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# DOES AN EX SITU CORAL NURSERY FACILITATE REEF RESTORATION IN SINGAPORE'S WATERS?

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***ABSTRACT.*** — Various techniques to assist in the rehabilitation and restoration of coral reefs have been developed. While in situ coral nurseries are increasingly used as a reef restoration tool, ex situ nurseries have not been utilised to the same extent. In this two-part study, the feasibility of rearing asexually propagated corals in an ex situ nursery was investigated using a flow-through filtered seawater facility at the Tropical Marine Science Institute on St John's Island, Singapore. The first compared the survivorship and changes in buoyant weight of three size classes of *Acropora digitifera*, *Psammocora digitifera* and *Porites lutea* fragments that were attached to cement tiles. *Porites lutea* and *P. digitata* fragments had high survivorship (100% and 98.9% respectively) while *A. digitifera* fragments had the lowest (5.7%), but mortality was not size-dependent for all three species. Buoyant weight increase was significantly greater for the large-sized *P. lutea* fragments compared to the mid-sized and small-sized ones, while those of *P. digitata* fragments of all three size classes were not significantly different from each other. The second part of the study recorded the time required by fragments of various hard coral species to self-attach to cement tiles. Initial self-attachment times ranged from four to 12 weeks for 19 of the 21 species investigated. The results show that the conditions in the flow-through facility are conducive to the survivorship and growth of many hermatypic species, and that an ex situ coral nursery phase can be used to complement in situ nurseries towards reef restoration in Singapore.

***KEY WORDS.*** — Ex situ coral nursery, fragments, survivorship, buoyant weight, self-attachment

## INTRODUCTION

The worldwide degradation of coral reefs by various stressors (Hughes & Connell, 1999) has necessitated remediation strategies using a plethora of approaches to rehabilitate and restore degraded reefs. Techniques include the induction of larval settlement (Lee et al., 2009), stabilisation of substrate (Raymundo et al., 2007), deployment of artificial reef structures (Clark & Edwards, 1994), and the transplantation of hard corals onto target sites (Clark & Edwards, 1995). The propagation of sexual (larvae) and asexual (nubbins) recruits for transplantation is also often employed as a reef restoration tool (Rinkevich, 1995), but a sheltered environment is invariably required to protect the coral propagules till they reach a suitable size for outplanting (Epstein et al., 2003). These measures to improve post-transplantation survivorship are achieved via the use of ex situ or in situ nurseries (Rinkevich, 2005).

Ex situ nurseries, sited on land, are considered short-term options. They provide a "preliminary foster period" (Epstein et al., 2003) before relocation of the coral material to in situ nurseries or eventual transplantation to target localities. In comparison, the costs for the construction and operation of in situ nurseries – comprising fixed and floating versions anchored in the sea – are relatively low (Shafir et al., 2010). In situ nurseries thus appear to be more widely employed, operated on a larger scale, and studied for the purposes of reef restoration (e.g. Epstein et al., 2001; Soong & Chen, 2003; Shafir et al., 2006; Amar & Rinkevich, 2007) than ex situ ones (e.g. Forsman et al., 2006; Petersen et al., 2008). Although ex situ coral rearing is considered as "specialist" (Shafir et al., 2010), the technique is still effective for preserving genotypes from reef locales facing impending obliteration and where there are constraints in the availability of coral material (Epstein et al., 2001). 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, there is a paucity of such data even though ex situ coral rearing is common in private and public aquariums, as much information from these practices are typically anecdotal and lack scientific scrutiny (Arvedlund et al., 2003; Olivotto et al., 2011).

Singapore's small reef area of 10km$^2$ hosts approximately 30% of the global coral diversity (Huang et al., 2009) but reef health has been impacted in recent decades by an assortment of anthropogenic disturbances such as shipping, land reclamation and dredging, all of which contribute to high sediment loads and extensive damage of the reefs (Chou, 2006). Attempts at reef restoration in Singapore have included the deployment of fibreglass reef enhancement units (Loh et al., 2006), the rearing of 'corals of opportunity' (i.e. naturally fragmented corals and coral juveniles that have recruited on loose rubble) and nubbins in in situ nurseries (Chou et al., 2009; Bongiorni et al., 2011), the transplantation of nubbins onto natural reefs (unpublished data), and the introduction of marine biodiversity to seawalls (Gunasingham, 2009). The use of ex situ coral nurseries to house asexually propagated corals in Singapore has, however, not been documented.

