

Re-fragmentation of the Coral Echinopora lamellosa (Esper 1795) for Mariculture

Chou LM, National University of Singapore, Singapore
Toh TC, National University of Singapore, Singapore
Kikuzawa YP, National University of Singapore, Singapore
Loke HX, National University of Singapore, Singapore
Ng CSL, National University of Singapore, Singapore
Sam SQ, National University of Singapore, Singapore
Afiq-Rosli L, National University of Singapore, Singapore
Toh KB, National University of Singapore, Singapore
Taira D, National University of Singapore, Singapore
Poquita-Du RC, National University of Singapore, Singapore

The Asian Conference on Sustainability, Energy and the Environment 2019
Official Conference Proceedings

Abstract

Repeated fragmentation of corals can increase the source material to re-stock a coral nursery and reduce dependence on wild stocks. However, the efficacy of this approach to generate coral cover remains limited. Live tissue growth and dead area of *Echinopora lamellosa* fragments and re-fragments were measured at an *in situ* nursery over one year. Overall, re-fragmented *E. lamellosa* generated less live tissue area (n = 10, 116.1 ± 114.1 cm²) than control fragments that were left intact (n = 20, 200.3 ± 130.9 cm²). Re-fragmenting corals at sixth month of nursery rearing resulted in 23% loss of coral tissue and it required almost four months to attain the size before re-fragmentation. The increase in initial dead area significantly reduced coral growth in both treatments. The results for this species demonstrated that while re-fragmentation can increase the number of coral fragments, it is negated by the reduction in overall growth.

Keywords: Coral propagation, Ornamental trade, Reef restoration, Coral nurseries, Singapore.

iafor

The International Academic Forum

www.iafor.org

Introduction

The trade of live corals increased tenfold to approximately 650 tonnes from 1985 to 1997, comprising more than half of the global coral trade in 1997 (Green & Shirley, 1999). The rising popularity of the live coral trade has fuelled concerns that unregulated harvesting could pose irreversible damage to coral reefs (Smith & Hughes, 1999; Bruckner, 2000). However, recent advancements in coral culture and husbandry have helped to reduce an over-reliance on harvesting from the wild (Pomeroy et al. 2006). Coral mariculture, comprising sexual and asexual propagation methods, is increasingly carried out to supplement efforts to rehabilitate degraded reefs (Epstein et al. 2003; Toh et al. 2014; Ng et al. 2016) and enhance the ecological value of man-made structures in the marine environment (Ng et al. 2015; Toh et al. 2017).

Asexual propagation involves excising fragments off parent coral colonies, and rearing them in nurseries to obtain the desired quantity of coral materials (Bowden-Kerby, 2001; Ng et al. 2012). Compared to sexual propagation, this approach is widely adopted due to its low cost and relative ease of use (Rinkevich 2005). However, the availability of healthy donor colonies may be limited in degraded reef habitats (Edwards & Clark, 1999; Bruno & Selig, 2007; Lirman et al. 2010). Additionally, coral fragmentation causes tissue lesions along the fractured edges of parent colonies, rendering them more susceptible to colonisation by fouling organisms and infections from pathogens (Cumming, 2002; Titlyanov et al. 2005; Casey et al. 2015). This in turn creates dead areas on the coral fragments that hinders coral growth (Osinga et al. 2011; Leal et al. 2014).

To reduce the impacts on wild colonies, corals that are already being reared in nurseries could be re-fragmented for further cultivation and re-stocking. This strategy was useful in increasing the production of *Acropora palmata* (Forrester et al. 2013). However, the viability of this approach has not been extensively tested for other species. A one-year investigation at an *in situ* nursery was conducted to determine the feasibility of re-fragmenting nursery-grown *Echinopora lamellosa* to generate new coral propagules. The objectives were to (1) examine the influence of initial live tissue area and dead area on the growth of the nursery-reared corals and (2) compare the growth of live tissue between nursery-grown corals that had been re-fragmented in the sixth month of nursery-rearing with those that remained intact.

Materials and Methods

Study species and nursery rearing

Echinopora lamellosa (Esper, 1795) is a foliose scleractinian widely distributed across Indo-Pacific reefs (Veron, 2000) and it can dominate shallow reef areas by forming large assemblages (Sheppard, 1980; Dai, 1993; Veron, 2000). Fragments of *E. lamellosa* that had been transplanted grew rapidly despite the stresses arising from fragmentation and transplantation (Dizon & Yap, 2006; Shaish et al. 2008), suggesting the suitability of this species as a candidate for examining the effects of repeated fragmentation.

