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Post-settlement survivorship of artificially supplied *Acropora* coral larvae in the Sekisei Lagoon

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ABSTRACT: Coral survivorship immediately after settlement remains relatively unknown despite many published reports on the larval recruitment of reef corals. This may be due to difficulties in the field observation of wild coral settlers. In this study, we compared *Acropora* coral survivorship immediately after settlement using a lattice-shaped plate that prevented fish grazing and sedimentation. To clarify the contribution of post-settlement survivorship toward the establishment of coral populations in different habitats, post-settlement survivorship was compared among 5 sites. Each site had different coral coverage: low in 3 sites and high in 2 sites. Six months after settlement, there was no significant difference in post-settlement survivorship between sites, except at one site where the settlement density was extraordinarily high. Extremely low survivorship was observed at that site probably because of a crowding effect. In terms of the substrate condition, macroalgae were abundant at sites where the coral cover was low. However, shading by macroalgae had a negative effect only on the growth of coral spats and not on their survivorship. The coral survivorship dropped sharply at one site 15 mo after settlement and 26 mo after settlement at another site. These results suggested that key factors associated with the high mortality of coral juveniles may vary temporally and spatially, even within a lagoon.

KEY WORDS: Lattice plate · Scleractinian coral · Larval recruitment · Macroalgae · Okinawa

INTRODUCTION

Larval supply and post-settlement mortality are 2 major processes that affect the population establishment of marine organisms. In particular, there are 2 hypotheses for the establishment of a distribution pattern of sessile organisms along an environmental gradient: random settlement and selective settlement. The former refers to a distributional pattern that is not determined by larval settlement, whereas the latter refers to larval selection of suitable habitats that determine the distributional pattern (Hatton 1938). For benthic marine invertebrates, both larval supply and post-settlement mortality may influence distribution at a small spatial scale, but post-settlement mortality has less influence at a larger scale (Hunt & Scheibling 1997, Bownes & McQuaid 2009). It is expected that the contribution of both processes to population establishment at a small scale differs among taxonomic groups. For example, for clonal ascidians, larval habitat selection determines the vertical zonation of recruits, and post-settlement mortality determines the density of juvenile colonies (Stoner 1990). In this study, the role of post-settlement mortality of larval settlers in coral recruitment was evaluated.

Acropora are one of the major dominant scleractinian corals in tropical and subtropical reefs of the Western Pacific and Indian Oceans, and they establish a zonation pattern along with wave exposure (Done 1982). Although the complex colony morphology of *Acropora* corals forms a unique sea-scape, all species start to grow from a single polyp after a larva settles. It takes approximately 1 yr for that one polyp to grow to a 1 cm diameter colony (Nozawa 2010).

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Coral spat mortality during this stage is very high (usually >99% yr^{-1}) (Suzuki et al. 2009, Trapon et al. 2013), which is mainly thought to be due to grazing by fish (Brock 1979, Baria et al. 2010, Penin et al. 2010) or echinoderms in the Caribbean (Sammarco 1980) as well as competition with epibenthic algae (Harrington et al. 2004, Arnold et al. 2010). Although this high mortality may differ by habitat and may critically influence the resilience of coral communities, there are few studies on coral spat mortality in the field.

One reason for limited field studies may be the difficulty in detecting coral spats on a natural substratum, particularly immediately after settlement. It is almost impossible to accurately count these coral spats because they are small (~1 mm in diameter) and commonly settle in small cavities on reef rocks (Harrison & Wallace 1990). To facilitate field observations, artificial plates have been adopted to identify coral spat mortality, and smooth plane plates were used in many cases (Harrison & Wallace 1990). Accordingly, coral spat mortality at 6 mo after settlement was estimated to be very high. However, this high mortality may be an overestimation because fish grazing, one of the largest contributors to spat loss, is maximized on smooth (no cavity) artificial plate surfaces (Nozawa 2008). To remove fish grazing pressure, cages were placed over plain plates in some experiments (Baria et al. 2010). However, caging treatments create an unnatural environment, particularly in terms of light availability.