The aquaria of the Tropical Marine Science Institute, National University of Singapore, on St John's Island (01°13'03.50"N, 103°50'59.50"E), have been instrumental in marine larval research (e.g. Lee et al., 2009; Neo et al., 2011). These facilities present an opportunity to investigate aspects of ex situ rearing of hard coral fragments to supplement reef restoration efforts in Singapore. In this study, we investigate the survivorship and compare growth rates of three size classes of hard corals (*Porites lutea*, *Psammocora digitata*, *Acropora digitifera*) in Singapore, selected to represent massive, submassive and branching growth forms respectively. We also report the times to initial self-attachment – referring to the growth of coral tissue onto substrate (Guest et al., 2011) – on cement tiles of 21 hermatypic species that are common on the reefs of Singapore. Such information will be useful for gauging the effort required to asexually propagate corals in an ex situ setting before they are ready for relocation to in situ nurseries or transplantation to the intended sites.

## MATERIAL AND METHODS

***Study site and preparatory work.*** — The study was carried out at the St John's Island outdoor aquarium facility, which consisted of several large tanks supplied with flow-through 20 µm filtered seawater. Temperature, salinity and light in the tanks averaged 29.5°C, 29 ppt, and 375 µmol m$^{-2}$ s$^{-1}$ respectively. Cement tiles made with equal parts of coarse sand, fine sand and cement were air-dried for two days and placed in flow-through seawater tanks for one month to allow biological conditioning to occur. These served as substrata for the attachment and growth of small coral fragments. *Trochus* spp. gastropods were introduced to the tanks as biological controls of macroalgae.

***Survivorship and growth.*** — Six colonies each of *Porites lutea*, *Psammocora digitata* and *Acropora digitifera* were collected from the coral reef fringing Raffles Lighthouse (01°09'36.50"N, 103°44'24.00" E) in September 2009. They were placed in the outdoor flow-through seawater system to acclimate for one month before fragmentation into three size classes: 10 ± 4 mm (Class 1), 25 ± 4 mm (Class 2), and 40 ± 4 mm (Class 3), with 30 fragments in each size class. For *Acropora digitifera*, only branch tips were used as source material. Adopting the method by Shafir & Rinkevich (2008), each coral fragment, upon excision from the parent colony, was attached to a cement tile with cyanoacrylate glue. All fragments were placed on suspended PVC trays and distributed between two circular flow-through seawater tanks that each contained approximately 4600L of filtered seawater with aeration. Circulation of water within each tank was ensured with the use of a 5000L/hour pump. Each tray held 25 fragments spaced 5 cm apart from each other, and the fragments were rotated weekly to reduce positional effects. Fragment survivorship was monitored weekly. As a proxy to growth, buoyant weight measurements were taken, and the change in buoyant weight of the live fragments was measured after three months. To compare monthly growth rates among size classes of each species, the changes in buoyant weight were log-transformed and analysed with a one-way ANOVA using SPSS ver 17.

***Self-attachment times.*** – Twenty scleractinian species and one non-scleractinian (*Heliopora coerulea*) were collected from the reef at Raffles Lighthouse and acclimated in the outdoor flow-through system for a month. The corals were then fragmented into pieces approximately 15 – 20 mm in size. Six fragments of each species were subsequently glued to cement tiles and placed in two flow-through tanks that held approximately 560L of filtered seawater each. The time taken for new coral tissue to grow over to attach (or self-attach) onto the cement tiles was observed.

## RESULTS

***Survivorship.*** — The overall survivorship for all *P. lutea*, *P. digitata*, and *A. digitifera* fragments after 12 weeks was 100%, 98.9% and 5.7% respectively (Table 1). As *P. lutea* and *P. digitata* fragments exhibited high survivorship throughout the study period, only the survivorship trend of *A. digitifera* fragments is shown (Fig. 1). Fragment survivorship decreased drastically after the third week for all size classes of *A. digitifera*. Tissue loss started at the base of many fragments and progressed to the apical tips rapidly.

Table 1. Survivorship of fragments from different size classes.

| | Survivorship (%) | | |
|---|---|---|---|
| Size class | *Porites lutea* | *Psammocora digitata* | *Acropora digitifera* |
| 1 (10 ± 4 mm) | 100 | 96.7 | 10 |
| 2 (25 ± 4 mm) | 100 | 100 | 6.7 |
| 3 (40 ± 4 mm) | 100 | 100 | 0 |

Table 2. Times to initial self-attachment of coral species onto cement tiles.

