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Caging enhances post-settlement survival of juveniles of the scleractinian coral *Acropora tenuis*

Maria Vanessa B. Baria [a,*], James R. Guest [b,1], Alasdair J. Edwards [b], Porfirio M. Aliño [a], Andrew J. Heyward [c], Edgardo D. Gomez [a]

[a] The Marine Science Institute, University of the Philippines, Diliman, 1101 Quezon City, Philippines
[b] School of Biology, Ridley Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom
[c] Australian Institute of Marine Science, Ocean Institute Building, University of Western Australia (M096), Crawley 6009, Western Australia, Australia

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ABSTRACT

Low cost, simple approaches leading to enhanced numbers of viable, mature corals on reefs are prerequisite to active reef rehabilitation at even modest spatial scales. Mass culture of coral larvae to settlement, utilising improved knowledge of major coral spawning events, promises to be relatively straightforward, but very significant mortality in the early post-settlement period remains a major hurdle. This study was conducted to examine the effect of herbivore exclusion on the survival of 6 week old coral spat of *Acropora tenuis* (Dana, 1846) reared *ex situ* at a site in north-western Philippines. Coral spat were placed on the reef approximately 6 weeks after settlement in three treatments, caged, open-sided cage and no cage at two depths (4 m and 9 m). Mean survival of coral spat was significantly higher at the deep sites compared to the shallow sites. Among treatments, survival was significantly lower in the uncaged treatment (4.7% ± 2.6% and 10.5% ± 4.5%, mean ± SE in shallow and deep respectively) compared to the open-sided cage (18.6% ± 5.0% and 22.5% ± 7.1%) and fully caged treatment (17.0% ± 4.5% and 33.0% ± 6.0%) after 3 months. The results indicate that removal of coral spat by grazers may have reduced survival in the uncaged treatment, although the fact that survivorship was not significantly reduced in the open-sided cage treatments suggests that the presence of the cage also had some effect on survival. It is possible that the open-sided cage prevented access by larger fish that may have actively removed coral spat or that shading provided by the cage enhanced spat survival. Further research is needed to see if survivorship in cages decreases at a later stage due to overgrowth by other biota and whether survivorship is enhanced if spat are settled on more complex surfaces that provide refuge from grazers. This study demonstrates that using cages to exclude herbivores and corallivores and/or to provide shading may be beneficial to survival during the early stages when rearing corals *in situ* for reef rehabilitation.

1. Introduction

Active biological rehabilitation is seen as one way to assist the process of recovery of degraded areas of reef where natural rates of recovery are slow. To date, most rehabilitation efforts have used asexual propagation, i.e. fragmentation, as a way of generating corals for transplantation (Rinkevich, 2005). More recently, there have been several investigations into the possibility of using sexually reared corals for reef rehabilitation (Heyward et al., 2002; Hatta et al., 2004; Okamoto et al., 2005). Larval rearing for rehabilitation aims to harness the high fecundity of corals and to bypass early natural mortality, reduce collateral damage to source colonies and increase the genetic diversity of transplanted corals. While the techniques for culturing coral larvae are well established (Heyward and Negri, 1999), to date they have primarily been used to produce relatively small numbers of larvae for experimental purposes. Mass production of larvae for coral reef rehabilitation is still experimental (Edwards and Gomez, 2007) and it is yet to be demonstrated that it can significantly enhance the rate of recovery of degraded reefs (Guest et al., 2010). Generally, when laboratory reared juvenile corals are outplanted to the reef at an early stage (i.e. within a few weeks after settlement), their survival is relatively low (<14% survival in the first 3–4 months) (Babcock and Mundy, 1996; Epstein et al., 2001; Wilson and Harrison, 2005; Nozawa et al., 2006). When reared corals are outplanted at a later stage, survival may be significantly higher. For example, juvenile *Pocillopora damicornis* with diameters ranging from 10–29 mm diameter had 47.5% survival 1 year after outplanting to the reef

* Corresponding author. Tel./fax: +63 2 4357417.
E-mail addresses: mv_baria@yahoo.com (M.V.B. Baria), james.guest@nus.edu.sg (J.R. Guest), a.j.edwards@ncl.ac.uk (A.J. Edwards), a.heyward@aims.gov.au (A.J. Heyward).
[1] Present address: Marine Biology Laboratory, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore.