Ten *E. lamellosa* colonies (25–50 cm in diameter) were collected in August 2014 from a reef fringing Sultan Shoal (an offshore island southwest of Singapore;

1°14'22.86"N, 103°38'59.1"E) and transferred to an *in situ* coral nursery established off Lazarus Island (1°13'41.76"N, 103°51'19.82"E). The colonies were fragmented with hammer and chisel into 48 pieces of similar sizes ($68.8 \pm 19.7 \text{ cm}^2$). The fragments were then secured to six PVC-mesh fixed nursery tables (50 cm × 50 cm and elevated 40 cm above bottom substrate) that were deployed at 4–5 m depth of the reef. All fragments were placed at least 5 cm apart to minimise competition and overgrowth (Edwards & Gomez, 2007; Shafir et al. 2010). These fragments were reared for seven months from August 2014 (referred to as 'Phase 1').

Re-fragmentation of coral fragments

At the end of Phase 1 in March 2015, 30 fragments were randomly selected for the re-fragmentation experiment. Ten were re-fragmented into 20 halves ($59.1 \pm 24.6 \text{ cm}^2$; mean diameter \pm s.d.) while the other 20 fragments were left intact as the control group ($155.0 \pm 38.8 \text{ cm}^2$; mean diameter \pm s.d.) (referred to as 'Phase 2'). The 20 re-fragmented and 20 control fragments were reattached to 10 nursery tables and cultivated for six months (referred to as 'Phase 3'). The nursery tables were regularly maintained to remove accumulated sediment, fouling organisms (e.g. barnacles, sponges, tunicates, macroalgae) and corallivorous snails (*Drupella* spp.) throughout the study.

Data analysis

All fragments were monitored monthly throughout the study and *in situ* photographs were taken directly above the corals together with a scale bar. The live-tissue area and dead area of each coral were measured using the software ImageJ (Schneider et al. 2012).

Coral growth was defined as the change in live tissue area for each fragment relative to the initial live tissue area at the start of each monitoring period: 'Phase 1' (before re-fragmentation), 'Phase 2' (re-fragmentation), 'Phase 3' (after re-fragmentation), and 'Overall' (change in live tissue area over the entire duration of the study). Adapting the methods from Forrester et al. (2013) and Lohr et al. (2015), net coral growth was defined as the combined live tissue area of each pair of re-fragmented corals originating from the same colony ($n = 10$). The same calculation was done for the changes in dead area.

To investigate the influence of initial live tissue area and dead area on coral growth in Phase 1, the data were first tested for normality using Shapiro-Wilk test and homogeneity of variances using Levene's test, followed by linear regression. To examine the differences in live tissue area and dead area at each phase between treatments, data were tested for normality and homogeneity of variances. Subsequently, student *t*-tests were performed accordingly to test for significant differences in live tissue area and dead area between re-fragmented and control coral fragments. All statistical analyses were performed using SPSS version 17.0.

Results

Live tissue area

In Phase 1, growth was similar between corals in the re-fragmented and control groups ($p > 0.05$; Table 1), with corals in the former group growing from $64.4 \pm 16.4 \text{ cm}^2$ to $135.1 \pm 43.7 \text{ cm}^2$ (mean \pm SD) and those in the latter group growing from 70.6

$\pm 20.9 \text{ cm}^2$ to $150.5 \pm 38.3 \text{ cm}^2$ (Figure 1a). The initial live tissue area was not a significant predictor of the change in live tissue area (Linear regression, $F = 0.025$, $p > 0.05$, $R^2 = 0.001$, Figure 2a).

Table 1. Change in mean live tissue area (LTA) and dead area (DA) of *Echinopora lamellosa* fragments in the re-fragmented treatment and control group. Standard deviations (SD) are shown in parentheses.

Treatment	n	Phase 1		Phase 2		Phase 3		Overall	
		LTA (cm ²)	DA (cm ²)	LTA (cm ²)	DA (cm ²)	LTA (cm ²)	DA (cm ²)	LTA (cm ²)	DA (cm ²)
Re-fragmented	10	70.7 (36.8)	15.0 (11.0)	-16.9 (27.5)	3.60 (5.17)	62.3 (99.4)	61.1 (31.9)	116.1 (114.1)	79.7 (35.4)
Control	20	79.9 (27.0)	16.1 (14.2)	4.45 (16.3)	1.77 (4.78)	115.9 (111.5)	47.2 (34.5)	200.3 (130.9)	65.1 (46.8)
<i>t</i> -value		-0.780	-0.22	-2.12	0.966	-1.29	1.06	-1.73	0.867
<i>p</i> -value		0.442	0.830	0.048*	0.342	0.209	0.297	0.0950	0.393