Based on these backgrounds, in this study, we monitored the post-settlement survivorship of *Acropora* settlers over a 2 yr period, to assess whether the post-settlement survival that was thought to be very low in the field was different among reef habitats. Fish grazing, light intensity, wave strength, and competition with benthic macroalgae are expected to be important factors that affect coral survivorship. We removed the effect of fish grazing by improving the shape of the artificial settlement plate, as fish grazing makes it difficult to monitor long-term post-settlement survivorship as stated above. Considering that other factors would differ depending on the position on inner or outer reefs, water depth, and coral coverage, some experimental sites were established in different habitats within a lagoon. In addition, we clarified a genetic clade composition of symbiotic algae in ambient water to test the hypothesis that the first acquisition of symbiotic algae from the environment by coral settlers influences their post-settlement survivorship.

Furthermore, to test the hypothesis that the recovery speed of *Acropora* population after a large disturbance such as bleaching would differ depending on the difference in post-settlement survivorship, we considered the period of coral recovery after past disturbances during our selection of the experimental sites. The recovery speed of *Acropora* corals differs by habitat, even within a relatively small area (i.e. a few km^2) (e.g. Connell et al. 1997). Indeed, *Acropora* populations recovered within several years in some areas, whereas few corals were found in some other areas for >20 yr. So far, whether selective settlement or post-settlement mortality makes a larger contribution to this difference in speed of coral recovery remains unknown. Based on a coral recruitment study, the number of settled corals (i.e. spats) differed widely among regions and habitats within a region (Hughes et al. 1999, Edmunds et al. 2015). For example, a comparison of spats on a mid-shelf (or lagoon) site with a fringing reef slope indicated that several dozens- or hundreds-fold higher number of spats were present on a fringing reef than on a mid-shelf reef (Fisk & Harriott 1990, Suzuki et al. 2008). In addition, more spats were found in shallow water than in deeper water (Wallace 1985). These settlement patterns are consistent with the distributional pattern of adult corals, suggesting that distribution is determined by the amount of larval settlement (Baird et al. 2003, Suzuki et al. 2008, 2012a). However, the hypothesis that high mortality of abundant settled corals is responsible for the delay of recovery has not been rejected yet. In this study, we tested this hypothesis to clarify the role of larval supply in building *Acropora* populations after large-scale disturbances.

MATERIALS AND METHODS

Study sites and setting of the lattice-shaped plates

This study was conducted in the Sekisei Lagoon (~300 km^2; 24.3° N, 124.0° E), which is located in the southern part of the Ryukyu Archipelago, Japan. Five sites were selected based on long-term observation of coral cover, favoring some sites with a period of low coral cover after disturbances, and reef type around the lagoon (Fig. 1a). To calculate coral cover, 3 transect lines (each line length was 5 m on the reef slope and 10 m in the lagoon) were set at each site in February 2015. A photograph of every 0.5 × 0.5 m quadrat placed on the line was taken. Coral cover was calculated using the Coral Point Count for Excel extension (Kohler & Gill 2006). The stratified random method (3 rows and 3 columns with 4 random points

