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CORAL LARVAL REARING IN SINGAPORE: OBSERVATIONS ON SPAWNING TIMING, LARVAL DEVELOPMENT AND SETTLEMENT OF TWO COMMON SCLERACTINIAN CORAL SPECIES

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ABSTRACT. — Small scale rearing of scleractinian coral larvae for experimental purposes has been practised for decades, but it is only in recent years that large scale larval rearing has been used as a means of providing corals for reef rehabilitation. This study aimed to develop optimal methods for rearing sexually propagated scleractinian corals from the broadcast spawning species *Acropora hyacinthus* and *Pectinia lactuca* in Singapore. Gravid *A. hyacinthus* was observed to spawn on the second to third day after full moon, while *P. lactuca* spawned on the fourth to sixth day after full moon. *P. lactuca* embryos displayed rapid development and the larvae settled within 48 h post-fertilization, with settlement peaks at 70 h and 118 h. *A. hyacinthus* propagules exhibited relatively slower embryonic development and later settlement, with the highest settlement rate at 142 h post-fertilization. Post-transplantation survivorship of the donor colonies for *A. hyacinthus* was 83% after 6 months and for *P. lactuca,* was 60% and 50% for colonies transplanted at Pulau Satumu and St. Johns Island, respectively.

KEY WORDS. — Scleractinian coral larvae, spawning, reef restoration, Singapore

INTRODUCTION

The discovery of the multispecific coral spawning event in the Great Barrier Reef, Australia in the 1980s (Harrison et al., 1984) has changed our understanding of coral reproductive biology and opened the door to studies of coral larval biology and early life history stages (Heyward & Babcock, 1986). An important prerequisite to successful propagation of sexually derived corals is an understanding of the reproductive season and timing of the scleractinian corals and in recent decades there has been a surge in knowledge about reproductive timing from previously understudied coral reef regions (Baird et al., 2009, Guest et al., 2010).

Coral larvae are now routinely reared at numerous coral reef locations around the world for a variety of experimental purposes. Early attempts at small-scale coral larvae rearing involved holding larvae *in situ* using floating bottles (Babcock & Heyward, 1986), which exposed delicate larvae to inclement weather. Now, typically, coral propagules are either collected directly from corals *in situ* using collecting devices placed over spawning colonies or by bringing colonies into land based aquarium tanks. Egg sperm bundles are then mixed, fertilised, cleaned of excess sperm and subsequently reared in volumes ranging from a few litres to large tanks with 1000s of litres of volume (Hatta et al., 2004; Guest et al., 2010). However, the maintenance of larvae culture after fertilization is one of the most critical stages in propagating sexually derived corals, since the developing larvae are very sensitive to variations in water quality (Baird et al., 2006) and mechanical disturbance (Guest et al., 2010; Heyward & Negri, 2012).

Over the past four decades, numerous reef restoration techniques and approaches have been developed (Edwards, 2010). Due to its relative ease and perceived low cost, transplantation of asexual coral fragments has become the most common approach used in the biological restoration of coral reefs (Rinkevich, 1995; Shafir et al., 2006). However, this method can result in collateral damage to the donor colonies (Yap & Gomez, 1985; Yap et al., 1992) and potentially reduce the genetic integrity of transplanted populations (Shearer et al., 2009). Recent advancement in coral reef restoration techniques have explored the use of sexually propagated corals as source materials for transplantation (Omori, 2008; Nakamura et al., 2011). This involves the collection of gametes from gravid coral colonies during the spawning period and the subsequent maintenance of the larval culture *ex situ* (Guest et al., 2010). While this approach can be more costly and labor intensive (Edwards, 2010), sexual propagation of corals can enhance the genetic variability of the transplanted population (Rinkevich, 1995) and due to the very high fecundity of corals, has the potential to be applied for large-scale restoration work.