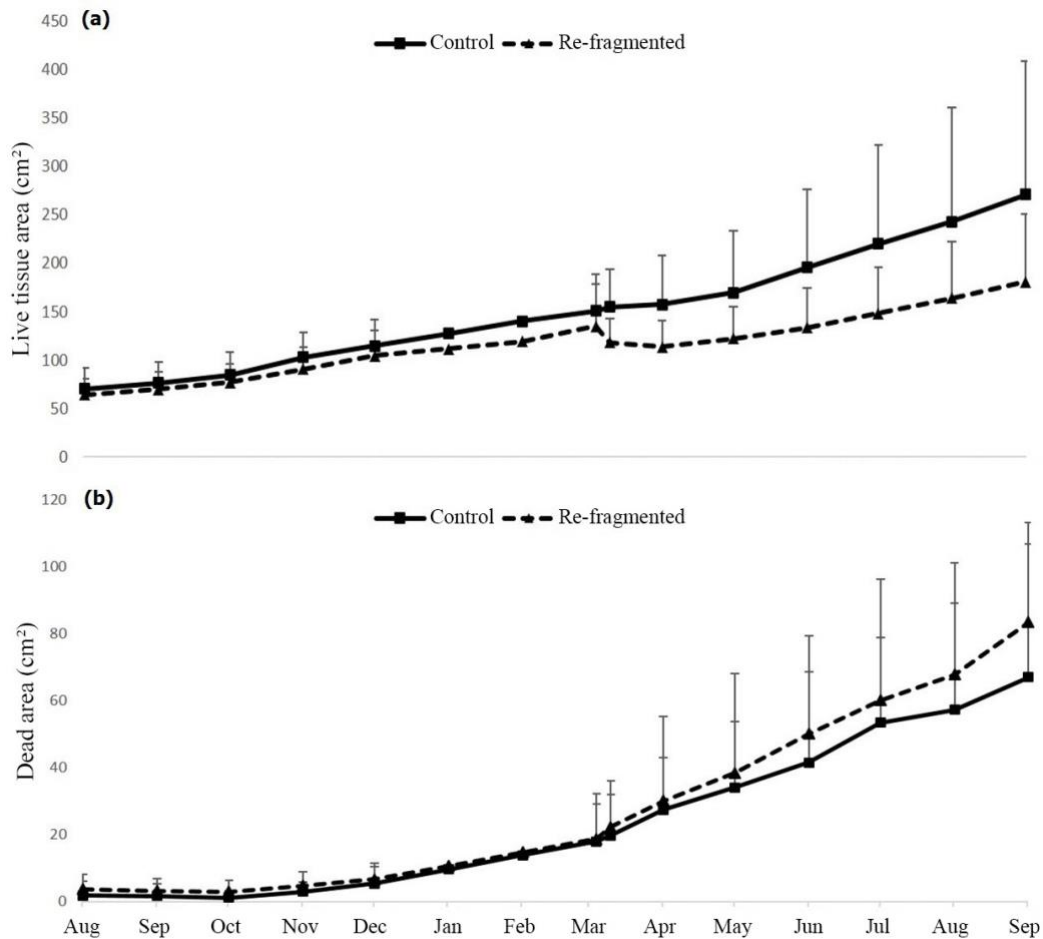


Figure 1. a) Mean live tissue area and b) dead area of *Echinopora lamellosa* fragments in the re-fragmented treatment ($n = 10$) and control group ($n = 20$) throughout the study (\pm SD). Re-fragmentation was carried out in March 2015.

