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Final report

Small research and development activity

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1 Acknowledgments

We thank ACIAR for essential project funding and associated support, and the staff and research students at the Bolinao Marine Laboratory, Marine Science Institute, University of the Philippines for research and logistical support. Thanks also to the many volunteers who supported the research for this project.

We note with great sadness the passing of our colleague Dr Ronald Villanueva shortly after this project was completed.

2 Executive summary

Coral reefs are among the most globally important marine ecosystems, having immense economic and ecological values and supplying fisheries and other essential resources to millions of people. The key problem is that coral reefs are facing intensifying anthropogenic impacts, and the crucial reef-building coral populations that provide the foundation of reefs are declining in most tropical reef regions including the Philippines. Restoring coral populations is therefore essential for reef recovery, but has not been successful at the larger reefal scales needed to restore essential goods and services. Therefore, the **aim** of this project was to quantify the effectiveness of using mass larval 'reseeding' to initiate restoration of damaged coral communities on reefs in the northern Philippines. The specific **objectives** were to: 1. Determine peak coral spawning periods, capture coral spawn and rear millions of coral larvae, 2. Reseed damaged reef areas via mass larval settlement, 3. Quantify larval settlement and recruitment, 4. Quantify juvenile coral growth and survival to assess the effectiveness of mass larval reseeding, 5. Use the results to develop guidelines and technical reports and peer-reviewed publications.

The project has achieved its aim and main objectives and has provided globally significant results. We have substantially increased knowledge of the main coral spawning periods for 16 species in the Bolinao-Anda reef region, northern Luzon. Major coral spawning periods occur from February to July each year, significantly extending the known period when mass coral larval rearing can occur for experiments and larval settlement on damaged reefs. Large multispecific coral spawning was recorded *in situ* during March 2015 enabling larger scale larval settlement in future. Experiments showed optimal fertilization requires sperm densities of $\geq 10^4$ ml^{-1}, and combining gametes from different colonies shortly after spawning is important for maximising fertilization success. Millions of coral larvae from multiple species were successfully reared at Bolinao Marine Laboratory, and experiments demonstrated that tiles made from natural dead coral skeletons are more effective for monitoring coral larval settlement and survival than artificial tile substrata.

Mass larval enhancement ('reseeding') trials were successfully completed on degraded reef sites at Magsaysay reef, Anda where the coral community had been destroyed by blast fishing etc. In 2013, ~1.6 million *Acropora tenuis* coral larvae were deployed in low-cost mesh enclosures resulting in high settlement rates (27±6.1 SE spat/tile) in larval settlement sites, whereas no corals settled in control sites. Recruit survival showed the expected pattern of initial post-settlement mortality, however, there was 100% survival on both recruitment tiles and natural reef substrata from 9 months to 2 years. Mean numbers of surviving corals on natural reef substrata and tiles after 2 years were 24±12 and 5±1.6, respectively. Growth of recruits significantly increased over time, and growth rates were comparable on natural reef substrata and tiles. In 2014, ~900,000 *Acropora granulosa* larvae were used to 'reseed' other nearby reef sites resulting in similar high settlement rates (25±13 spat/tile), with no recruits in control sites. Recruit survival subsequently decreased, while surviving juveniles grew to visible size at 8 mo. Monitoring showed very low natural recruitment rates especially of branching *Acropora*, indicating that these reef sites will have very slow rates of recovery without active intervention. These outcomes clearly demonstrate that coral recruitment can be significantly enhanced using mass larval settlement to rapidly initiate coral population restoration and fast growth even on degraded reef areas. High survival of juvenile corals after 9 months shows that mass larval enhancement is a viable and effective active restoration option for initiating coral population recovery where natural recruitment is limited. The success of the low-cost mass larval settlement techniques demonstrated in this project will enable this approach to be used in initiating coral restoration in a wide range of other degraded reefs in the Philippines and around the world. It is recommended that expanded larval enhancement trials be used to initiate reef restoration at larger reef scales in future, further build research training and capacity, and examine socio-economic impacts of reef restoration.

3 Introduction and background

Coral reef importance and global decline

Coral reefs are internationally recognised as globally important marine ecosystems due their immense ecological, socio-economic and cultural values, and are worth $billions to national economies (reviewed by Birkeland 1997, Harrison and Booth 2007, Wilkinson 2008). These reefs are highly productive centres of marine biodiversity that supply essential fisheries and other resources and ecosystem services to hundreds of millions of people in tropical coastal communities (Burke et al. 2011, de Groot et al. 2012). A key problem is that coral reef ecosystems are highly sensitive to disturbance and are declining in many reef regions around the world (Bruno and Selig 2007, Wilkinson 2008, Burke et al. 2011, De'ath et al. 2012, Jackson et al. 2014). More than 60% of the world's coral reefs are under immediate and direct threats from human activities including overfishing, destructive fishing, coastal development and associated pollution impacts (Wilkinson 2008, Burke et al. 2011). Furthermore, nearly 95% of coral reefs in SE Asia are threatened and these threats are increasing (Burke et al. 2011).

The Philippines' coral reefs form part of the Coral Triangle that has the highest diversity of corals, fish and other reef biota in the world (Carpenter et al. 2011). However, the Philippines is characterised by having about 1.4 million ha of degraded reefs from a total of about 2.6 million ha (Wilkinson 2008). This represents the world's second largest area of threatened coral reefs behind Indonesia, with a high proportion of reefs in the high or very high threat categories (Burke et al. 2011), so further declines and loss of reefs in the Philippines are likely to occur in future. When coral reefs are degraded, fisheries production and other goods and services decline or are lost and therefore their economic values diminish in proportion to the loss of resources and ecosystem function. Given that average total ecosystem values of healthy coral reefs are estimated to be worth about US$350,000/ha/yr (de Groot et al. 2012), the costs of the reef degradation are immense. Key threats to these coral reefs are destructive fishing and overfishing, watershed-based pollution and ongoing coastal development arising from increasing coastal populations (Wilkinson 2008, Burke et al. 2011). Reef fish and other fish provide a major part of the diet for many Philippine communities and blast and poison fishing and other forms of unsustainable fishing have resulted in reef degradation in many areas (Alcala and Russ 2006, Nanola et al. 2011). Blast and poison fishing have been halted in some reef areas such as the Northern Luzon region, which is important as this makes it feasible to initiate coral and reef restoration in this region. In addition, increasing numbers of locally managed marine protected areas (MPAs) are being developed to support reef recovery (Alcala and Russ 2006, Nanola et al. 2011).

Importance of reef-building corals as foundation species

Reef-building scleractinian corals are foundation species on reefs and build the complex three-dimensional reef structure and form essential habitats for many thousands of other species including important fisheries species (Harrison and Booth 2007). Successful reproduction by these corals enables natural recovery of damaged reefs over time, thereby enhancing their resilience (Harrison and Wallace 1990, Richmond 1997, Harrison 2011). However, corals have limited tolerance to environmental changes and pollution stressors hence these foundation species and the reefs they create are particularly vulnerable to chronic human disturbances and climate and ocean change (Hoegh-Guldberg et al. 2007, Knowlton and Jackson 2008, Pandolfi et al. 2011). Periodic mass mortality or chronic declines in coral communities can result in coral-dominated reef systems undergoing phase shifts to algal-dominated systems (Knowlton and Jackson 2008, Pandolfi et al. 2011). The collapse of healthy coral reef ecosystems creates significant problems for local communities through loss of essential food and other

resources, reduced economic and other values (Birkeland 1997, Burke et al. 2011, de Groot et al. 2012), and natural reef recovery can take many decades (Edwards and Gomez 2007, Hoegh-Guldberg et al. 2007).

The global decline in coral reefs and periodic catastrophic loss of corals due to mass bleaching and other mortality events has become a major focus for coral reef research and management around the world, and intense public and scientific concern (e.g. Hoegh-Guldberg 1999, 2004, Hughes et al. 2003, Burke et al. 2011). A number of international and national coral reef initiatives have developed to increase our understanding of coral reef responses to climate change and coastal habitat disturbances, in order to better manage these essential resources in future. However, the rate of natural recruitment and regeneration on many reefs around the world has failed to keep pace with the ongoing decline in reef health and periodic die-off of corals and associated reef food resources. Therefore, active reef restoration is increasingly viewed as an essential tool for managing coral reefs (e.g. Edwards and Gomez, 2007, Consensus Statement from World Ocean Conference 2009 in Indonesia, Obura and Grimsditch 2009, Edwards 2010).

Coral restoration and project rationale

Traditional small-scale coral reef restoration techniques usually involve transplanting small broken sections of corals from nearby reefs to provide some coral cover on damaged reefs. In some cases this can lead to an enhanced coral community, but this technique is relatively costly. Mass coral larval reseeding has the potential to more rapidly and effectively initiate coral community restoration on damaged reefs and therefore provides a more cost-effective and broader scale solution to the problem of restoring damaged reefs. Accordingly, this project is globally significant.

Active coral restoration can enhance coral cover, but current restoration methods have not yet been shown to be effective at larger scales needed to halt or reverse the decline in reef coral communities (Rinkevich 2005, Edwards 2010, Young et al. 2012). Most coral restoration studies have used smaller-scale asexual fragmentation of adult colonies in combination with transplantation and sometimes nursery techniques, but these are relatively labour-intensive and expensive and in many cases these projects have failed to restore self-sustaining coral populations (Cabaitan et al. 2008; Shaish et al. 2010, Young et al. 2012, Barton et al. 2015). Fragmentation also damages donor colonies and increases disease risk, and coral fragments have limited genetic diversity that may constrain their resistance to future stress disturbances (Edwards 2010, Young et al. 2012, Barton et al. 2015).

An alternative restoration approach is to use mass coral larval enhancement (‘reseeding’) by rearing and settling millions of larvae resulting from large-scale spawning events of highly fecund corals (e.g. Harrison et al. 1984, Babcock et al. 1986, Harrison and Wallace 1990, Vicentuan et al. 2008). This approach has great potential for scaling-up restoration efforts to ecologically meaningful reefal scales (Harriott and Harrison 1984, Richmond 1997). Mass larval enhancement can more rapidly and effectively initiate coral community restoration on damaged reefs and therefore provides a more cost-effective and broader scale solution to the problem of restoring damaged reefs. This approach also has advantages of greater genotypic diversity among recruits that is likely to improve adaptive potential and increase resistance to future disturbances, thereby strengthening coral community resilience and recovery rates (van Oppen et al. 2015).

To date, results from only a few small-scale *in situ* field trials using mass coral larval settlement have been published in refereed journals, and these studies used reef areas adjacent to healthy coral communities in Marine Protected Areas (Heyward et al. 2002, Cooper et al. 2014, Edwards et al. 2015). *Acropora* larvae in coral spawn slicks resulting from a large multispecific spawning event were captured and reared in small floating culture ponds then used to ‘seed’ small 1.8 m^2 replicate reef areas at Ningaloo Reef, Western Australia (Heyward et al. 2002). After six weeks, more than 6,500 acroporid coral recruits were recorded growing on conditioned terracotta tiles in the larval ‘seeded’ areas,

whereas control tiles had 100-to 1000-fold lower natural recruitment rates. Heyward et al. (2002) concluded that spawning slicks and field harvesting of coral spawn provide great opportunities for very large-scale coral larval culture that can be used for reef restoration management over larger reef scales. However, the longer-term outcomes from their study could not be determined because there was no subsequent monitoring of post-settlement survival or growth of the recruits derived from cultured larvae.

A small-scale study by Cooper et al. (2014) used brooded *Porites astreoides* larvae that were settled onto small 10 x 10 cm reef areas in Biscayne National Park, Florida. High rates of mortality resulted in <1% recruit survival after 5 months and choice of substrata and post-settlement caging experiments did not significantly influence mortality (Cooper et al. 2014). In a more recent study in Palau, ~1 million *ex situ* cultured *Acropora digitifera* larvae were used to 'seed' replicate 3 m^2 concrete pallet balls for 24 hours resulting in significantly higher density of acroporid recruits on fiber-cement tiles attached to 'seeded' pallet balls compared with tiles on unseeded controls after 5 weeks (Edwards et al. 2015). However, monitoring of recruit survival after 30 weeks and then at ~13 months showed no significant differences in acroporid recruit densities between the 'seeded' and the control treatments due to high post-settlement mortality of 'seeded' recruits and high rates of natural acroporid coral recruitment in this healthy reef system (Edwards et al. 2015).

Other published papers on larval restoration practices provide general accounts of releasing reared coral larvae without quantifying the effects on recruitment (e.g. Nonaka et al. 2003), monitored growth (Omori et al. 2008) or sexual reproduction (Iwao et al. 2010, Baria et al. 2012) of transplanted colonies reared from settled larvae, or focus on larval rearing for smaller scale public aquarium management (e.g. Petersen et al. 2006). Suzuki et al. (2012) examined the effect of larval density on settlement and survival of two species of *Acropora* larvae settled onto artificial polycarbonate surfaces. They found that initial larval settlement was proportional to different larval densities, but that survival rates were best in medium larval density treatments compared with high and low densities.

In summary, these previous studies indicate that larval enhancement is feasible but have had mixed results and outcomes resulting from their very different experimental designs and methods used, and mostly small-scale approaches and short-term monitoring of the outcomes. Therefore, further detailed and longer-term research on mass larval enhancement is needed in order to fully assess the ecological and cost-effectiveness of this technique for coral reef restoration. Furthermore, previous studies have not been done on degraded reef systems where coral restoration is most urgently needed.

Therefore, the main focus of this project was to complete a larger-scale and longer-term study of the effectiveness of using mass larval settlement enhancement to initiate coral restoration on degraded coral reef areas in the Northern Luzon region of the Philippines. The key issues the project intended to address included: quantifying the timing of sexual reproduction in a range of ecologically important corals on reefs in the Bolinao-Anda reef province to determine the extended periods during which coral larvae could be reared and used for the larval enhancement experiments; capturing coral spawn and rearing millions of coral larvae for use in larval settlement experiments at BML and *in situ* on degraded reef areas; and quantifying patterns of coral larval settlement and early recruitment in replicate reef areas provisioned with larvae versus control areas without larvae. Importantly, the project was also designed to quantify the longer-term rates of post-settlement survivorship and growth in juvenile recruits after the larval enhancement process, as this critically important phase of the coral reproductive cycle had not been adequately assessed in previous research.

4 Objectives

The overall **aim** of this project is to quantify the effectiveness of mass larval reseeding to restore damaged coral communities on reefs in the Philippines, in order to assess its use in future global reef restoration management strategies.

The specific **objectives** of this research are to:

1. Determine peak coral spawning periods on study reefs in the Philippines (at Bolinao, in northern Luzon) and then capture coral spawn and rear millions of coral larvae in floating larval enclosures during their development period,

2. Reseed replicate areas of damaged reefs by facilitating mass settlement of coral larvae from the rearing enclosures,

3. Quantify patterns of coral larval settlement and early recruitment in replicate reseeded reef areas versus natural areas of similar reef habitat to determine the enhanced recruitment rates,

4. Quantify rates of settled juvenile coral growth and survival to assess the ecological and cost-effectiveness of mass larval reseeding in reef restoration,

5. Use the results of this research to develop guidelines and technical training reports and peer-reviewed publications in high-ranking international refereed journals to enhance restoration of damaged coral reef around the world in future.

5 Methodology

The methods used for this project are based on those developed and used by Harrison and colleagues over many years of extensive successful research on coral reproduction, larval development and recruitment studies in many coral reef regions around the world (e.g. Harrison et al. 1984, Harrison 1988, 2006, Babcock et al. 1986, Hayashibara et al. 1993, Willis et al. 1985, 1997, Ward and Harrison 1997, Wilson and Harrison 1998, 2005, Heyward et al. 2002, Reichelt-Brushett and Harrison 2000, 2004, Harrison and Ward 2001, Nozawa and Harrison 2002, 2005, 2007, Mangubhai and Harrison 2008, Mangubhai et al. 2007, Villanueva et al. 2008, 2012, among others). The first section 5.1 outlines the general methods used in a range of experiments for this project, and then more detailed methods used for the major mass larval enhancement experiments are described in section 5.2.

5.1 General methods

Coral collection

Coral reefs in the Bolinao-Anda Reef Complex (BARC) have experienced a significant loss of corals over recent decades resulting from a combination of anthropogenic impacts and other disturbances including blast fishing and overfishing, coastal pollution including eutrophication from extensive development of fish farms, crown-of thorns seastar outbreaks, coral bleaching and impacts from typhoons (e.g. dela Cruz et al. 2014). As a result, there are relatively few locations where healthy patches of coral communities exist. However, blast fishing is no longer tolerated in this region hence it is feasible to initiate coral restoration on these degraded reefs. Corals used in the work for this project were mainly collected from reef areas at Caniogan Reef and the 'coral garden' near Magsaysay Reef, and other nearby reef areas (Fig. 5.1).

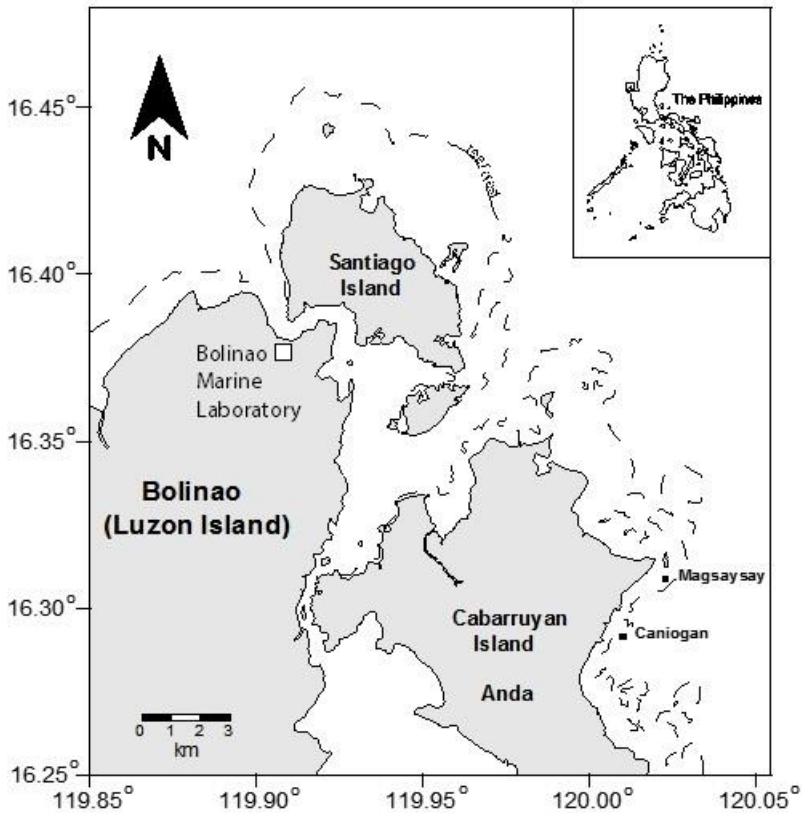

Figure 5.1. Map showing the locations of the Bolinao Marine Laboratory, collection sites for corals at Caniogan Reef, and the location of the experimental larval-enhanced and control sites at Magsaysay Reef in the Bolinao-Anda Reef Complex, northwestern Philippines.

Coral spawning, brooding and fertilization

During the project a wide range of gravid broadcast-spawning *Acropora* branching corals and massive brain coral species, and the brooding species *Porites cylindrica* and the blue coral *Heliopora coerulea* were collected using hammers and chisels while scuba diving or on snorkel from nearby reef areas containing healthy populations of these species. Prior to collection, broadcast-spawning colonies were sampled by carefully breaking a few branches or polyps in massive corals to determine the presence of mature oocytes, as indicated by their colouration that develops in the last few weeks prior to spawning (Harrison et al. 1984, Babcock et al. 1986). The colonies were transferred to large plastic tubs filled with clean seawater in boats and were carefully transported to the Bolinao Marine Laboratory (BML) hatchery where they were transferred into large concrete or plastic tanks and were maintained in a healthy condition with flow-through seawater and aeration.

Colonies were monitored periodically from about 1800 h to 2200 h at night with red torchlight to check for setting and spawning behaviours before and during predicted coral spawning periods and the timing of spawning was recorded (Harrison et al. 1984, Babcock et al. 1986, Vicentuan et al. 2008). In most cases, coral colonies were returned alive to their collection sites and reattached to reef areas using cable ties or a mixture of cement and non-toxic epoxy materials. Where species identification was uncertain, small portions of the colony were removed and labelled with plastic Dymo labels prior to returning the colony to the reef area, and samples were bleached in 10% sodium hypochlorite solution to remove the tissues so that the taxonomically important skeletal characters could be used to confirm species identifications.

Following spawning, egg and sperm bundles were collected from the water surface (Fig. 5.2) and either kept separate from those of other colonies if used for controlled fertilization experiments, or bundles from all colonies of a species were combined and gently mixed in containers with filtered seawater to maximise fertilization and outcrossing rates among offspring (e.g. Harrison 1988, 2006, Willis et al. 1997). Sperm concentrations were checked by taking subsamples of the sperm and seawater in the fertilisation tubs and viewed using a haemocytometer viewed under a high-power microscope (Willis et al. 1997, Harrison and Ward 2001). For routine larval rearing, sperm concentrations were adjusted to about $\geq 10^4$ ml^{-1} to ensure high rates of fertilization and to prevent polyspermy (Willis et al. 1997, Harrison and Ward 2001). After about 1 hour, seawater containing excess sperm was siphoned off (sperm-washing) beneath the floating eggs and new filtered seawater (FSW) was slowly added, and this process was repeated three times to remove excess sperm that may degrade water quality during larval culture.