Extensive coastal development in Singapore since the 1950s has resulted in the loss of over 60% of its coral cover (Hilton & Manning, 1995), with 70% of the remaining reefs in various degraded states (Chou, 2000). Land reclamation in particular, has increased sedimentation rates to as high as 44.64 mg cm^{-2} day^{-1} (Low & Chou, 1994), affecting larval recruitment and reducing in coral diversity (Dikou and van Woesik, 2006). While local coral reef restoration research has been carried out since the 1990s, research involving the sexual propagation of scleractinian corals is limited (Tay et al., 2011; Erftemeijer et al., 2012). This study aims to optimize the technique for coral larvae rearing in Singapore for future reef restoration applications by examining (1) the *ex situ* spawning timing and duration of two species of scleractinian corals: *Acropora hyacinthus* and *Pectinia lactuca,* (2) the embryonic development and settlement competency periods of the coral larvae and (3) the post-transplantation survivorship of the donor colonies.

MATERIAL AND METHODS

Study species. — *Acropora hyacinthus* and *Pectinia lactuca* are common Indo-Pacific scleractinian corals that tend to colonize clear shallow waters of upper reef slopes and turbid lower reef slopes, respectively (Veron, 2000). Both species are hermaphroditic broadcast spawners (Baird et al., 2009) and have been documented to participate in the multi-specific synchronous coral spawning events in Singapore three to five days after the March or April full moon (Guest et al., 2005).

Collection of coral larvae. — A pre-spawning sampling trip was conducted one week before the full moon in March 2010 (30th March) to determine the maturity of the coral gametes in the field by fragmenting part of the colony to check for the presence of pigmented eggs (Guest et al, 2002). One to two days after the full moon, a total of six gravid adult colonies of *A. hyacinthus* and 13 colonies of *P. lactuca* were collected from the fringing reefs off Pulau Satumu (1°9'36"N, 103°44'27"E), Kusu Island (1°13'25"N, 103°51'38"E) and St John's Island (1°13'44"N, 103°50'73"E) using SCUBA. Only corals spaced at least 5 m apart were selected to reduce the chances of collecting genetically identical colonies, which could otherwise compromise fertilization success (Heyward and Babcock, 1986). Colonies that were more than 40 cm in diameter were fragmented to facilitate transportation. All specimens were maintained in an outdoor holding tank (volume approx. 1800 L) with flow-through sand-filtered seawater (SFSW) at the Tropical Marine Science Institute (TMSI) mariculture facility on St John's Island, Singapore.

One hour before sunset (approx. 1900 h), smaller corals were transferred and isolated in floating plastic pots (volume approx. 20 L) suspended in the holding tank (Fig. 1A), with at least 30 cm of water column above the colonies, to facilitate gamete collection. This method allowed precise control over the genetic crosses, although it is possible to allow multiple colonies to spawn together in a tank. Larger colonies were transferred to 40 L tanks without flow-through seawater and aeration to prevent premature dissociation of the egg-sperm bundles and mixing of gametes during spawning. Light is thought to be one of the final synchronising cues for spawning (Penland et al., 2004) therefore all lights near the aquarium tanks were switched off. Visual monitoring of colonies was carried out with a dim red light not more than once every half an hour thereafter, to check for signs of egg-sperm bundles "setting" on the polyp mouths (Fig. 1B) or the presence of bundles in the water column (Guest et al., 2010). Spawning colonies were left untouched until the majority of eggs sperm bundles had been released. Once all colonies had completed spawning, the positively buoyant egg-sperm bundles were collected from at least two colonies of each species by gently skimming the water surface using 100 mL polyethylene cups (Fig. 1C) and were immediately transferred to 36 L fertilization tanks filled with 0.2 µm UV filtered seawater (FSW). Once sufficient numbers of bundles had been harvested, they were broken apart with gentle agitation (Fig. 1D) and left standing for 30 to 60 min for fertilization. Three water changes were done to remove excess sperm using the same scooping method described above and five 50 mL subsamples were taken out of the tanks to estimate the total number of propagules. The embryos were subsequently transferred to 450 L rearing tanks containing 0.2 µm UV filtered seawater (FSW) in covered aquaria at ambient air temperature and the stock cultures were maintained at concentrations not exceeding 350 propagules per L throughout the experiment. Approximately 50% water changes were carried out after 24 h, every one to two days by gently siphoning out water from the rearing tank using a 100 µm sieve attached to polyvinyl chloride pipes (Fig. 1E). Dead embryos and any other floating material was removed by skimming the water surface with polyethylene plastic wrap.