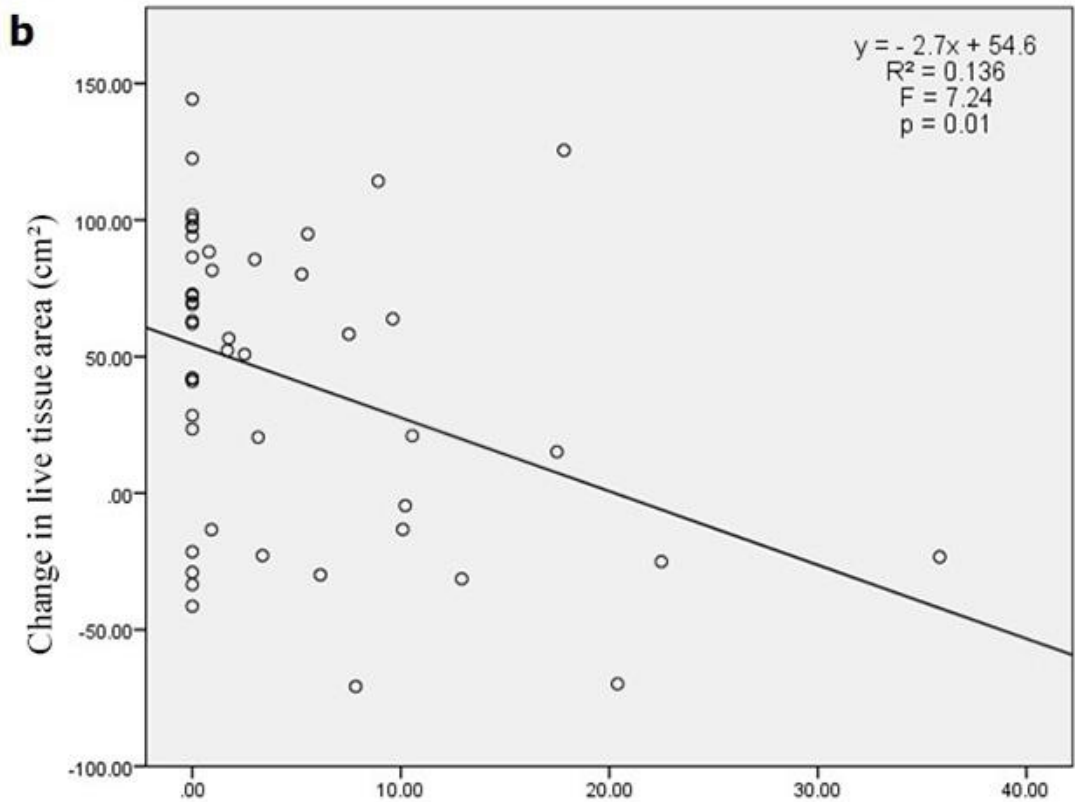
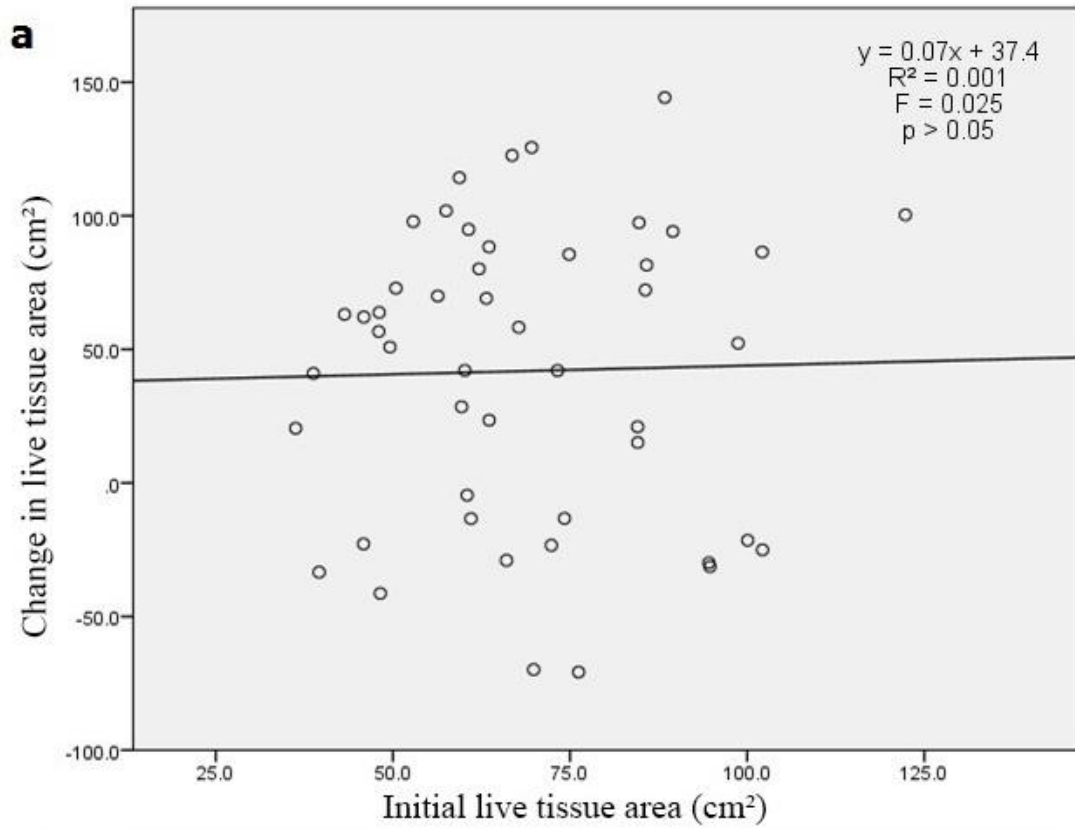


Figure 2. Linear regression predicting the change in live tissue area of *Echinopora lamellosa* fragments in Phase 1 using (a) initial live tissue area and (b) initial dead area (n = 48).