Figure 5.2. Collection of spawned egg-sperm bundles from the water surface following coral spawning in the BML hatchery (Images: P. Harrison)

For controlled fertilization experiments, spawned egg-sperm bundles were collected from isolated colonies of *Acropora millepora* and *A. tenuis* branching corals and the brain coral *Montastrea colemani*. Eggs and sperm were then separated using a fine plankton mesh filter and eggs were triple rinsed with sperm free seawater (SFSW) to remove sperm from the same colony following standard protocols (Willis et al. 1997, Harrison and Ward 2001). Sperm concentrations were adjusted to a graded concentration series by adding clean filtered seawater after quantifying initial sperm density, then eggs were added and fertilization responses were analysed over time to determine optimal sperm concentrations (after Willis et al. 1997). Additional related experiments examined the effects of delaying combining eggs and sperm from 30 minutes to 10 hours after spawning to determine optimum times for gamete combination for these three species.

Embryo and larval rearing

Subsamples of fertilized eggs, embryos and eggs were collected periodically from larval rearing containers after gametes were combined and examined under a stereomicroscope to determine percentage fertilization. Developing embryos were transferred into large rearing tanks for 24 h and gently agitated periodically to maintain embryos and early larvae in a healthy condition. After 24 hours when embryos were sufficiently developed and less likely to disintegrate or malform due to agitation, gentle aeration was supplied and 50 L of new filtered seawater was added daily to tanks with developing larvae. Larval cultures were kept healthy during their larval development phase by maintaining larval densities between 250 and 500 embryos L^{-1} and removing unhealthy or dead larvae and surface scums. Larval development and swimming activity were periodically monitored to determine when larvae were competent to settle, at which time they were used for larval settlement experiments in the BML aquarium system or transported to the field reef restoration sites (see Section 5.2 below).

Larval settlement and post-settlement survival experiments

A wide range of larval settlement experiments were completed during this project including quantifying larval settlement rates of different coral species using a range of different natural and artificial settlement tile surfaces, the effect of different larval densities on settlement and survival, and the effects of different conditioning times for settlement tiles to optimise settlement.

<u>Effect of artificial versus natural coral tile substrata on larval settlement</u>

During April 2012 larvae of *Acropora pulchra*, *A. intermedia, A. tenuis, A. granulosa* and the brain coral *Favites abdita* were reared following spawning at BML (Fig. 5.2), and competent larvae were provided with a choice of settlement on four 10 x 10 cm fibre-cement artificial tiles (used in previous larval settlement experiments at BML prior to this project) and four natural 10 x 10 cm tiles cut from dead *Acropora* coral skeletons. The dead *Acropora* coral plates were collected from a shallow reef area near Cory rubble bar near Magsaysay Reef (Fig. 5.1) where the region's history of dynamite fishing, coral bleaching and typhoon impacts has resulted in large shoals of dead coral rubble. For each species, five replicate tubs were set up and four tiles of both types were randomly added to each tub together with 7,200 larvae (Fig. 5.3). The tiles were placed in an ~45° angle to maximise the tile surface areas available for larval settlement, and tiles were supported by inserting the pointed end of a glass pipette tip into a hole bored in the centre of each tile. Tiles were randomly positioned in each tank and gentle aeration was supplied to maintain water quality during the 5-day settlement period.

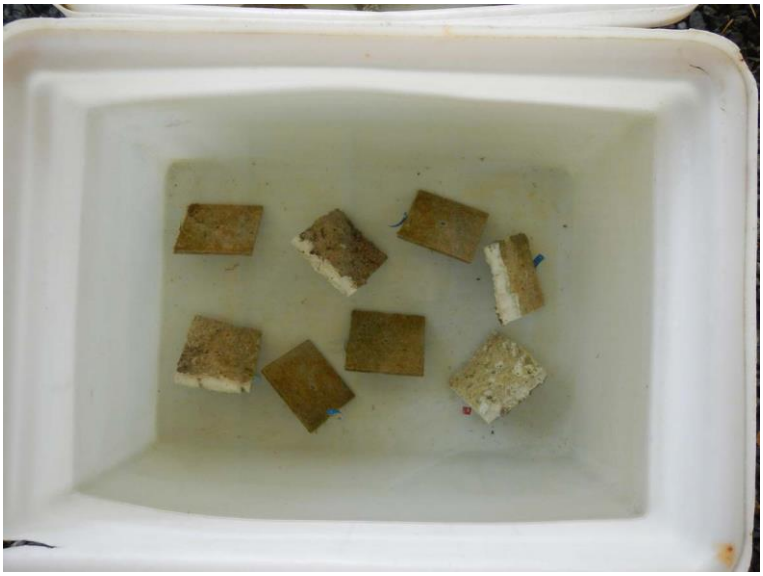

Figure 5.3. Larval settlement design to compare settlement rates on tiles made from dead *Acropora* coral skeletons and artificial settlement tiles (Image: P. Harrison).

Larval settlement on each tile monitored under stereomicroscopes illuminated with fibre-optic lights and settlement data were standardised to 0.1 m^2 of tile surface to account for different surface areas of the different tile types. Settlement data were analysed using One-way ANOVA. Mean settlement rates of *A. granulosa* and *A. pulchra* larvae were significantly higher on the natural tiles versus the fibre-cement tiles, and although low rates of *A. intermedia* larval settlement occurred on natural coral tiles compared with those of *A. pulchra*, settlement was still significantly higher on the natural coral tiles compared to artificial tiles on which no larvae settled. No significant differences in larval settlement were recorded between the two tiles types for *A. tenuis* and *F. abdita*. Therefore, most subsequent larval settlement experiments and the mass larval enhancement field restoration experiments (see Section 5.2) used the natural *Acropora* skeleton tiles, as these provide more appropriate settlement surfaces to monitor larval settlement and subsequent survivorship of corals over time.

In 2013, a similar experiment was done using larvae of *A. millepora*, the brooding coral *Porites cylindrica* and the blue coral *Heliopora coerulea* to examine settlement preferences among three types of tiles: dead *Acropora* skeletons, and two types of artificial tiles (terra cotta and fibre-cement, Fig. 5.4). Five gravid coral colonies of *A. millepora* were collected and larvae were reared as described above. Ten colonies of *P.*

cylindrica and *H. coerulea* were collected and placed in tanks with flow-through seawater and aeration. Water flow was stopped at dusk and brooded larvae that had been released overnight were collected on the following morning. The experimental design consisted of 5 replicate tubs per species, and approximately 7,200 competent larvae of *A. millepora* and 1,500 brooded planula of *H. coerulea* and *P. cyclindrica* were transferred separately in each of the 5 tubs per species. Each tub contained 4 randomly arranged tiles each of the dead *Acropora* coral skeletons, fibre-cement, and terracotta tiles, and tiles were randomly positioned within each tub and orientated at an ~45° angle using a glass pipette inserted through the centre hole of each tile, with gentle aeration supplied to each tub. Larval settlement was monitored after the 5-day settlement period and settlement rates were standardised per 0.1 m^2 of tile surface as described above. Data were analysed using Two-way ANOVA with subsequent *post hoc* comparisons conducted using Newman-Keuls tests for the three tile types.

Figure 5.4. Juvenile coral settlement and survival experiment comparing larval settlement rates on dead coral skeleton tiles and two types of artificial settlement tiles in the BML hatchery (Image: D. dela Cruz).

Effect of different larval density on settlement and survival on natural versus artificial tiles

In May 2012, the effect of different densities of *Acropora tenuis* larvae on settlement rates and subsequent survival were determined experimentally. Larvae were reared for four days at BML following spawning, and four densities of larvae were used, with 1,800, 3,600, 7,200 and 14,400 larvae transferred to each of the 70 L tubs. Four replicate tubs were used per density treatment and within each tub four 10 x 10 cm natural dead *Acropora* tiles and four 10 x 10 cm fibre-cement artificial tiles were randomly positioned and orientated at an ~45° angle using a glass pipette inserted through the centre hole of each tile, with gentle aeration supplied to each tub (Fig. 5.5).

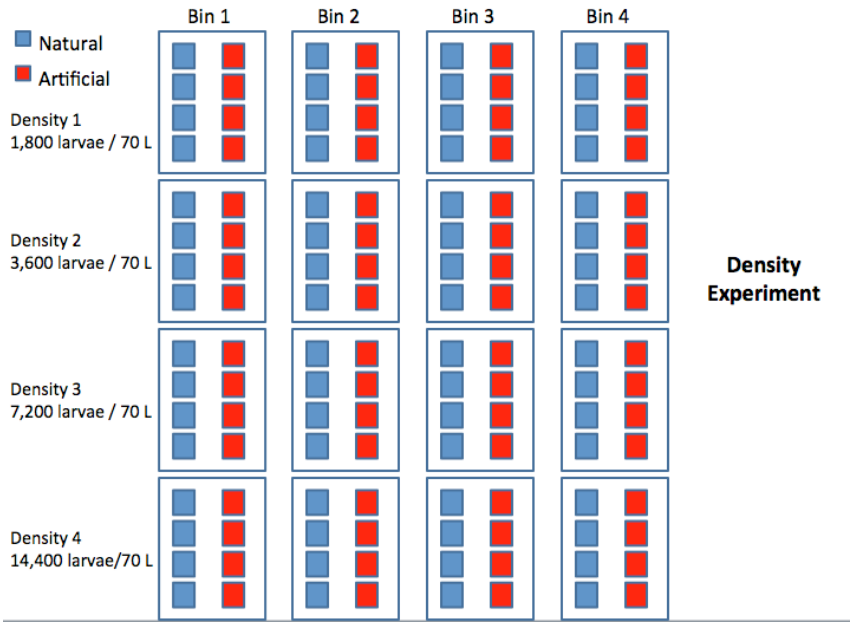

Figure 5.5. Design of the larval density and settlement tile experiment using tiles made from dead *Acropora* skeleton or fibre-cement tiles.

Larval settlement was monitored after a 5-day settlement period by viewing tiles under a stereomicroscope illuminated with fibre-optic lights, and total numbers of larvae that had settled (alive + dead with remnant skeleton present) and survived (alive during the monitoring) were recorded and standardised per 0.1 m^2 of tile surface. Data were analysed using Two-way ANOVA with initial settlement as the dependent variable and tile types and densities as the categorical predictors, with subsequent *post hoc* comparisons using Newman-Keuls tests to determine any significant differences between tile types and larval densities.

Experiment with 3D tiles versus natural coral tile substratum

Subsequent experiments included preliminary experiments using 3D printed tiles with SCU Honours student Katy Horan. In May 2015, larvae were reared from *Montastrea colemani* that spawned 4 nights after the full moon (nAFM). A set of four 10 x 10 x 3 cm tiles were cut from plate *Acropora* and these were 3D scanned and then replica tiles were printed by a 3D printer, in each of the two most commonly used 3D printing plastics: polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS). Scanning and printing was done by '3D Go' in Manila. *Acropora* tiles were then scrubbed, bleached and baked in sunlight to destroy settlement cues on these natural tiles before being returned to aquaria at BML along with PLA and ABS replica tiles on 10 May 2015 for 5 days of biological conditioning prior to the experiment.

Competent *M. colemani* larvae were exposed to the conditioned substrata on 15 May 2015. One replicate tile of each material: the original *Acropora* tile and replicas printed in PLA and ABS were placed haphazardly at an ~45° angle within each of four 40 L tubs by inserting a glass pipette into the central hole in each tile to maximise available tile surfaces for potential larval settlement. FSW was added to each tub and gentle aeration was supplied to maintain the larvae and conditioned tile surfaces in a healthy condition. Approximately 1000 larvae were added to each tub each containing the three types of tiles. The desired larval density was achieved by adding 2 L of larval culture with a larval density of 0.5 ml^{-1} to 38 L of FSW. After 5 days settlement, tile surfaces were monitored under stereomicroscope for larvae that were attached (larvae still elongate and attached to substratum at one end), settled (larvae attached to substratum with slightly flattened

spherical shape) and metamorphosed (larvae attached to substratum with flattened disc-like shape and observable mesenteries forming).

A related experiment was done in June 2015 using *A. gemmifera* larvae. Colonies of *A. gemmifera* were collected in the week before the full moon on the 3rd June 2015 and these spawned on 8 June 2015. Gamete collection, fertilisation and larval rearing followed standard methods. A nested experimental design was used in which competent larvae were offered a choice of different tile material (dead *Acropora* skeleton, PLA or ABS) within one of two biological conditioning treatments (reef conditioned or BML hatchery conditioned, Fig. 5.6). Replicate tiles of each material (n=5) were conditioned in either reef seawater near the Magsaysay Reef area or in hatchery water in tank facilities at BML for two weeks. Prior to conditioning all tiles were bleached in 10% sodium hypochlorite solution for 24 hours, rinsed and soaked in running seawater for 2 days and then dried in sunlight. Conditioning treatments could not be tested together as water soluble settlement cues may have been transferred to the water column or between tiles from different conditioning treatments. Within each conditioning treatment one coral tile and its two matching 3D printed replicas in PLA and ABS were placed in a 40 L tank filled with FSW. Tiles were suspended vertically on a stainless steel rod allowing settlement on all tile surfaces. Approximately 1000 larvae were added to each tank 4 days after spawning. Tile surfaces were monitored after 3 days settlement under a stereomicroscope for larvae that were attached, settled or metamorphosed.

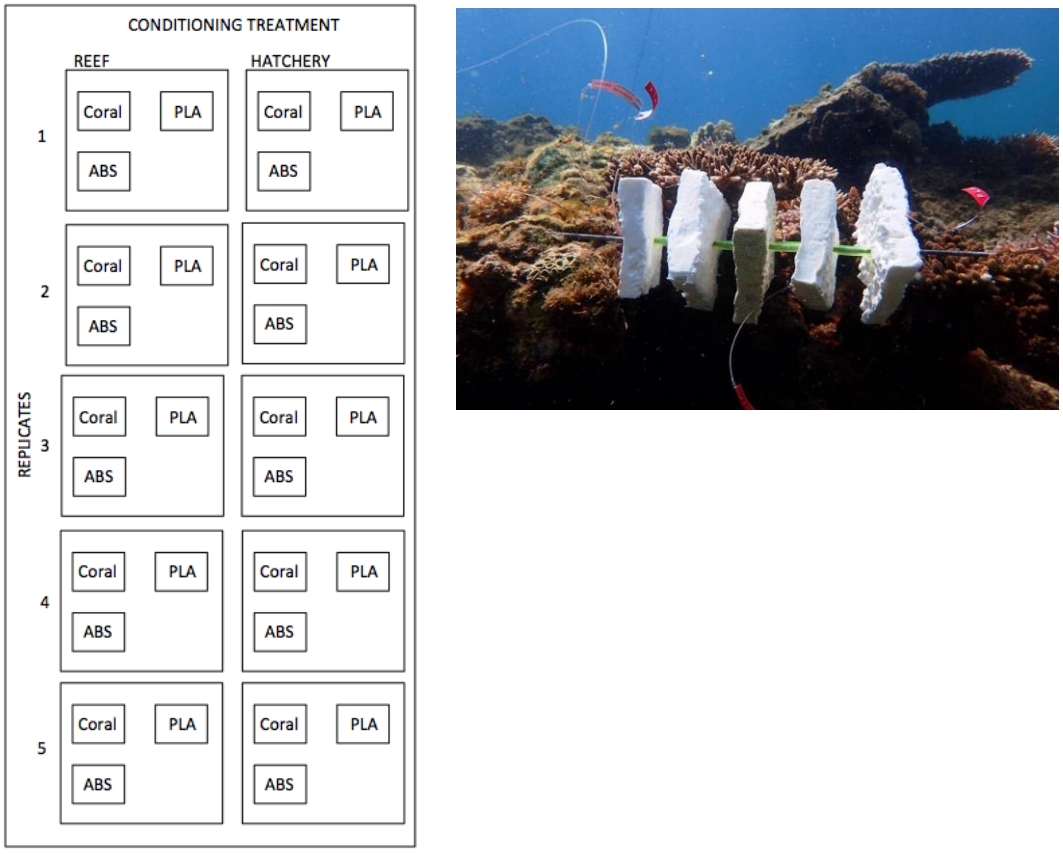

Figure 5.6. Diagram (left) of nested experimental design comparing *A. gemmifera* larval settlement on tiles from dead *Acropora* skeletons (coral), and 3D printed replicas in PLA and ABS conditioned in reef water or in the BML hatchery, and (right) image of tiles being conditioned on the reef (Image: D. dela Cruz).

Optimising biological conditioning times for larval settlement

The effect of varying degrees of biological conditioning of the settlement tiles was also examined in a detailed experiment in May 2015 with Honours student Katy Horan. Larvae were reared following spawning of *A. tenuis* on 1 May 2015 and *M. colemani* on 8 May 2015 (full moon was on 4 May 2015). Gamete collection, fertilization and larval culture followed standard methods described above. Fertilization was allowed to occur for 1 hour after which embryos were collected from the water surface and transferred to 1000 L rectangular rearing tanks. Rearing tanks contained FSW and were lightly aerated after 24 hours, and larval density was maintained at ~500 larvae L^{-1}. Larvae were monitored for settlement competency from 2 days post-fertilisation by exposing 10 larvae to a small (~0.5 cm^{-1}) chip of crustose coralline algae (CCA) in 15 mL FSW (*n*=6). Settlement rates were quantified by observing the number of larvae attached and metamorphosed onto the rubble or side of the culture well vessel under a stereomicroscope. Settlement of *A. tenuis* and *M. colemani* larvae peaked at about 7 days and 5 days post-spawning, respectively, at which time the larvae were introduced into tubs with the experimental tiles.

Settlement tiles were cut from dead plate *Acropora* skeletons to sizes of 10 x 10 x 3 cm and were autoclaved to ensure all biochemical settlement cues were removed prior to the experiment. Sets of 10 settlement tiles were sequentially added to flow through tanks at BML at intervals of 7, 4, and 2 months prior to the predicted May 2015 spawning event. Tanks were supplied with sand-filtered seawater pumped from the adjacent marine environment offshore from the BML hatchery to allow biofilms to develop on the tile substrata. In addition two sets of 5 tiles were conditioned for only 24 hours prior to each larval settlement experiment for comparison with the longer conditioning times.

Competent larvae of *A. tenuis* and *M. colemani* were exposed to the conditioned tiles on 8 and 13 May 2015, respectively. One replicate tile from each conditioning treatment (7, 4, 2 months and 24 hours) was placed haphazardly at an ~45° angle within each of five 40 L tubs to allow potential settlement on all tile surfaces. Tubs were lightly aerated and ~1000 larvae were added to each tub. The desired larval density was achieved by adding 2 L of larval culture with larval density of 0.5/ml to 38 L of FSW. Tile surfaces were monitored under stereomicroscopes for larvae that were attached (larvae still elongate and attached to substratum at one end), settled (larvae attached to substratum with slightly flattened spherical shape) and metamorphosed (larvae attached to substratum with flattened disc-like shape and observable mesenteries forming). Monitoring took place after 3 days settlement for *A. tenuis* and 5 days settlement for *M. colemani.* One-way analysis of variance (ANOVA) was used to analyse the effects of tile substrata conditioning time on larval settlement with subsequent *post hoc* comparison with Tukey's HSD test.

5.2 Mass larval enhancement experiments

Experimental Design

These major project experiments were designed to test the effect of supplying large numbers of *Acropora* larvae on replicate degraded reef areas during a five-day larval settlement period to compare initial larval settlement, and subsequent survival and growth of coral recruits over time in larval enhanced sites versus control areas without larval provision.

Acropora tenuis experiment initiated in April 2013

Site selection and reef benthic community status

Prior to the larval enhancement experiment, degraded reef sites at 3-4 m depth were identified at the Magsaysay reef, Anda, Pangasinan (16°19'36" N, 120°02'01" E; Fig. 5.1).

The coral community in the area was destroyed by a Crown-of-thorns starfish outbreak in 2007 and previous blast fishing which no longer occurs. A total of eight 6 x 4 m reef sites (~2-4 m apart) were haphazardly selected on the reef and demarcated using steel bars (Fig. 5.7A). Four randomly selected sites were treated with larvae (larval-enhanced) and the other four sites were controls – without provision of larvae. Prior to the larval enhancement experiment, photographs of the benthic communities within each site were taken using a 1 × 1 m frame and analysed using CPCe (Kohler and Gill 2006) to quantify benthic cover of corals and other benthos and to determine the state of the reef community in the area (Fig. 5.7B). A total of 10 random points were generated and scored in each of the 24 frames taken in each of the sites. There were no *A. tenuis* adult colonies or visible recruits from previous spawning events present inside the sites prior to the reseeding activity. All living corals inside the sites were carefully covered with plastic mesh to avoid the matting from becoming caught and ripping and damaging the coral tissues (Fig. 5.7C).

