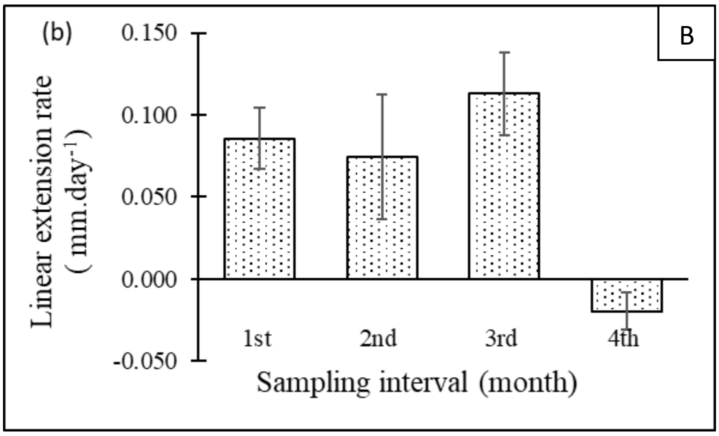


Figure 5. Mean growth rate of *A. digitifera* at **(A)** Pulau Bidong (n=24) and **(B)** mesocosm (n=20) system in AKUATROP hatchery. Error bars represent standard error.

Discussion

Survival rate

In this study, small nubbins (<5cm) had higher survivorship compared to large nubbins (>5cm). Unavoidable handling procedures during fragmentation and retransplant caused tissue damage to the nubbins which affected the survivorship (Lirman et al., 2010b). This observation was also reported by Ng et al. (2012) where *A. digitifera* nubbins experienced tissue loss that started at the base and progressed to the upper tips rapidly. However, in this study, only large nubbins experienced those situations with high mortality. This could be because the large nubbins did not recover as effectively from tissue lesions compared to small nubbins. The active growth tissue area of smaller nubbins suggests the faster ability to recover (Rogers et al., 1982). The smaller size could be more resilient to the changing environment and have a higher recovery rate due to the efficiency in energy sharing among adjunction of coral polyps (Allen and Steene, 1994). Likewise, tissue regeneration ability also varies among corals (Bak et al., 1981) or intra- and inter-colony variation in stress handling (Lirman et al., 2010a). Thus, size selection does play an important role in post transplantation survivorship.

Higher survivorship in *ex-situ* than *in-situ* further indicates that it is feasible to asexually propagate coral nubbins and grow them in *ex-situ*. Their treatment in a stable, controlled environment aids in improving the survivorship of corals. Moreover, there are fewer threats in the controlled environment of compared to the wild. Threats include corallivorous predator grazing, high light intensity and temperature fluctuations (Loke et al., 2016). Similar high survival rates (above 60%, Figure 3) were achieved for *ex-situ* coral colonies reported in short (<3months) and long (>6 months) term studies (Forsman et al., 2006; Shafir et al., 2010). Moreover, mesocosm systems allow closer monitoring of the responses of the organisms (Yap and Molina, 2003) as it is not possible to achieve in the wild environment.

Growth rate

Typically, growth rates for *Acropora* species are highly variable (Epstein et al., 2001). The linear extension rate of *A. digitifera* in Pulau Bidong was found to be 1.5 to 2 times higher (Figure 5(A) than the growth rate of the wild *A. digitifera* (34.7 to 42.2 mm/year) population in Maldives, Indian Ocean (Morgan and Kench, 2012). This indicated that *A. digitifera* at Pulau Bidong was in healthy condition. Other studies reported that the growth rate of *Acropora* could range between 30 mm/year and 200 mm/year (Wabnitz et al., 2003; Lesser, 2004), with the fastest growth reported to be 500 mm/year for *Acropora cervicornis* (Griffin et al., 2012). In the wild, the growth of coral is known to be strongly affected by environmental conditions (Lough and Barnes, 2000). A variety of abiotic and biotic factors may influence individual coral growth (Pratchett et al., 2015). Specifically, seawater temperature factor would have a significant influence on the growth performance of coral (Anderson et al., 2017). The seawater temperature profile in this study site (Pantai Pasir Cina, Pulau Bidong) showed minimal thermal stress (annual mean = 29.4 ± 0.898 °C) during the study period (July to November) (Tan & Kamarudin, 2018). The stable seawater temperature could be the reason for continuous growth of corals in this study. Thus, possibly optimum stable temperature is one of the important parameters for governing optimum coral growth in an *in-situ* environment.

On the other hand, slow growth in mesocosm system (Figure 5B) showed that corals grew significantly slower in the initial phase of transplant (Lirman et al., 2010b, Hernández-Delgado et al., 2014, Lohr and Patterson, 2017). The study reported that the slower rates of linear extension were only temporary due to a shift of energy toward recovery and possibly due to stress in adapting to a new environment (Edward and Clark, 1999). As the fragmented coral nubbins were transferred from the wild into a mesocosm environment, corals need more energy to adjust their metabolic activity to adapt to new surroundings. Therefore, this is why newly fragmented nubbins in the mesocosm tank was observed to grow at a slower pace compared to those in the wild. A study by Epstein et al. (2001) also found that isolated nubbins grew up to 10 times slower than whole colonies.

Besides, corals also invest energy in extending extra tissue at the base (see Figure 4). This tissue is named "basal-attachment disc" for self-attachment, which provides stability to the newly transplanted coral nubbins (Guest et al., 2011). According to Stearns (1989), these life history strategies for better survival are related to trade-offs of energy for maintenance. Therefore, instead of continuing growing linearly upwards with new and secondary fillings of calcium carbonate, corals use their energy to focus on growing tissue at the base and for maintenance from fragmentation. In the wild, it is an important strategy for their survival, especially to avoid further abrasion (from rolling on the substrate) of their tissue, which can lead to fatality. This is consistent with a previous study reported by Lirman et al. (2014) where they recorded that colonies get thicker at the base as they grow with a reduction in growth.

Conclusion

Growth and survival rates are two important physical indicators of a healthy reef. The survivability of either donor colonies in the wild or fragmented nubbins in the mesocosm system is highly affected by their life history strategy. In the early stages the newly transplanted corals are prone to have higher mortality and slow growth. This is because coral needs extra energy to repair tissues and to adapt to a new environment. However, *ex-situ* condition provides a stable environment with a lower number of threats compared to the wild. Furthermore, smaller (<5cm) nubbins are recommended for coral transplant as they are better in recovery. Thus, selection of a suitable environment and coral fragment sizes are necessary for better survivability.

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