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# Direct seeding of mass-cultured coral larvae is not an effective option for reef rehabilitation

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## ABSTRACT

Large scale rearing of coral larvae during mass spawning events and subsequent direct introduction of competent larvae onto denuded reefs (“larval seeding”) has been proposed as a low-tech and affordable way of enhancing coral settlement and hence recovery of degraded reefs. While some studies have shown positive short-term effects on settlement, to date, none have examined the long-term effects of larval seeding for a broadcast spawning coral. Here we test whether larval seeding significantly increases coral recruitment rates both in the short (5 weeks) and longer (~6 mo to 1 year) term. Larvae of *Acropora digitifera* were reared *ex situ* and approx. 1 million larvae were introduced to 7 artificial reefs (ARs) while 7 others were left unseeded. Settlement tiles deployed on both seeded and control ARs were retrieved for examination 5 and 30 weeks after seeding. In addition, the presence of visible coral recruits on the AR surfaces was monitored before and for ~13 months post-seeding. Density of acroporid spat was significantly higher on seeded tiles than controls 5 weeks after seeding but this effect had vanished by 30 weeks. Comparison of the densities of new visible *Acropora* recruits between seeded and control ARs showed no significant difference ~13 months after seeding. Larval seeding therefore had no long term effect due to high post-settlement mortality (which appeared to be density related). Results suggest that reef rehabilitation methods that aim to harness coral sexual reproduction might better focus on rearing juveniles through early post-settlement mortality bottlenecks.

**KEY WORDS:** Larval seeding, Coral reef rehabilitation, Larval rearing, *Acropora digitifera*, Mass spawning, Density-dependent mortality, Palau

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## INTRODUCTION

The role that active reef rehabilitation can play in the recovery of degraded coral reefs systems is contentious. Measures to actively rehabilitate reef ecosystems are expensive (Haisfield et al. 2010) and are not practised at geographical scales relevant to the scales of degradation (i.e., tens to thousands of square kilometres (Wilkinson, 2008)). Proponents of active rehabilitation argue however that more traditional management measures have largely failed and therefore we must explore and research active methods (Rinkevich 2005, 2008). Active reef rehabilitation typically involves artificially increasing the cover of hard corals on denuded reefs by transplanting asexually propagated corals, usually via an intermediate nursery phase using techniques analogous to those of silviculture for reforestation on land (Epstein et al. 2003, Rinkevich 2006, Shafir et al. 2006).

More recently, sexual propagation methods have received increasing attention (see review by Omori & Iwao 2014). The potential advantage of sexual over asexual propagation techniques is that the former should result in much greater genotypic diversity of transplanted corals. In most cases, sexual coral propagation involves careful nursery rearing until corals have attained a “refuge” or “escape” size (i.e. a few cm in diameter) at which a high proportion of transplants will survive to become reproductively mature adults (Omori et al. 2008, Baria et al. 2010, Nakamura et al. 2011, Villanueva et al. 2012, Guest et al. 2014). The ability however to directly enhance larval settlement rates by introducing high densities of competent coral larvae (“larval seeding”) to targeted areas of reef (Babcock & Mundy 1996, Richmond et al. 1997, Heyward et al. 2002, Nonaka et al. 2003, Omori et al. 2004) has led to discussion of whether such techniques could be effective in reef rehabilitation (Richmond 1997, Hatta et al. 2004, Omori & Fujiwara 2004, Amar & Rinkevich 2007, Edwards & Gomez 2007, Edwards 2010, Suzuki et al. 2012, Omori & Iwao 2014).

Previously, Heyward et al. (2002) and Omori et al. (2004) showed that mass rearing of millions of coral larvae from spawning slicks was feasible without laboratory facilities and could be used to enhance natural coral recruitment directly onto reef habitat by 10- to 100-fold, albeit at small spatial scales of several square metres of reef. In those studies millions of coral larvae were raised from embryos in floating culture ponds until competent to settle and then released inside mesh enclosures placed over natural areas of reef (Heyward et al. 2002) or concrete blocks placed on the sea bed (Omori et al. 2004). The attraction of a method that involves directly enhancing coral larval settlement is that it does not require the costly and labour intensive husbandry required to rear corals to a transplantable size (Hatta et al. 2004, Omori 2005, Omori et al. 2008). A potential disadvantage of this approach is that there is little control over the factors affecting early post-settlement mortality. Like many benthic invertebrates (Hunt & Scheibling 1997), broadcast spawning corals have very high mortality rates during the early post-settlement period (Type III survivorship, sensu Deevey 1947), therefore, larval seeding is likely to be successful only if variations in recruitment to the adult population are determined primarily by settlement density rather than the extent of early post-settlement mortality (Hughes 1990). If, on the other hand, the extent of early post-settlement mortality is related to initial settlement density, then methods that aim to enhance settlement alone may have little effect on adult abundance. Furthermore, if only about one in  $10^4$  settled larvae survive to reproduce (Harriott 1985), then releasing tens to hundreds of thousands of larvae onto patches of reef is unlikely to be either an ecologically viable or a cost-effective option for reef rehabilitation.

The primary aim of this study was to test whether supplying high densities of mass cultured coral larvae directly to denuded substrate would have any long-lasting effect on coral recruitment compared to control substrates with only natural supply. To achieve this aim we

compared short-term levels of coral settlement (5 weeks after larval seeding) on tiles attached to artificial reefs and longer term recruitment (after ~6 months to one year) to both tiles and 14 replicate artificial reefs, half of which received high densities of larvae and half of which received ambient larval supply during a mass coral spawning event.

## MATERIALS AND METHODS

### Experimental design

Standardized artificial substrates ('pallet balls', Reef Ball Foundation, Inc.) were used as experimental units to mimic areas of denuded reef and avoid confounding effects of highly variable natural reef substrates on the settlement of corals. Pallet balls are 1.2 x 0.9 m (base diameter x height) concrete structures with exposed embedded limestone aggregates. Each pallet ball is divided into three panels, demarcated by humps produced by the mould used in the construction. The exposed, monitorable surface area of the pallet ball including rim was estimated at 2.94 m<sup>2</sup> (0.98 m<sup>2</sup> per panel). In January 2007, 14 pallet balls were deployed 3-5 m apart on an area with sandy-rubby substrate at 5-8 m depth adjacent to a natural reef at Iou Lukes reef, Palau (7°17.3'N 134°30.0'E). To provide a baseline against which to evaluate the larval seeding experiment in 2008, rates of coral recruitment to tiles and "visible" recruitment (Wallace 1983) to pallet ball surfaces were monitored both in the year before and after the experiment at approximately 6 monthly intervals. Seven of the 14 pallet balls were chosen at random to be treated with high densities of competent *Acropora* larvae in 2008, thus treated and control pallet balls were spatially interspersed on the study reef.