Embryonic development and larval settlement competency periods. — To document the embryonic developmental stages of the propagules after fertilization, subsamples of

Table 1. *Ex situ* spawning duration of gravid *Acropora hyacinthus* and *Pectinia lactuca* and the diameter of the propagules at release.

	Spawning			Egg diameter (µm)			
Species	**Proportion**	**Duration***	**Time**	**Mean**	**S.E.**	**Range**	**n**
Acropora hyacinthus	6 of 6	2 - 3	20.00 h – 22.30 h	650	19.4	550 - 750	10
Pectinia lactuca	9 of 13	4 - 6	21.30 h – 23.30 h	348	7.17	300 - 375	10

*Days after full moon

approximately 20 propagules of each species were obtained from the stock rearing tanks and viewed under a stereo-dissection microscope. Photomicrographs were taken (Canon S90) through the objectives of the microscope from one hour post-fertilization to metamorphosis (46 h for *P. lactuca* and 94 h for *A. hyacinthus*).

The time taken for different species of coral larvae to settle varies considerably across location and environmental conditions (Guest et al., 2010), influencing the time of which settlement substrates should be introduced. To determine the larval settlement competency periods, approximately 20 larvae from each species were introduced into each well of a 6-well plate (BD Falcon™) filled with 6 mL of FSW and a 25-mm^2 crustose coralline algae (CCA) chip. The wells were placed in a covered aquarium at ambient temperature and each well was treated as one replicate following Heyward and Negri (1999, 2010). This was repeated every 22 to 24 h (8 time points spread over 190 h after fertilization) and the proportion of larvae that settled in each well was recorded under a stereo-dissection microscope and categorized into unattached, attached and metamorphosed larvae; where attachment and metamorphosis were observed, the larvae were considered to have settled (Guest et al., 2010). The scoring criteria used in this study followed that used in Heyward and Negri (1999), i.e. attachment was defined as the development of the coral larvae from the free swimming stage to the firmly attached pear-shaped forms, while metamophosis was defined as coral larvae that had changed from the pear-shaped forms to disc-shaped structures with pronounced flattening of the primary polyp.

Transplantation of donor colonies. — Adult coral colonies used for the experiment were transplanted back to their donor sites within two weeks after spawning to minimize the stress exerted on the corals. Due to the proximity of the donor sites, both *P. lactuca* colonies collected from Kusu Island were transplanted back to the reefs off St John's Island. Prior to transplantation, the substrata were cleaned by manually removing algae and sediments. The corals were spaced 50 to 200 cm apart, stabilized using commercial two part marine epoxy and the survivorship was monitored over the following six months after transplantation.

RESULTS

Duration of spawning. — All of the *A. hyacinthus* colonies collected spawned *ex situ* from the 2nd to the 3rd day after March full moon. Setting of the first egg-sperm bundle was documented as early as 2000 h, approx. 30 to 45 min after sunset. The duration between setting and the commencement of propagule release was between 60 and 80 min, and spawning ceased within 20 to 30 min after propagule release. Nine of 13 *P. lactuca* colonies collected spawned *ex situ* from the 4th to the 6th day after full moon. Spawning was observed to commence at 2130 h and ceased within 30 to 45 min after propagule release (Table 1).