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(Raymundo and Maypa, 2004). Similarly, survival may be significantly enhanced when modifications that protect newly settled spat from fish grazing and/or predation are introduced (Omori, 2005; Nozawa, 2008).

Caging coral colonies is commonly used as an experimental method to understand the effect of herbivore exclusion on coral health at scales ranging from individual juvenile and adult coral colonies (Miller and Hay, 1998; Lirman, 2001; Box and Mumby, 2007) to several square metres of reef (Hughes et al., 2007). However, to the best of our knowledge, no caging studies have been conducted to examine the effect of herbivore exclusion on recently settled coral spat. Caging may have two possible effects on the survival of coral spat. Firstly, survival may be reduced by caging as macro-algal growth is enhanced and this may promote competitive interactions between algae and corals. Secondly, survival may be enhanced if caging prevents removal of juvenile corals either accidentally during grazing or deliberately by predation. The factors affecting coral post-settlement survival still remain poorly understood and several factors may be important at different stages. Some reports suggest that grazing fish such as acanthurids and small scarids, actively avoid removing small corals (Birkeland, 1977) while other studies show that juvenile corals are removed by grazers, either deliberately or accidentally and that this may be a significant source of post-settlement mortality (Bak and Engel, 1979; Sato, 1985; Christiansen et al., 2009). Survival of juvenile corals being reared *in situ* therefore may benefit from exclusion of herbivores and corallivorous fish. The aim of this experiment therefore was to test the hypothesis that caging of 6 week old hatchery-reared coral spat of the broadcast spawner *Acropora tenuis* would significantly enhance survival following outplanting to the reef compared to uncaged controls.

2. Materials and methods

This experiment was carried out at the Bolinao Marine Laboratory (BML) in north-western Luzon, Philippines (16°22′N 119°54′E). *Acropora tenuis* colonies were sampled in April and May 2007 (Vicentuan et al., 2008) to establish the timing of spawning (see Baird et al., 2002 for methods). During sampling in April 2007, 96% of sampled colonies (n = 24) contained large visible oocytes in fractured branches. Eighteen colonies of *A. tenuis* were collected from two fringing reef sites (2–5 m depth) approx. 9 km and 15 km from the marine laboratory around the day of the full moon (2 April 2007). Colonies were transported to the BML hatchery facility and kept in flow-through seawater in 400 l fibre-glass tanks. Each night, water flow was shut off just before sunset and colonies were isolated in 60 l plastic tubs and checked for spawning activity periodically until 22:30 or until spawning occurred. Eleven colonies spawned on April 27 and 29 but only gametes collected from four colonies that spawned synchronously on 27 April were used in this experiment.

Larval culture techniques followed Heyward and Negri (1999). Egg-sperm bundles from the 4 colonies were scooped from the water surface and transferred to a 20 l fertilisation tank containing 1 μm filtered, UV treated seawater. Egg-sperm bundles were separated by gentle agitation to allow cross-fertilisation to occur. After 2 h, excess sperm were removed by gently pouring embryos onto a submerged 100 μm mesh. Embryos were then transferred to clean filtered seawater at densities between 250 and 300 embryo l^{-1} of seawater. Developing embryos were left for 24 h without aeration then water changes were done every 12 h. After 48 h, larvae were transferred to several seawater tanks (60–400 l) for settlement, maintaining densities at 250–300 larvae l^{-1}.