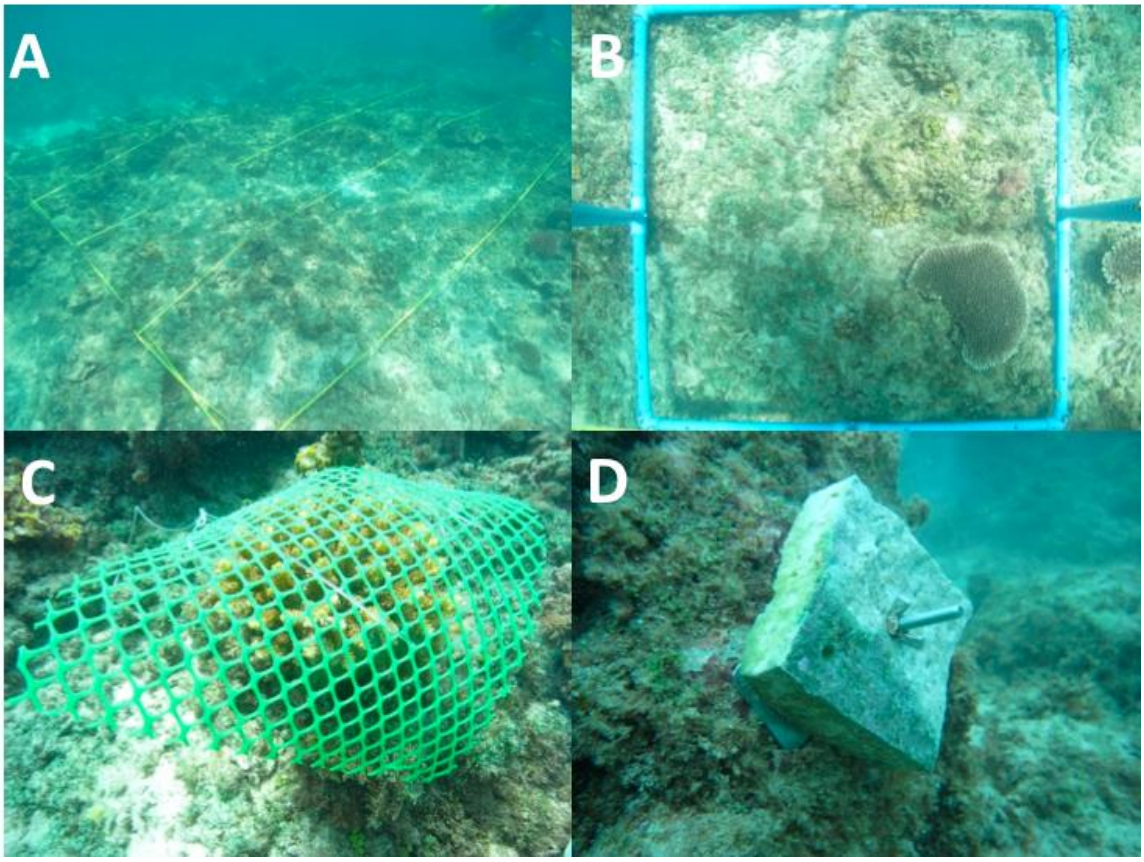

Figure 5.7. Preparations of the experimental sites prior to the larval enhancement experiment. A. Demarcated 4 x 6 m experimental site. B. Sample 1 x 1 photo of a site for reef community analysis using CPCe. C. A coral covered with plastic mesh inside a site to protect the larval enclosure. D. Settlement tile of dead *Acropora* coral deployed inside a site prior to provision of larvae. (Images D. dela Cruz).

Ten biologically conditioned 10 x 10 cm natural recruitment tiles cut from dead table *Acropora* were deployed inside each of the eight sites just prior to the larval enhancement experiment to quantify the settlement rates of the *A. tenuis* larvae (Fig. 5.7D). The tiles were directly attached to the substratum using stainless steel base plates as described by Mundy (2000). Dead table *Acropora* pieces used to produce the tiles were collected from the intertidal zone beside Cory reef rubble bar near the experimental sites (Fig. 5.1). Recruitment tiles were conditioned for a month in aquaculture tanks with flow-through seawater and aeration at the Bolinao Marine Laboratory (BML) of The Marine Science

Institute (University of the Philippines) prior to use on the reef sites. Each tile had a coded aluminium tag attached to the top left hand corner to ensure correct placement and orientation of the tiles within each site, and correct placement on the reef following each monitoring period.

To estimate the surface area of the irregular surfaces of the recruitment tiles, 30 representative tiles were 3D scanned using a David® SLS-1 structured white light technology scanning system. The scanner has a mesh density up to 1,200,000 vertices per scan with resolution precision up to 0.1% of scan size down to 0.06 mm. Each tile was scanned and shot in all angles using a 360^{o} turntable and each shot was fused to form a digitized 3D object. The formed 3D object was then converted to a stereolithography (STL) file that was further refined using 3D modelling software (viz., Zbruzh™ and Blender™). The surface area of the 3D image of the tiles was analysed and calculated using netFabb® software. As there was minimal variation in the surface area of the scanned tiles (mean: 360 ± 3.7 SE cm2), larval settlement and recruitment was expressed as numbers of recruits per tile.

Coral collection and larval culture

Thirty-two gravid colonies of *Acropora tenuis* (minimum diameter of 15-20 cm) were collected from the reef ~2-4 m deep in Caniogan, Anda, Pangasinan (Fig 5.1; 16°19'36" N, 120°02'01" E) a week before the full moon of April 2013. This species was selected for this study because it is common in the Bolinao-Anda Reef Complex (BARC) in the northwestern Philippines, and larvae from this species have been successfully cultured in previous experiments (e.g. Lane and Harrison 2002, Nishikawa et al. 2003, Baria et al. 2010, Omori 2011). Prior to collection, colonies were sampled by carefully breaking a few branches to determine the presence of mature oocytes, as indicated by their pink coloration (Harrison et al. 1984). Gravid colonies were carefully transported in seawater to the BML aquaculture facility for *ex situ* spawning and gamete collection.

Colonies were maintained in concrete tanks with flow-through seawater and aeration and were monitored periodically at night to check for setting and spawning behaviours. Gametes from major spawning events involving 30 colonies that spawned between 1830 and 1900 h on April 29 and 30 2013 (3 and 4 nights after full moon, respectively) were collected for use in this experiment. Many thousands of spawned egg-sperm bundles were skimmed off the water surface and transferred to a fertilization container in 10 L of 1 µm filtered seawater. Gamete bundles were gently agitated to facilitate egg and sperm separation and to maximize subsequent cross-fertilization (Willis et al. 1997). After 1 hour, seawater containing excess sperm was siphoned off (sperm-washing) beneath the floating eggs and new filtered seawater was slowly added. This process was repeated three times to remove excess sperm that may degrade water quality during larval culture and to prevent polyspermy (Willis et al. 1997). Subsamples of embryos and eggs were collected and examined after a further hour under a stereomicroscope to determine percentage fertilization.

Developing embryos were transferred into 13 large rearing tanks (each containing >1,000 L of seawater) at water surface densities between 4-5 embryos cm^{-2} for 24 h. To maintain embryos and larvae in a healthy condition, aeration was supplied 24 h after fertilization and 50 L of new filtered seawater was added daily to tanks with developing larvae.

At 4 days post-fertilization, competent actively swimming larvae were collected using a plankton net sieve (60 µm mesh size) and transferred to a holding basin. The total number of larvae was estimated using three 60 mL sub-samples taken from the basin after the larvae had been thoroughly mixed throughout the water column, and then the larvae were distributed equally into twelve strong 40 x 50 cm 20 L plastic bags. Oxygen was supplied into each bag before it was sealed for transport to the field for the larval enhancement experiment at Magsaysay Reef (Fig. 5.1).

Coral larval enhancement

Organza-hapa mesh enclosures were used to retain the larvae inside the sites during the 5-day larval settlement period (Fig. 5.8). The organza cloth (100-150 µm mesh opening) was sewn to form 6 x 4 m enclosures, with hapa nylon net (500 µm mesh) as a second, outer layer for additional support (Fig. 5.8A). The advantage of organza cloth as a mesh enclosure material is its low cost relative to plankton mesh, yet it is strong enough to withstand occasional strong wave surges on the reef. This matting assembly can effectively retain *A. tenuis* larvae whose diameters are 300–500 µm (Nishikawa et al. 2003). Cylindrical 20 g weights (1.75 x 4 cm) were inserted along the lower edge of the mesh to firmly hold the matting in place on the reef and prevent larvae from escaping during the settlement period (Fig. 5.8B). The corners of the matting were secured using steel reinforcing bars driven into the reef substratum. Larvae were introduced into the mesh enclosures on each of the larval-enhanced sites through re-sealable openings distributed over the upper areas of the mesh enclosures (Fig. 5.8C). Control sites were also covered by organza-hapa mesh enclosures during this period but no larvae were introduced (Fig. 5.8D).

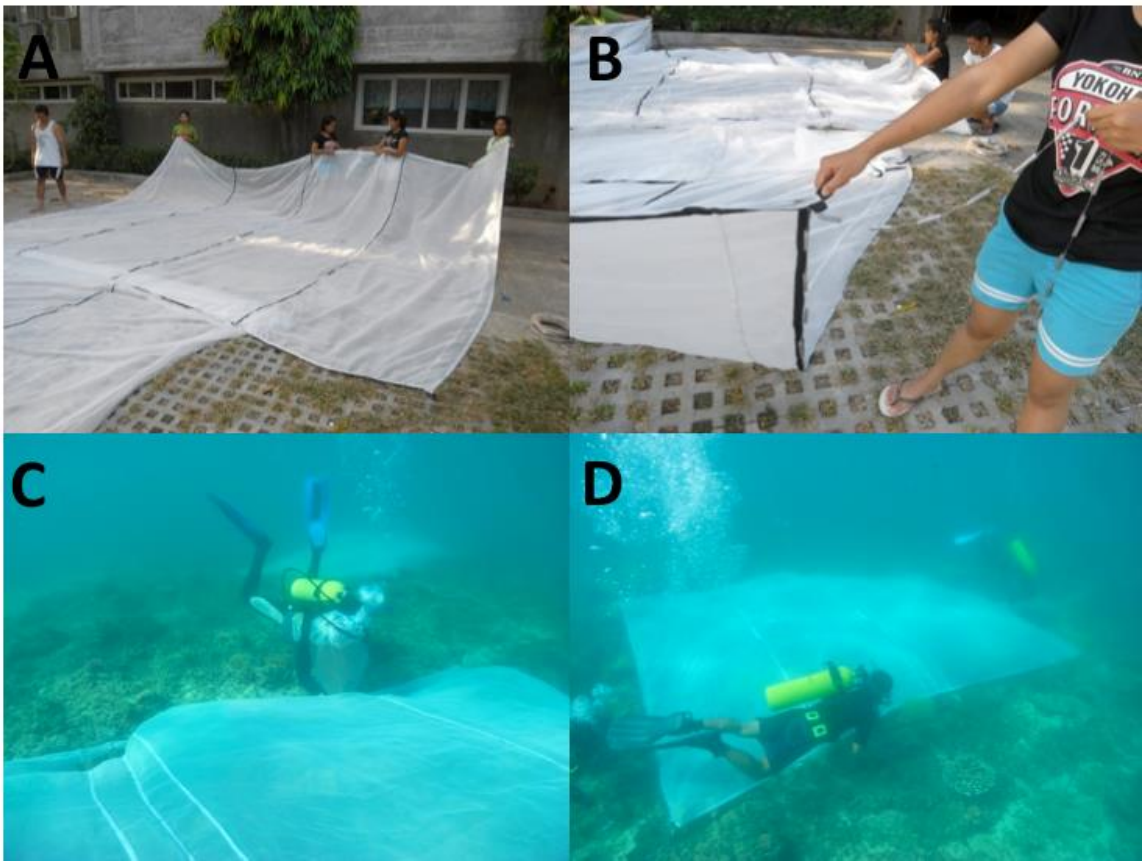

Figure 5.8. A. Mesh matting enclosures used in retaining the larvae inside the sites. B. Lead weights inserted in the edge of the matting. C. Introduction of larvae inside the matting. D. Control site with matting but no larval provision. (Images: D. dela Cruz).

Five days after the provision of larvae inside the mesh enclosures, the enclosures were removed from all of the larval-enhanced and control sites. All settlement tiles were then carefully collected and kept submerged in clean seawater while carefully taken to the BML laboratory to monitor the initial numbers of settled coral spat on each tile under a stereoscope and were illuminated with fibre-optic lights to avoid heat stress to the settled polyps. Tiles were kept submerged during monitoring and each coral recruit was recorded

and carefully mapped to facilitate repeated monitoring during the Project and beyond. Recruitment tiles were returned immediately to the experimental sites after each monitoring period and reattached to their designated locations within each site.

Sample fragments from colonies of *A. tenuis* inside the larval enhancement sites that are most likely derived from larvae that settled during the larval provisioning period were collected for DNA analysis and for comparison with the DNA samples collected from the parent colonies that spawned in the BML hatchery. These analyses will be used to confirm the genetic identity of the *A. tenuis* recruits inside the control plots (i.e. are they derived from larvae from the larval enhancement experiment, or subsequently settled 'wild' recruits that arrived after the matting enclosures were removed). In addition, the genetic analyses will enable identification of the genotypes that have successfully survived and grown on the restoration sites, and the diversity of parent types that produced these successful recruits.

Coral recruits on reef substrata

Settlement refers to the initial attachment of competent larvae and their metamorphosis into a juvenile coral polyp (spat), while recruitment refers to the subsequent life stage of the juvenile coral when they survive and grow to become new members of the community, initially as small invisible cryptic recruits and subsequently as larger visible recruits (Harrison and Wallace 1990). *Acropora tenuis* recruits that had settled on natural reef substrata inside the larval-enhanced sites became visible underwater 9 months after the larval enhancement activity. The concurrent size of the juvenile *A. tenuis* on the recruitment tiles was used as the basis for identification of *A. tenuis* recruits on natural substrata inside the plots that originated from the larval enhancement experiment. Identified *A. tenuis* juveniles on the natural reef substrata were mapped and tagged with a numbered aluminium tag for growth and survivorship monitoring together with the juveniles on the recruitment tiles after the larval enhancement. *In situ* growth monitoring commenced at this time, with the length (l), width (w) and height (h) of each of the juvenile corals on recruitment tiles and natural substrata measured using callipers. The ecological volume (EV) was calculated using the volume formula: $EV = \pi r2h$, where $r = (l+w)/4$ (Shaish et al. 2008). Growth rates (ecological volume change per month) were also calculated over time, using the formula $Gr = [EVf – EVi]/m$, where Gr is standardized growth rate, EVf and EVi are final and initial ecological volumes, respectively, and m is the number of months elapsed (dela Cruz et al. 2015). Mean planar diameter was calculated from the maximum and minimum diameters measured for each colony.

Natural recruitment rates in the restoration area were monitored by deploying and replacing ten 10 x 10 cm natural coral recruitment tiles quarterly in each of the control sites for 24 months. Retrieved tiles were bleached in 10% sodium hypochlorite solution for at least 48 hours and then air-dried. Tiles were examined under a stereomicroscope and each coral spat recorded was categorized as acroporid, pocilloporid, poritid, others or unidentified (for broken and damaged unidentifiable recruits) based on skeletal morphology (Babcock et al. 2003).

Sea temperature monitoring

During the larval enhancement experiment period, sea temperatures were monitored to quantify environmental conditions that could affect the growth and survival of coral recruits and other corals in the reef area. Sea Surface Temperature (SST) data were obtained from coralreefwatch@noaa.gov, and these data were supplemented with *in situ* data recorded on a Stowaway temperature data logger deployed at 3 m depth near the experimental sites from February to July 2015 (Fig. 5.9).

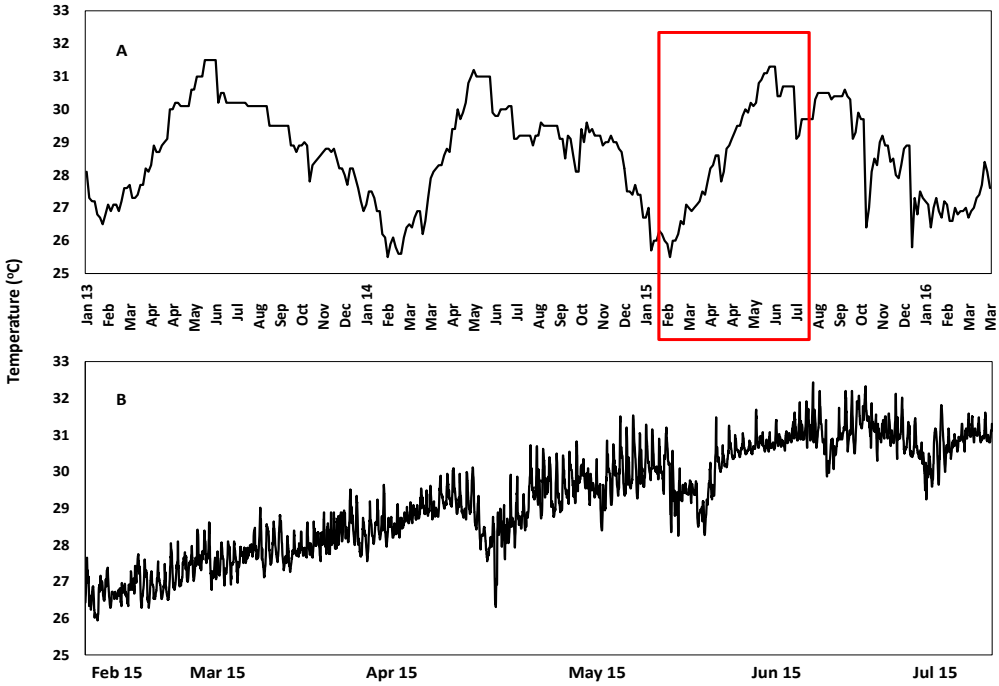

Figure 5.9. **A**. Sea surface temperature profile in the Bolinao region during this study from January 2013 to March 2016 (coralreefwatch@noaa.gov); **B**. temperatures recorded *in situ* at the Magsaysay Reef study site using a data logger from February 2015 to July 2015, corresponding to the period indicated within the red box in A.

Monitoring for onset of sexual reproduction

The *A. tenuis* colonies that recruited onto coral tiles and natural reef substratum were monitored by carefully breaking small branches to determine if gametes were present at age 10 and 20 months prior to the predicted spawning periods after 1 and 2 years of growth. After two years of growth, the *A. tenuis* colonies resulting from settled larvae ranged in size from 3 to 23 cm mean diameter, with an overall mean diameter 11 (±1) cm, which was below the minimum size and age threshold for *Acropora* colonies to become sexually reproductive in other studies (Iwao et al. 2010, Baria et al. 2012).

Statistical Analysis

All data are reported as mean values ± 1 standard error. Analysis of similarities (ANOSIM) was performed to test for similarities and significant differences in the benthic cover composition (e.g., sand, rubble, macroalgae, coral) between larval enhancement and control sites before the larval experiment. Significant differences in the initial settlement patterns on different tile surfaces on natural tiles from the larval-enhanced sites after five days of larval settlement was tested using One-way ANOVA. Tukey's HSD test was conducted *post hoc* to determine significant differences in settlement patterns among tile surfaces.

Survivorship of coral recruits on different surfaces of tiles was analysed using survival analysis, a non-parametric pairwise comparison test based on the Kaplan–Meier function (Lee 1992). The same analysis was used to determine any significant difference in survival patterns of juvenile corals on natural substrata and on tiles from nine to 24 months after the larval enhancement. Significant increases in growth of juvenile corals through time were determined using Repeated Measures ANOVA. One-way ANOVA was used to compare growth rates of juvenile corals between recruitment tiles and natural

substrata, and to determine the variation in natural recruitment of acroporids in the area versus recruitment of *A. tenuis* from the larval enhancement activity.

Acropora granulosa experiment initiated in June 2014

A second major larval enhancement experiment was initiated in June 2014 using larvae of *A. granulosa*. Methods for this study followed those successfully trialled in 2013 for the *A. tenuis* larval enhancement and restoration experiment. Twenty five colonies of *A. granulosa* were collected from Balingasay and Caniogan reefs prior to the predicted spawning period and were maintained in flow-through seawater tanks at the BML hatchery. Spawning occurred on 16 and 17 June 2014, and gametes were collected and larvae reared using standard methods detailed above. After four days larval development, larvae were collected using a fine plankton mesh sieve and the total number of larvae was estimated using three 60 mL subsamples taken from the water column. Larvae were transferred into six strong 20 L plastic bags, supplied with oxygen and then sealed for transport to the larval settlement sites on Magsaysay Reef (Fig 5.1).

Prior to the larval enhancement activity, replicate 4 x 6 m sites were demarcated on Magsaysay Reef in the vicinity of the sites used for the *A. tenuis* experiment. Photo-quadrats were used to quantify the coral cover and status of the benthic reef community in each site prior to the larval enhancement activity, and ten 10 x 10 cm dead *Acropora* skeleton settlement tiles were attached to each site just prior to the larval enhancement experiment. These tiles had been biologically conditioned in the BML hatchery for one month prior to use.

Using the same larval mesh enclosure technique used for the *A. tenuis* experiment, ~300,000 *A. granulosa* larvae were added to each of three replicate 4 x 6 m mesh enclosures on reef sites with three replicate control sites that were not provisioned with larvae. As about 900,000 larvae were available for this experiment only three larval enhancement and three control sites were used in 2014 to ensure sufficient larvae were available for substantial settlement in each replicate site during the 5-day larval settlement period.

Larval mesh enclosures were removed from each of the six sites after the 5-day settlement period and the settlement tiles were carefully collected and transported to the BML facility where the initial larval settlement patterns on each tile were recorded under stereomicroscopes. Tiles were then returned to their correct location and orientation at each reef site, and the survival and growth of settled spat on tiles was monitored over time. When the surviving *A. granulosa* recruits that had settled on reef substrata had grown large enough to become visible for *in situ* monitoring on the reef at about 8 months after settlement, each colony was identified with a numbered aluminium tag placed nearby to facilitate repeated *in situ* monitoring of growth and survival.