Propagule size and embryonic development. — Immediately after propagule release, subsamples of the eggs from both species were measured under a dissecting microscope (n = 10). The diameter of the eggs was 650 ± 19.4 µm for *A. hyacinthus* and 348 ± 7.17 µm (mean ± S.E.) for *P. lactuca* respectively (Table 1). Fertilized eggs from both species commenced first cleavage one hour post-fertilization and continued to cleave, taking on the distinctive 'prawn chip' shape at five hours post-fertilization (Fig. 2). Subsequently, embryonic development was accelerated in *P. lactuca* embryos and gastrulation was completed from 9 to 18 hours post-fertilization, and planulae were observed to be elongated and motile at 18 h (Fig. 2A). *A. hyacinthus* completed gastrulation at 24 h post-fertilization and planulae were motile at 48 hours (Fig. 2B).

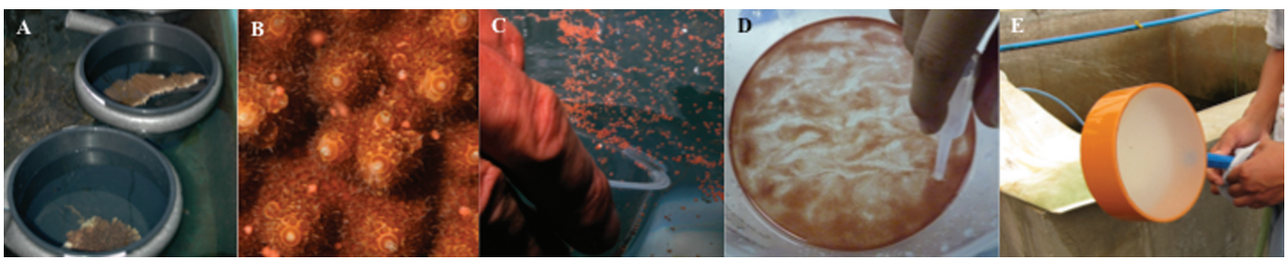

Fig. 1. Procedures in coral larval rearing. (A) Isolation of coral colonies in plastic pots one hour prior to sunset. (B) Observation for the setting of egg sperm bundles on coral polyps; (C) Skimming egg sperm bundles off the water surface; (D) Gentle agitation of the egg sperm bundles to promote fertilization; (E) Water change using a 100 µm sieve.

Larvae settlement competency periods. — *A. hyacinthus* began attaching to the CCA chip at 70 h post-fertilization and metamorphosis (Fig. 2A) was observed at 94 h. The percentage of larvae settling continued to increase and peaked at 71.8 ± 5.45% (mean ± S.D.), 142 h post-fertilization. For *P. lactuca,* larval attachment and metamorphosis were observed as early as 46 h post-fertilization (Fig. 2B) and the settlement rate were between 79.1 ± 5.98% and 83.4 ± 2.98% (mean ± S.D.) at 70 h and 118 h post-fertilization, respectively (Fig. 3).

Transplantation of donor colonies. — Survivorship of *A. hyacinthus* was 100% for the first 2 mo and stabilized at 83% (5 of 6 colonies survived) after 6 mo. Survivorship of *P. lactuca* at both transplant sites decreased steadily from 1 to 4 mo post-transplantation and stabilized to 60% (3 of 5 colonies survived) and 50% (4 of 8 colonies survived), at P. Satumu and St Johns island respectively (Fig 4).

DISCUSSION

Prediction of spawning timing. — Spawning timings for many scleractinian coral broadcast spawning species are predictable to within a few nights of the year for many localities (Baird et al., 2009). Further detailed information on spawning timing is essential to coral larval rearing activities (Guest et al., 2010), facilitating the allocation of resources in large-scale larval culture efforts and influencing the choice of coral species. The present study documented, with relative precision, the spawning timing and duration of *A. hyacinthus* and *P. lactuca,* and this information can be used for future larval culture work. As observed from this study, despite the general synchrony between species, subtle variations in spawning timings exist. For *P. lactuca,* nine of 13 colonies spawned throughout the monitoring period and spawning was spread over two to three days (Table 1), whilst all six colonies of *A. hyacinthus* spawned within two days. Hence,