Fibre-cement tiles (100 mm × 100 mm × 6 mm) were used as settlement substrates (n = 310). Tiles were conditioned in flow-through seawater tanks for 1 month and accumulated sediment was cleaned just prior to use. Tiles were suspended vertically in the settlement tanks and separated by 2 cm lengths of plastic hose. Settlement competency was monitored by placing at least 10 larvae in 12 15 ml culture wells with a 0.5 cm^2 chip of crustose coralline algae (CCA) (see Heyward and Negri, 1999 for methods). Settlement began two days post-fertilisation (mean settlement = 39.4% SD ± 19.6%, n = 12) and settlement peaked at day 4 (91.0% SD ± 10.0%, n = 12). Larvae were introduced to tiles during the peak settlement period and tiles were left for several days, during which time water changes were carried out daily, before being transferred to flow-through seawater tanks (400 l). Tiles were maintained in flow-through seawater with constant aeration until deployment in the field. The number of corals settled on each tile was counted and mean settlement was 104 spat per tile (SD ± 241, n = 310). Tiles with 20 or more coral spat (at least 1 cm apart) were selected (n = 120). Corals were removed from some tiles using a scalpel so that each tile had exactly 20 coral juveniles (10 on each side) prior to transplantation.

The field experiment was conducted at a fringing reef site at the Malilnep channel (16°25′N 119°56′E). Tiles were placed in one of the three treatments: i) fully caged (to exclude grazers), ii) open-sided cage (to control for cage effects) and iii) uncaged controls (Fig. 1). Each treatment was replicated ten times and each replicate was placed approx. 20 m apart. Treatments were also set up at two depths (4 m and 9 m) to examine whether the effects of caging varied between shallow and deep sites. The cages (400 mm × 400 mm × 400 mm) were constructed of 1 cm^2 PVC mesh. Cages were attached using monofilament line to four angle-iron bars hammered into the substrate. For each treatment, two vertically oriented tiles were placed 20 cm apart on a stainless steel rod suspended between two of the angle-iron bars 10 cm above the substrate. The 120 tiles, each with 20 coral spat, were deployed on June 8 and 13, 2007 (4 m and 9 m, respectively), 42–47 days (6–7 weeks) after fertilisation. Cages were cleaned once a week for the duration of the experiment to remove fouling organisms from the exterior of the cages.

Survival of coral spat was examined 3 months after deployment on 8 and 13 September 2007 (4 m and 9 m, respectively). All surviving coral spat on each tile were counted immediately after retrieval. Filamentous and fleshy algae were scraped from each tile using a scalpel, rinsed with fresh water to remove salts, dried in an oven at 70 °C for 1 week and then weighed on an analytical balance. Percentage

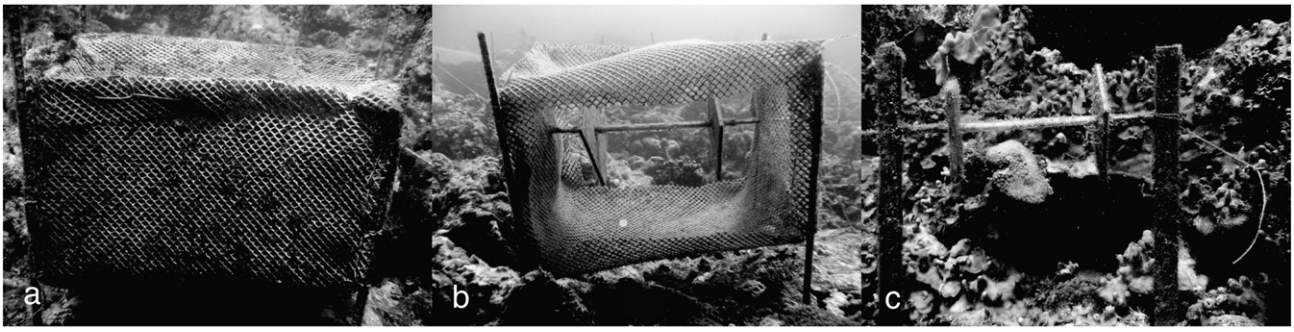

Fig. 1. The experimental set up showing a) full cage, b) open-sided cage and c) no cage (control).