### Larval rearing

The larval seeding experiment was carried out during the April/May mass coral spawning in 2008 using the aquarium facilities of the Palau International Coral Reef Centre (PICRC). Gravid colonies (i.e. containing deeply pigmented oocytes in fractured branches) of *Acropora digitifera* with geometric mean diameters ranging from 18-35 cm were collected from reefs adjacent to the pallet ball site and Uchul a Chei reef (7°13.8'N 134°26.8'E) between 14 and 21 April 2008 from depths of 1-4 m. *A. digitifera* was locally very common, with predictable spawning times, facilitating the larval rearing work. Colonies were removed from the reef by divers using a 2 kg hammer and cold chisel and were transported to land based aquaria in plastic coolers (>50 l) filled with seawater, where they were subsequently maintained in aerated, flow-through seawater tanks (approx. 1000 l). Several colonies were placed together in each tank so that gamete bundles would be immediately mixed if several colonies spawned synchronously. Each evening, water flow and aeration in the tanks was turned off at approx. 17:30 h (approx. 30-40 minutes before sunset), artificial lighting in the aquarium was switched off and colonies were monitored for signs of spawning approximately every 30 min until 22:30 h or until spawning occurred.

*In situ* observations and evidence from sampling indicates that a large mass spawning event occurred on 20 April involving a high proportion of mature *A. digitifera* at Iou Lukes reef, whereas colonies at Uchul a Chei reef spawned predominantly between 22 and 24 April (P. Mumby pers. comm, C. Boch unpublished data). Colonies kept in the tanks at PICRC spawned on 20, 22, 24, or 25 April. Bundle setting was observed between 19:00 and 19:30 h on nights of spawning, colonies were noted to start spawning between 20:20 and 20:50 h and gametes were mixed for fertilisation between 21:00 and 21:30 h. All colonies used for the experiment were returned to the reef post-spawning.

Larval culture methods followed those described by Heyward & Negri (1999). When spawning of all colonies in the tank had finished, buoyant gamete bundles were scooped from

the surface of buckets using plastic cups and transferred to a 100 l polycarbonate fertilisation tank. Oocyte density was then estimated to establish the correct stocking density for the rearing tanks by counting numbers in 10 replicate 15-ml samples under a dissecting microscope. Samples were retained so that estimates of fertilisation success could be made approximately 2 h after mixing gametes. After 1 hour excess sperm were removed by gently scooping buoyant eggs from the water surface and transferring to clean seawater in a 50 l polycarbonate tank; these were immediately transferred to larger rearing tanks (one 1000 l fibre glass aquarium tank and three 4000 l inflatable paddling pools) at densities of not more than 300 larvae l<sup>-1</sup>. Rearing tanks were left static for at least 24 hours after which mild aeration was introduced and partial water changes were carried out periodically. Estimations of larval densities in each tank were done every day by stirring the culture to distribute embryos evenly while taking 10 replicate 50 ml samples and counting the number of larvae present under a dissecting microscope. Settlement competency of larvae was estimated by pipetting approx. 20 larvae into each of three 10-ml culture wells containing filtered sea water and a small chip of crustose coralline algae (approx. 5 x 5 mm) at 12 h and subsequently every 24 h after fertilisation. The number of larvae settled and/or metamorphosed in culture wells or on the chips was counted every 24 h post-fertilisation and the larval seeding experiment was started when >50% of larvae were competent to settle. Due to inadequate shading which led to excessive water temperatures, the first batch of larvae (20 April spawning) being reared in an inflatable paddling pool were lost. Thus only three rearing ponds were available for larval seeding.

Table 1. Numbers of *Acropora digitifera* larvae seeded and mean density of acroporid spat on 3-mo conditioned tiles 5 weeks after seeding.

Pallet ball#	Rearing pond	Date seeded	Approx. # larvae	Spat per 0.1 m <sup>2</sup>
Enhanced 1	2	29/04/2008	200,000	459.8
Enhanced 2	2	29/04/2008	200,000	305.8
Enhanced 3	1	27/04/2008	40,000	119.8
Enhanced 4	1	28/04/2008	40,000	54.6
Enhanced 5	1	28/04/2008	40,000	160.7
Enhanced 6	3	29/04/2008	260,000	158.5
Enhanced 7	3	29/04/2008	260,000	175.4

### Larval seeding

Competent larvae were introduced to the pallet balls on 27, 28 and 29 April (Table 1). Larvae were transferred from rearing tanks to a 50 l plastic cooler just prior to being transported to the study site. To contain larvae around the pallet balls, a hole was cut in the plastic ground-sheets of generic camping tents and the inner mesh tent (polyester NoSeeUm mesh, nominal pore size 250 µm x 250 µm) was placed over each of the 7 treatment pallet balls (Fig. 1). The mesh, similar in size to the 200 µm mesh of a WP2 smaller mesozooplankton sampling net (Anonymous 1968), while possibly not an absolute barrier to coral larvae, particularly older more plastic forms, was expected to largely contain the introduced cohort. The tent base was reinforced and weighted down with a metal 're-bar' base and lengths of rubber hose pipe were placed over the tent frame to make it flexible but durable. The tents were anchored to the substrate using the guy ropes attached to re-bar stakes hammered into the surrounding substrate. Competent larvae of *Acropora digitifera* that had been reared in tanks (see Table 1 for numbers and dates) were transported in the plastic coolers and poured directly into the mesh tents from the deck of a boat by connecting a length of flexible plastic hose to a valve

on the top of each (Heyward et al. 2002). Mesh tents were left over each pallet ball for 24 h before being carefully removed. Seven of the 14 pallet balls were seeded and 7 controls were left untouched. For logistical reasons, no procedural control was done to test the effects of placing the mesh tents over the pallet balls for 24 h but not seeding with larvae.