In Phase 2, corals in the re-fragmented group had significantly lower combined live tissue area than those in the control group (Table 1; $p < 0.05$). It took approximately four months for corals in the re-fragmented group ($118.2 \pm 41.3 \text{ cm}^2$) to attain a combined live tissue area of $133.3 \pm 68.0 \text{ cm}^2$, a size that was similar to the corals prior to re-fragmentation ($135.1 \pm 43.7 \text{ cm}^2$) (Figure 1a).

The reduction in live tissue area of the corals upon re-fragmentation did not result in significant changes in growth throughout Phase 3 compared to corals in the control ($p = 0.209$) (Table 1). Overall, the re-fragmented corals were smaller ($180.5 \pm 112.6 \text{ cm}^2$) than those left intact ($270.9 \pm 137.8 \text{ cm}^2$; 1a) and coral growth in the re-fragmented treatment was 84.2 cm^2 lower than those in the control (Table 1; Figure 3).

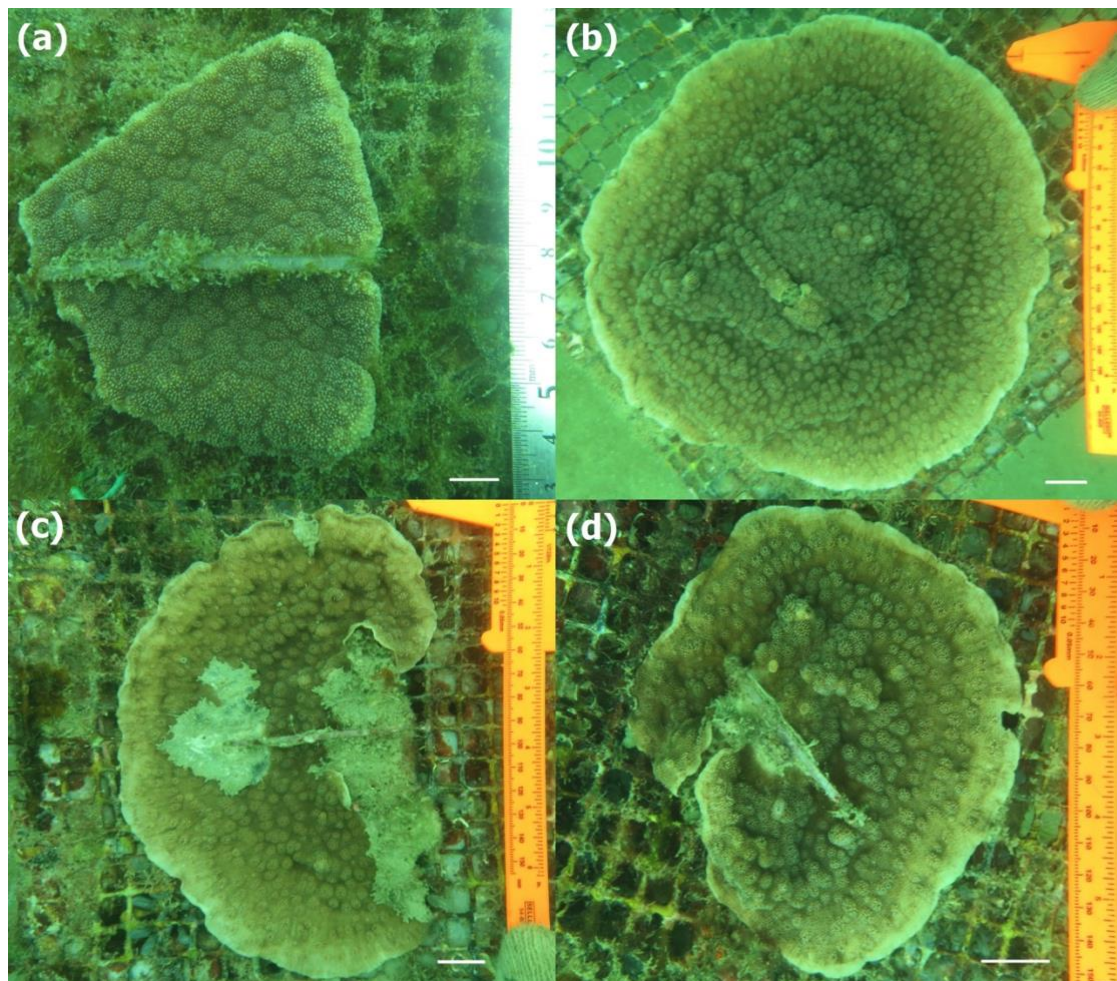


Figure 3. Representative photographs of the *Echinopora lamellosa* fragments (a) after Phase 1 and the corals in the (b) control group (c,d) re-fragmented group after Phase 3. Scale bar = 2 cm.

Dead area

At the end of Phase 1, dead area was $18.7 \pm 10.4 \text{ cm}^2$ for re-fragmented corals and $18.0 \pm 14.1 \text{ cm}^2$ for control corals (Figure 1b) and the change in dead area was similar between the two treatments (Table 1). The initial dead area was a significant predictor of the change in live tissue area during Phase 1 (Linear regression, $F = 7.237$, $p = 0.01$, $R^2 = 0.136$; Figure 2b).

In Phase 2, the change in dead area was slightly higher for corals in the re-fragmented group compared to the controls ($p > 0.05$; Table 1). In Phase 3, dead area increased by $47.2 \pm 34.5 \text{ cm}^2$ for corals in the re-fragmented group and $61.1 \pm 31.9 \text{ cm}^2$ for control corals ($p > 0.05$; Table 1; Figure 1b; Figure 3). At the end of Phase 3, two re-fragmented corals from different colonies were dead. Overall, corals in the re-fragmented group had higher change in dead area ($79.7 \pm 35.4 \text{ cm}^2$) than the control corals ($65.1 \pm 46.8 \text{ cm}^2$; Figure 1b) but the difference was not significant ($p > 0.05$; Table 1).

Discussion