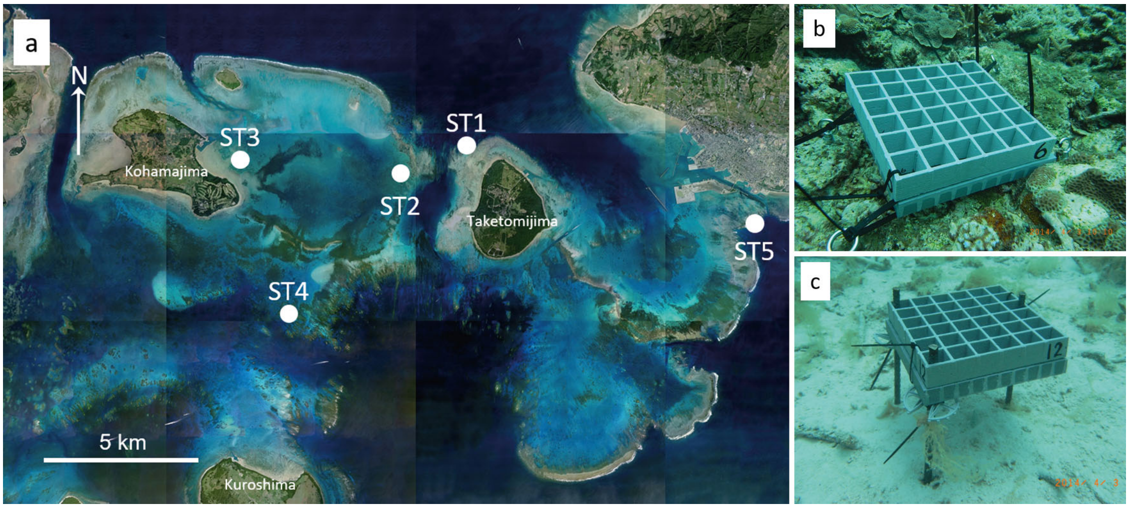

Fig. 1. (a) Study area at Sekisei Lagoon, Japan. Lattice-shaped plate fixed on (b) the seafloor and (c) a sandy bottom

in each cell for a total of 36 points on each image of quadrat) was used to determine the distribution of random points on each quadrat. Corals (or no coral area) at the points were identified to species or genus level, and the ratio of the number of occupied points was calculated as an estimate of the coverage.

To monitor the long-term post-settlement survivorship, lattice-shaped settlement plates (Fig. 1b,c) were used in this study. This type of plate reduces the incidental mortality of coral settlers due to fish grazing, and the many vertical surfaces not only ensure adequate sunlight but also avoid sedimentation, which leads to high survivorship in coral settlers under optimal environmental conditions (Suzuki et al. 2011). In addition, because each vertical wall of this plate has a smooth surface, the observation of coral settlers is easy when some walls (i.e. sides of lattice) are cut out from the plate. Eight lattice-shaped plates (6 × 6 cells, 4 cm pitch of inner spacing, 4 cm height) made of fiber-reinforced plastic were fixed to the seafloor at each site (40 plates in total) by a stainless anchoring bolt with plastic cable ties and epoxy resin adhesive (only in the wave-exposed habitats) in March 2014 (Fig. 1b,c). The site features are summarized in Table 1. ST1 (north of Taketomijima) is located on a gentle reef slope facing the open sea (East China Sea); it has a healthy coral community that mainly consists of tabular *Acropora* corals. Four plates were fixed at 3 m depth, and the other 4 plates were fixed at 6 m depth. ST2 (west of Taketomijima) is a shallow lagoon <5 m deep with a low-density distribution of small coral colonies, although branching *Acropora* corals had dominated until the 1980s when they were wiped out by crown-of-thorns sea stars. This site has a sandy bottom with sparsely distributed reef rocks; 4 plates were fixed on the rocks and the other 4 plates were fixed on the sandy bottom using an iron rod. ST3 (east of Kohamajima) is also in the shallow lagoon (<5 m), and the corals here have not yet recovered from the disturbance caused by coral-eating sea stars. Plate setting was the same as that described for ST2. ST4 (north of Kuroshima) is also located within Sekisei lagoon with a sandy bottom, but the cover of branching *Acropora* coral is high here. Plate setting was the same as that described for ST2. ST5 (Sakuraguchi) is located on the reef slope facing the open sea (Pacific Ocean) near Ishigaki port (i.e. city area); hard corals disappeared due to the 1998 bleaching event, and soft corals have recently dominated this site. Four plates were fixed at 3 m deep, and the other 4 plates were fixed at 8 m deep.

Collection of coral gametes and larval settlement

To supply a certain number of coral larvae to the plate, reared larvae from artificially collected eggs were used in this experiment. By allowing the same number of larvae to settle on each plate, we could compare the settlement and survivorship after settlement. *Acropora tenuis* and *A. selago* larvae were used in this study because both species dominated or were commonly distributed at all study sites. Ten mature colonies (size of approximately 20 × 20 cm) of each species were collected in May 2014 and artificially spawned in an aquarium by adding 1 mM hydrogen peroxide (Hayashibara et al. 2004). Collected egg–sperm bundles were gently mixed for