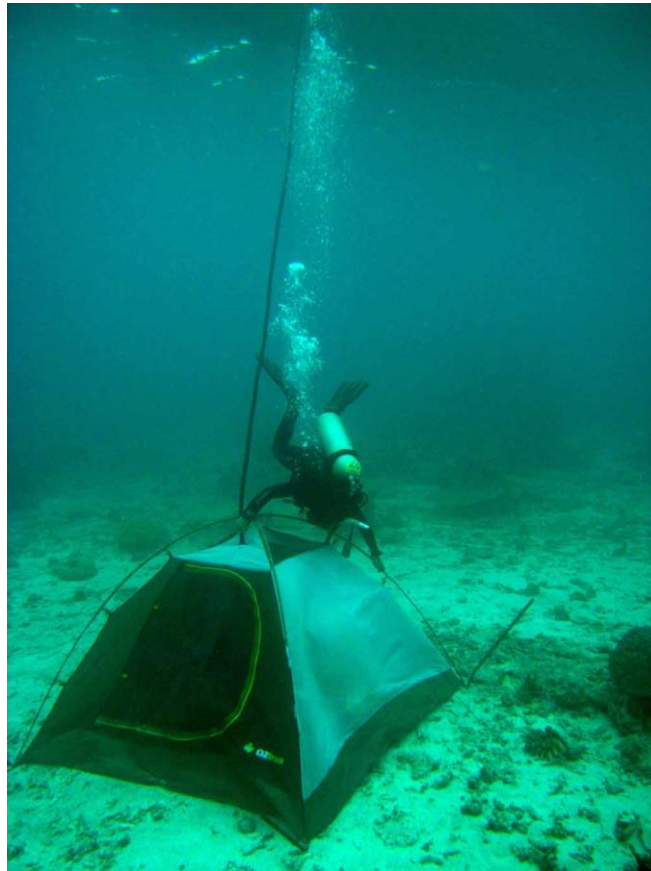


Fig. 1. Diver inspects inner mesh tent used to contain coral larvae around a pallet ball on Iou Lukes Reef, Palau on 28 April 2008. Flexible plastic hose used to introduce high densities of competent *Acropora digitifera* larvae can be seen running to surface.

### Settlement on recruitment tiles

To examine differences in coral settlement between treatments,  $10 \times 10 \times 0.6$  cm fibre cement tiles (Flexboard™) were attached with wing-nuts to stainless steel base plates which were attached to the pallet balls with Panduit™ masonry push plugs (Mundy 2000). On each pallet ball, there were twelve base plates arranged in 3 rows, c. 5, 25, and 45 cm below the pallet ball rim, with 4 plates equally spaced in each row. In mid-January each year from 2007-2009, at least 3 mo prior to expected peak in spawning (week following April full moon: Penland et al. 2004) recruitment tiles were attached to base plates on each pallet ball (Table 2). This allowed tiles to biologically condition (Baird et al. 2003, Webster et al. 2004, Segal et al. 2012) for three months before peak spawning. Six recruitment tiles were attached to base plates on each pallet ball in 2007 and 4 per ball in 2008 and 2009 in the top and middle rows of each pallet ball. These tiles were retrieved 5-6 wk after the annual peak of coral spawning (Table 2). In 2008, a further 4 tiles were attached to base plates on the top and middle rows of each pallet ball 3 mo prior to larval seeding and retrieved 30 wk after seeding. In addition in 2008, to investigate the effect of biological conditioning on coral larval settlement rates, four tiles were attached to base plates on the bottom row in each pallet ball only one week prior to

larval seeding. In three treated and three control pallet balls, these tiles were retrieved one week after treatment, while those on the remaining pallet balls were retrieved 5 wk after treatment. The recruitment of corals to these tiles conditioned for 1 wk was compared to those that had been conditioned for 3 mo in order to discover whether conditioning duration had a significant effect on the number of coral spat that settled. Retrieved tiles were bleached in a 0.5% sodium hypochlorite solution for at least 48 h and then air-dried. Tiles were examined under a stereomicroscope for coral recruits, which were classified as either acroporid or non-acroporid (pocilloporid, poritid, others) using family-specific morphological features of the skeleton as specified in Babcock et al. (2003).

Table 2. Numbers of recruitment tiles deployed on 14 pallet balls (7 of which were seeded with *Acropora digitifera* larvae in April 2008).

Year	Total tiles	Tiles/pallet ball	Time after spawning retrieved
2007	84 (3-mo conditioning)	6	6 wk
2008	56 (3-mo conditioning)	4	5 wk
	56 (1-wk conditioning)	4	1 wk (24 tiles); 5 wk (32 tiles)
	56 (3-mo conditioning)	4	30 wk
2009	56 (3-mo conditioning)	4	6 wk

### Coral recruitment to pallet balls

In addition to examining settlement on tiles, “visible” coral recruitment on the exposed, monitorable surface of two randomly selected panels (total area 1.96 m<sup>2</sup>) of 7 treated and 7 control pallet balls was censused during four occasions – before (November 2007) and after (June, November 2008 and May 2009) larval seeding. The coordinates (angle and distance from a fixed point at the centre of the panel rim) of each recruit were determined using a protractor and tape. This allowed new recruits to be distinguished from ones previously recorded at each monitoring survey and growth and mortality of individual recruits to be followed through time. The identity of each new recruit was determined to the lowest possible taxon, but is reported here as either *Acropora* spp. or non-*Acropora* recruit. The greatest and least diameter of each recruit (and where feasible height) was also measured at each survey.

### Statistical analysis

#### Recruitment to tiles

To avoid pseudo-replication (Hurlbert, 1984), counts from the 4-6 tiles retrieved from each pallet ball were combined to provide a single estimate of density of acroporid and non-acroporid coral spat settling on tiles on each of 7 control and 7 treatment pallet balls. Since the total area available for settlement on tiles on each ball was approx. 0.1 m<sup>2</sup> (0.09 m<sup>2</sup> for 4 and 0.13 m<sup>2</sup> for 6 tiles), densities of recruits are expressed per this unit of area. A few of the fibre-cement tiles were damaged by parrotfish grazing and using counts per unit area allowed data from such tiles to be included. Data were normalised by a logarithmic transformation, which also achieved reasonable homoscedasticity (Levene’s test,  $P > 0.15$ ), prior to parametric analyses (e.g. ANOVA) in Minitab v.16.

#### Recruitment to pallet ball surfaces

Although recruit numbers are expressed per unit area to allow comparison to other studies, analysis was carried out on counts as the same surface area (1.96 m<sup>2</sup>) was surveyed on each pallet ball. Count data were normalised using a square root transformation, which also achieved homoscedasticity (Levene’s test,  $P = 0.947$ ), prior to parametric analyses (e.g. ANOVA) in Minitab v.16.