Optimising propagation and rearing processes in coral nurseries is essential for effective reef restoration. While substantial efforts have been devoted to enhancing the yield of sexually and asexually derived coral propagules (Forrester et al. 2013; Toh et al. 2012), few have explored the viability of re-fragmenting nursery-reared corals for re-stocking. Re-fragmentation could have the potential to support mariculture efforts and reduce collection from the wild (Forrester et al. 2013). In this study, we tested this approach by re-fragmenting nursery-grown *E. lamellosa* colonies. Our results demonstrated that despite generating more fragments, the increase in live-tissue area of the re-fragmented corals was about half that of the control fragments after one year.

The loss of coral tissue during the re-fragmentation process (Phase 2) was a drawback of this strategy. The re-fragmented *E. lamellosa* required approximately four to five months of growth to regenerate to their initial sizes, translating to a longer period needed for rearing this species in a nursery and a higher operational cost to maintain the nursery (Toh et al. 2017). This is unlike fragmenting other coral species such as *Acropora*, which can recover faster after fragmentation (Shaish et al. 2008; Raymundo & Maypa, 2004), and exhibit rapid and indeterminate growth (Highsmith, 1982). There was no evidence in this study to show that the initial live issue area affected growth, but smaller sizes can reduce coral survival (Highsmith, 1982). While this effect was not tested in the present study, we did observe that two re-fragmented corals died while those in the control were all alive after six months.

Additionally, the greater the extent of initial tissue mortality, the slower *E. lamellosa* fragments grew in the nursery. We observed that dead regions of the colony were rapidly colonized by fouling organisms (e.g. algae), corroborating the observations reported by Fishelson (1973). These organisms compete with the coral for space and further damage adjacent tissues (Fishelson, 1973; Toh et al. 2013). As more resources are needed to facilitate wound healing, coral growth can be delayed (Fong & Lirman, 1995; Lirman, 2000; Henry & Hart, 2005). Tissue necrosis also reduces coral immunity and increases their susceptibility to coral diseases (Sheridan et al. 2013). Consequently, tissue necrosis can spread rapidly (Titlyanov et al. 2005; Casey et al. 2015) and hinders the growth of coral fragments especially at the circumference (Osinga et al. 2011; Leal et al. 2014). This could account for why initial fragment live tissue area was a poor predictor of growth in this study. To mitigate the impact of tissue necrosis on growth, dead portions of cultivated corals should be removed to prevent spread of disease and reduce further mortality (Shafir et al. 2010; Sheridan et al. 2013).