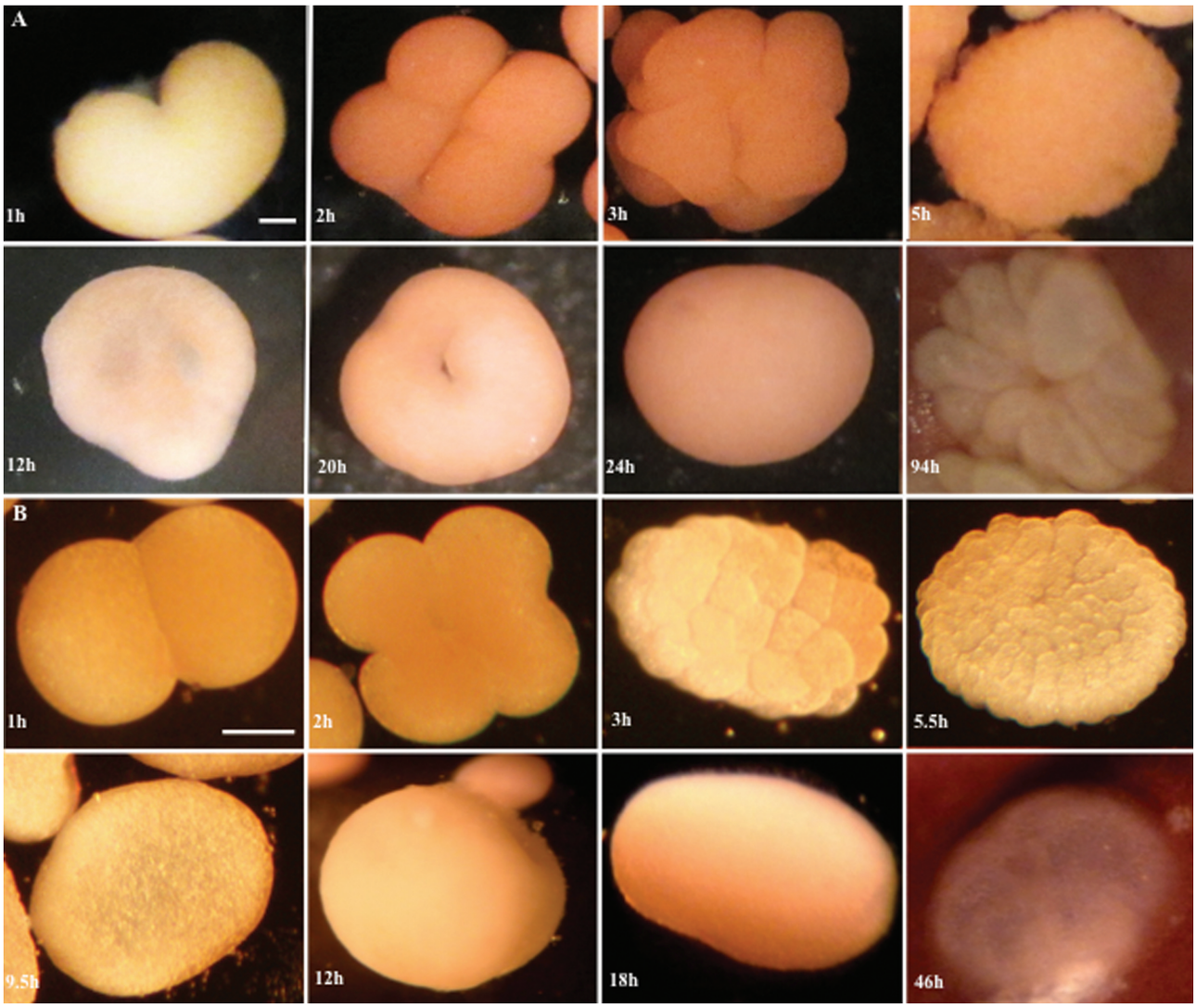

Fig. 2. Embryonic and larval developmental stages of: (A) *Acropora hyacinthus*; and (B) *Pectinia lactuca* from 1h post-fertilization to metamorphosis. Scale bars: approximately 100 µm.

to ensure sufficient gametes for fertilization and larvae for subsequent rearing, more than six gravid colonies of at least 20 cm in diameter should be collected for each species (Guest et al., 2010). In addition, the highest proportion of spawning *P. lactuca* and *A. hyacinthus* colonies on any given night, were 69% and 83% respectively, suggesting that more colonies of *P. lactuca* should be collected to maximise fertilization success.