Table 1. Description of each study site

Site	Local name	Latitude (°N)	Longitude (°E)	Depth (m)	Reef type	Corals
ST1	North of Taketomijima	24.347	124.077	3 6	Reef slope	Tabular *Acropora* dominant Branching *Acropora* dominant
ST2	West of Taketomijima	24.335	124.053	3	Lagoon	No corals
ST3	East of Kohamajima	24.336	124.009	3	Lagoon	No corals
ST4	North of Kuroshima	24.301	124.016	7	Lagoon	Branching *Acropora* dominant
ST5	Sakuraguchi	24.322	124.167	3 8	Reef slope	Soft corals dominant

each species, and eggs only were moved to a 100 l plastic container filled with fresh filtered seawater once fertilization was confirmed. The developed planula larvae could settle after 4 d of rearing (Morse et al. 1996). At that point, 1000 larvae of each species (2000 larvae in total) were packed in plastic bags (40 bags in total) and taken to each site by a boat. To induce larval settlement on the conditioned lattice-shaped plates at each site, a previously fixed plate was detached and sealed in a plastic bag with 2000 larvae underwater. After 48 h, the plate was retrieved from the bag and re-fixed to the seafloor.

Because the larval seeding was conducted 1 wk after a mass spawning event of wild *Acropora* corals, naturally developed larvae could settle on the lattice-shaped plate. The amount of natural recruitment varies annually among sites considerably. To estimate natural coral recruitment in each study site, 5 sets of 2 flat-shaped settlement plates (8 × 8 cm, square concrete panel) were set next to each lattice-shaped plate at each site in 2014 and 2015. To avoid direct fish grazing, 2 plates were put on top of one another with a 1 cm gap using a stainless-steel bar screw (i.e. a set of 2 plates). All plates were set for 2 mo before the *Acropora* mass spawning event, which usually occurs around the full moon in May in this study area. They were retrieved 2 wk after spawning. Each plate was observed under a microscope, and the number of coral recruits was counted. Although the mortality would be high for settled corals on flat-shaped plates, this type of plate is generally used to estimate the number of coral recruits (Edmunds et al. 2015) and is suitable for estimating short-term recruitment. That is, because the flat-shaped plate has a different structure from the lattice-shaped plate and consists of different materials (concrete or FRP), the post-settlement mortality of larval settlers on the flat-shaped plate would be higher than that on the lattice-shaped plate in case of long-term monitoring (>3 mo) as stated above. However, it could be useful to compare the relative amount of short-term recruitment (i.e. retrieved 2 wk after spawning) among sites.

Survivorship and growth of coral settlers

Each lattice-shaped plate was processed by making slits in the corner of the lattice in advance of fixing to the seafloor, which allowed us to cut out at most 15 pieces of lattice from the plate. The size of each piece was 3 × 4 cm. To estimate the number of corals that settled and survived on the lattice plates, 5 pieces were collected from the interior sections of each plate 3 times (72 h, 1 mo and 3 mo) after settlement. The number of settled corals was estimated by counting the number of corals on the pieces that were collected 72 h after larval seeding (i.e. 72 h after that the plates were re-fixed to seafloor). The collected pieces were kept submerged in seawater while being transferred to the laboratory. Subsequently, the number of live versus dead corals (only skeletons remaining) were counted on the pieces using a stereo microscope. The number of settled corals was estimated as the total of live and dead corals. The average density of settled corals (i.e. the number of settled corals per cm^2) was calculated for each plate and multiplied by the total surface area to estimate the total settlers on the plate. Coral survivorship was estimated using the same method at 1 and 3 mo after larval seeding. The survivorship was estimated as the number of surviving corals divided by the number of settled corals at 72 h after settlement on each plate.

In addition, at 6, 15, and 26 mo after larval settlement, divers counted the number of juvenile corals (>2 mm diameter) on the lattice plates at each site with the naked eye to estimate the number of corals that survived on the whole plate. At 15 and 26 mo after settlement, >20 corals were haphazardly selected on each plate, and the long diameter (i.e. the largest width) of the colony was measured using a caliper underwater.

Estimation of macroalgal cover on the lattice-shaped plates

The macroalgal cover on the experimental plates was calculated from photos that were taken from directly overhead in December 2014 (6 mo after settlement), August 2015 (15 mo after settlement), and July 2016 (26 mo after settlement). The number of cells of the lattice plate covered by macroalgae was counted on each plate, and the proportion of covered cells to the total cells was calculated as algal cover.