Conclusion

Despite the reduced growth, the re-fragmentation method has the potential to produce more coral individuals. This can be useful in situations where healthy donor colonies are scarce, especially for endangered coral species (Lirman et al. 2010). Further research is needed to determine the time required for the secondary fragments of various coral species to recover and grow sufficiently before the next re-fragmentation (Soong & Chen, 2003; Forrester et al. 2013; Lohr et al. 2015). This will help optimize nursery coral production and reduce the overall dependence on natural donor colonies for propagation.

Acknowledgements

This study was carried out as part of the project “Enhancing Singapore’s Coral Reef Ecosystem in a Green Port”, funded by the Maritime and Port Authority of Singapore (Grant number R347-001-215-490).

References

- Bowden-Kerby, A. (2001). Low-tech coral reef restoration methods modeled after natural fragmentation processes. *Bulletin of Marine Science*, 69(2), 915-931.
- Bruckner, A. W. (2000). New threat to coral reefs: trade in coral organisms. *Issues in Science and Technology*, 17(1), 63-68.
- Bruno, J. F., & Selig, E. R. (2007). Regional decline of coral cover in the Indo-Pacific: timing, extent, and sub regional comparisons. *PloS One*, 2(8), e711.
- Casey, J. M., Connolly, S. R., & Ainsworth, T. D. (2015). Coral transplantation triggers shift in microbiome and promotion of coral disease associated potential pathogens. *Scientific Reports*, 5, 1-11.
- Cumming, R. (2002). Tissue injury predicts colony decline in reef-building corals. *Marine Ecology Progress Series*, 242, 131-141.
- Dai, C. F. (1993). Patterns of coral distribution and benthic space partitioning on the fringing reefs of southern Taiwan. *Marine Ecology*, 14(3), 185-204.
- Dizon, R. T., & Yap, H. T. (2006). Effects of coral transplantation in sites of varying distances and environmental conditions. *Marine Biology*, 148(5), 933-943.
- Edwards, A. J., & Clark, S. (1999). Coral transplantation: A useful management tool or misguided meddling? *Marine Pollution Bulletin*, 37(8-12), 474-487.
- Edwards, A. J., & Gomez, E. D. (2007). *Reef restoration concepts and guidelines: making sensible management choices in the face of uncertainty*. Coral reef targeted research & capacity building for management programme. St Lucia, Australia.
- Epstein, N. R., Bak, R. P. M., & Rinkevich, B. (2003). Applying forest restoration principles to coral reef rehabilitation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 13(5), 387-395.
- Fishelson, L. (1973). Ecological and biological phenomena influencing coral-species composition on the reef tables at Eilat (Gulf of Aqaba, Red Sea). *Marine Biology*, 19(3), 183-196.
- Fong, P., & Lirman, D. (1995). Hurricanes cause population expansion of the branching coral *Acropora palmata* (Scleractinia): wound healing and growth patterns of asexual recruits. *Marine Ecology*, 16(4), 317-335.
- Forrester, G., Dauksis, R., & Ferguson, M. (2013). Should coral fragments collected for restoration be subdivided to create more, smaller pieces for transplanting? *Ecological Restoration* 31(1), 4-7.
- Green, E., & Shirley, F. (1999). *The global trade in coral*. Cambridge, UK: World Conservation Press.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. *Marine Ecology Progress Series*, 7(2), 207-226.

Leal, M. C., Ferrier-Pagès, C., Petersen, D., & Osinga, R. (2014). Coral aquaculture: applying scientific knowledge to ex situ production. *Reviews in Aquaculture*, 6, 1-18.

Lirman, D. (2000). Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. *Journal of Experimental Marine Biology and Ecology*, 251(1), 41-57.

Lirman, D., Thyberg, T., Herlan, J. Hill, C., Young-Lahiff, C., Schopmeyer, S., Huntington, B., Santos, R., & Drury, C. (2010). Propagation of the threatened staghorn coral *Acropora cervicornis*: methods to minimize the impacts of fragment collection and maximize production. *Coral Reefs* 29(3), 729-735.

Lohr, K. E., Bejarano, S., Lirman, D. Schopmeyer, S., & Manfrino, C. (2015). Optimizing the productivity of a coral nursery focused on staghorn coral *Acropora cervicornis*. *Endangered Species Research*, 27(3), 243-250.

Ng, C. S. L., Ng, S. Z., & Chou, L. M. (2012). Does an ex situ coral nursery facilitate reef restoration in Singapore's waters? In: K. S. Tan (Ed.), *Contributions to Marine Science* (pp. 95-100). Singapore: National University of Singapore.