Larval size and settlement competency. — Differences in coral propagule sizes across different species can be attributed to the varying lipid content of the egg, influencing its buoyancy (Harii et al., 2002) and the amount of energy reserves available for the larvae (Arai et al., 1993). The propagules of *A. hyacinthus* were positively buoyant and the sizes measured in this study falls within the size range of other acroporid species highlighted in Babcock et al., 2003. *P. lactuca* possessed smaller eggs of 348 ± 7.17 µm and which were neutrally buoyant after the dispersion of the egg-sperm bundle (pers. obs.); an indication of lower lipid content (Harii et al., 2002). Even though the embryonic developmental stages observed for both species were similar to that in Okubo and Motokawa (2007), the differences in energy reserves translated to rapid development as seen in this study (Fig. 2) where *P. lactuca* exhibited faster embryonic development than *A. hyacinthus*, presumably to facilitate the early onset of settlement. *P. lactuca* exhibited settlement peaks at 70 h and 118 h post-fertilization, while *A. hyacinthus* had peaks at 142 h post-fertilization (Fig. 3). While it is beyond the scope of the present study to examine the changes in coral energy budget at different morphological stages, our observations support the hypothesis that the lipid content is a determining factor in influencing the settlement competency periods of coral larvae (Richmond, 1987) and this influences the timing in which substrates should be introduced for settlement.

Post-transplantation survivorship of donor colonies. — Corals allocate significant amount of energy into repairing damaged tissues and they respond differently to fragmentation and transplantation stress (Yap et al., 1992; Yap et al., 1998). While branching acroporid corals are known to exhibit high survivorship and growth rates after fragmentation (Rinkevich, 1995; Shafir and Rinkevich, 2008) and transplantation (Yap et al., 1992), transplantation of *P. lactuca* has not been attempted before. Here, fragmentation of donor colonies selected for spawning was reduced to the minimum to mitigate against tissue damage and stress. However, *A. hyacinthus* exhibited higher post-transplantation survivorship than *P. lactuca* when subjected to the same pre and post-transplantation procedure (Fig. 4). This difference can be attributed to the different levels of mucus discharge in response to mechanical disturbance between the two species (Benson and Muscatine, 1974). *P. lactuca* is known to be copious mucus producers and they tend to produce large quantities of mucus after fragmentation and mechanical stress (pers. obs.). Repeated agitation of the corals translates into a significant loss of energy through mucus production (Benson and Muscatine, 1974), reducing the corals' capacity for innate recovery and repair of damaged tissues. This could explain the high mortality rates after transplantation. While a study on the detailed responses of *P. lactuca* to fragmentation and transplantation is lacking, our results have emphasized the importance of adopting different methods of gamete collection for different species and the need to monitor the post-transplantation survivorship of the donor colonies, to minimise collateral damage to existing coral population.

Implications for using sexually propagated corals for reef restoration in Singapore. — Intensive coastal development in Singapore over the past six decades has resulted in significant decrease in coral cover. Apart from habitat destruction and a decline in species diversity and richness, existing coral populations have to overcome challenges arising from chronic sedimentation. Sedimentation can interfere with the population dynamics of scleractinian corals at various stages of development; fertilization (Erftemeijer et al., 2012), larval settlement (Babcock & Davies, 1991; Gilmour, 1999; Babcock & Smith, 2000) and post-settlement survivorship