## RESULTS

### Settlement on tiles

The primary question being addressed by the recruitment tile study was whether the seeding with *Acropora digitifera* larvae in 2008 was effective in terms of initially increasing acroporid settlement. However, since some of the 1-week conditioned tiles were retrieved one week and some five weeks after larval seeding, we first checked to see if the 4-week difference in retrieval time had affected acroporid spat densities recorded on these tiles. An analysis of variance using a general linear model with treatment and retrieval time as factors showed no significant effect of the 4-week difference in retrieval time ( $F_{1,11} = 2.08$ ,  $P=0.177$ ) so we were able to pool data from the 1-wk conditioned tiles in the subsequent analysis. Comparison between treated and control pallet balls of the densities of acroporid coral spat on both 1-wk and 3-mo conditioned tiles (Fig. 2) showed significantly increased settlement on seeded tiles in both cases ( $P=0.017$  and  $P=0.002$  respectively; two-sample  $t$ -test on log-transformed data, variances not assumed to be equal). Mean density of acroporids was 7.7 times higher on 1-wk conditioned tiles from seeded pallet balls compared to those retrieved from controls (mean = 28.6 vs. 3.7 spat per 0.1 m<sup>2</sup>, respectively), whereas it was 4.1 times higher on 3-mo conditioned tiles (mean = 204.9 vs. 50.4 spat per 0.1 m<sup>2</sup>, respectively). A two-way analysis of variance showed that both conditioning (3-mo vs. 1-wk) and treatment (larval seeding) had a significant effect on acroporid settlement (conditioning:  $F_{1,24} = 68.20$ ,  $P<0.001$ ; treatment:  $F_{1,24} = 25.02$ ,  $P<0.001$ ) but that there was no significant interaction ( $F_{1,24} = 0.44$ ,  $P=0.515$ ). Tiles conditioned for 3 months had on average from 7.2 times (seeded tiles) to 13.6 times (controls) the density of acroporid spat as those conditioned for only one week.

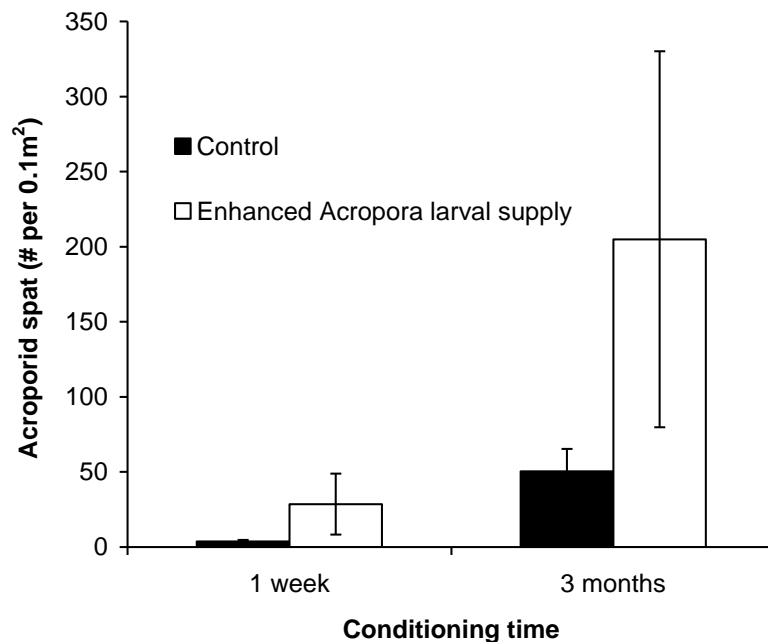


Fig. 2. Effect of larval seeding and conditioning time on mean density ( $\pm 95\%$  CI) of acroporid coral larvae settling on tiles deployed on 7 control pallet balls and 7 pallet balls treated with high densities of mass cultured *Acropora digitifera* larvae in 2008. 4 tiles were deployed on each pallet ball for each level of conditioning.

To allow assessment of the 2008 experiment in the context of the natural variability of coral recruitment after mass-spawning events at the Palau study site, settlement on tiles was also monitored in 2007 and 2009 (Fig. 3). The preliminary study in 2007 established that there was



substantial natural settlement of acroporid larvae (mean density on tiles = 71 spat per 0.1 m<sup>2</sup> or ~16 spat per tile) on 3-mo conditioned tiles with the mean density of all coral spat being 118 per 0.1 m<sup>2</sup>. This latter figure compares to an average of 175 coral spat 0.1 m<sup>-2</sup> yr<sup>-1</sup> (range 15-459 coral spat 0.1 m<sup>-2</sup> yr<sup>-1</sup>) from several studies using settlement plates on the Great Barrier Reef compiled by Glassom et al. (2004: see their Table 6). 78.5% of acroporid spat were found on cryptic lower surfaces of the tiles, 11.9% on the edges and only 9.6% on the exposed outer surfaces. Over the three year study, on control pallet balls (with only natural larval supply), the density of acroporid spat on 3-mo conditioned tiles collected 5-6 wk after peak spawning varied significantly among years (ANOVA,  $F_{2,18} = 29.38$ ,  $P < 0.001$ ), with Tukey pairwise comparisons showing that settlement in 2007 > 2008 > 2009. Among years densities ranged from a mean of 23.1 to 76.6 spat per 0.1 m<sup>2</sup>.

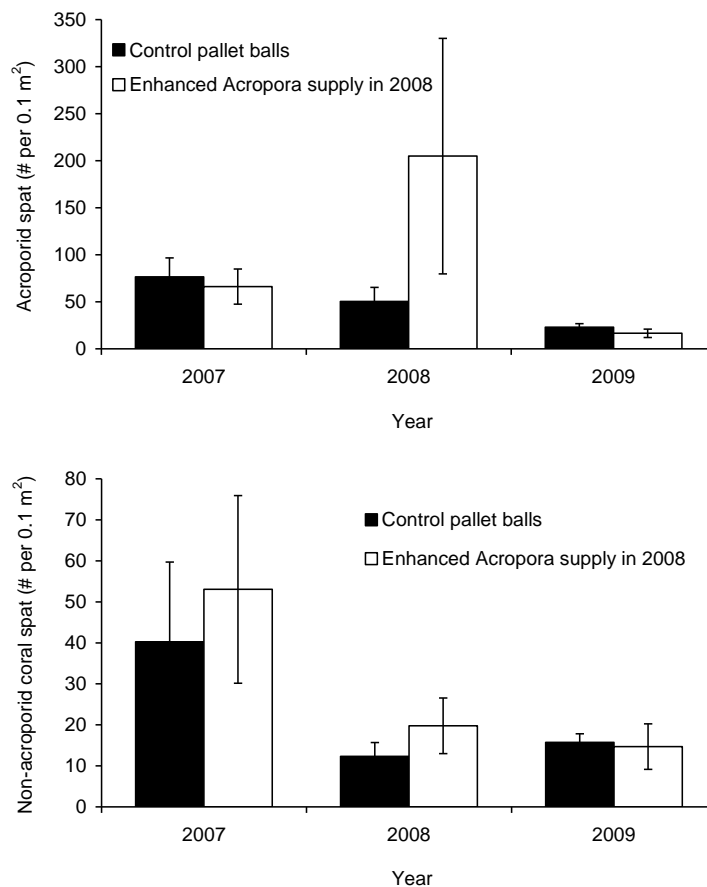


Fig. 3. Mean density ( $\pm 95\%$  CI) of acroporid and non-acroporid coral spat on 3-mo conditioned tiles deployed on 7 control pallet balls and 7 pallet balls treated with high densities of mass cultured *Acropora digitifera* larvae in 2008. 6 tiles were examined per pallet ball in 2007 and 4 tiles per ball in 2008 and 2009. Tiles were retrieved 5-6 wk after peak spawning in each year.