6 Achievement against activities and outputs/milestones

The project has achieved its aim and the achievements against the project objectives are described below with further specific details provided in Section 7.

Objective 1. Determine peak coral spawning periods on study reefs in the Philippines (at Bolinao, in northern Luzon) and then capture coral spawn and rear millions of coral larvae in floating larval enclosures during their development period.

The project has substantially increased scientific knowledge of the specific timing and patterns of major coral reproduction periods for 18 scleractinian reef-coral species in the Bolinao-Anda reef region, northern Luzon, Philippines. These include a range of ecologically important corals from different families and with different modes of reproduction. Importantly, these project results have also expanded the months during which gravid corals are now known to spawn, which provides extended opportunities for reliable access to gametes and larvae.

Information on spawning periods and the specific timing of gamete release were recorded over multiple years from 2012 through 2015 for 16 species (13 *Acropora* branching coral species and three massive brain coral species). Reproductive patterns of two brooding species were also examined (a branching *Porites* and the blue octocoral *Heliopora*). Field observations of synchronized multi-specific coral spawning were also completed in March 2015 coinciding with coral spawning periods observed in the BML aquarium system.

Together these new data enabled improved planning for routine access to many millions of spawned gametes from gravid corals that could be captured and used to successfully rear millions of coral larvae. Due to periods of inclement weather, the coral spawning, fertilization and embryo and larval rearing work was done mainly in the BML aquarium system where conditions could be best controlled during the primarily nocturnal coral spawning and planula release periods.

Fertilization experiments were completed with gametes of three broadcast-spawning coral species to determine the optimum sperm concentrations and periods for crossing eggs and sperm. These experiments showed that sperm concentrations at or above 10^4 ml^{-1} resulted in the highest rates of fertilization, and that fertilization rates and health status of egg cultures declined after 2-4 hours after spawning. These results were used to optimise sperm concentrations and gamete combination periods to maximise fertilization rates and the health status and numbers of embryos and larvae reared for experiments.

A series of experiments were also done to determine the most suitable types and design of larval settlement tiles for monitoring settlement and recruitment patterns, including testing of innovative 3D printed plastic tiles. The effects of different larval densities on settlement and initial post-settlement survival, and effects of different conditioning times on settlement patterns were also examined to optimise the coral larval settlement responses.

Testing of different types of settlement tiles and larvae from different species showed a range of responses, however significantly higher settlement rates occurred on tiles cut from dead plate *Acropora* skeletons compared with artificial fibre-cement or terra-cotta tiles for three of the *Acropora* species tested, with no significant differences detected for larval settlement of two *Acropora* and a brain coral species. Therefore, the natural *Acropora* skeleton tiles were selected as the standard tile type for the mass larval enhancement experiments in the field and for most of the subsequent larval settlement experiments.

Additional experiments comparing tiles printed using two types of common commercially available 3D printing plastics and *Acropora* skeleton tiles showed that larval settlement rates were similar among tile types. Therefore these plastics are not toxic to the two types of coral larvae tested and hence 3D printing has very good potential for designing new types of larval settlement tiles to optimise coral larval settlement and recruitment in future reef restoration studies. A final series of experiments examined the influence of different periods of immersion time and biologically conditioning of settlement tiles on larval settlement. The results demonstrated that the period of biological conditioning strongly affects larval settlement rates, with increased settlement on tiles conditioned for two months and maximum settlement on tiles with four months immersion and conditioning.

An experiment was also completed to quantify the effect of providing different densities of larvae on settlement rates and initial survival. The results showed increased settlement with increased larval density, however survival decreased at the two highest densities. Therefore the effects of density on settlement and recruitment are important factors to consider in designing future larval settlement and reef restoration experiments.

Objective 2. Reseed replicate areas of damaged reefs by facilitating mass settlement of coral larvae from the rearing enclosures.

This objective was achieved through two major field experiments. In April 2013 approximately 1.6 million larvae of *Acropora tenuis* were reared and used in the first mass larval enhancement experiment on degraded reef areas on Magsaysay Reef. Approximately 400,000 larvae were added to and retained in low-cost organza cloth and hapa matting larval enclosures in each of four replicate degraded reef sites on Magsaysay Reef. The experiment was highly successful and resulted in relatively high rates of larval settlement on conditioned coral skeleton tiles (mean of 27 spat per tile) that were used to quantify the initial settlement patterns. Larval settlement was significantly higher on side surfaces compared to bottom and top tile surfaces. As expected, no *A. tenuis* larvae settled on tiles in the four control sites that were enclosed by mesh nets but not provisioned with larvae during the five day settlement period.

A second major field experiment was initiated in June 2016 using larvae of *Acropora granulosa*. Approximately 900,000 larvae were successfully reared and about 300,00 larvae were added into each of three of the organza-hapa larval mesh enclosures for the five day settlement period. Settlement responses were similar to those of A. tenuis, with relatively high rates of settlement on conditioned coral skeleton tiles, with a mean of 25 spat per tile. No corals settled on tiles in the three control sites that were not provisioned with larvae.

Together, these experiments demonstrate that the provision of large numbers of competent coral larvae of two *Acropora* species can significantly increase coral larval settlement on damaged reef areas, satisfying Objective 2.

Objective 3. Quantify patterns of coral larval settlement and early recruitment in replicate reseeded reef areas versus natural areas of similar reef habitat to determine the enhanced recruitment rates.

This objective was achieved through two forms of repeated monitoring of the experiments from Objective 2. The initial patterns of survival and recruitment of coral spat that were too small to be visually censused were quantified by repeated monitoring of coral spat that settled on tile surfaces, until the surviving corals grew large enough to become visible recruits that could be monitored *in situ* at about 8-9 months after settlement. At this stage the visible coral recruits were also able to be seen on the natural reef substrata, hence patterns of recruitment could also be directly monitored and quantified on the degraded reef sites at Magsaysay Reef.

The survivorship of the *A. tenuis* recruits on tiles in the larval enhancement sites showed the expected rate decline over time during the first 6-9 months after settlement. In contrast the *A. granulosa* juveniles exhibited a survivorship rate after settlement. In both species, the rates of early recruitment continued to be significantly higher in the larval enhanced sites compared with the natural control reef sites that were not provisioned with larvae.

Objective 4. Quantify rates of settled juvenile coral growth and survival to assess the ecological and cost-effectiveness of mass larval reseeding in reef restoration.

This Objective follows on from Objectives 2 and 3, and was achieved by continued monitoring of the patterns of growth and survival of juvenile coral recruits that had settled on the tiles and on the natural reef substrata, once they became visible recruits at 8-9 months after settlement.

There was remarkably high survival of visible recruits, with 100% survival of *A. tenuis* recruits on both the settlement tiles and on the natural reef substrata during the period from 9 months until 2 years after settlement. A slight decrease in survivorship was recorded for the *A. granulosa* visible recruits between 9 and 10 months after settlement, but recruitment was still significantly higher in the larval enhanced sites compared with the control reef sites.

Repeated monitoring of surviving recruits also showed relatively rapid growth and no significant differences in size or average growth rates between corals that settled on tiles versus those that settled on natural reef substrata. *Acropora tenuis* recruits grew to visible size at about 9 months after larval settlement and continued to grow quickly to an average size of 13 cm mean diameter by two years of age. *Acropora granulosa* recruits grew to a visible size at about 8 months after settlement.

Together, the results of the two major field experiments using *Acropora* larvae clearly demonstrate that mass larval settlement can be effective at initiating mass larval settlement and high rates of recruitment even on degraded reef areas using low-cost techniques. Therefore this approach to initiating coral population and reef restoration can be ecologically effective and cost-effective.

Objective 5. Use the results of this research to develop guidelines and technical training reports and peer-reviewed publications in high-ranking international refereed journals to enhance restoration of damaged coral reef around the world in future.

The results of this research have been used to develop improved protocols for guiding the collection and rearing of millions of coral larvae from a range of coral species and for field trials using mass larval enhancement. The main project results have been presented at national and international conferences and a paper was submitted to the high-ranking international journal *Science*. The project outcomes have also been used in training a range of other workers to increase skills that can be applied to other reef restoration projects in future.

An additional important outcome from the project is that the results have been used to modify the recent ‘Memorandum of Understanding between the Philippines and Australia on Coral Reefs’. This MoU acknowledges that “Australia and the Philippines share a common view on the protection and conservation of coral reefs” and recognises the important roles coral reefs play in the marine ecosystem, their very significant economic benefits, and the “benefits arising from cooperating and exchanging information on key issues associated with the protection and conservation of coral reefs”. The revised MoU also states that “Australia and the Philippines are collaborating on projects that seek to restore coral reefs in the Philippines using coral larval reseeding”, which specifically refers to, and highlights, the results from this SRA project. The MoU also notes that the

participants undertake to “share knowledge, expertise, research skills and technical training relating to coral rearing and reef restoration techniques” resulting from collaborative projects.

7 Key results and discussion

The project has achieved its aim and the main research objectives and has provided globally significant results and impacts that are detailed below, arranged in order of the objectives and the coral reproductive life stages.

7.1 Coral spawning and larval release

Research during this project has substantially increased knowledge of the specific timing and patterns of major coral spawning and brooding periods for 18 ecologically important coral species in the Bolinao-Anda reef region, northern Luzon, Philippines (Fig. 7.1). Data on spawning periods and timing of gamete release were recorded for 16 scleractinian reef-coral species including 13 *Acropora* branching coral species (Fig 7.2), three species of brain corals (Figs. 7.3, 7.4). Data on planulae release was also obtained for two brooding species, the branching coral *Porites cylindrica* and the non-scleractinian blue octocoral *Heliopora coerulea* (Fig. 7.1).

Significant coral spawning periods are now known to occur over an extended period from February through to July each year (Fig. 7.1), which corresponds to a period between monsoons when less rainfall occurs in the region. This new information significantly advances our knowledge of the timing of spawning for these species and extends the period during which millions of spawned coral gametes can be reliably obtained and used for rearing millions of coral larvae for manipulative experiments including mass larval enhancement experiments on damaged reefs. Large multispecific coral spawning was also recorded *in situ* during March 2015 at the 'Coral Garden' on Magsaysay Reef resulting in a surface coral spawn slick containing many millions of coral embryos. These larger-scale synchronized spawning events are important, as they will enable larger scale larval rearing and settlement experiments to be completed on local reefs in future.

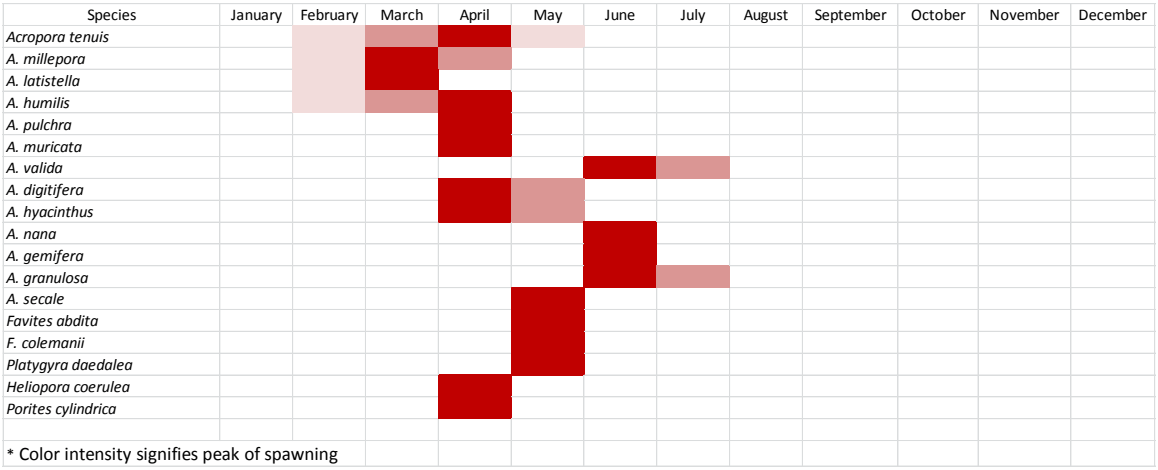

Figure 7.1. Summary of coral spawning periods recorded for 18 coral species from the Bolinao-Anda reef region.

Figure 7.2 *Acropora valida* branching coral spawning egg-sperm bundles in the BML aquarium facility in April 2012 (Image: P. Harrison).

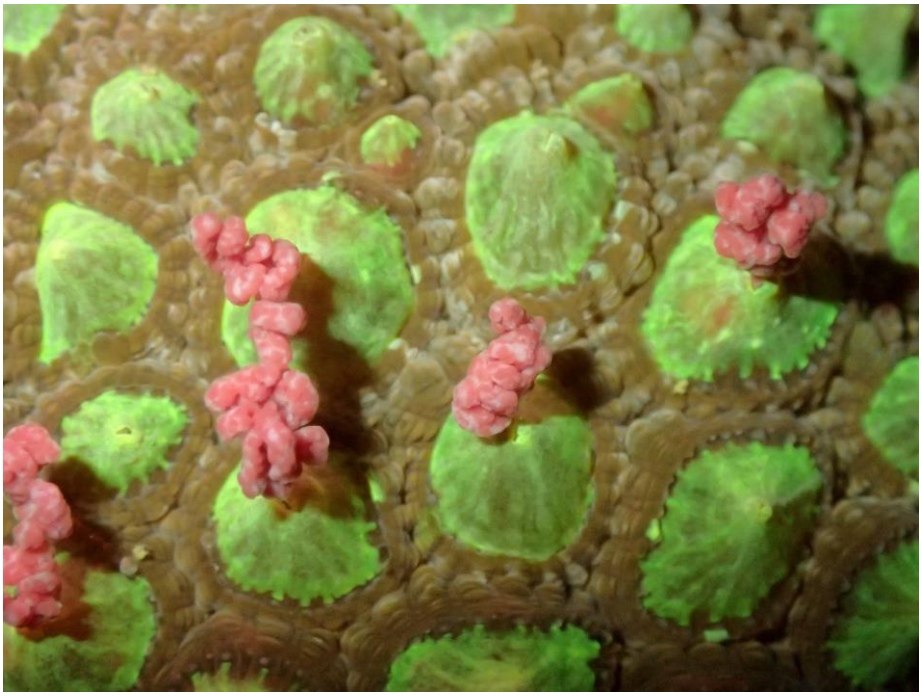

Figure 7.3 *Montastrea colemani* brain coral spawning egg-sperm bundles in the BML aquarium facility in May 2015 (Image: P. Harrison).

Figure 7.4. *Favites abdita* brain coral colony spawning egg-sperm bundles in the BML aquarium facility in May 2015 (Image: P. Harrison).

The new data obtained during this project extend the earlier records of coral spawning in the Bolinao region by Vicentuan et al. (2008) who noted that multispecific coral spawning occurred after full moon periods from March to June, with a maximum of 13 species recorded spawning together after the full moon in May 2007. The patterns of coral spawning in this region are similar to those in some other tropical reef areas (reviewed by Harrison and Wallace 1990, Richmond and Hunter 1990, Baird et al. 2009, Harrison 2011) and are characterised by relatively predictable and discrete spawning periods for three brain coral species and some *Acropora* spp., with split-spawning (*sensu* Willis et al. 1985) and more variable spawning patterns over two or more lunar cycles for other *Acropora* species (Fig. 7.1).

7.2 Fertilization and larval rearing

Experiments were completed using spawned eggs and sperm of *A. millepora* and *A. tenuis* and the brain coral *M. colemani* to determine the optimal ranges of sperm concentrations and temporal response patterns when crossing of eggs and sperm was delayed. These experiments were designed to optimise fertilization responses in these corals for maximising the success of embryo and larval cultures. The results showed that sperm concentrations below 10^3 ml^{-1} resulted in low rates of fertilization for all species, but that sperm concentrations at 10^4 ml^{-1} resulted in high rates of fertilization for the *Acropora* spp. and near maximum fertilization rates for *Montastrea* (Fig. 7.5a). At sperm concentrations of 10^5-10^7 ml^{-1} maximum fertilization was achieved for all three species (Fig. 7.5b). Therefore optimizing fertilization success in these corals requires sperm densities of $\geq 10^4$-10^5 ml^{-1}. Accordingly, sperm concentrations in larval cultures were adjusted to maximize fertilization rates and hence the numbers of embryos and larvae reared for experiments.

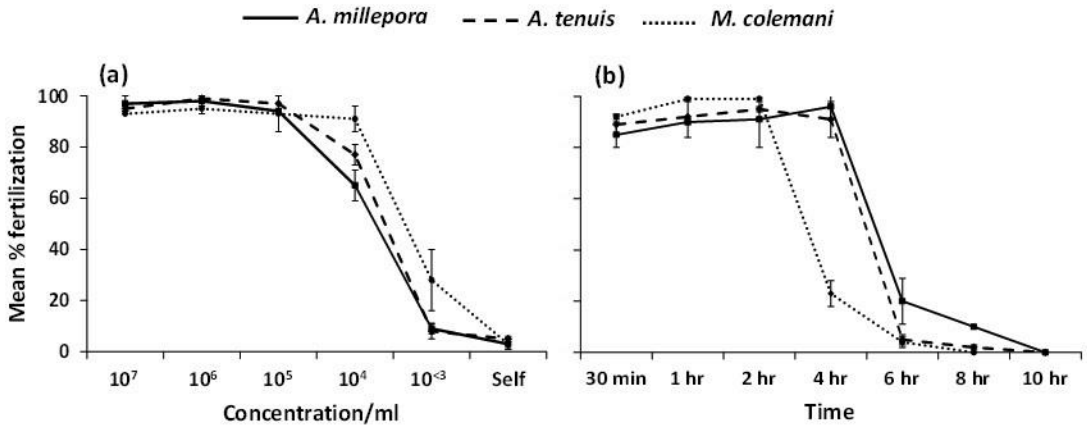

Figure 7.5. Fertilization responses (mean ± SD) for three coral species using, (a) different concentrations of sperm, and (b) delayed crossing of eggs and sperm for periods up to 10 hours after spawning.

Similar fertilization response curves have been recorded in other species of *Acropora* and brain corals (Willis et al. 1997, Nozawa et al. 2015) hence sperm density is an important consideration for successful coral embryo and larval production. Furthermore, given the ongoing decline in coral populations on many reefs around the world and particularly in the Philippines and some other regions of Southeast Asia, populations of corals may face increasing sperm limitations (Levitan and Petersen 1995, Yund 2000) and therefore impaired reproductive success in future as the numbers of gravid corals that can spawn synchronously decreases. This reinforces the need to re-establish large numbers of coral colonies of the same species during coral restoration projects to increase the likelihood of successful fertilization and production of large numbers of coral larvae that can contribute to larval settlement and recruitment to maintain coral populations in future.

Delayed fertilization experiments were also completed to examine the effects of gamete ageing and minimum contact periods required to optimize fertilization success in these three species. Very high fertilization rates were achieved when eggs and sperm were combined after 30 minutes and up to 2 hours for all three species and up to 4 hours for *A. millepora* and *A. tenuis* gametes (Fig 7.5b). Fertilization rates were significantly reduced after a delay of 4 hours for *M. colemani* and after 6 hours for the *Acropora* spp., with no fertilization after a delay in combining gametes for 10 hours (Fig 7.5b). These trends mirror the health status of the eggs of the three species, with a high proportion of healthy eggs maintained in *Montastrea* cultures for only 2 hours and up to 4 hours for *Acropora* spp. cultures with increasing numbers of deformed or lysing eggs present after these times (Fig. 7.6). A previous study on the effects of gamete age of *A. millepora* from the GBR by Willis et al. (1997) reported similar trends with about 95% fertilization up to 6 hours after spawning and a decline thereafter.

The fertilization responses recorded in the present study show that gamete age is an important factor when rearing coral embryos and that gametes should be combined within the first few hours after spawning to achieve maximum rates of fertilization and healthy embryo cultures. Accordingly, this project routinely combined gametes within two hours after spawning events and with a minimum of 1 hour gamete contact time to enable high rates of fertilization before SFSW was used to remove excess sperm from the embryo cultures to avoid polyspermy and to maintain the health of eggs and sperm. The importance of gamete contact time has also been demonstrated in a recent study by Nozawa et al. (2015) comparing fertilization responses of two *Acropora* and four brain coral species. They found that highest rates of fertilization were achieved using gamete contact times of 60 minutes compared with shorter periods of 10 and 30 minutes.