| Family | Species | Time to initial self-attachment (weeks) |
|---|---|---|
| Acroporidae | *Acropora digitifera* (Dana, 1846) | 4 |
| | *Acropora valida* (Dana, 1846) | 4 |
| Helioporidae | *Heliopora coerulea* (Pallas, 1766) | 6 |
| Oculinidae | *Galaxea fascicularis* (Linneaus, 1767) | 6 |
| Siderastreidae | *Psammocora obtusangula* (Lamarck, 1816) | 6 |
| | *Psammocora digitata* Milne Edwards & Haime, 1851 | 12 |
| Poritidae | *Porites sillimaniana* Nemenzo, 1976 | 8 |
| | *Porites lobata* Dana, 1846 | 10 |
| | *Porites lutea* Milne Edwards & Haime, 1851 | 10 |
| | *Goniopora lobata* Milne Edwards & Haime, 1860 | 12 |
| Pocilloporidae | *Pocillopora damicornis* (Linneaus, 1758) | 10 |
| Merulinidae | *Hydnophora exesa* (Pallas, 1766) | 10 |
| | *Hydnophora rigida* (Dana, 1846) | 10 |
| Faviidae | *Cyphastrea serailia* (Forskål, 1775) | 6 |
| | *Favia speciosa* Dana, 1846 | 12 |
| | *Favites abdita* (Ellis & Solander, 1786) | 12 |
| | *Goniastrea minuta* Veron, 2000 | 12 |
| | *Goniastrea pectinata* (Ehrenberg, 1834) | 12 |
| | *Platygyra sinensis* (Milne Edwards & Haime, 1849) | 12 |
| | *Caulastrea furcata* Dana, 1846 | Does not form any attachment to substrate |
| Dendrophylliidae | *Turbinaria peltata* (Esper, 1794) | Does not form any attachment to substrate |

***Growth.*** — Buoyant weight for *Acropora digitifera* fragments was not calculated due to the high mortality rates across all size classes.

The change in buoyant weight was significantly different among the three size classes of *Porites lutea* fragments (F = 8.218, df = 2, p = 0.001). The largest fragments (Class 3) showed the greatest increase in buoyant weight per month (0.336 ± 0.027 g), while those of the mid-sized (Class 2) and smallest (Class 1) fragments were not significantly different (0.235 ± 0.010 g and 0.237 ± 0.013 g respectively).

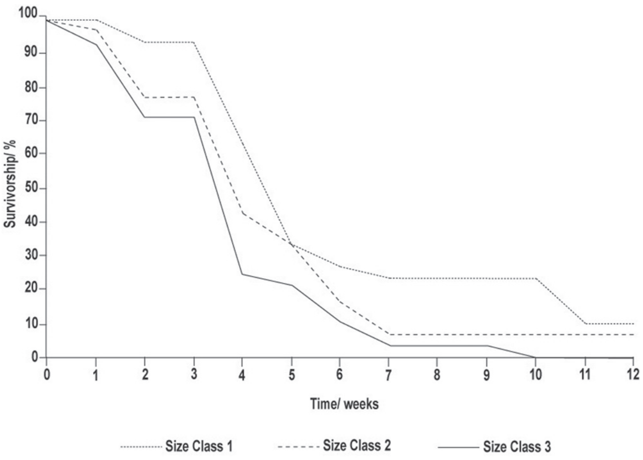

Fig. 1. Survivorship of *Acropora digitifera* fragments over twelve weeks.

*Psammocora digitata* fragments had a greater increase in buoyant weight per month than fragments of *P. lutea*, but the change in buoyant weight was not significantly different among the three size classes of *P. digitata* fragments (F = 0.020, df = 2, p = 0.980). The average increment in buoyant weight per month from the largest to the smallest size class was 0.780 ± 0.048 cm, 0.765 ± 0.042 g and 0.766 ± 0.037 g respectively.

***Self-attachment times.*** — Fragments of the various species began forming new tissue onto the cement tiles from as early as four weeks to as late as twelve (Table 2). All fragments had visible increases in fragment size or corallite number, but two scleractinian species, *Caulastrea furcata* and *Turbinaria peltata*, did not exhibit self-attachment even after the rest of the other species did.

## DISCUSSION

While the disadvantages of ex situ coral propagation commonly include the lack of genetic diversity and the incurring of substantial logistical costs (Epstein et al., 2001), such nurseries can confer stable environments to improve the survivorship and growth of the coral material as well as facilitate the control of corallivory and fouling organisms. This method will still present a viable option in the case of localities that are prone to extreme weather conditions or experience chronic stresses which impact in situ coral rearing such that overall propagule survivorship is less than ideal (e.g. Shaish et al., 2010; Bongiorni et al., 2011). As a supplement to other reef restoration endeavours, the results of this study indicate that it is feasible to asexually propagate certain hard coral species and achieve appreciable survivorship and growth in an ex situ flow-through aquarium facility.