For tiles on those pallet balls that received an enhanced supply of *A. digitifera* recruits in 2008, mean acroporid spat density per 0.1 m<sup>2</sup> was 66.6 in 2007 and 16.6 in 2009, compared to a mean of 204.9 spat per 0.1 m<sup>2</sup> when seeded in 2008. Thus seeding led to 3.1-12.3 times more settlement than in years with only natural settlement. There was similar inter-annual variability in the density of non-acroporid coral spat with mean density ranging from 12.3 to 53.0 spat per 0.1 m<sup>2</sup> for control and treatment pallet balls among years. Settlement again varied significantly among years (ANOVA,  $F_{2,39} = 25.85$ ,  $P < 0.001$ ) and Tukey pairwise

comparisons showed that non-acroporid settlement in 2007 was also significantly higher than in the other two years (which were not significantly different).

To rule out the possibility of bias in settlement on control or treatment pallet balls due to hydrological or other confounding factors, spat densities on tiles on control and treated pallet balls were compared for acroporids in 2007 and 2009 (when there was no larval seeding) and for non-acroporids in all years (as their settlement should not have been affected by the *A. digitifera* seeding). Because of the significant inter-annual variability in natural settlement shown above (Fig. 3), comparisons between tiles from treated and control pallet balls were conducted using nested ANOVA (treatment nested within years). This indicated no significant difference in coral spat densities on tiles between treated and control group pallet balls within years (acroporids:  $F_{2,24} = 3.10$ ,  $P > 0.05$ ; non-acroporids,  $F_{3,36} = 2.03$ ,  $P > 0.05$ ). That is, only for acroporid settlement in 2008 was any treatment effect observed, ruling out any settlement bias due to confounding factors.

Tiles (conditioned for 3 mo) were retrieved from pallet balls 5 weeks and 30 weeks after larval seeding in 2008. From mean acroporid spat densities of 204.9 spat per 0.1 m<sup>2</sup> (treated) and 50.4 spat 0.1 per m<sup>2</sup> (controls) on tiles retrieved 5 weeks after enhancement, spat densities fell to 59.7 and 32.9 spat per 0.1 m<sup>2</sup> respectively by 30 weeks (Fig. 4). By this time, acroporid spat densities on treated and control tiles were no longer significantly different (two-sample *t*-test,  $P = 0.137$ ). The decline in acroporid spat density over ~6 months was highly significant on tiles from the treated pallet balls (two-sample *t*-test,  $P = 0.006$ ), but that on the controls was not ( $P = 0.127$ ). Non-acroporid spat densities also showed no significant change over the same period (two-sample *t*-test,  $P = 0.497$  controls;  $P = 0.614$  treated;  $P = 0.874$  for all tiles), averaging 16.0 and 16.7 spat per 0.1 m<sup>2</sup> at 5 wk and 30 wk respectively. The inferred mean “survival” rate (persistence of 5-week acroporid corallites assuming negligible additional settlement) on tiles from control pallet balls was 71% whereas that on the tiles from treated pallet balls was only 31%. Comparison of numbers of acroporid spat on lower cryptic and exposed outer surfaces of tiles at 5 wk and 30 wk, using a two-way contingency table, showed no significant difference in ‘survival’ rate between the two surfaces for tiles from both seeded ( $X^2 = 0.548$ ,  $P = 0.459$ ) and control ( $X^2 = 0.087$ ,  $P = 0.768$ ) pallet balls.

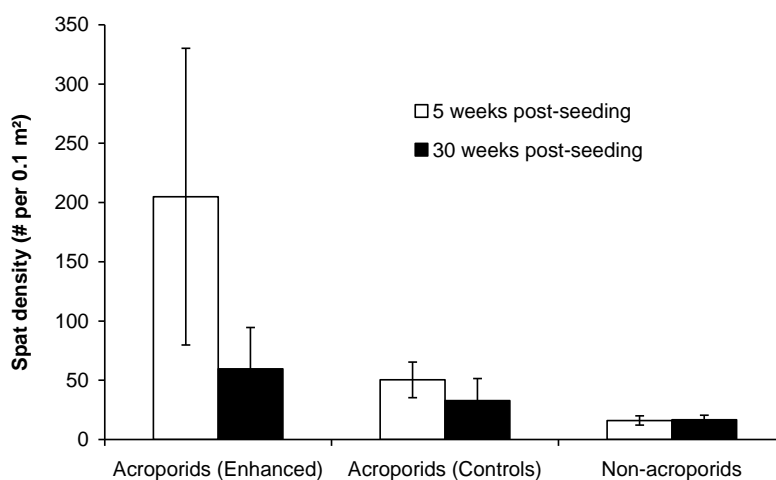


Fig. 4. Mean density ( $\pm 95\%$  CI) of acroporid and non-acroporid coral larval spat on 3-mo conditioned tiles between 5 wk and 30 wk post-seeding in April 2008. Densities of acroporid recruits on tiles deployed on 7 pallet balls seeded with high densities of mass cultured *Acropora digitifera* larvae in 2008 (enhanced) are compared with those on 7 pallet balls with only natural larval supply (controls). In addition, densities of non-acroporid recruits on tiles from all 14 pallet balls are shown. 4 tiles were retrieved off each pallet ball 5 wk and 30 wk after larval seeding.

To investigate further the relationship between acroporid density at 30 weeks post-seeding and that at 5 weeks, acroporid spat densities on tiles ( $n=4$  per pallet ball) on each of 7 control and 7 treatment pallet balls are compared in Figure 5. The dashed line shows the 30 week densities that would be expected if “survival” was density-independent and equal to the mean acroporid “survival” rate (71%) on control tiles with natural levels of settlement ( $H_1$ ). The divergence from this model and significant fit ( $P<0.05$ ) to a quadratic model ( $H_2$  - based on linear “density-dependent” mortality: *sensu* Holm 1990) suggests that “mortality” had a density-related – see discussion of terminology in Sale and Tolimieri (2000) – component at the higher settlement densities achieved on tiles attached to the pallet balls subject to larval seeding.