Environmental source of symbiotic algae

Acropora coral larvae do not have symbiotic algae during the developmental stage; these are acquired from the ambient water during the swimming stage or after settlement (Baird et al. 2009). Symbiotic algae are mainly comprised of one of the major dinoflagellate groups, *Symbiodinium* spp., and several genetic clades have been identified (Clades A–I) (Pochon & Gates 2010). Considering that the clade composition of *Symbiodinium* algae in the environmental pool may have an influence on the initial survivorship and growth of coral settlers, we collected seawater samples from each site 1 mo after starting the experiment and compared the genetic composition among the sites. At each site, a diver collected a 10 l seawater sample 1 m above the seafloor near the lattice plates and returned it to the laboratory for processing. DNA of environmental *Symbiodinium* cells was extracted following the method of Yamashita et al. (2013). Namely, 3 l of the collected seawater samples were filtered through a 20 µm sieve to remove large size contaminants; it was then re-filtered through a 0.8 µm PC filter. The environmental *Symbiodinium* cells trapped on PC filters were then subjected to the TE (Tris-EDTA buffer) boiling method to extract the DNA (Koike et al. 2007). The nuclear internal transcribed spacer (ITS) total region (ITS1-5.8S rRNA-ITS2) was amplified by the polymerase chain reaction method using *Symbiodinium*-specific primers (Yamashita & Koike 2013). The amplicons were cloned and sequenced using the same method as Yamashita et al. (2014). In the present study, ~20 clones for each sample were sequenced, and the *Symbiodinium* clade was identified by checking a database. The sequences obtained in the present study were deposited in the GenBank/EMBL/DDBJ database (accession numbers LC368832 to LC368951).

Statistical analyses

Corals were classified into 3 groups for coral coverage analyses: *Acropora*, other hard corals (mostly Family Scleractinia), and soft corals (mostly Family Alcyonacea); the percent cover of each group was compared using a 1-way ANOVA among sites (also between depths in ST1 and ST5). The null hypothesis (H_0) was that there was no difference in coral coverage among sites. If a significant difference was detected ($p < 0.05$), multiple comparisons were conducted using the Tukey-Kramer method. The same statistical analyses were applied for the comparison of number of naturally settled corals. For the number of initial settlers on the lattice plates, a 3-way ANOVA was used that treated site (ST1 to ST5), depth (shallow or deep), and fixed place (on sandy or rocky substratum) as factors. Multiple comparisons (Tukey-Kramer method) were conducted for factors for which a significant difference was detected. For the post-settlement survivorship of settlers on the plates, the survival rate at 1, 3, 6, and 15 mo after settlement to the number of initial settlers was calculated for each plate, and a 3-way ANOVA and multiple comparisons were conducted in the same way with the initial settlers. At 26 mo after settlement, the survivorship from 15 mo to 26 mo was calculated, and the same statistical analysis was used. For the colony size at 15 and 26 mo after settlement, the measured data were pooled for each site and a 3-way ANOVA and multiple comparisons were conducted as for the settlement number and survivorship. Algal coverage on the plates was also compared by the same 3-way ANOVA, and multiple comparisons were conducted using data calculated from each plate. For the genetic clade composition of symbiotic algae, Bray-Curtis similarities among samples were calculated, and a cluster analysis was conducted. To estimate the relationship between coral survivorship, colony size, and other environmental factors, multiple regression analysis was conducted. First, survivorship was treated as an explained variable, and colony size, depth, algal cover, and *Acropora* cover were treated as explanatory variables. Next, colony size was treated as an explained variable, and survivorship and other same factors were treated as explanatory variables. Akaike's Information Criterion (AIC) was used for model selection. Statistical analyses were conducted using R software (v. 3.1.2).

RESULTS

Coral coverage

Coral coverage was significantly different among the sites (ANOVA, df = 6, F = 20.793, p < 0.001). Multiple comparisons revealed that coral coverage was higher at ST1, ST4, and ST5 than that at the other 2 sites (Tukey HSD: p < 0.05), but only soft corals were found at ST5 (Fig. 2). *Acropora* corals occupied approximately half of all hard corals at ST1 and 80% of all hard corals at ST4. There were almost no corals at ST2 (average coverage: 0.3%) and ST3 (average: 0.26%).