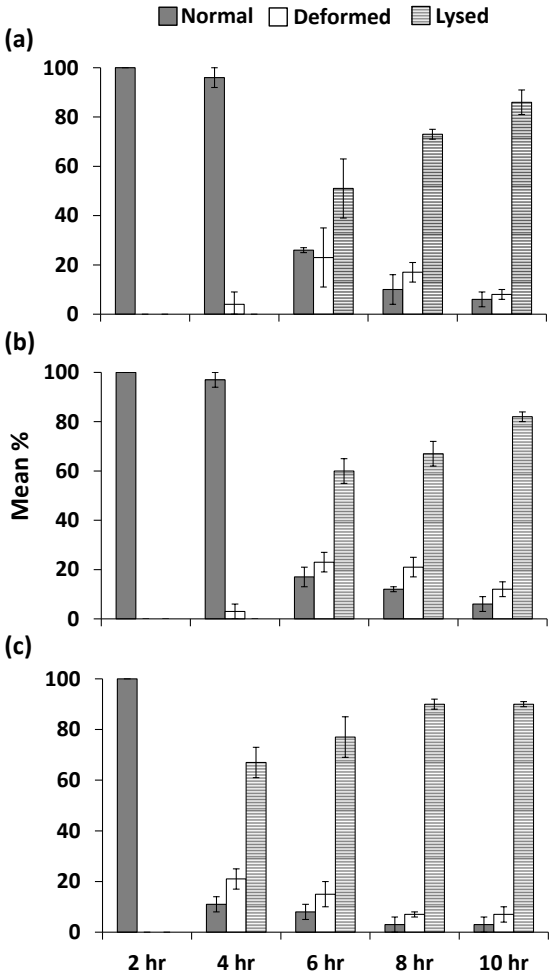

Figure 7.6. Effects of delayed fertilization on the health status of coral eggs in cultures of (a) *A. millepora*, (b) *A. tenuis*, and (c) *M. colemani*.

7.3 Larval settlement experiments

Coral larvae are discriminating in their choice of settlement sites, and the choice they make during this period of their life cycle is critically important for subsequent survival and growth because they become permanently sessile after they metamorphose from the free-swimming planktonic planula larval stage and settle permanently as benthic juvenile coral polyps (Harrison and Wallace 1990). Most coral larvae require biologically conditioned substrata to induce metamorphosis and settlement, but a wide range of settlement preferences and responses have been reported among different coral species in relation to different types of substrata, microtopography and orientation, biofilm components and presence of crustose coralline algae (CCA) or algal competition, larval age and competency periods, and environmental factors including temperature, light, salinity, and water quality (e.g. Harrison and Wallace 1990, Babcock and Mundy 1996, Morse et al. 1996, Ward and Harrison 1997, Wilson and Harrison 1998, Heyward and Negri 1999, 2010, Nozawa and Harrison 2002, Harrington et al. 2004, Webster et al. 2004, Gleason and Hofmann 2011, Edmunds et al. 2014, Whalan et al. 2015, Tebben et al. 2015). Therefore a range of coral larval settlement experiments was completed during this project to determine the most suitable type of larval settlement tiles for monitoring settlement and recruitment patterns, the effect of larval density on settlement and initial survival, and effects of different conditioning times on settlement patterns to optimise settlement responses.

Effects of natural coral skeleton versus artificial tile substrata on larval settlement

A series of experiments were completed to examine the settlement responses of larvae from different coral species to tiles made from natural coral *Acropora* skeletons and various artificial tile substrata. The first experiments examined settlement responses of *A. pulchra*, *A. intermedia, A. tenuis, A. granulosa* and *F. abdita* larvae to tiles made from coral versus fibre-cement. Significantly higher mean settlement was recorded for *A. granulosa* larvae on natural coral tiles (1867.7 ±472.6) compared to fibre-cement tiles (351.6 ±49.6; $F = 10.18$, $P = 0.01$; ANOVA) (Fig 7.7). Mean settlement of *A. pulchra* larvae was also significantly higher on the natural tiles (211.9 ±94.8) versus the fibre-cement tiles (43.07 ±19.3; $F = 13.36$, $P = 0.006$, ANOVA) (Fig. 7.7), and although lower rates of *A. intermedia* larval settlement were recorded, settlement was still significantly higher on the natural coral tiles (3.2 ±3.2) compared to artificial tiles on which no larvae settled. No significant differences in larval settlement were recorded between the two tiles types for *A. tenuis* (natural coral = 1735.3 ±88.1; fibre-cement = 2208.5 ±418.9; ($F = 1.22$, $P = 0.3$; ANOVA) and *F. abdita* (natural coral = 1027.6 ±204.8; fibre-cement = 981 ±317.6; ($F = 0.02$, $P = 0.9$; ANOVA).

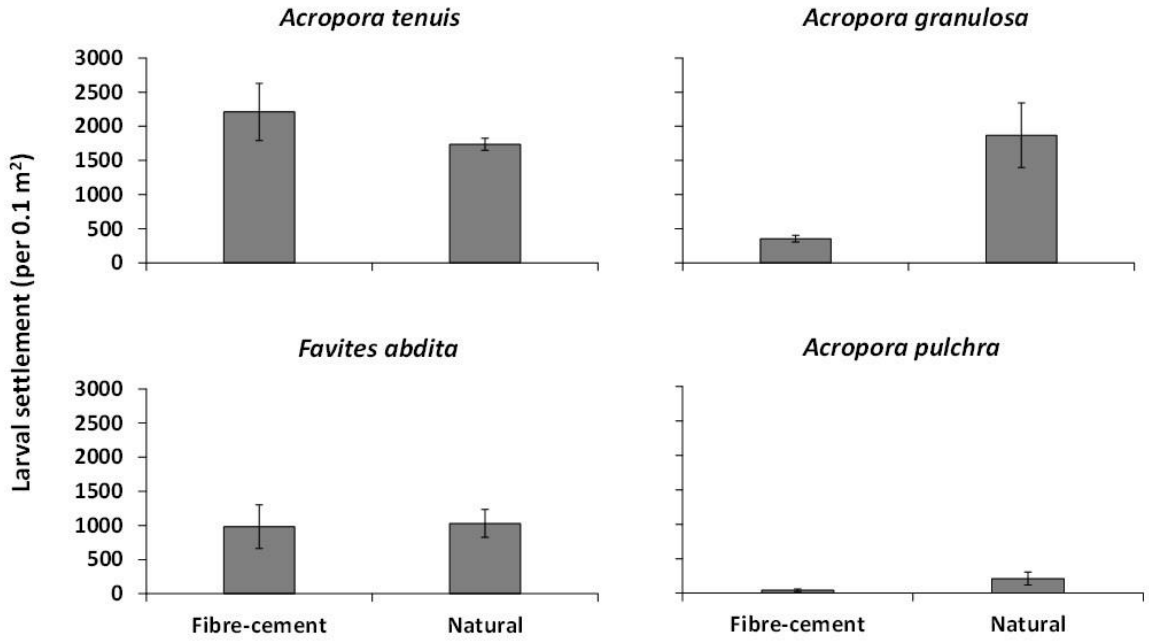

Figure 7.7. Larval settlement responses (mean ± SE) on natural *Acropora* skeleton tiles and fibre-cement tiles for four coral species.

Subsequent experiments testing coral, fibre-cement and terracotta tiles showed significantly different responses among larvae of *A. millepora* and brooded larvae from *P. cylindrica* and the blue coral *H. coerulea* ($F = 5.91$, $P = 0.02$; $F = 18.38$, $P = 0.000$; and $F = 16.80$, $P = 0.0003$, respectively; Two-way ANOVA) (Fig. 7.8). For *A. millepora* larvae mean settlement was significantly higher on both natural coral tiles and fibre-cement compared to terracotta tiles (natural = fibre-cement > terracotta). For brooded *P. cylindrica* and *H. coerulea*, larvae of both species had significantly higher settlement on fibre-cement tiles compared to both natural and terracotta tiles (fibre-cement > natural = terracotta) (Fig. 7.8).

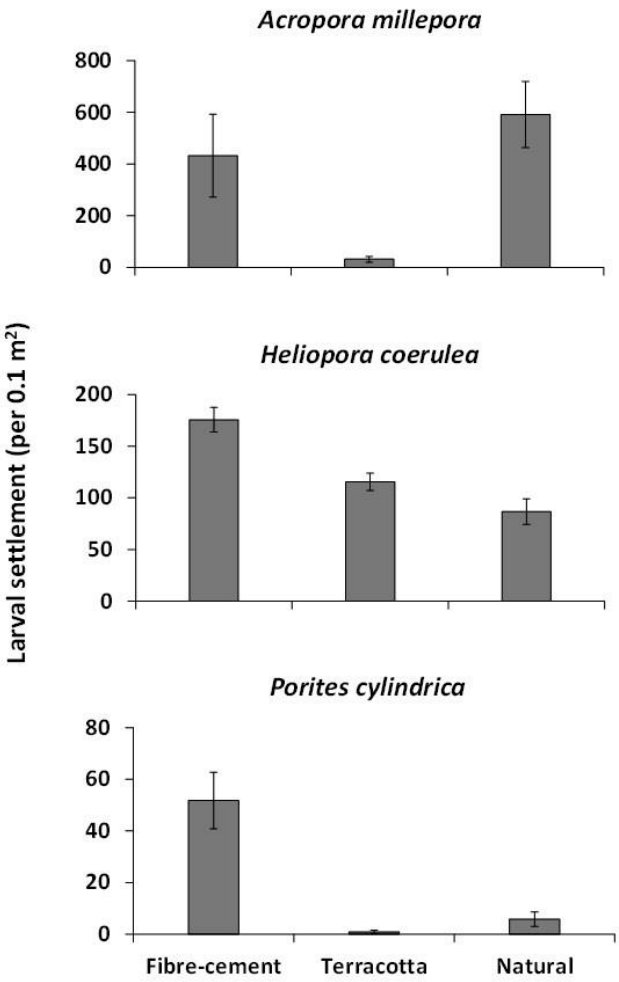

Figure 7.8. Larval settlement responses (mean ± SE) on natural *Acropora* skeleton tiles terracotta and fibre-cement tiles for two reef coral species and the blue coral *Heliopora*.

Based on these results with significantly higher larval settlement on coral skeleton tiles versus artificial tiles for three *Acropora* species and no significant differences in two other *Acropora* and one brain coral species, the natural *Acropora* skeleton tiles provide more appropriate settlement surfaces to monitor larval settlement and subsequent survivorship of corals over time. The natural coral tiles also have a range of irregular surface structures and micro-crevices that may provide potential refuges for settling corals. Therefore these natural *Acropora* skeleton tiles were used as the standard larval settlement and recruitment unit for most of the subsequent larval settlement experiments and the mass larval enhancement field restoration experiments (see Section 7.4).

Larval density experiment

The density of coral larvae available for settlement and subsequent recruitment is likely to have important ecological consequences for coral recruitment, but relatively few studies have been done on the effect of different coral larval densities on settlement responses. Therefore, an initial experiment was done in May 2012 to quantify settlement rates of *A. tenuis* larvae using four larval densities and natural coral versus fibre-cement tiles to examine the initial patterns of post-settlement survival. After a five day settlement period, significant differences in settlement rates were recorded among different density treatments but not between tile types ($F = 22.63$, $P = 0.000$ and $F = 0.51$, $P = 0.486$, respectively; Two-way ANOVA). Mean larval settlement increased with increasing densities of larvae (Fig. 7.9), indicating a clear density-dependent effect. Mean settlement

rates were similar on natural coral and fibre-cement tiles, similar to responses of *A. tenuis* larvae in the previous experiment (Fig. 7.7). However, there were significant differences in the proportion of larvae remaining alive between densities and between tile types (F = 13.35, P = 0.000 and F = 12.67, P = 0.001, respectively; Two-way ANOVA). A decreased percentage of recruits were alive at the two higher density treatments and lower survival was recorded on fibre-cement tiles versus natural coral tiles (Fig. 7.9).

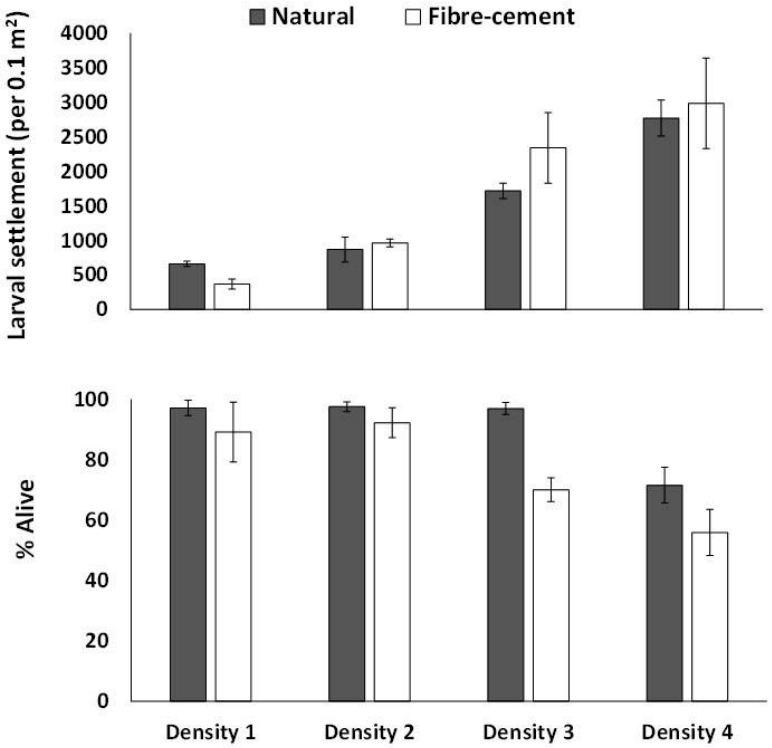

Figure 7.9. Effect of different *Acropora tenuis* larval densities in 70 L settlement tubs (1 = 1,800, 2 = 3,600, 3 = 7,200, 4 = 14,400) on larval settlement responses (mean ± SD) on natural *Acropora* skeleton tiles and fibre-cement tiles, and percentages of living settled larvae on tiles after five days (lower graph).

These results indicate that although provision of increased densities of *A. tenuis* larvae during the larval settlement period can increase initial settlement rates, post-settlement survival may be compromised at higher densities (Fig. 7.9). A similar conclusion was reached by Suzuki et al. (2012) who examined the effect of using three densities of *A. muricata* and *A. tenuis* larvae on larval settlement and survival. They found that initial larval settlement on artificial polycarbonate grids coated in fine sand grains was proportional to larval density, but that survival rates were much lower in the high-density treatment. After three months most experimentally settled corals had died in the low density treatment, hence they concluded that the optimal density for 'seeding' these *Acropora* larvae on artificial grid plates was the mid-density treatment using about 5,000 larvae per m^2 (Suzuki et al. 2012).

Larval settlement experiments with 3D tiles versus natural coral tile substratum

Many factors influence coral larval settlement responses including the type of substratum and its rugosity and the presence of small refugia that may enhance settlement responses in some corals (e.g. Edmunds et al. 2014, Whalan et al. 2015). The emerging 3D printing technology enables new experimental approaches to examining the effects of different coral larval settlement surfaces including replicating natural coral and reef surface shapes and rugosity and the presence of micro-settlement pores and refugia. However, some

plastics can leach toxic compounds hence it is not known whether commonly used 3D plastics will affect coral larvae and their settlement responses. Therefore two innovative settlement choice experiments were completed testing the effects of using two types of common commercially available 3D printing plastics (PLA and ABS) on coral larval settlement to determine whether the use of 3D printed tiles may be useful in optimising coral larval settlement and restoration activities.

Larvae of *M. colemani* were offered a choice of settlement on natural *Acropora* skeleton tiles and replica plastic tiles printed in PLA or ABS from scans of the original coral tiles. After a five day larval settlement period, low numbers of larvae had settled on all three types of tiles and there were no significant differences in mean settlement among the three tile types (Fig. 7.10). The settlement responses indicate that the PLA and ABS materials did not inhibit coral larval settlement, and slightly higher settlement rates occurred on these plastic replica tiles compared with the original coral skeleton tiles (Fig. 7.10), despite the relatively short five days biological conditioning period prior to use in the experiment.

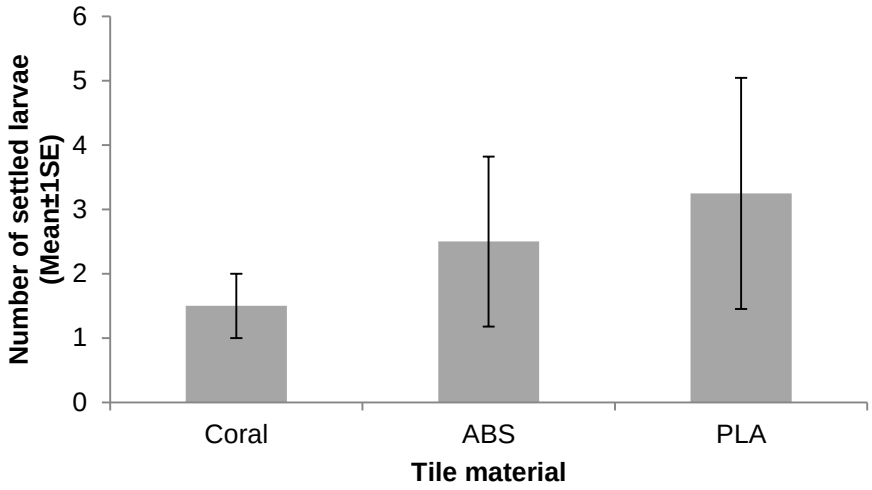

Figure 7.10: Settlement of *Montastrea colemani* larvae on tiles cut from *Acropora* coral and replicas 3D printed in PLA and ABS from 3D scans of the original coral tile. Data are means ± 1 SE (*n* = 4).

A subsequent experiment using *A. gemmifera* larvae examined the effects of these three tile materials and two biological conditioning treatments (reef conditioned or BML hatchery conditioned) on larval settlement responses. There were no significant differences in mean larval settlement among the two plastic replica tile treatments and conditioning site, however settlement rates were higher on coral tiles that had been biologically conditioned in the BML flow-through seawater system for four weeks compared to coral tiles that been conditioned on the natural reef environment (Fig. 7.11). The results indicate that conditioning settlement tiles in the BML hatchery for fours weeks provides sufficient biological conditioning appropriate for coral larval settlement experiments and that the use of the plastics PLA and ABS does not inhibit *A. gemmifera* larval settlement. Together the results from the two experiments with 3D printed plastic tiles indicate that PLA and ABS are not toxic to two types of coral larvae and that 3D printing offers good potential for designing improved coral settlement and recruitment tiles for future reef restoration research.

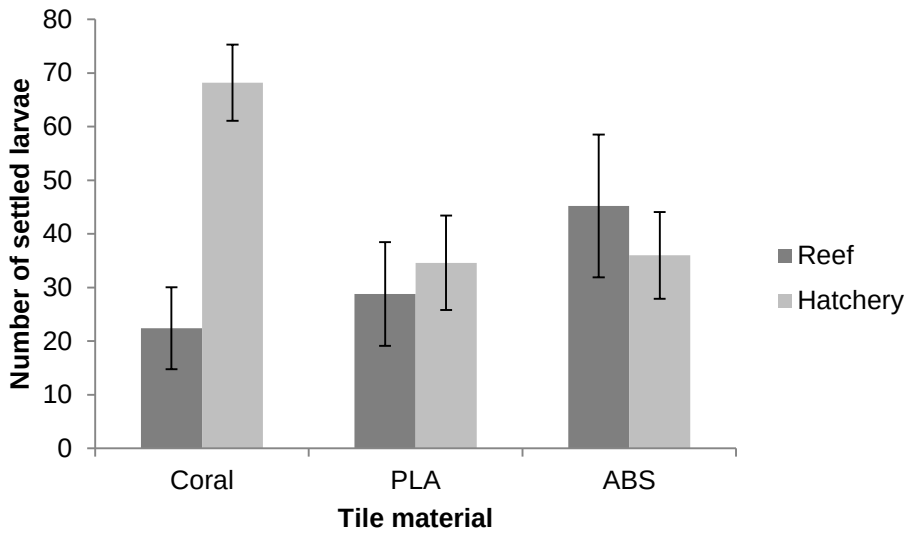

Figure 7.11. Settlement of *Acropora gemmifera* larvae on tiles cut from *Acropora* coral and replicas 3D printed in PLA and ABS from 3D scans of the original coral tile. Replicate tiles of each material were conditioned in either reef seawater or in flow-through seawater at the BML hatchery. Data are means (*n*=5) ± 1 SE.

Optimising biological conditioning times for larval settlement

It has long been known that most coral larvae require some form of biological conditioning on abiotic substrata to promote successful attachment and metamorphosis from the planktonic larval stage to the settled juvenile polyp stage (earlier research reviewed in Harrison and Wallace 1990). More recent research has highlighted the complexity of larval responses to microbial and algal biofilms and the importance of CCA in inducing metamorphosis and final settlement in larvae of some species (e.g. Heyward and Negri 1999, Webster et al. 2004, Tebben et al. 2015). One of many areas of uncertainty in coral larval settlement ecology is the period required for optimal biological conditioning of settlement surfaces to maximise larval settlement and survival.

Therefore, experiments were done with larvae of two coral species to quantify settlement responses to varying periods of natural coral skeleton tile immersion and biological conditioning in the BML hatchery. Similar settlement responses were evident in larvae of *A. tenuis* (Fig. 7.12) and *M. colemani* (Fig. 7.13), with very low levels of settlement on tiles that had been conditioned for only 24 hours. Significantly increased settlement occurred on tiles conditioned for 2 months, with highest settlement rates on tiles conditioned for 4 months, and intermediate settlement rates on tiles conditioned for 7 months (Figs. 7.12, 7.13). These results indicate that tile conditioning time does have a significant influence on larval settlement choice for these two species, with the optimum conditioning time or coral skeleton tiles in the BML hatchery being about 4 months. Longer 7 month conditioning times resulted in tiles becoming overgrown with other benthic organisms including sea anemones that covered much of the tile surfaces. It is likely that the higher density of competing organisms settled on the 7 month conditioned tiles reduced the area of available substrata for the coral larvae to settle on, or some of the benthos may have preyed on settling larvae or otherwise impaired their settlement responses, resulting in decreased settlement rates compared with 4 month conditioned tiles.