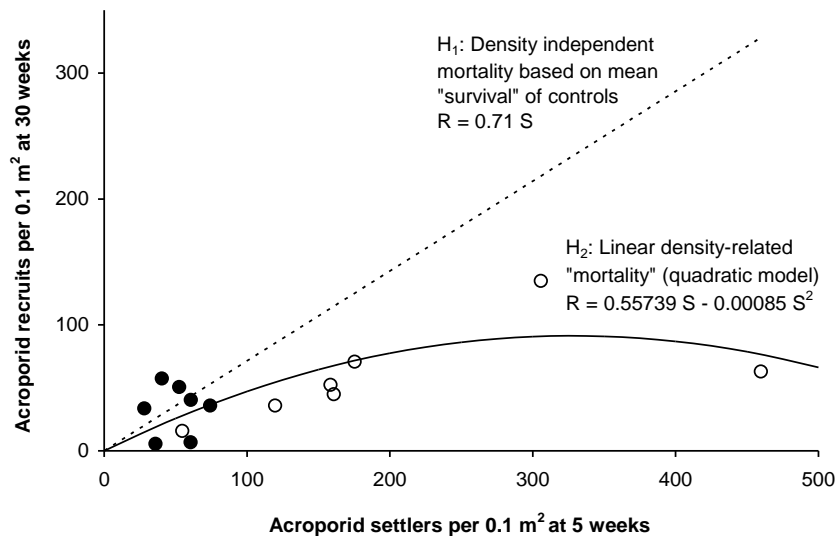


Fig. 5. Comparison of mean densities of acroporid spat on tiles ( $n=4$  per pallet ball at each time) retrieved 5 wk and 30 wk post-seeding on each of 14 pallet balls. The dashed line ( $H_1$ ) shows the expected densities at 30 wk if net losses are density-independent and equal to 1 – the inferred mean “survival” rate of acroporid spat which settled at natural densities on the 7 control pallet balls (71% over 25 wk). The black curve is the line of best fit based on a quadratic model ( $H_2$ ) under the assumption of linear density-related mortality (Holm 1990). (Black circles – controls; white circles – treated pallet balls; R = recruits at 30 weeks; S = settlers at 5 weeks.)

### Visible recruitment to pallet balls

The primary question being addressed by the study of “visible” recruitment (i.e. monitoring of later-stage recruits visible to the naked eye in the field: see Wallace 1983) to the pallet balls was whether the larval seeding had a longer term effect. The mean density of live coral recruits (both *Acropora* and non-*Acropora*) on pallet ball surfaces rose from  $11.2 \text{ m}^{-2}$  in November 2007 (~10 mo after deployment of the pallet balls) to  $42.7 \text{ m}^{-2}$  in May 2009 (~28 mo after deployment). The smallest visible recruits recorded had a greatest diameter of 2 mm and over 95% were  $<25$  mm in geometric mean diameter (GMD) when first recorded in the approximately half-yearly surveys. The average GMD of recruits when first recorded was 11.2 mm (SD  $\pm 7.6$  mm, median 9.5 mm, interquartile range 6.5-13.4 mm,  $n=1638$ ). The proportion of recruits on the pallet ball surfaces that were *Acropora* spp. ranged from 18-30% among surveys and mean densities of live *Acropora* on the 14 pallet balls rose from  $1.9 \text{ m}^{-2}$  to  $12.0 \text{ m}^{-2}$  from November 2007 until May 2009. Approximately 44% of *Acropora* recruits recorded at the first survey in November 2007 were still alive 1.5 years later.

For the purposes of this study, what we were interested in was whether recruitment was enhanced on those pallet balls seeded with large numbers of *A. digitifera* larvae in April 2008.

That is, would there be significantly more new *Acropora* recruits on treated pallet balls surveyed in November 2008 or May 2009 (~7 mo and ~12.7 mo after seeding) than on control pallet balls? (The June 2008 survey would be too early to see ‘visible’ recruits from the April seeding.)

Between *Acropora* mass-spawning in April 2007 and the survey in June 2008, visible *Acropora* recruits reached a median GMD of 14.8 mm (interquartile range 9.9-18.7 mm), whereas between the mass-spawning in April 2008 and the survey in May 2009, new (first recorded after June 2008) visible *Acropora* recruits reached a median GMD of 15.5 mm (interquartile range 9.5-26.2 mm). Although a total of 53 live *Acropora* recruits from the April 2007 mass-spawning were visible by November 2007 (~7 mo later), the November 2008 survey (~7 mo after seeding) might have been too early to detect effects of enhancement but by May 2009 (~12.7 mo after seeding) any significant effect should have been clearly detectable.

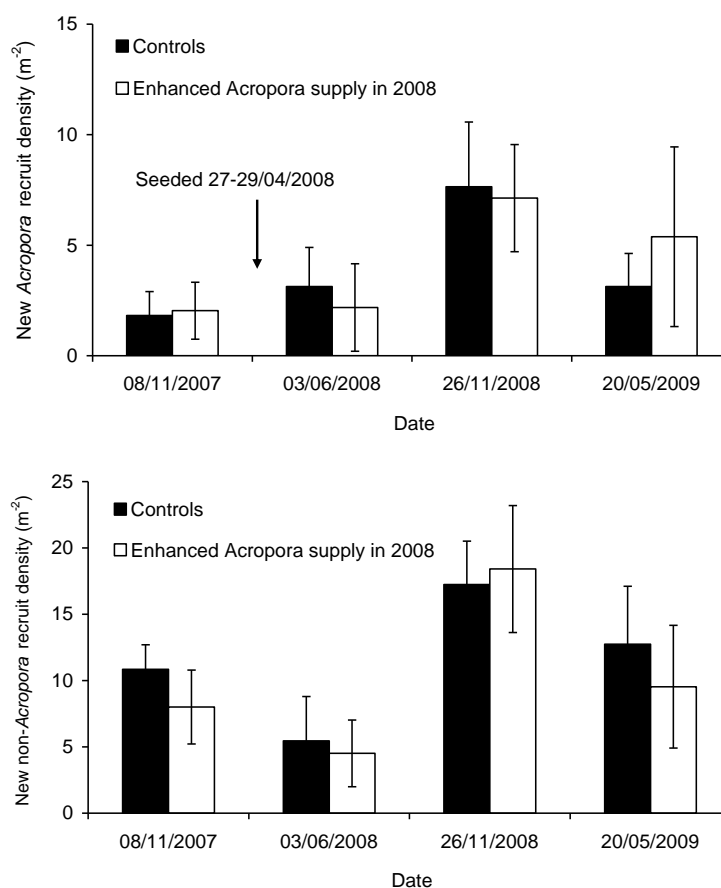


Fig. 6. Comparison of mean density ( $\pm 95\%$  CI) of new *Acropora* spp. and non-*Acropora* coral recruits visible on surfaces of 7 control pallet balls and 7 pallet balls seeded with high densities of mass cultured *Acropora digitifera* larvae in 2008.