Settlement, post-settlement survivorship, and size of artificially induced coral larvae

The number of settled larvae was approximately 25 to 30 ind. 100 cm^{-2}, except at ST1 where an extremely high density of recruits was found (average ± SE of 150 ± 14 ind. 100 cm^{-2}; ANOVA, df = 4, F = 98.113, p < 0.001, and post hoc Tukey HSD, ST1 vs. other sites: p < 0.001) (Fig. 3). This high-density population may have been derived from natural settlement because natural recruitment was significantly high at ST1 in 2014 (ANOVA, df = 6, F = 7.452, p < 0.001) (Fig. 4). In addition, the larval seeding on the plates was performed at the same time as the settlement of natural settlers (1 wk after mass spawning). No settlement intensification (Pineda et al. 2010) was found because the number of settlers was not significantly different depending on depth and substratum.

The survivorship at 1 mo after settlement was significantly different among sites (ANOVA, df = 4, F = 7.811, p < 0.001); the lowest survivorship was at ST1 (~20%), where the settlement density was extremely high, whereas the survivorship was the highest at ST2 and ST3 (80%) (Fig. 5). More than 60% of the settlers survived at Sites ST4 and ST5. At 3 mo after settlement, the survivorship values were 35 to 70% at all sites, but it was only 4% at ST1. Also, at 6 mo after settlement, ~20% of the settlers had survived at all sites, except ST1. However, at 15 mo after settlement, the survivorship had significantly declined at ST5 (1% survivorship; post hoc Tukey HSD, p < 0.05) as well as at ST1. The survivorship levels at the other 3 sites at that time were 13 to 15%. At 26 mo after settlement, approximately half of the corals that had

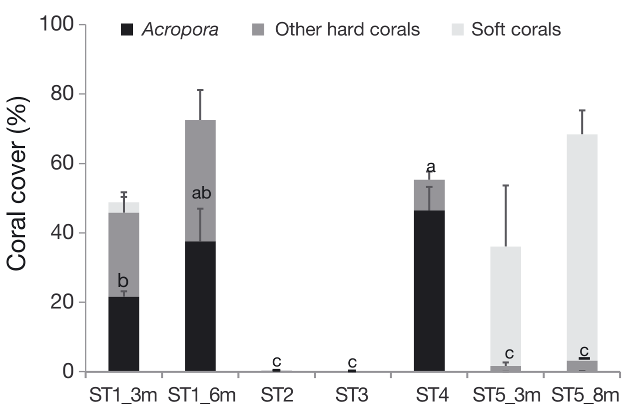

Fig. 2. Coral coverage at each site. Different lowercase letters represent significant differences among sites for *Acropora* coverage (Tukey-Kramer test, p < 0.05). Error bars indicate standard error

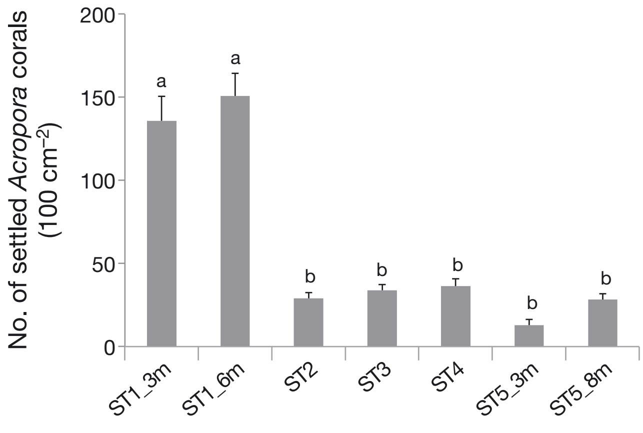

Fig. 3. Number of *Acropora* coral settlers on lattice-shaped plates by site. Different lowercase letters represent significant differences among sites (Tukey-Kramer test, p < 0.001). Error bars indicate standard error