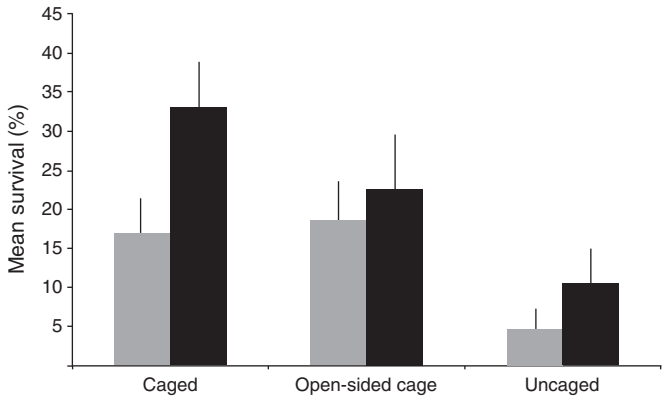

Fig. 2. Mean survival (%) of *Acropora tenuis* juveniles in caged, open-sided cage and uncaged treatments at two depths (n = 10). Grey bars = 4 m depth, solid bars = 9 m depth. Error bars are standard error.

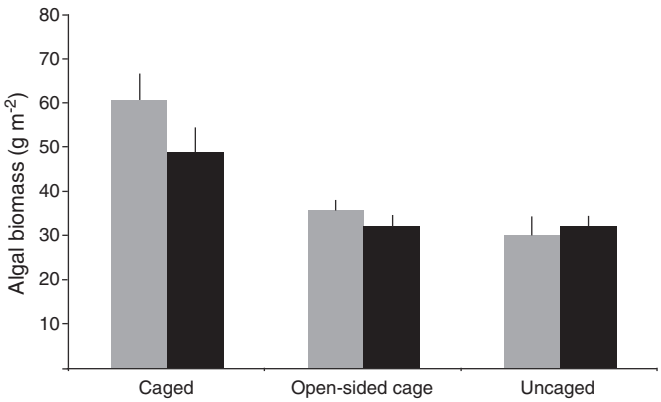

Fig. 3. Mean algal biomass (g dry weight m^{-2}) on settlement plates in caged, open-sided cage and no cage treatments at two depths (n = 10). Grey bars = 4 m depth, solid bars = 9 m depth. Error bars are standard error.

survivorship of coral spat and algal biomass (g dry weight m^{-2}) on the tiles among treatments and between depths were compared using a 2-factor ANOVA followed by Student–Newman–Keuls tests (SNK) (GMAV5, University of Sydney). Tests for heterogeneity of variances were not significant (Cochran's test, $p>0.05$) so data were not transformed prior to analyses.

3. Results

At the shallow site (4 m), the caged and open-sided cage treatments had mean survival of 17.0% (SE ± 4.5%) and 18.6% (SE ± 5.0%) respectively, whereas only 4.7% (SE ± 2.6%) survived in the uncaged treatment. Similarly, at the deep site (9 m), the caged and the open-sided cage treatments had mean survival of 33.0% (SE ± 6.0%) and 22.5% (SE ± 7.1%) respectively, whereas only 10.5% (SE ± 4.5%) survived in the uncaged treatment (Fig. 2). Survival was significantly different among treatments and depths, while the interaction term was not significant (Table 1). SNK tests comparing differences between treatments revealed that survivorship was significantly higher in the caged and open-sided cage treatments compared to the uncaged treatment, but survival between caged and open-sided cage treatments was not significantly different (Table 1). At the end of the study, the number of polyps comprising the juvenile coral colonies ranged from 1–21 (mean = 4.85 SD ± 3.83) and the average geometric mean diameter was 2.20 mm (SD ± 0.54 mm, n = 29 juveniles) (measurements from images were made using Vidana 1.2.1 software, University of Exeter).