The mean density of new “visible” *Acropora* recruits on pallet balls ranged from  $1.9 \text{ m}^{-2}$  to  $7.4 \text{ m}^{-2}$  for the four ~6 monthly surveys (Fig. 6). Combining data on new *Acropora* recruits ( $n=320$ ) from the two post-seeding surveys (within 13 mo of seeding), the mean density of new *Acropora* recruits was  $12.5 \text{ m}^{-2}$  ( $95\% \text{ CI} \pm 5.59$ ) on treated vs  $10.8 \text{ m}^{-2}$  ( $95\% \text{ CI} \pm 3.11$ ) on control pallet balls, with no evidence for significantly greater densities of new *Acropora* recruits on treated pallet balls (one-tailed two-sample *t*-test:  $P=0.273$ ). At the final survey in May 2009 (Fig. 6) the mean density of new *Acropora* recruits ( $n=117$ ) was  $5.4 \text{ m}^{-2}$  ( $95\% \text{ CI} \pm$

4.06) on treated vs  $3.1 \text{ m}^{-2}$  (95% CI  $\pm 1.50$ ) on control pallet balls, and differences were again not significant (one-tailed two-sample  $t$ -test:  $P=0.119$ ).

Although there was no significant increase in visible recruitment to pallet ball surfaces as a result of the larval seeding, analysis of all surveys using a one-way ANOVA showed significant differences among surveys in recruitment of both *Acropora* and non-*Acropora* juveniles ( $F_{3,52} = 12.77$ ,  $P<0.001$ ;  $F_{3,52} = 21.78$ ,  $P<0.001$ , respectively) with Tukey pairwise comparisons showing significantly greater densities of both new *Acropora* and new non-*Acropora* recruits in November 2008 than at other surveys (Fig. 6).

## DISCUSSION

Despite its potential as a method for elucidating the role of larval recruitment in coral population dynamics (Heyward et al. 2002) and as a means of enhancing recovery of coral cover on degraded reefs (Amar & Rinkevich 2007), few attempts have been made to artificially manipulate natural larval settlement rates on coral reefs (Suzuki et al. 2012, Omori & Iwao 2014). The paucity of such studies may in part have been due to the lack, until recently, of reliable methods for rearing large numbers of broadcast spawned larvae ex situ and delivering competent larvae to areas of reef. In this study we reared approximately one million coral larvae using low tech methods (i.e. coral larvae reared in plastic paddling pools) and successfully delivered these to replicate artificial reefs in situ. Heyward et al. (2002) found great variability in initial settlement on tiles retrieved 6 weeks after seeding with larvae reared in situ in floating ponds utilising natural *Acropora* spawning slicks. Six weeks after seeding they achieved coral spat densities on treated tiles that ranged from 100-1000 times the low natural background levels of settlement (only  $\sim 1$  spat per  $0.1 \text{ m}^2$ ) at their study site at Coral Bay in Western Australia but did not report the long term effects of the larval seeding. The background levels of coral larval settlement at our site in Palau were within the range recorded from sites on the Great Barrier Reef (Glassom et al. 2004) but approximately 500 times those at the Coral Bay, Ningaloo Reef site of Heyward et al. (2002). Thus scope for enhanced settlement was greatly reduced. Indeed, we achieved only about a fourfold increase in settlement compared to controls with only natural larval supply on the 3-mo conditioned tiles, despite achieving similar settlement rates to them on seeded tiles.

Webster et al. (2004) using biofilms of different ages developed on glass slides and larvae of *Acropora microphthalma* showed that 2-week-old biofilms induced metamorphosis in less than 10% of coral larvae but that 41% were induced to metamorphose when exposed to 8-week-old microbial films. As part of our study, we compared settlement of acroporid larvae on tiles with 1-week-old and 3-month-old biofilms. This showed an even larger relative difference with  $\sim 14$  times greater settlement on the 3-mo biofilms at natural larval densities (tiles on control pallet balls), reducing to  $\sim 7$  times greater settlement on 3-mo biofilms on tiles with an enhanced larval supply. The halving of the conditioning effect on the seeded tiles suggests that the limited biofilm development on the 1-week conditioned tiles was partially mitigated at high larval densities. This study reinforces the importance of controlling the biological conditioning of settlement tiles or other artificial substrates used in recruitment studies (Heyward et al. 2002, Baird et al. 2003, Webster et al. 2004, Segal et al. 2012).

In our study, the larval seeding appeared initially successful with acroporid settlement densities  $\sim 4$  times higher (3-mo conditioning) to  $\sim 8$  times higher (1-wk conditioning) on tiles from seeded pallet balls compared to those from controls with only natural larval supply. However, after 30 weeks there was no significant difference in acroporid spat densities between tiles from seeded and control pallet balls. Furthermore, monitoring of new visible *Acropora* recruits on the pallet ball surfaces showed no significant augmentation of numbers

on seeded ones compared to controls in the 13 months after larval seeding. One possible explanation for the lack of any detectable difference in the number of visible recruits on control versus seeded pallet balls is that early post-settlement mortality is related to initial settlement density.

Newly settled corals are known to be extremely vulnerable and corals, like many marine invertebrates (Hunt & Scheibling 1997), have typical Type III survivorship curves (sensu Deevey, 1947), with high early mortality and a greater probability of survival with increasing age and size (Vermeij & Sandin 2008). Several factors are involved in early post-settlement mortality including competitive interactions with macroalgae (McCook et al. 2001, Birrell et al. 2008, Tebben et al. 2014), sedimentation (Fabricius et al. 2003), and accidental removal by grazing fish or direct predation (Sammarco & Carleton 1981, Rylaarsdam 1983, Vermeij 2006, Ritson-Williams et al. 2009, Baria et al. 2010, Traçon et al. 2013). Less is known about the role of initial spat density in later survival (Vermeij & Sandin 2008) although Suzuki et al. (2012) found that larval survival rates were significantly lower on specially designed plates seeded with high densities of *Acropora* larvae than those seeded with low or medium densities.