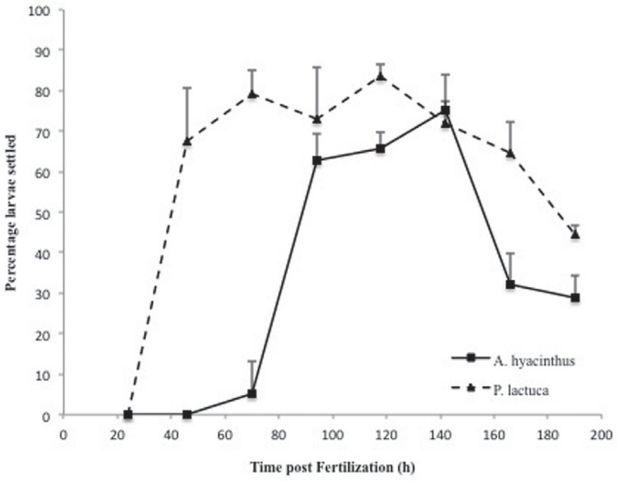

Fig. 3. Settlement competency periods of *Acropora hyacinthus* and *Pectinia lactuca.* The graph shows the percentage of coral larvae attached/settled after every 24 h, calculated using approx. 20 larvae in each of the 6 replicate wells at each time point (error bars = S.D.).

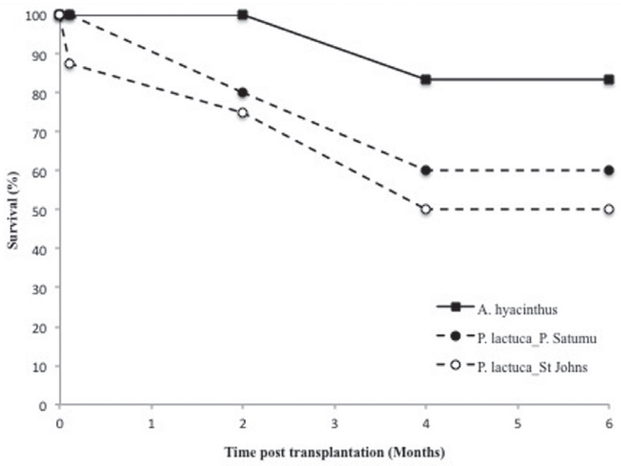

Fig. 4. Post-transplantation survivorship of *A. hyacinthus* and *Pectinia lactuca* donor colonies. Six colonies of *Acropora hyacinthus* and five colonies of *P. lactuca* were transplanted at Pulau Satumu and the remaining eight colonies of *P. lactuca* were transplanted at St John's Island.

(Hunte and Wittenberg, 1992). The establishment of coral mariculture can overcome this problem by facilitating large-scale propagation of corals and mediating their subsequent transplantation of corals (Raymundo, 2001), increasing the survival of the coral transplants used for reef restoration (Gilmour, 2002). In particular, propagation of sexually derived coral juveniles can help to maintain, or even increase, the genetic diversity of the existing coral community and the enhancement of locally endangered populations of coral that do not respond well to fragmentation. The presence of chronic sedimentation, however, tends to restrict the choice of techniques used for gamete collection to *ex situ* due to reduced visibility, and the need for an extended *ex situ* nursery phase to grow corals to a substantially larger size to help them withstand the effects of sedimentation.

In this study, it has been shown that coral larvae would require at least 24 h to complete the entire embryonic developmental stages and thus mechanical agitation during this period of time should be minimal. This reduces physical damage on the embryos, which would otherwise result in developmental abnormalities and associated larval mortality. Based on the settlement rates of both species observed in this study, it is recommended that settlement substrates should be introduced from 36 h to 160 h post fertilization to ensure optimal settlement. The substrates should also be inspected daily for the presence of settled coral spats, and substrates with spats should be replaced to prevent aggregation of spats that could compete for resources such as space. The variation in spawning timings observed in this study has emphasized the need to collect sufficient colonies to maximize rearing success, but has also highlighted the importance of establishing effective transplantation procedures and monitoring regimes to minimise damage to the donor colonies. Clearly, large-scale coral larvae rearing is technically feasible in Singapore, and future research should focus on improving the post-settlement survivorship of the coral spats and exploring avenues to improve the cost effectiveness of the techniques used.

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