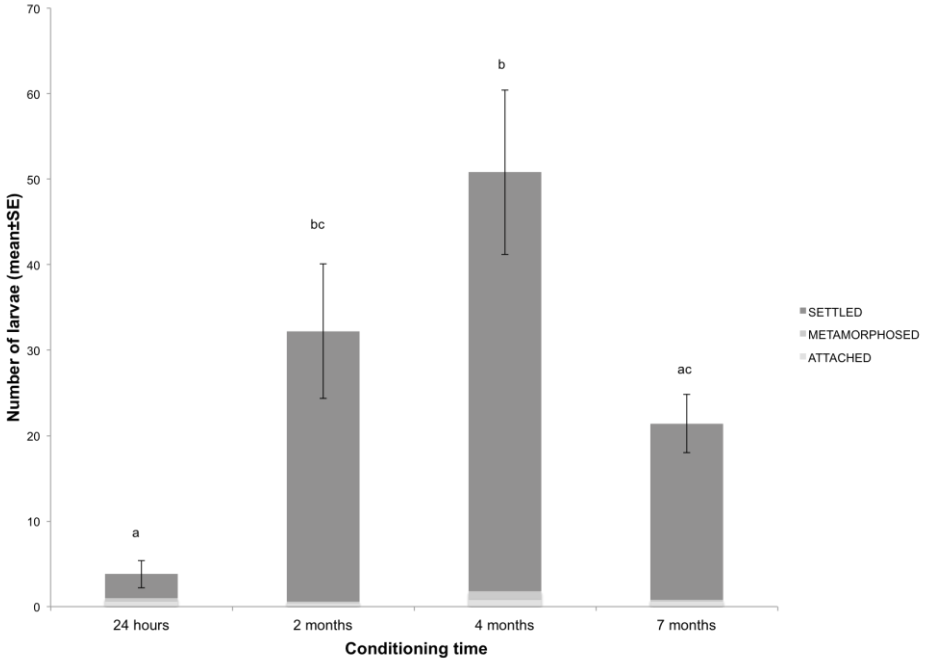

Figure 7.12. Number of settled, metamorphosed and attached *Acropora tenuis* larvae on *Acropora* skeleton tiles conditioned for 24 hours, 2, 4 and 7 months. Numbers are mean ± 1 SE (*n*=5). Means with different letters are significantly different (Tukey's HSD, P<0.05).

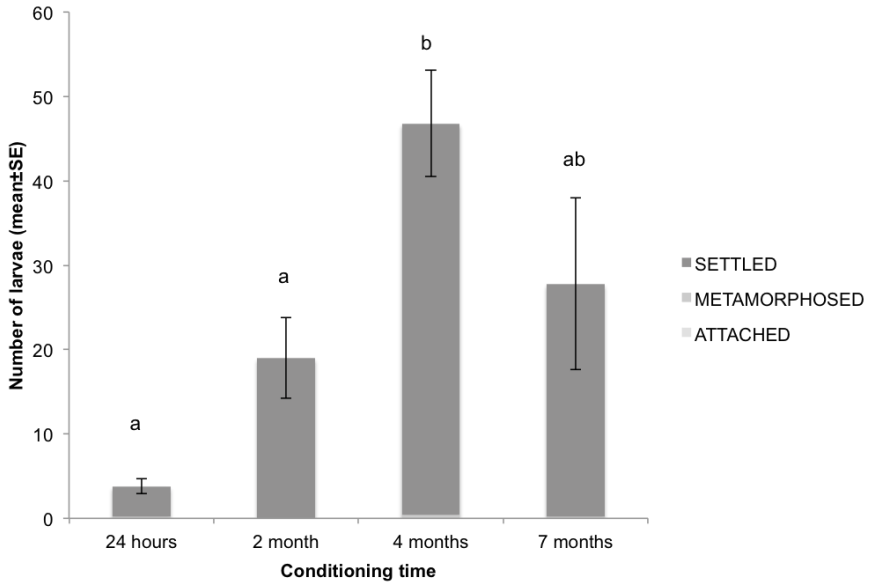

Figure 7.13. Number of settled, metamorphosed and attached *Montastrea colemani* larvae on *Acropora* skeleton tiles conditioned for 24 hours, 2,4 and 7 months. Numbers are mean ± 1 SE (*n*=5). Means with different letters are significantly different (Tukey's HSD, P<0.05).

7.4 Mass larval enhancement experiments

Two major field experiments were initiated during this project to quantify the effectiveness of using mass larval enhancement to initiate coral recovery on degraded reef areas in Magsaysay Reef.

Acropora tenuis larval enhancement experiment initiated in April 2013

Results of the benthic community analyses in the eight 6 x 4 m experimental reef sites established at Magsaysay Reef prior to the larval enhancement experiment showed that benthic cover and reef community status were comparable and not significantly different (*R*: 0.04, *P* = 0.40, ANOSIM) in the four replicate larval enhancement sites and the four control sites (Fig. 7.14). All sites were characterised by low mean living scleractinian coral cover of 15.6% (±1.6% SE), which equates to the category of very poor coral cover (*sensu* Wilkinson 2008), highlighting the degraded status of these reef areas. Other benthos including soft corals, sponges, macroalgae and dead coral covered with algae comprised 56.8% (±2.9% SE) of the mean benthic cover. Dead coral and coral rubble surfaces that were potentially available for coral larval settlement equated to 27.6% (±3.7% SE) of mean cover within these sites.

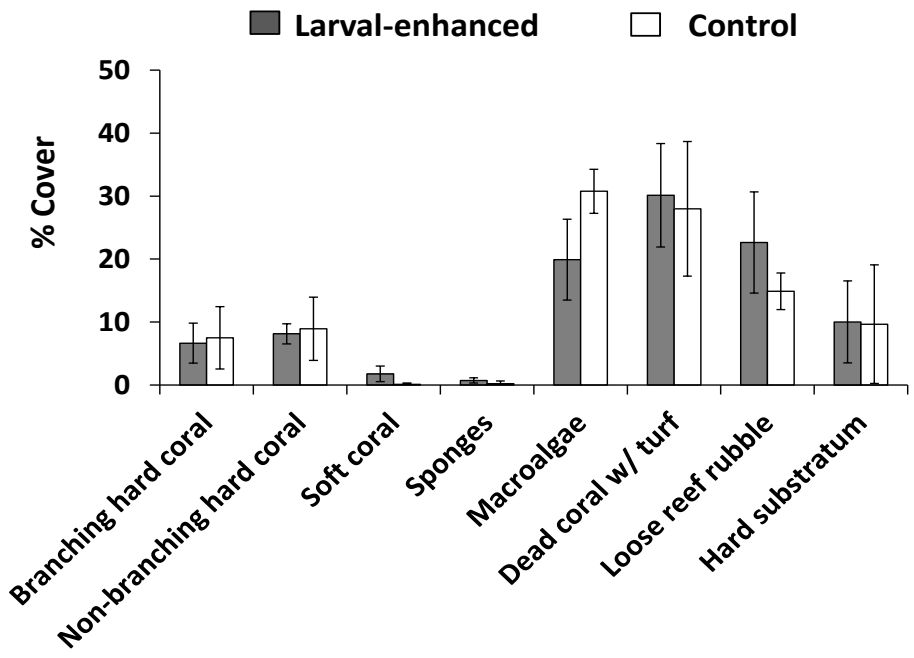

Figure 7.14. Percentage cover (mean ±SE) of benthic categories in larval-enhanced and control sites before the experiment.

After the five day settlement period in the larval mesh enclosures, a total of 1,021 *A. tenuis* spat settled on the biologically conditioned natural *Acropora* skeleton recruitment tiles that were attached to reef surfaces in the larval enhancement treatment sites (Fig. 7.15). Mean settlement of 27 (±6.1 SE) spat per tile in larval enhancement treatment sites was significantly higher than for control sites in which no *A. tenuis* spat settled on tiles (Fig. 7.16). Mean larval settlement was significantly higher on side surfaces of tiles (18.3, ±5.0 SE) compared to bottom (7.9, ±2.4 SE) and top surfaces (0.9, ±0.3 SE) (α = 0.05, Tukey's test, sides > bottom = top, Fig. 7.16).

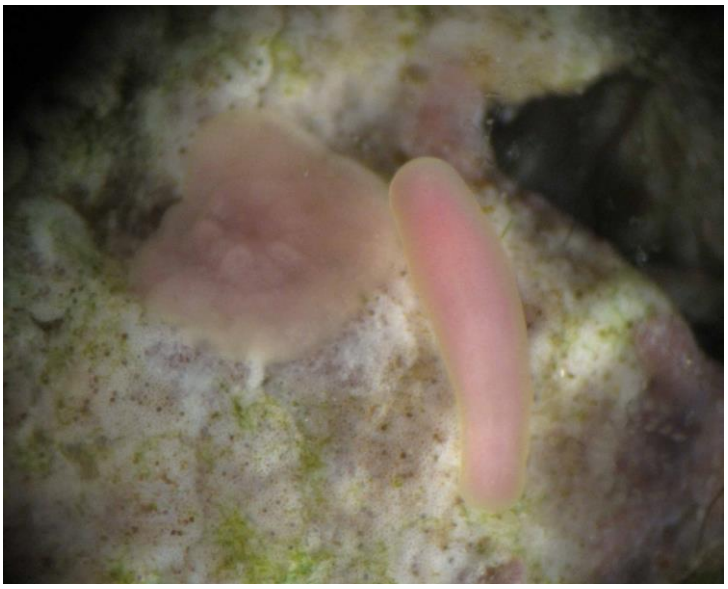

Figure 7.15. Newly settled *Acropora tenuis* juvenile polyp and elongated larva searching for suitable settlement site (Image: P. Harrison).

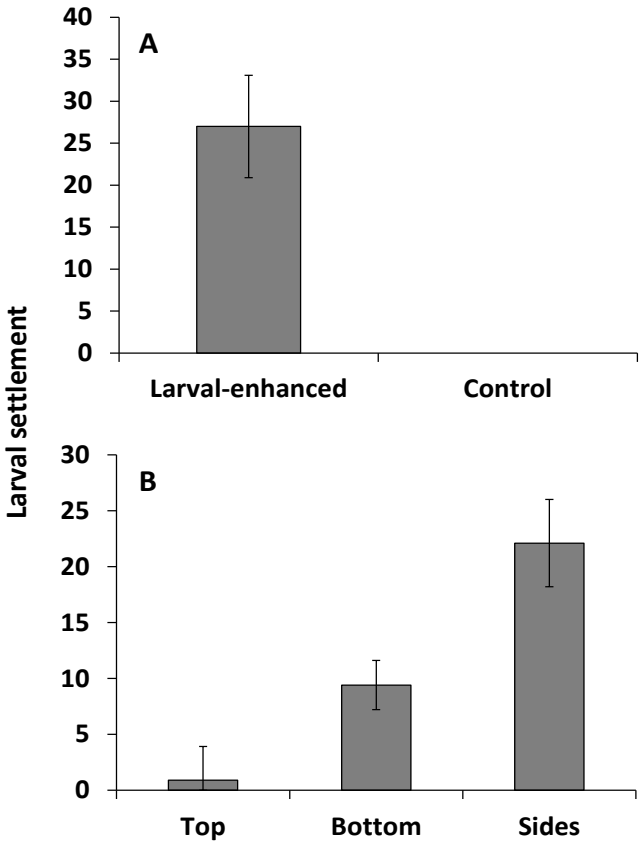

Figure 7.16. (A) Initial *A. tenuis* larval settlement (mean ±SE) on all tile surfaces in the larval-enhanced and control sites after 5 days, and (B) settlement on the different surfaces of tiles (mean ±SE) in the larval-enhanced sites.

Subsequent monitoring showed a decline in survivorship of settled juvenile corals on tiles during the first nine months after settlement (Fig. 7.17), approaching a Type III survivorship curve that is typical of many broadcast spawning marine invertebrates including corals (Wilson and Harrison 2005, Vermeij and Sandin 2008, Suzuki et al. 2011). However, survivorship stabilized after nine months which coincided with the period when the previously cryptic juvenile *A. tenuis* corals that had settled on the natural reef substrata had grown large enough to become visible recruits at 11.0 cm^3 (±4.0 SE) ecological volume, and could be identified and tagged for subsequent *in situ* growth and survivorship monitoring (Fig. 7.18). Remarkably, there was 100% survival of tagged settled juvenile *A. tenuis* recruits on the natural substrata and on recruitment tiles during the subsequent monitoring periods from nine months to two years after the experiment was initiated (Fig. 7.17).

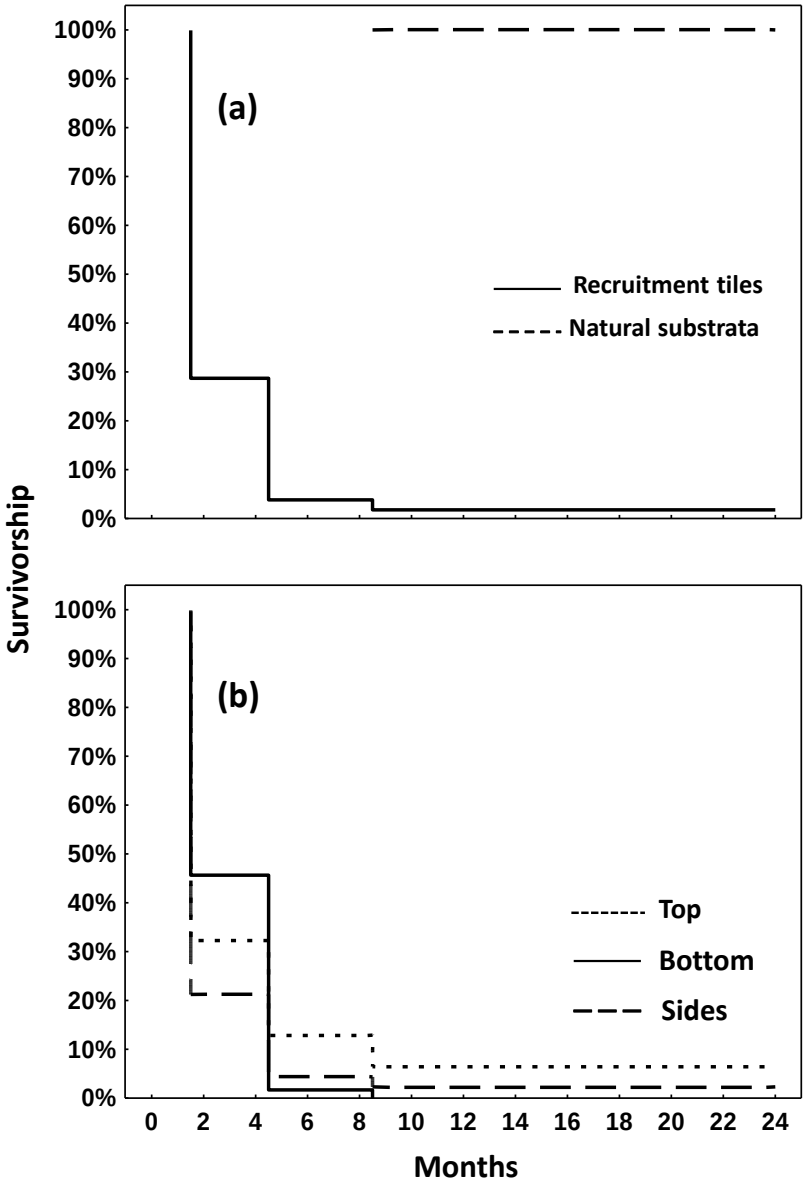

Figure 7.17. Kaplan-Meier survivorship over 24 months for (a) *A. tenuis* recruits settled on tiles, and for visible recruits on natural substrata starting at 9 months post-settlement, and (b) *A. tenuis* recruits on different tile surfaces.

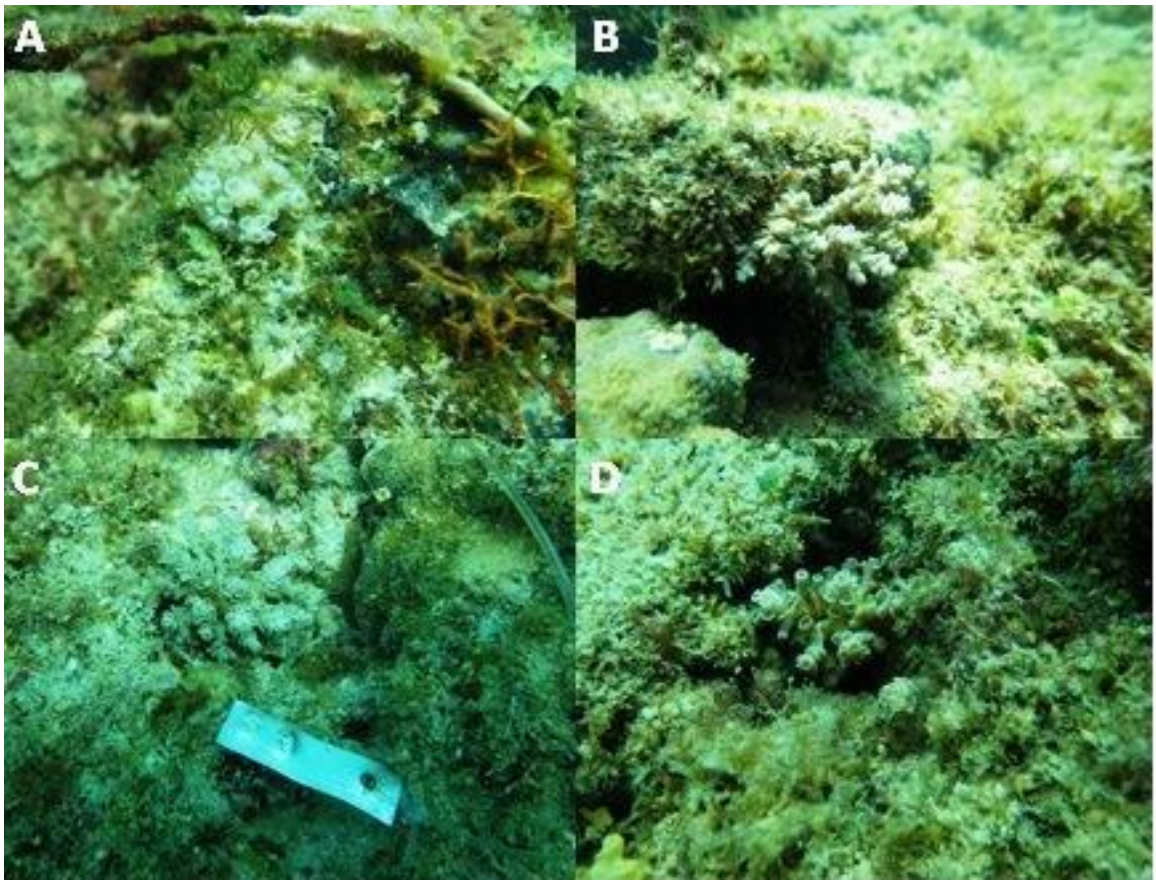

Figure 7.18. Juvenile *A. tenuis* corals that have grown large enough on settlement tiles (A, B) and natural substrata (C, D) to become visible recruits within the larval enhancement sites (Images: D. dela Cruz).

The stable survivorship of visible recruits on both the natural reef substrata and on the tiles occurred despite a recorded anomalous increase in sea surface temperature to 32°C and partial bleaching of some corals on nearby reef areas at 26 months after settlement (Fig. 5.9). Hence, the surviving recruits are likely to be those best adapted to the local environmental conditions on these reefs (van Oppen et al. 2015). This high survivorship after nine months may reflect the importance of size-escape thresholds in survival and recruitment processes once recruits attained visible size (Doropoulos et al. 2012).

There was no significant difference in the survival patterns of *A. tenuis* recruits that settled on tiles versus those that settled on natural reef substrata from February 2014 to May 2015 (Log-rank test, χ^2 = -26.02, p = 0.20). Conversely, a significant difference was found in the survivorship of recruits from May 2013 to May 2015 among different tile surfaces (Log-rank test, χ^2 = 52.09, p = 0.01; top = sides > bottom; Fig. 7.17). After two years, the mean number of surviving corals in each plot was 5 (±1.6 SE) on tiles and 24 (±12 SE) on natural reef substrata.

Initially, the ecological volume of the juvenile *A. tenuis* recruits on natural substrata (7.3 ±2.3 cm^3 SE) was slightly larger than on the recruitment tiles (3.2 ±1.3 cm^3 SE). However, after two years the ecological volume of corals growing on tiles versus those growing on natural reef substrata did not differ significantly in any of the monitoring periods (Repeated Measures ANOVA; p = >0.05) (Fig. 7.19). Average growth rates of *A. tenuis* recruits monitored on natural substrata from nine months to two years post-settlement reached

40.9 (±6.8) cm^3 mo^{-1} and were not significantly different ($F_{3,6}$ = 2.36, P = 0.17, ANOVA) from growth rates of recruits that settled on tiles (59.3 ±9.8 cm^3 mo^{-1}).