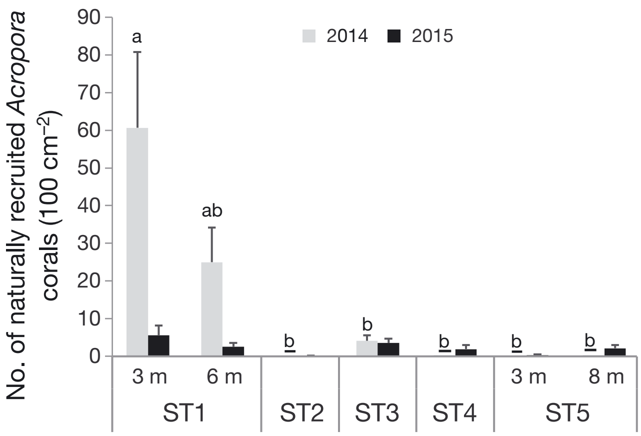

Fig. 4. Density of naturally recruited corals at each site. Different lowercase letters represent significant differences among sites (Tukey-Kramer test, p < 0.05), and no significant differences were detected in 2015. Error bars indicate standard error. The result was obtained from the flat plates installed directly in the field. These plates were retrieved 2 wk after mass spawning

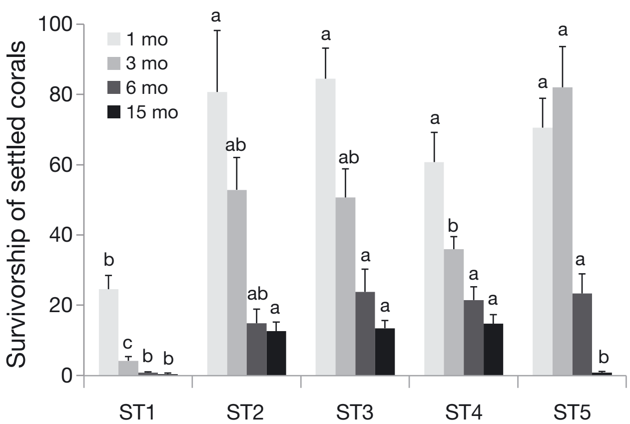

Fig. 5. Survivorship of *Acropora* juveniles on lattice-shaped plates from 1 to 15 mo after settlement. Error bars indicate standard error. Different lowercase letters represent significant differences among sites when compared at the same time interval (Tukey-Kramer test, $p < 0.05$)

survived up to 15 mo at ST3 and ST4 remained alive, whereas most corals died in ST2 (Fig. 6).

Several lattice-shaped plates were lost at ST5; the remaining plates were 1 in the shallow waters and 2 in deep waters at 26 mo after settlement (2 plates had already been lost at 3 mo after settlement at each depth). At ST1, 2 plates were lost at 6 mo after settlement. These 2 sites were in the outer reef (i.e. wave-exposed habitat). No plates were lost at the other 3 stations (ST2, ST3, and ST4), which are in the lagoon.

Comparing the size of settled corals at 15 mo after settlement, the long diameter (average ± SE) was 14.2 ± 0.6 mm at ST1 (n = 87), 9.5 ± 0.4 mm at ST2 (n = 157), 11.6 ± 0.3 mm at ST3 (n =250), 13.8 ± 0.3 mm at ST4 (n = 423), and 8.1 ± 0.9 mm at ST5 (n = 14). The coral size was significantly larger at ST1 and ST4 (ANOVA, df = 4, $F = 3.938$, $p < 0.05$) (Fig. 7). A multiple regression analysis showed that only *Acropora* coral cover was a significant explanatory variable ($p < 0.05$) when the colony size was an explained variable. No significant explanatory variables were found when the survivorship was an explained variable. The best model was colony size at 15 mo = 0.104 × *Acropora* cover + 0.113 × survivorship at 15 mo + 8.565 (AIC = 69.99, $R^2 = 0.368$, $p < 0.001$). At 26 mo after settlement, the average colony size had almost doubled at all sites, although there were no significant differences among ST1, ST2, ST3 and ST4.