Mean algal biomass at the shallow site in the open-sided cage and uncaged treatments was 35.72 g m^{-2} (SE ± 2.24) and 30.17 g m^{-2} (SE ± 4.15) respectively, whereas mean algal biomass in the caged treatment was 60.74 g m^{-2} (SE ± 6.09). At the deep site, open-sided cage and uncaged treatments had mean algal biomasses of 31.90 g m^{-2} (SE ± 2.73) and 32.12 g m^{-2} (SE ± 2.27) respectively, whereas the caged treatment had 48.88 g m^{-2} (SE ± 5.65) (Fig. 3). Algal biomass was significantly different among treatments but not between depths (Table 2). SNK tests revealed that algal biomass in the caged treatment was significantly higher than that of the uncaged and open-sided cage treatments, however algal biomass was not significantly different between uncaged and open-sided cage treatments (Table 2). No direct observations of herbivory were done during the experimental period, however extensive grazing scars were noted on the majority of the tiles in the uncaged and open-sided cage treatments indicating that herbivores actively grazed in both treatments.

4. Discussion

In situ caging of 6–7 week old laboratory reared scleractinian coral spat resulted in significantly more corals surviving for the next 3 months compared to uncaged corals but not compared to corals in open-sided cages. There was also a significant difference in survival between depths, but there was no significant treatment × depth interaction.

Mean survival of the uncaged corals (4.7% SE ± 2.6% and 10.5% SE ± 4.5% at the shallow and deep sites respectively) was comparable to most other studies using reared corals on artificial settlement plates deployed on the reef and exposed to grazing. In the Red Sea, 3 month old *Stylophora pistillata* had 5.0% survivorship after 1 month on the reef (Epstein et al., 2001). In Japan, for 3-day old spat of *Pocillopora damicornis*, survivorship ranged from 0.0–16.0% after ~6 months (Sato, 1985) while for 1.5 month spats of *Acropora solitaryensis*, *Cyphastrea serialia*, *Favia favus* and *A. japonicum* survivorship ranged from 0.0–12.0 % after 3 months (Nozawa et al., 2006). In the sub-tropical Solitary Islands, Australia, *A. lordhowensis*, *Goniastrea australensis*, and *Montastraea curta* had 8.0–13.0% survivorship after 4 months on the reef (Wilson and Harrison, 2005) while at Magnetic Island, Australia, survivorship was 0.5 and 3.9% for *Platygyra sinensis*

Table 1
Results of 2-factor ANOVA for comparison of coral spat survivorship between depths and among treatments. Asterisks denote significant differences, * = $P<0.05$, ** = $P<0.01$, NS = not significant, SNK = Student–Newman–Keuls.

	df	MS	F	P
Depth (D)	1	1098.2	4.15	*
Caging treatment (C)	2	1632.5	6.17	**
C × D	2	212.2	0.80	NS
Residual	54	264.6		
Total	59			
Cochran's test	C = 0.3167, P > 0.05			
SNK test (depth)	Shallow < deep			
SNK test (treatment)	No cage (control)* < open-sided cage = caged			

Table 2
Results of 2-factor ANOVA for algal biomass. Asterisks denote significant differences, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$, NS = not significant, SNK = Student–Newman–Keuls.

	df	MS	F	P
Depth (D)	1	314.2	1.81	NS
Caging treatment (C)	2	3360.6	19.41	***
C × D	2	240.0	1.39	NS
Residual	54	173.1		
Total	59			
Cochran's test	C = 0.3565, P > 0.05			
SNK test (depth)	Shallow = deep			
SNK test (treatment)	No cage (control) = open-sided cage** < caged			

and *Oxypora lacera*, respectively after 4 months (Babcock and Mundy, 1996). In the Philippines, 1 month old *P. damicornis* spat (≤3 mm) showed less than 5% survivorship after 4 months of deployment and none survived on the reef after 1 year (Raymundo and Maypa, 2004). That study highlighted size-specific mortality where larger juveniles showed higher survivorship 1-year after deployment on the reef; 10.1–29 mm diameter juveniles had the highest survivorship (47.5%), while other size classes (≤3 mm, 3.1–6 mm, 6.1–10 mm) had lower survivorship — 0%, 2.5% and 16.3%, respectively. Two studies conducted in Bolinao close to the site used in the present study found that spat survival of *Seriatopora caliendrum* after 81 days was 11% (Villanueva et al., 2005) while survival of *P. damicornis* was 19% and 17%, respectively (Villanueva et al., 2006).