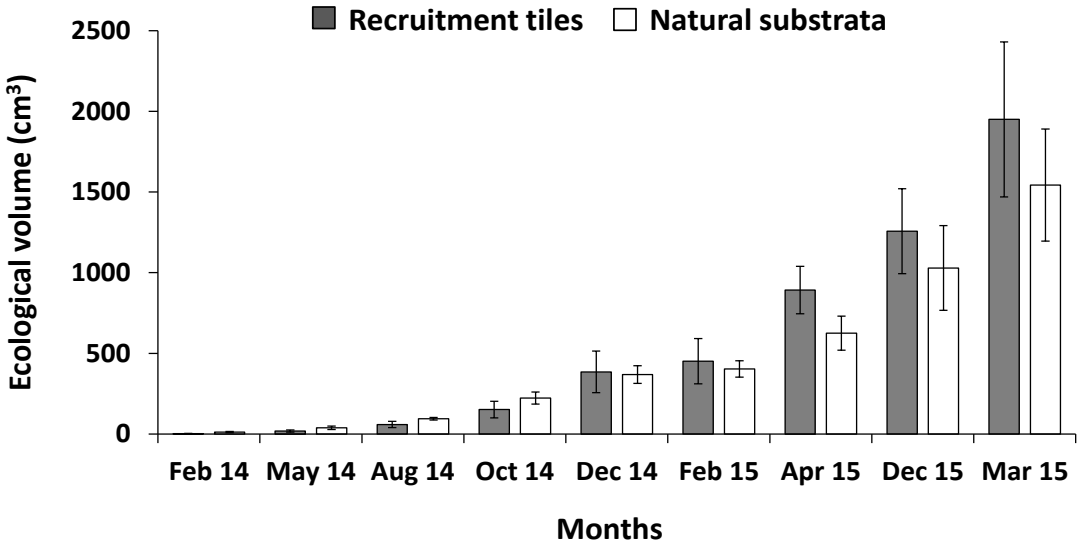

Figure 7.19. Growth in ecological volume (mean ± standard error) of juvenile *A. tenuis* on recruitment tiles and natural substrata inside the larval enhancement sites.

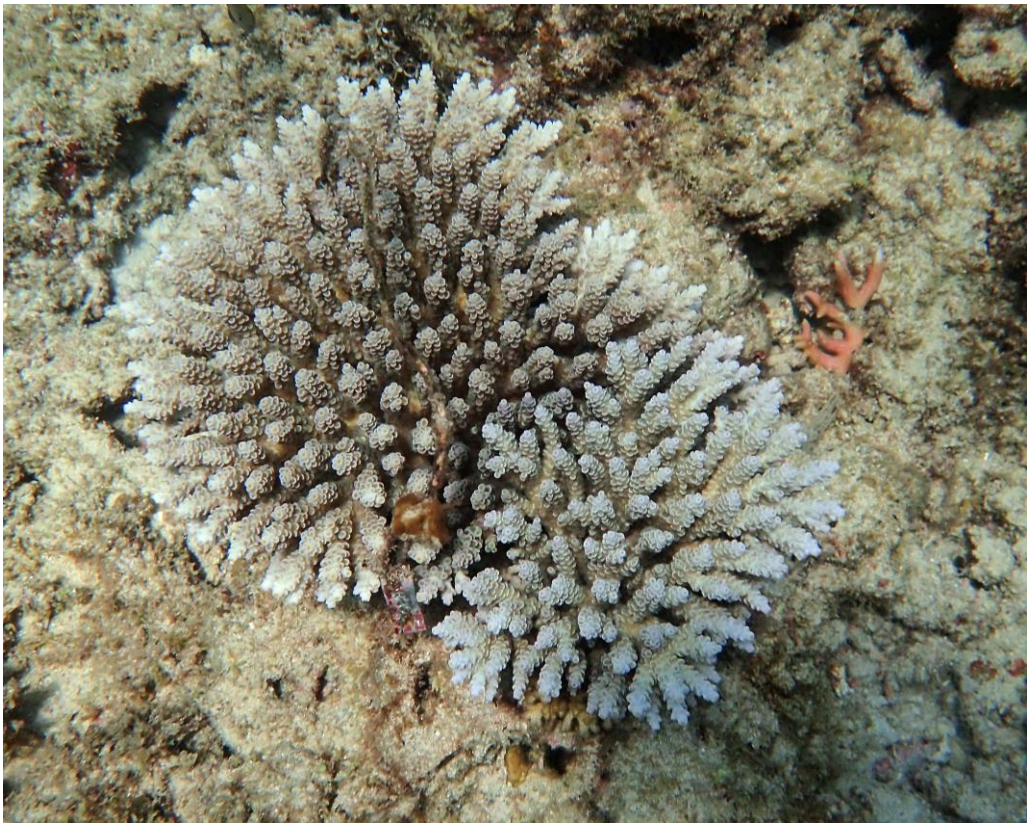

Figure 7.20. Two year old *Acropora tenuis* colonies photographed in April 2015 that were grown from larvae settled in larval enhancement sites in 2013 (Image: P. Harrison).

The *A. tenuis* recruits that settled on tiles and on natural reef substrata in this field experiment grew faster than cultured *A. tenuis* that were settled onto artificial substrata in

an outdoor hatchery and subsequently transplanted to reef areas in subtropical Okinawa, Japan (Omori et al., 2008). The *A. tenuis* transplanted in Okinawa reached a diameter of 20 cm (4 cm yr^{-1}) after 4 years of transplantation while in the present study the corals grew to a mean diameter of 13 ± 1.70 cm (6.5 cm yr^{-1}) in just two years (Fig. 7.20). The faster growth rate of *A. tenuis* colonies in the present study suggests that the surviving recruits were well adapted and acclimatized to the local environmental conditions at Magsaysay Reef despite the degraded reef status. This suggests that growth rates and possibly recruit survival may be even higher in reef sites with better water quality and healthy coral communities.

Age at first reproduction varies widely among scleractinian corals with brooding corals typically requiring shorter time periods of 1-2 years, whereas broadcast spawning corals vary from at least 3-5 years for faster growing branching *Acropora* species and up to 4-7 years for massive species (Wallace 1985, Harrison and Wallace, 1990, Iwao et al., 2010). The only published data on the onset of sexual reproduction for *A. tenuis* is from Okinawa, Japan where some cultured corals reared from larvae became sexually reproductive at 4 years of age (Iwao et al., 2010). Baria et al. (2012) reported that spawning of *A. millepora* colonies that were cultured and reared from larvae can occur at 3 years of age provided that the colony mean diameter is ≥ 12.3 cm. After two years growth, the *A. tenuis* colonies resulting from this study were not sexually reproductive, but colonies ranged in size from 8.43 ± 2.71 to 15.1 ± 1.19 cm mean diameter (Fig. 7.21). Hence many of these colonies are predicted to become sexually reproductive by 3 years.

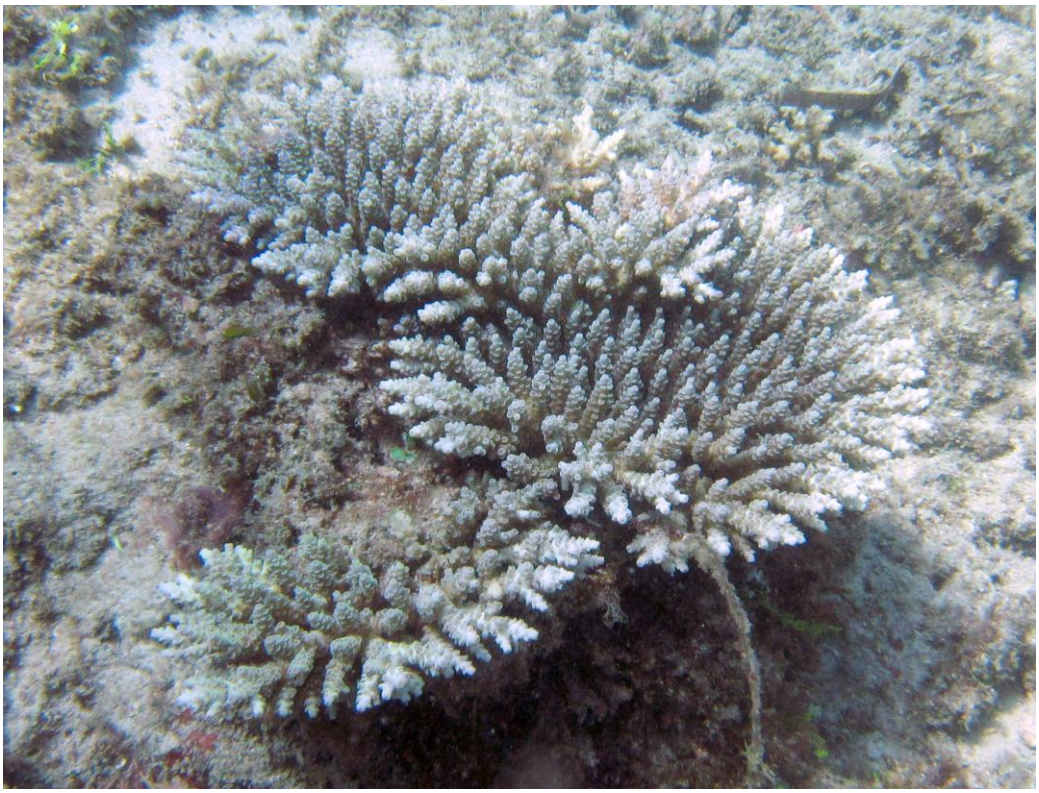

Figure 7.21. Cluster of two year old *Acropora tenuis* colonies of varying sizes photographed in April 2015 that were grown from larvae settled in larval enhancement sites in 2013 (Image: P. Harrison).

A total of 4,197 natural coral recruits were recorded on 307 recruitment tiles that were monitored at the Magsaysay Reef over two years (Fig 7.22). Natural recruitment was dominated by pocilloporids (88.5%), with other recruit categories comprising 2.7% acroporids, 2.3% poritids, 3.8% other recruits, and 2.8% were broken and damaged unidentifiable recruits (Figs. 7.22, 7.23). The highest mean number of recruits per tile was recorded in February 2014 (23 ± 2.5) and the lowest recruitment rates occurred in August 2013 (2.1 ± 0.3; Fig. 7.22). Pocilloporid corals were consistently the highest recruit category in all periods, and all of the taxonomic categories of coral recruits were present in each monitoring period except for acroporids that were absent in November 2013 and 2014 (Fig. 7.22).

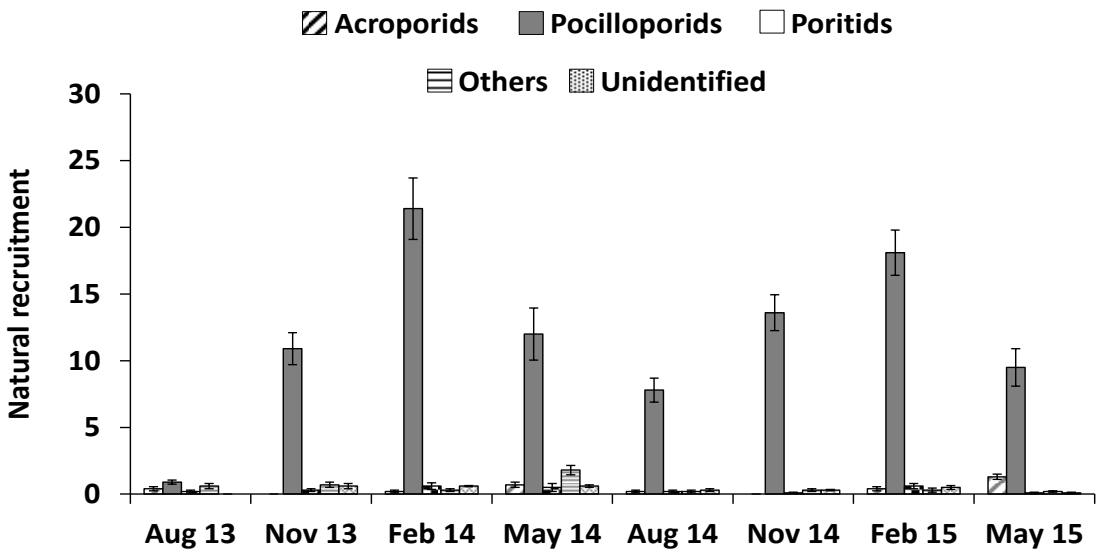

Figure 7.22. Natural coral recruitment patterns (mean ±SE) on recruitment tiles in the larval enhancement study area.

Monitoring of adult corals and recruits inside all of the reef sites over two years showed that no *A. tenuis* were present in the control sites. *Acropora tenuis* recruitment from the larval settlement experiment was significantly higher (70 times greater) than natural acroporid recruitment during any of the monitoring periods ($F_{1,6}$ = 19.20, P = 0.001, ANOVA; Tukey's test, α <0.05). This indicates that natural acroporid recruitment is currently limited at these degraded reef sites hence these reef areas will have very slow rates of *Acropora* population recovery without active intervention. No other visible natural coral recruits appeared at any of the larval enhancement or control sites when monitored over two years during this project.

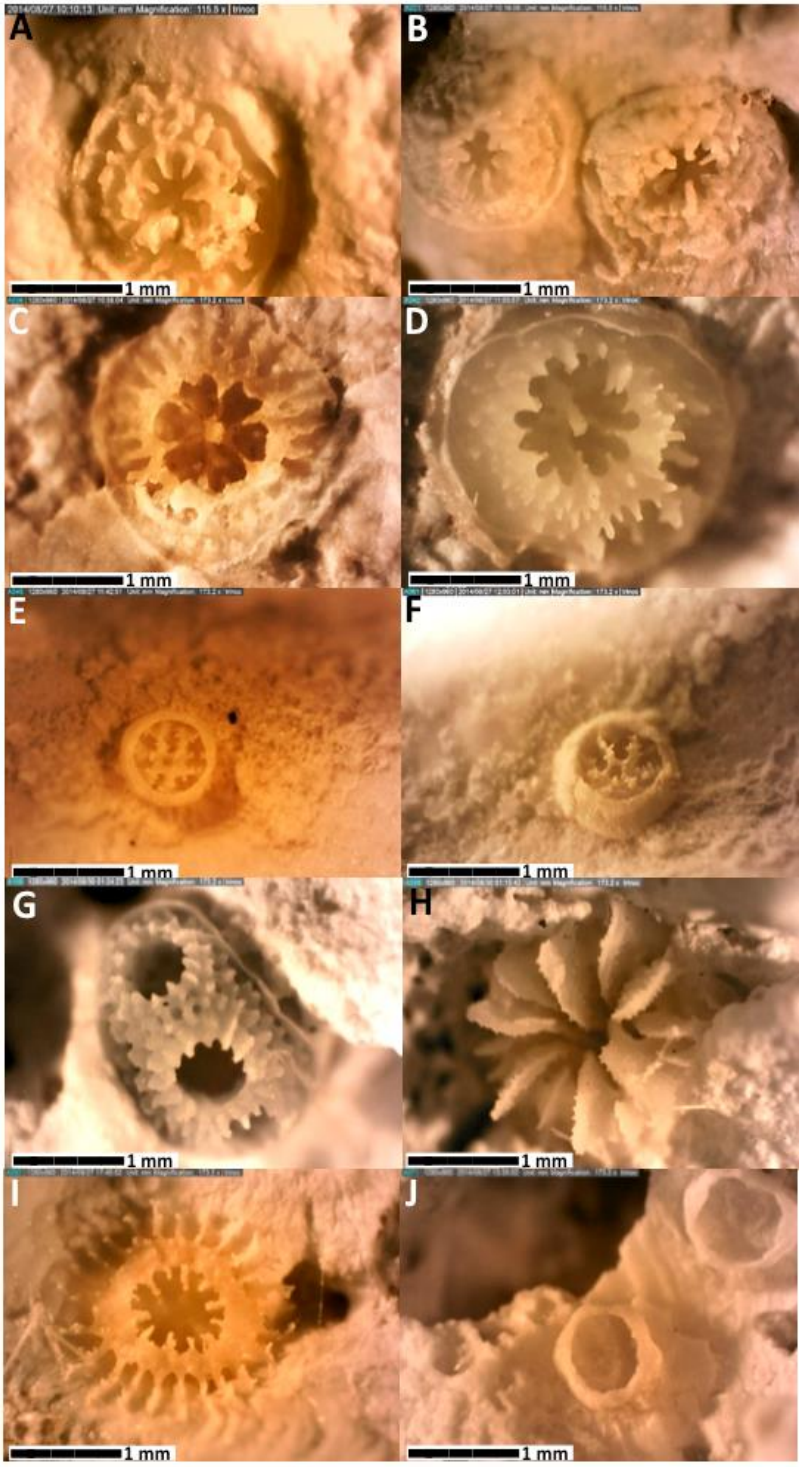

Figure 7.23. Images of taxonomic groups of natural coral recruits monitored on tiles in the larval enhancement study area. Acroporids (A and B), pocilloporids (C and D), poritids (E and F), other taxa (G to J) (Images: D. dela Cruz).

Acropora granulosa larval enhancement experiment initiated in June 2014

A second larval enhancement experiment was initiated at Magsaysay Reef in June 2014 using ~900,000 *A. granulosa* larvae. Similar initial settlement responses were recorded for *A. granulosa* larvae (Fig. 7.24) compared to the *A. tenuis* larval settlement (Fig 7.16). Significantly higher larval settlement occurred inside the larval enhancement sites averaging 25 (±13 SE) settled corals per tile, compared to zero larval settlement in the control sites.

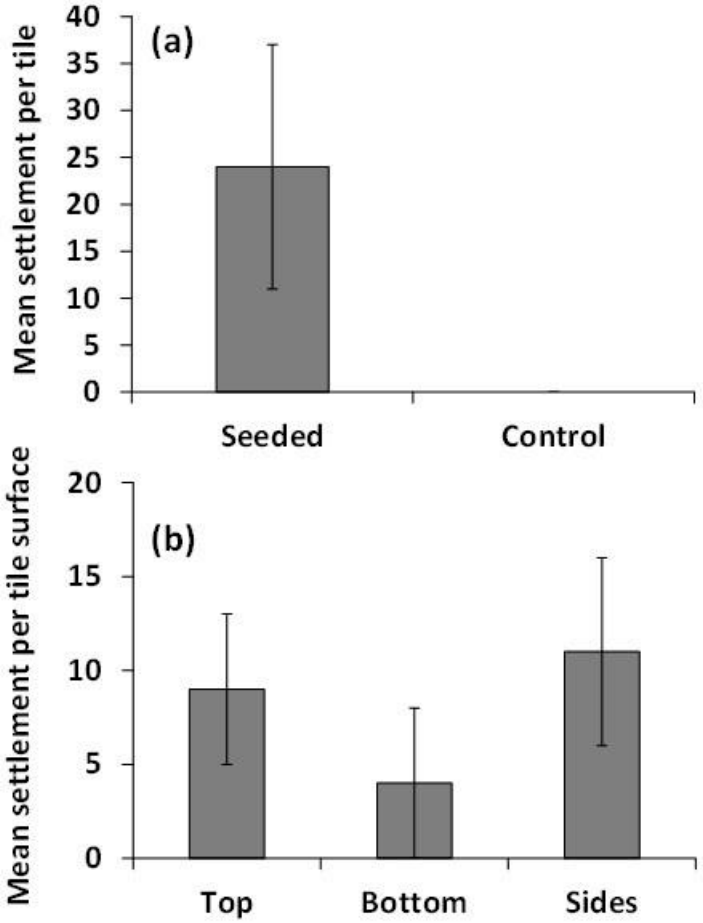

Figure 7.24. (A) Initial *A. granulosa* larval settlement (mean ±SE) on all tile surfaces in the larval-enhanced and control sites after 5 days, and (B) settlement on the different surfaces of tiles (mean ±SE) in the larval-enhanced sites.

Subsequent monitoring of *A. granulosa* recruits over ten months showed ongoing mortality in subsequent months after settlement as expected (Fig. 7.25), but the survivorship trend was initially higher than for the *A. tenuis* recruits (Fig. 7.17). Kaplan-Meier survival analysis showed a significant difference in survivorship of recruits between tile surfaces (Log-rank test, $\chi^2 = 90.59$, $p = 0.00$; bottom > top = sides) with survivorship being initially higher on the bottom surfaces of the tiles, similar to the initial pattern observed for the *A. tenuis* recruits (Fig. 7.17). Settled *A. granulosa* juveniles on the recruitment tiles and natural reef substrata grew large enough to become conspicuous for *in situ* observation and monitoring after 8 months growth (Fig. 7.26). Compared to *A. tenuis* where no mortality was recorded in visible recruits observed on the natural substrata, a decrease in survivorship was recorded for *A. granulosa* visible recruits after the first *in situ* monitoring period (Fig. 7.25). However, *A. granulosa* recruits that settled and grew on the natural reef substrata still had significantly higher survivorship than the visible recruits that settled and

grew on the recruitment tiles from eight months onwards (Log-rank test, $\chi^2 = 10.21$, $p = 0.00$).