Ng, C. S. L., Lim, S. C., Ong, J. Y., Teo, L. M. S., Chou, L. M., Chua, K. E., & Tan, K. S. (2015). Enhancing the biodiversity of coastal defence structures: transplantation of nursery-reared reef biota onto intertidal seawalls. *Ecological Engineering*, 82, 480-486.

Ng, C. S. L., Toh, T. C., & Chou, L. M. (2016). Coral restoration in Singapore's sediment-challenged sea. *Regional Studies in Marine Science*, 8, 422-429.

Osinga, R., Schutter, M., Griffioen, B., Wijffels, R. H., Verreth, J. A. J., Shafir, S., Henard, S., Taruffi, M., Gili, C., & Lavorano, S. (2011). The biology and economics of coral growth. *Marine Biotechnology*, 13(4), 658-671.

Pomeroy, R. S., Parks, J. E., & Balboa, C. M. (2006). Farming the reef: is aquaculture a solution for reducing fishing pressure on coral reefs? *Marine Policy*, 30(2), 111-130.

Raymundo, L. J., & Maypa, A. P. (2004). Getting bigger faster: mediation of size-specific mortality via fusion in juvenile coral transplants. *Ecological Applications*, 14(1), 281-295.

Rinkevich, B. (2005). Conservation of coral reefs through active restoration measures: recent approaches and last decade progress. *Environmental Science and Technology*, 39(12), 4333-4342.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671-675.

Shafir, S., Edwards, A., Rinkevich, B., Bongiorno, L., Levy, G., & Shaish, L. (2010). Constructing and managing nurseries for asexual rearing of corals. In: Edwards, A. J. (Ed.), *Reef rehabilitation manual* (pp. 49-72). St Lucia: Coral Reef Targeted Research & Capacity Building for Management Program.

Shaish, L., Levy, G., Gomez, E., & Rinkevich, B. (2008). Fixed and suspended coral nurseries in the Philippines: Establishing the first step in the “gardening concept” of reef restoration. *Journal of Experimental Marine Biology and Ecology*, 358(1), 86-97.

Sheppard, C. R. C. (1980). Coral cover, zonation and diversity on reef slopes of Chagos atolls, and population structures of the major species. *Marine Ecology Progress Series*, 2(3), 193-205.

Sheridan, C., Kramarsky-Winter, E., Sweet, M., Kushmaro, A., & Leal, M. C. (2013). Diseases in coral aquaculture: causes, implications and preventions. *Aquaculture*, 396, 124-135.

Smith, L., & Hughes, T. (1999). An experimental assessment of survival, re-attachment and fecundity of coral fragments. *Journal of Experimental Marine Biology and Ecology*, 235(1), 147-164.

Soong, K., & Chen, T. A. (2003). Coral transplantation: regeneration and growth of *Acropora* fragments in a nursery. *Restoration Ecology*, 11(1), 62-71.

Titlyanov, E. A., Titlyanova, T. V., Yakovleva, I., Nakano, Y., & Bhagooli, R. (2005). Regeneration of artificial injuries on scleractinian corals and coral/algal competition for newly formed substrate. *Journal of Experimental Marine Biology and Ecology*, 323(1), 27-42.

Toh, T. C., Guest, J., & Chou, L. M. (2012). Coral larval rearing in Singapore: observations on spawning timing, larval development and settlement of two common scleractinian coral species. In: K. S. Tan (Ed.), *Contributions to Marine Science* (pp. 81-87). Singapore: National University of Singapore.

Toh, T. C., Ng, C. S. L., Guest, J., & Chou, L. M. (2013). Grazers improve health of coral juveniles in ex situ mariculture. *Aquaculture*, 414, 288-293.

Toh, T. C., Ng, C. S. L., Peh, J. W. K., Toh, K. B., & Chou, L. M. (2014). Augmenting the post-transplantation growth and survivorship of juvenile scleractinian corals via nutritional enhancement. *PloS one*, 9(6), e98529.

Toh, T. C., Ng, C. S. L., Loke, H. X., Taira, D., Toh, K. B., Afiq-Rosli, L., Du, R. C. P., Cabaitan, P., Sam, S. Q., Kikuzawa, Y. P., Chou, L. M., & Song, T. (2017). A cost-effective approach to enhance scleractinian diversity on artificial shorelines. *Ecological Engineering*, 99, 349-357.

Veron, J. (2000). *Corals of the World, vol. 1-3*. Townsville: Australian Institute of Marine Science.

Contact email: lokemingchou@gmail.com