The decline in spat densities on tiles between 5 weeks and 30 weeks after larval seeding shows that early stage corallites were not a permanent record of settlement. On the one hand, if a coral recruit died soon after laying down skeleton then the skeleton may have dissolved or been eroded (e.g. by sponges) over the ~6 months between surveys. On the other hand grazing by fish or echinoids may have removed corallites among the 21.5% of spat on tiles that were accessible to such grazers (Hutchings 1986). The relationship between recruits present at 30 weeks and settlers at 5 weeks can be expressed as follows:

$$\begin{aligned} \# \text{ Recruits (30 wk)} &= \# \text{ Settlers (5 wk)} - \# \text{ Losses (Predation|Erosion|Dissolution)} \\ &+ \# \text{ New settlers} \end{aligned}$$

From this equation, it can be seen that interpretation of the decline in acroporid coral skeleton densities (Figs. 4 and 5) between tiles collected at 5 weeks and 30 weeks requires caution. We do not know whether coral spat are definitely alive or dead when tiles are retrieved. However, if we make the reasonable assumptions that (1) the processes of loss of spat via predation, erosion or dissolution, and (2) the rate of increase by new settlement are unbiased among tiles on enhanced and control pallet balls, then any differences in relative persistence of coral skeletons should be proportional to survival. Thus, the data may be treated as a proxy for post-settlement mortality processes. For broadcast spawning acroporids, additional settlement between May and November was expected to be relatively low (Penland et al. 2004) and thus processes leading to losses of coral skeletons were expected to dominate. For non-acroporids, one might expect monthly contributions of new spat from planulating pocilloporids (~15% settlers), which may explain the absence of any net decline in non-acroporid spat numbers between 5 and 30 weeks. Although the study was not designed specifically to look at density-related mortality, the inferred “survival” of 30-wk recruits as a function of 5-wk settlers (Fig. 5) appears more consistent with linear density-related mortality (Holm 1990), than density-independent mortality at the rate observed on tiles subject to natural levels of larval supply. Holm (1990) shows that if mortality is linearly related to settler density then one might expect a parabolic curve (Fig. 5) to describe the relationship between recruits surviving and initial settlers. We have no clues as to the cause of this mortality, but the lack of any difference in inferred “survival” rates between exposed outer surfaces and cryptic lower surfaces of tiles suggests that it was not grazing.

In their study in the Ryukyus, Suzuki et al. (2012) showed that only one month after settlement *Acropora* larval survival rates at high densities were already significantly lower

than those at medium and low densities whereas our inferred poor “survival” was not apparent until 30 weeks post-enhancement. In their study they collected L-shaped pieces of their grid plates which were transferred to the laboratory submerged in seawater. Only live corals were then counted whereas we were counting coral skeletons. If the time course of post-settlement mortality in Palau was similar to that recorded by Suzuki et al. (2012), at 5 weeks many (or even the majority) of our acroporid spat may already have been dead but the skeletal record of their settlement was still intact. By 30 weeks however, these corallites were lost due to erosion or dissolution with the first to die (smallest) presumably being the most vulnerable to loss. This again illustrates the caution that is needed in interpreting settlement tile data where tiles are bleached and dried before examination. Also, while we infer that there was density-related mortality of *Acropora* spat on the tiles and that the same process could account for the lack of any detectable increase in recruitment to the pallet ball surfaces in the ~13 months post-seeding, we do not know that post-settlement mortality processes on the pallet ball surfaces mirrored those on the tiles.

Without true replication of the different larval densities we were unable to make a valid analysis of the relationship between density of spat settled and number of larvae supplied (Table 1), however, the data appear to be in accord with the findings of Suzuki et al. (2012) that intermediate larval densities are optimal for larval seeding.

For reef rehabilitation to have impacts at meaningful scales, it needs to be low-tech and affordable. Here we show that with care large numbers of larvae can be reared through to competency and directly released onto areas of reef (in this study, artificial reefs). The method was successful in significantly enhancing settlement in the short term; however, there was no detectable effect on recruitment after 13 months. While the idea of directly seeding areas of reef with competent larvae is appealing because of the relatively low investment required, very high levels of post-settlement mortality represent a formidable challenge. Survival rates might have been higher using different species or more complex substrate types (Suzuki et al. 2011), but low cost, high volume methods to remove the early post-settlement, high mortality bottleneck on reef substrates are yet to be demonstrated. In the present study, natural interannual variation in recruitment of *Acropora* and non-*Acropora* juveniles to pallet ball surfaces exceeded the insignificant differences in recruitment between seeded and control pallet balls. For seeding to justify the costs involved and be a practical proposition for reef rehabilitation it would need not only to have a significant long-term effect but also compete with alternative techniques such as sexual (Omori et al. 2008, Baria et al. 2010, Nakamura et al. 2011, Villanueva et al. 2012, Guest et al. 2014) and asexual nursery rearing (Epstein et al. 2003, Rinkevich 2006, Shafir et al. 2006). At present, we find no evidence that larval seeding succeeds on either count but accept that it could possibly still have a role at sites with very low background recruitment such as the Ningaloo study site of Heyward et al. (2002). Even at such sites, where the reef is actually degraded and with high seaweed cover (like many reefs in need of rehabilitation) settlement may be inhibited by certain species of turf and macroalgae (e.g. Birrell et al. 2005, Kuffner et al. 2006). Further, Dixson et al. (2014) have recently shown that on such reefs coral larvae may be deterred, possibly by chemical cues, from even attempting to settle, thus simply supplying larvae may be futile.

If larval rearing methods are going to be used to rehabilitate ecologically significant areas of reef then it will require more investment in husbandry during the early life stages to overcome the bottleneck of post-settlement mortality. These methods should allow large numbers (at least 10,000s) of spat to be reared from millions of larvae to a “refuge size” (size beyond which survival dramatically improves) before being transplanted to degraded reefs. Working with planulae from the brooding coral *Pocillopora damicornis*, Raymundo & Maypa (2004) showed markedly better one-year survival (47.5%) of 10.1-29 mm juveniles compared to 6.1-

10.0 mm juveniles (16.3%) outplanted from aquaria to the reef, with no survivors found from a cohort of  $\leq 3$  mm colonies. Similarly, Guest et al (2014) found that rearing *Acropora millepora* from settlement for 19 months before transplanting to the reef led to overall greater survival and significantly lower costs compared to rearing corals for just 7 and 14 months. Determining the optimal economic size at which to outplant reared corals of different species remains a key challenge for rehabilitation research.

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