Almost all fragments of *Porites lutea* and *Psammocora digitata* survived over the study period (100% and 98.9% respectively), demonstrating that an ex situ nursery phase can maintain and improve fragment survivorship significantly (Epstein et al., 2001; Forsman et al., 2006). From the lack of trends in size-specific mortality, fragments of both species 40 mm and below can be expected to survive well in an ex situ environment. Fragments of both species also grew throughout the study, as observed by the increase in buoyant weights for all size classes. While buoyant weight increment was not related to the size of *P. digitata* fragments, the positive relationship between size and growth of *P. lutea* fragments – consistent with that reported for congeneric (*P. lobata*) fragments grown in an ex situ setting (Forsman et al., 2006) – indicates that shorter rearing times will be needed if larger fragments of *P. lutea* are used. Similar to initial nubbin sizes (10 – 14 mm) of *P. lutea* and *P. digitata* employed in an in situ nursery in Singapore (Bongiorni et al., 2011), fragments as small as 10 mm can thus be excised from donor colonies of both species and grown in an ex situ nursery without influencing survivorship significantly. Such knowledge on the patterns of survivorship and growth of different sized fragments will be useful for deciding the amount of source material to obtain from donor colonies, as prudent collection will reduce collateral damage to donor areas (Edwards & Clark, 1999).

In contrast, survivorship of *Acropora digitifera* was the lowest of the three species studied, and all size classes fared poorly in the aquaria (0 – 10% survival). As a result, even though self-attachment was fairly rapid (about four weeks; see Table 2), any changes in buoyant weight could not be analysed. The characteristic loss of tissue which began from the base and spread upwards for many fragments suggests that rather than a response to high light or heat (Takahashi et al., 2004), or inadequate water flow (Nakamura et al., 2005), this mass mortality could likely have resulted from one of the pigmented band diseases that commonly afflicts branching *Acropora* colonies (Raymundo et al., 2008), with the severity of the spread exacerbated by the confines of the mariculture tanks. While Guest et al (2011) reported low mortality rates of *A. digitifera* fragments in an in situ nursery, the rapid decline in health of this species in the current study indicates that other techniques should be explored to facilitate the ex situ mariculture of this species.

Unsecured small fragments in an ex situ nursery face a much reduced risk of burial or abrasion compared to ones strewn unconsolidated in the wild; however, adhering them to a stable substrate allows for easier identification and repositioning within the tank, and also decreases the chances of the fragments getting pushed about by grazing herbivores (pers. obs.). In nurseries where nubbins or fragments are glued to an artificial media such as cement tiles or plastic pins (e.g. Shafir & Rinkevich, 2008), information on times to self-attachment, which is currently limited (Bowden-Kerby, 2001), would help in projecting the amount of time required for them to suitably cover the substrate so that they do not easily detach and are ready for transplantation. The self-attachment exhibited by the various species in this study indicated that the overall environment in the aquaria was conducive for hard coral growth. Additionally, the trends in self-attachment times appear consistent to those investigated by Guest et al (2011) in an in situ nursery and also with general scleractinian growth trends (Gladfelter et al. 1978; Clark & Edwards, 1995; Riegl & Purkis, 2012) – the acroporids attached the fastest, while the faviids were slowest. Species which self-attached faster (e.g. *Heliopora coerulea*, *Galaxea fascicularis*, *Cyphastrea serailia*) tended to have softer skeletons, and therefore are more likely to be damaged if transplanted prematurely. More time should probably be given for them to grow sufficiently large before relocation from ex situ nurseries. Finally, the increase in the number of corallites on *Caulastrea furcata* and *Turbinaria peltata* fragments over the study period corresponded to an expansion in fragment size but not self-attachment over the cement tiles, making such propagation methods unsuitable if these species are intended for use in restoration.

This study showed that flow-through aquaria can function as an ex situ nursery for the rearing of hard coral fragments. Of the three species studied, *P. lutea* and *P. digitata* fragments 40 mm and smaller were able to survive and grow in the aquaria, and can thus be deemed more suitable for rearing in an *ex situ* nursery than *A. digitifera*. The nursery conditions were also suitable for fragments of 18 scleractinian and one non-scleractinian species to self-attach within three months of artificial adhesion on cement tiles. Although the experimental period was short, the study illustrated that the option of ex situ coral rearing may be considered as a step to improve fragment survivorship in locations which experience chronic stresses that can impact restoration outcomes (e.g. Bongiorni et al., 2011). The relatively stable environment within the outdoor aquarium tanks was advantageous to the survival of coral fragments.

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