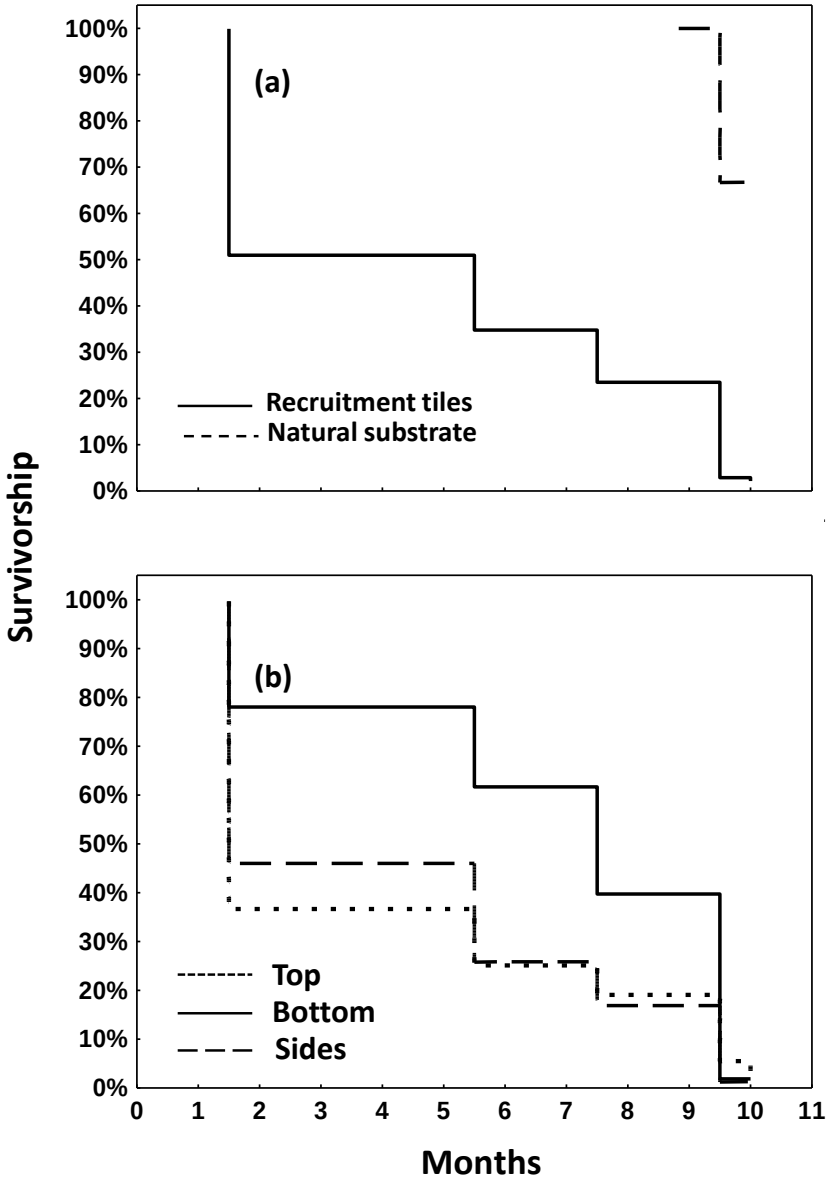

Figure 7.25. Kaplan-Meier survivorship over 10 months for (a) *A. granulosa* recruits settled on tiles, and for visible recruits on natural substrata starting at 8 months post-settlement, and (b) *A. granulosa* recruits on different tile surfaces.

Figure 7.26. Juvenile colony of *Acropora granulosa* at 10 months of age growing on natural reef substrata (Image: P. Harrison).

Overall, the results of these larval enhancement field experiments clearly demonstrate that *Acropora* larval settlement and recruitment can be significantly enhanced compared with natural background levels using mass larval settlement to rapidly initiate coral population restoration and fast growth, even on degraded reef areas. The natural loss of juvenile recruits following settlement can be ameliorated through the use of increased concentrations of competent coral larvae to substantially increase settlement rates and recruitment overall. In addition, future research should examine selective breeding of more stress-tolerant genotypes that are likely to have higher survivorship in the critical initial period after settlement to increase overall recruitment. For example, breeding from local corals that are likely to be best adapted to the environmental conditions on reefs where the larval enhancement experiments occur may improve survivorship, or breeding from corals on nearby reefs have survived previous coral bleaching events and that have photo-protective fluorescent pigments (e.g. Salih et al. 2000) and heat-tolerant clades of *Symbiodinium* microalgae that are more tolerant of thermal anomalies (e.g. Berkelmans and van Oppen 2006) would be likely to confer higher stress tolerance in progeny and enhance their survival. Assisted evolution using offspring of corals that naturally hybridise (e.g. Willis et al. 1997) may also provide greater survivorship among recruits due to hybrid vigour (van Oppen et al. 2015). These enhanced breeding outcomes combined with the remarkably high survival of visible recruits after attaining a critical size threshold at nine months post-settlement found in this project, indicate that mass larval enhancement is a viable and effective active restoration option for initiating coral population recovery where environmental conditions are suitable for reef corals to survive and grow, but natural recruitment is limited (i.e. degraded but recoverable coral reef habitats). Furthermore, the success of the low-cost mass larval settlement techniques demonstrated in this project will enable this approach to be used in initiating coral restoration in a wide range of other degraded but recoverable reef areas around the world including developing nations where most reefs are under increasing threat.

8 Impacts

8.1 Scientific impacts now and in 5 years

This project has provided some globally significant scientific outcomes that are relevant to scientists and coral reef managers throughout the Philippines and Southeast Asia, as well in many other nations including Australia that have responsibilities for understanding and managing large areas of coral reefs and the increasing need for restoring damaged coral communities and the reef ecosystems corals create.

The main scientific impacts from this project include substantial new knowledge of the biology and ecology of reef coral reproductive processes and factors that enhance or limit coral reproductive success, and successful field experiments to initiate coral restoration using mass larval enhancement. The project outcomes have led to improved techniques for maximising fertilisation rates and successful rearing of millions of embryos and larvae from a range of ecologically important corals that can be used to increase the diversity of corals used for larval restoration. The use of millions of larvae in field experiments using low-cost larval mesh enclosures resulted in significantly increased rates of coral larval settlement and subsequent recruitment to initiate coral population restoration on damaged reef areas in the Northern Luzon region of the Philippines. The key results of the research have been presented at national and international conferences and research workshops and will be published in peer-reviewed international journals in the near future so that the scientific discoveries and new information can be made available for application by other reef scientists, managers and other stakeholders around the world. It is anticipated that the scientific impacts from this project will continue to grow rapidly in the next few years as more researchers adopt these larval enhancement methods for reef restoration projects in other regions.

8.2 Capacity impacts now and in 5 years

This project and associated research equipment have resulted in substantial capacity building impacts through increased knowledge and skills among the many research students, technicians and research volunteers who have been associated with this project, mostly from the Philippines. These include PhD student Dexter dela Cruz from the Philippines who is enrolled at Southern Cross University (SCU) and has been actively mentored by Harrison and Villanueva, and Katy Horan who is an Honours student from SCU who initiated innovative research with us using 3D printed settlement tiles and related experiments at BML in 2015.

Throughout the project many Masters students and Research Assistants from the University of the Philippines (UoP) and other institutes benefitted directly from capacity building training on research techniques, coral reproduction and larval enhancement methods and access to tanks, microscopes and other research equipment at BML provided through this project.

Fifteen Masters research students from the University of the Philippines – Marine Science Institute (UP-MSI) participated or volunteered with coral spawning research and larval experiments from 2012-2014 including:
Miguel Azcunya, Janine Erica Dayao, Rico Duco, Mikko Garcia, Gabriele Grace Mendoza, Doreen Kate Minoza, Emilio Paul Candelaria, Leomir Diaz, Janice Loreato, Joemark Narciso, Sephanie Faith Ravelo, Minin Sinsona, Mikhael Clotilde Tanedo, Andrew Torres, Darryl Anthoy Valino.

In addition, three UP-MSI Research Assistants were trained under this project:

Emmeline Jamodiong, Tracy Dofeliz Tabalanza, Charlon Ligson

Some of the other research students and on-the-job trainees from other Institutions who benefitted from capacity building through this ACIAR project and the coral restoration activities from 2012-2014 include:

Ateneo de Manila University
Antoni Andreu Martija, Caryll Ivy Uy, Dustin Jan Cruz

Don Mariano Marcos Memorial State University
Giselle Caquioa, Karla Jeanne Aruelo, Karen Benter, Sylviane Manlapaz, Janica Peralta

Palawan State University
Stephen Wallace, Jerimae Cayanan

Philippine Science High School
Catherine Ann Tan, Rose Marie Butaran, Andrew Patrick Juat, Raphael Carlos Malixsi

Graduate Research Volunteers at BML in 2012 and 2015
Tiffany Harrison, graduate from Monash University
Daron Willison, graduate from the University of Miami

This capacity building training was designed to increase the research, technical and management capacity of these participants in order to sustain the outcomes from this project after it was completed, and to support the application of this knowledge to other reef restoration and coral mariculture projects in future.

8.3 Community impacts now and in 5 years

The project research outcomes have already had impacts beyond the scientific sphere including the modification of the 'Memorandum of Understanding between the Philippines and Australia on Coral Reefs', to include specific reference to the results from this project noting that "Australia and the Philippines are collaborating on projects that seek to restore coral reefs in the Philippines using coral larval reseeding" and that the participants undertake to "share knowledge, expertise, research skills and technical training relating to coral rearing and reef restoration techniques" resulting from collaborative projects.

Presentation of the scientific outcomes from the larval restoration experiments in the Philippines has led to detailed discussions with reef managers and research coordinators from the Great Barrier Reef Foundation and the Great Barrier Reef Marine Park Authority about the application of these methods for reef restoration in the Great Barrier Reef region in future. Furthermore, presentation of the research results at the Society for Ecological Restoration (SER) international conference in Manchester in 2015 resulted in plans for inclusion of a coral reef restoration theme within the SER Australasian Chapter.

8.3.1 Economic impacts

The economic impacts from this Small Research Activity project are not yet known as quantitative evaluation of socio-economic impacts was beyond the scope of this project. However, local businesses in Bolinao and in other centres including Manila benefited from the expenditure on research support and infrastructure during this project, and local communities benefited from hiring local staff to support the research work and maintenance of the tanks in the BML seawater system. In addition, the initiation of new coral communities in degraded reef areas on Magsaysay Reef will result in an improved

status of the coral and reef communities as these corals continue to grow and develop in the likely absence of major disturbances such as blast fishing. This is predicted to result in some improvement in ecosystem services from the restored reef patches including the provision of new coral larvae from the new recruits that are likely to become sexually mature within 3-4 years of age, which could increase coral recruitment on other reef areas in future. This in turn could promote improved fisheries production and other reef resources for local communities. In the absence of major disturbances, this could lead to increased food security in future, and potential employment and new enterprise opportunities for local people from growing juvenile corals for sale and use in other reef areas in future, based on the successful and rapid growth of juvenile corals in this project.

An indication of the potential economic values of restored reef areas was provided by de Groot et al. (2012) who completed a meta-analysis of global estimates of the values of coral reefs and other ecosystems and their services. They concluded that the average total economic values of healthy coral reefs are worth about US$352,250 per hectare each year. In contrast, Cruz-Trinidad et al. (2011) estimated that the economic value of the Bolinao-Anda coral reefs in their present degraded state is about $1,900 per hectare per year, therefore future improvements in the status of these coral reefs is likely to have significant economic benefits.

8.3.2 Social impacts

As noted above, the social impacts from this SRA project could not be quantified, however it is well documented that healthy coral reefs provide a wide range of goods and services that are socially and economically important at local and regional scales. Coral reefs in the Philippines are particularly important to local coastal communities, as they provide essential fish food and other resources for dependent people (e.g. Alcala and Russ 2006, Cruz-Trinidad et al. 2011). The current degraded status of the Bolinao-Anda coral reefs and concomitant loss of production values of local reefs are likely to have a direct negative effect on the well being and potentially on the health of local people who depend heavily on these reefs. The initiation of new coral colonies on some areas of these degraded reefs is predicted to lead to improved reef status at small scales in future and if the restored coral communities continue to grow and reproduce then the larval ‘spill-over’ may catalyse the recovery of other reef areas. This in turn could increase food resources and other ecosystem services in future, leading to some social benefits such as fishers spending more time onshore in the community and with families due to reduced travel times for fishing.

8.3.3 Environmental impacts

The main beneficial environmental impacts from this project are the initiation of new coral colonies now growing on degraded reef patches in Magsaysay Reef resulting from the larval enhancement experiments and mass larval settlement of *A. tenuis* larvae in 2013 and *A. granulosa* larvae in 2014. The very high survival of recruits once they attained visible sizes and relatively rapid growth of the young coral colonies, particularly the *A. tenuis* colonies, means that coral cover is predicted to continue to increase in the larval enhancement sites in future leading to restoration of local populations of these species. In addition, recruits from these and other coral species used in related larval settlement experiments during this project have been outplanted onto nearby reefs, and at least some colonies are likely to survive and grow to produce mature reproductive colonies in the near future. Therefore this project has had positive environmental impacts by initiating the restoration of some key foundation coral species on degraded reef areas. Based on current trends it seems likely that significant numbers of these new colonies will continue to grow and become sexually reproductive, thereby enhancing the coral cover and health status of these reef patches. The corals will also become increasingly important habitats

for reef fish and other coral-associated reef organisms, leading to improved biodiversity on these reefs in future.

8.4 Communication and dissemination activities

The key results from this project have been communicated to a range of different stakeholders including scientists, managers and other reef workers and more broadly to the public through presentations at workshops and national and international conferences, and through targeted media releases. The first major paper was submitted to *Science*, and other publications are being completed for submission to other international peer-reviewed journals to disseminate the new findings from this project to other researchers and reef workers. It is anticipated that these publications will result in substantial renewed interest in using mass larval settlement to initiate coral restoration in other reef regions.

Project results have been presented at the following scientific conferences to promote uptake of the larval restoration approaches among other coral reef research scientists and managers:

- Philippine Association of Marine Science national conference in Tacloban City, Leyte, 2013

- Society for Ecological Restoration International Conference in Manchester, 2015

In addition, the results from ongoing monitoring of survival and growth of the *A. tenuis* larval restoration experiment over three years will be presented at the International Coral Reef Symposium in Hawaii in 2016, in a Session chaired by Harrison and colleagues on Coral Reef Restoration.

The project outcomes were featured in the Australia Unlimited Austrade media story promoting selected Australian expertise and success internationally: http://www.australiaunlimited.com/environment/international-coral-sexpert-peter-harrison

The outcomes from the larval restoration experiments have also been highlighted in Southern Cross University Annual Research Reports, and through presentations to the Great Barrier Reef Foundation.

9 Conclusions and recommendations

9.1 Conclusions

This project has achieved its aim and the main research objectives and has provided some globally significant results that provide a strong foundation for future larger-scale reef restoration work. Research during the project substantially increased information about the main coral spawning periods for a range of ecologically important coral species in the Bolinao-Anda reef region, and demonstrated that coral spawning periods occur over an extended period from February to July each year, with some species spawning over two or three lunar cycles. These results are important as they extend the known period when spawning and access to millions of spawned gametes can facilitate mass coral larval rearing for experiments and larval settlement on damaged reef sites. In addition, large multispecific coral spawning was recorded *in situ* during March 2015 resulting in a surface slick containing millions of embryos, which provides scope for larger scale *in situ* larval rearing and settlement experiments in future.

Experiments confirmed that optimal fertilization rates require sperm densities of $\geq 10^4$ ml^{-1}, and that combining gametes from different colonies shortly after spawning is important for maximising fertilization success and outbreeding potential. High rates of fertilization and maintenance of good water quality during embryo development enable millions of coral larvae from different coral species to be successfully reared for use in experiments.

Experiments testing different types of settlement tiles and larvae from different species showed that significantly higher settlement rates occur on tiles cut from dead plate *Acropora* skeletons compared with artificial fibre-cement or terra-cotta tiles for three *Acropora* species, with no significant differences detected for three other species. Therefore, the natural *Acropora* skeleton tiles are the most suitable standard tile type for quantifying larval settlement and subsequent survivorship and growth of juvenile recruits for mass larval enhancement experiments in the field. Experiments comparing replica tiles printed using two types of common commercially available 3D printing plastics and *Acropora* skeleton tiles showed that larval settlement rates were similar among tile types and that the plastics are not toxic to the coral larvae tested. Therefore 3D printing has very good potential for designing new types of larval settlement tiles to optimise coral larval settlement and recruitment in future reef restoration studies.

Further experiments demonstrated that the length of the biological conditioning period strongly affects larval settlement rates, with increased settlement on tiles conditioned for two months and maximum settlement on tiles with four months immersion and conditioning. Therefore, optimising biological conditioning is important for quantifying larval settlement responses. An experiment using different densities of larvae showed increased settlement with increased larval density, however survival of the newly settled spat decreased at the two highest densities. Therefore larval settlement and reef restoration experiments need to be designed carefully to consider the effects of larval density on initial settlement rates and subsequent survival for maximising recruitment.

The two major mass larval settlement experiments at degraded reef sites on Magsaysay Reef using larvae from *A. tenuis* and *A. granulosa* resulted in high larval settlement rates on conditioned tiles, with initial post-settlement survivorship decreasing over time as expected. However, remarkably high survivorship and growth rates were recorded on both tiles and reef substrata once the juvenile corals grew large enough to become visible recruits at 8 to 9 months after settlement. The natural loss of juvenile corals following settlement can be ameliorated through the use of increased numbers of competent coral larvae to substantially increase settlement rates and recruitment overall. Detailed monitoring of natural coral recruitment patterns at Magsaysay Reef showed very low

natural recruitment rates especially of branching *Acropora*, which indicates that these degraded reef sites will have very slow rates of recovery without active intervention through coral restoration. The outcomes from these experiments and ongoing monitoring clearly demonstrate that coral recruitment can be significantly enhanced using mass larval settlement to rapidly initiate coral population restoration and fast growth even on degraded reef areas. High survival of juvenile corals after 9 months shows that mass larval enhancement is a viable and effective active restoration option for initiating coral population recovery on degraded but recoverable coral reef areas where environmental conditions are suitable for reef corals to survive and grow, but where natural coral recruitment is limited. The success of the low-cost mass larval settlement techniques demonstrated in this project will enable this approach to be used in initiating coral restoration in a wide range of other degraded reefs in the Philippines and in many other regions around the world where the foundation coral communities and reef health are declining, including the Great Barrier Reef.

9.2 Recommendations

The following actions are recommended to increase the impacts of this project and build on the successful coral reef restoration work. In particular, it is recommended that mass larval settlement trials be significantly expanded to initiate coral restoration at larger reef scales in future, and that monitoring of the outcomes from this project be continued to determine the longer-term impacts of the larval settlement and mass larval enhancement experiments. Genotyping of parent corals and new recruit colonies is also recommended.

Further research is also needed to determine coral reproduction periods for other ecologically important groups of corals that have not yet been well studied in the Bolinao-Anda region, to increase the range of corals that can be used for restoration work and the diversity and resilience of the restored coral communities. These groups include massive *Porites*, *Montipora* and locally abundant stress-tolerant corals including *Turbinaria*, *Merulina* and *Pavona* that may be better able to cope with future environmental changes. Further work is also needed to determine peak periods of multispecific coral spawning and development of coral spawn slicks on local reefs in order to maximise access to millions of spawned gametes from a wider range of coral species for *in situ* embryo and larval rearing and subsequent larval settlement.

Research should also focus on reducing juvenile coral mortality during the critical initial period after settlement to increase survivorship and growth rates and overall recruitment. This could be achieved through selective breeding of locally adapted corals, including the large number of surviving corals grown from larvae during this project once they become large enough to be sexually reproductive at about 3 to 4 years of age. Breeding from stress-tolerant genotypes that are likely to likely to confer higher stress tolerance in larvae and have higher survivorship is also recommended and could be achieved by breeding from corals on nearby reefs that have survived previous coral bleaching events, colonies with photo-protective fluorescent pigments and culturing heat-tolerant clades of *Symbiodinium* microalgae for uptake by larvae or settled juveniles. Experiments should also be done to determine the optimal densities of larvae for use in larger-scale settlement experiments on reefs, and the potential for aggregated larval settlement to increase growth and survival and the development of chimeras among juvenile recruits. Provision of different types of food during the critical early stages of life for juvenile corals after settlement should also be evaluated to optimise their survival and growth. Research on the effects of innovative tile surfaces using 3D printing and microhabitats is also needed as these factors are likely to strongly influence survival and growth of recruits.

These recommendations would significantly expand the impacts from this project, further build research training and capacity, and enable the socio-economic impacts of reef restoration to be examined in detail.

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10.2 List of publications produced by project

Previous publications by other workers on research using coral larval restoration have highlighted conclusions based only on initial larval settlement responses or relatively short-term monitoring of survivorship patterns, but this has precluded detailed analysis of the overall success of the larval restoration approach for reef restoration. Therefore, we delayed submission of the first manuscript from the larval enhancement experiments until the longer term outcomes from the experiment were clear, to more strongly emphasise the conclusion that mass larval settlement is an effective approach for successfully initiating coral restoration on degraded reef areas. Details of the first paper that was submitted are provide below, and remaining papers are now being prepared for submission to other international refereed journals.

D. dela Cruz and P. L. Harrison (submitted). More larvae on reefs: mass larval settlement enhances coral recruitment and reef restoration. Submitted to *Science*.

Proposed titles for additional papers arising from this Project:

- Enhanced settlement of *Acropora granulosa* coral larvae leads to population restoration on degraded reefs.
- Reproductive patterns of reef corals in the northern Philippines
- Effects of sperm density and gamete combination time on fertilization responses of three coral species
- Comparison of coral larval settlement rates on natural and artificial tile surfaces
- Effects of larval density and biological conditioning on settlement and early survival of coral recruits
- Designing innovative coral settlement tiles using 3D printing for coral reef restoration
- Patterns of coral recruitment on degraded reefs in the northern Philippines show natural recruitment will not enable recovery