In the present study, significantly higher survival of caged juvenile corals may have been caused by exclusion of fish or invertebrate grazers (e.g. sea urchins). However, as survival was also significantly higher in the open-sided cage control, some factor related to the presence of the cage may also have affected coral survival. The pattern among treatments was consistent between depths for survival and algal biomass, however, survival was significantly higher at the deeper site while algal biomass did not differ significantly between depths. One possibility is that shading by the PVC mesh on the upper part of the cage and lower irradiance at the deep site had a positive effect on the survival of coral spat. High irradiance may be detrimental to corals because of photochemical damage to symbionts and other studies have suggested that exposure to high irradiance may cause elevated mortality in juvenile corals. For example Mundy and Babcock (2000) found that the pattern of survivorship of *Goniastrea aspera* and *Oxypora lacera* among depths depended on whether corals were settled on top or bottom surfaces suggesting that different levels of exposure to irradiance explained differences in survivorship.

Another possibility is that the type and intensity of grazing between uncaged and open-sided cage treatments differed as the latter treatment may have excluded large grazers such as parrotfishes that could deliberately or accidentally remove coral juveniles. Miller and Hay (1998) also found that coral predation by fish was reduced in open-sided cages compared to uncaged corals and inferred from this that larger coral eating fish were deterred by the open-sided cages. There are a number of observations that fish and invertebrates graze (accidentally or deliberately) on coral juveniles, however, it is still not clear whether this is a major source of juvenile mortality (see review by Mumby, 2009). Sato (1985) and Sammarco (1980) observed that fish and urchin grazers accidentally damaged coral spat during grazing. Similarly, Bak and Engel (1979) inferred that mortality of *Agaricia* sp. juveniles (≤40 mm) resulted from individuals being bitten off and scraped from the substratum. Recently, direct observation on the effect of the epilithic algal matrix grazing of blenny *Salarius fasciatus* in controlled conditions showed that coral spat at the single polyp stage were more susceptible to algal grazing than corals with more than one polyp (Christiansen et al., 2009). Other studies have demonstrated that modifications that exclude grazing fish enhance survival of reared coral spat. Omori (2005) reported that the success of mass culture of corals was impeded by fish grazing, leading to the development of open water coral culture techniques involving floating cages that excluded fish grazers but contained the top shell *Trochus niloticus* to control algal growth. In that study, all corals that were caged without topshells had died within 4 months, however no data were reported on the survival of uncaged corals. Similarly, Nozawa (2008) showed that the survival of coral spat is enhanced on settlement plates with micro-crevices that may serve as a refuge from grazing. The coral spat on the tiles that were used in the present study had just a single polyp when they were outplanted to the reef so it seems likely that they were in a size class most susceptible to grazing.

In conclusion, caging appears beneficial for survivorship of early stage corals (approx. 6–20 weeks after fertilisation), however it is not clear whether grazer exclusion, shading or a combination of these factors contributed to increased survival. The effects of shading and grazing need to be further investigated by *in situ* observations on the behaviour of herbivores in open-sided cages, measurements of the extent of shading inside different cage treatments and include additional control treatments such as open-top cages to separate the effects of caging from shading. In addition, further studies are needed to determine if survivorship in cages decreases at a later stage due to overgrowth by other biota and whether survivorship is enhanced if spat are settled on more complex surfaces that provide refuge from grazers. If the results of this study apply more generally, then the effort involved in caging corals and maintaining the cages during the initial few months of rearing corals *in situ* for reef rehabilitation may allow a much greater proportion of juvenile corals to reach a transplantable size.

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