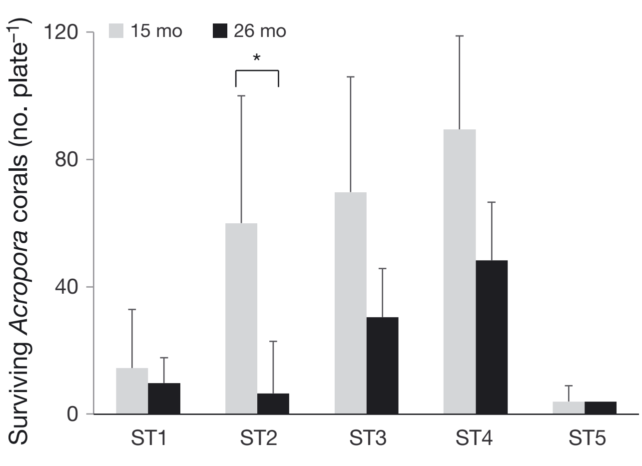

Fig. 6. Change in the average number of *Acropora* juveniles from 15 to 26 mo after settlement. Error bars indicate standard error. The asterisk represents significant difference from the other sites ($p < 0.001$)

Algal coverage on the plate

Macroalgal coverage on the plates was significantly different among sites throughout the experimental period (ANOVA, df = 4, $F = 34.384$, $p < 0.001$); it was very high at ST2 and ST3 (40 to 50 %), whereas few algae were observed at the other sites (Fig. 8). Therefore, algal cover was high only at those sites with low coral cover in the lagoon. The major macroalgal taxonomic group was the brown algae *Phaeophyta*, specifically the brown sea fan *Padina minor* and the forked ribbons *Dictyota* spp.

Composition of symbiotic algae in ambient waters

Five of the 9 *Symbiodinium* algae genetic clades (A, C, D, F, and G) were detected in the ambient water

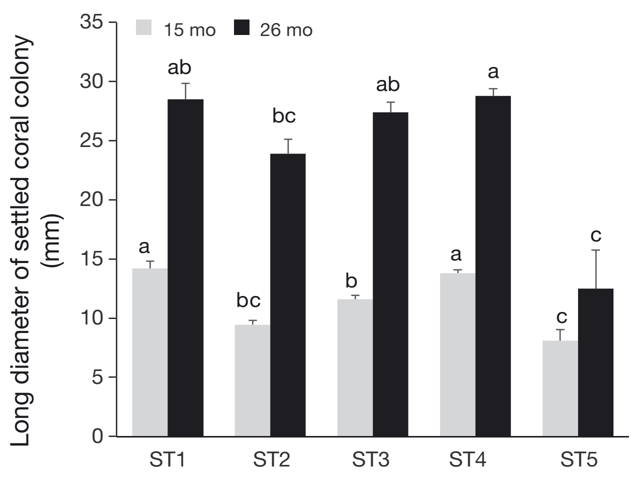

Fig. 7. Comparison of juvenile *Acropora* colony size among sites. Different lowercase letters represent significant differences among sites; colony sizes at 15 and 26 mo after settlement were separately compared with each other (Tukey-Kramer test). Error bars indicate standard error

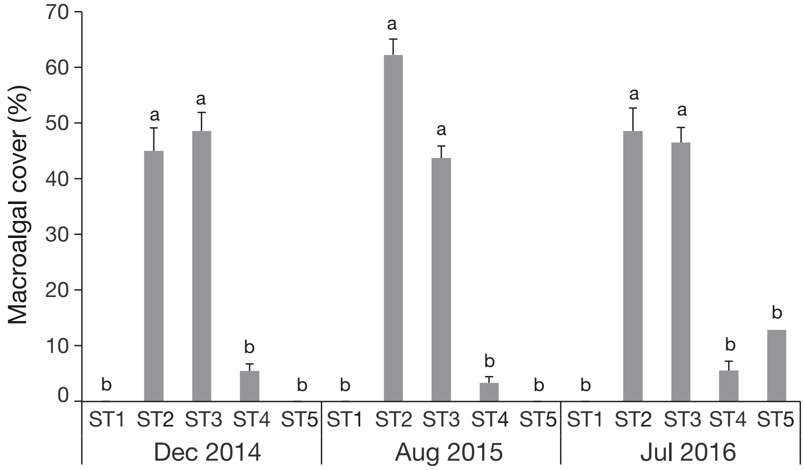

Fig. 8. Macroalgal cover on the lattice-shaped plates from 6 to 26 mo after starting the experiment. Different lowercase letters represent significant differences among sites (Tukey-Kramer test, p < 0.05). Error bars indicate standard error

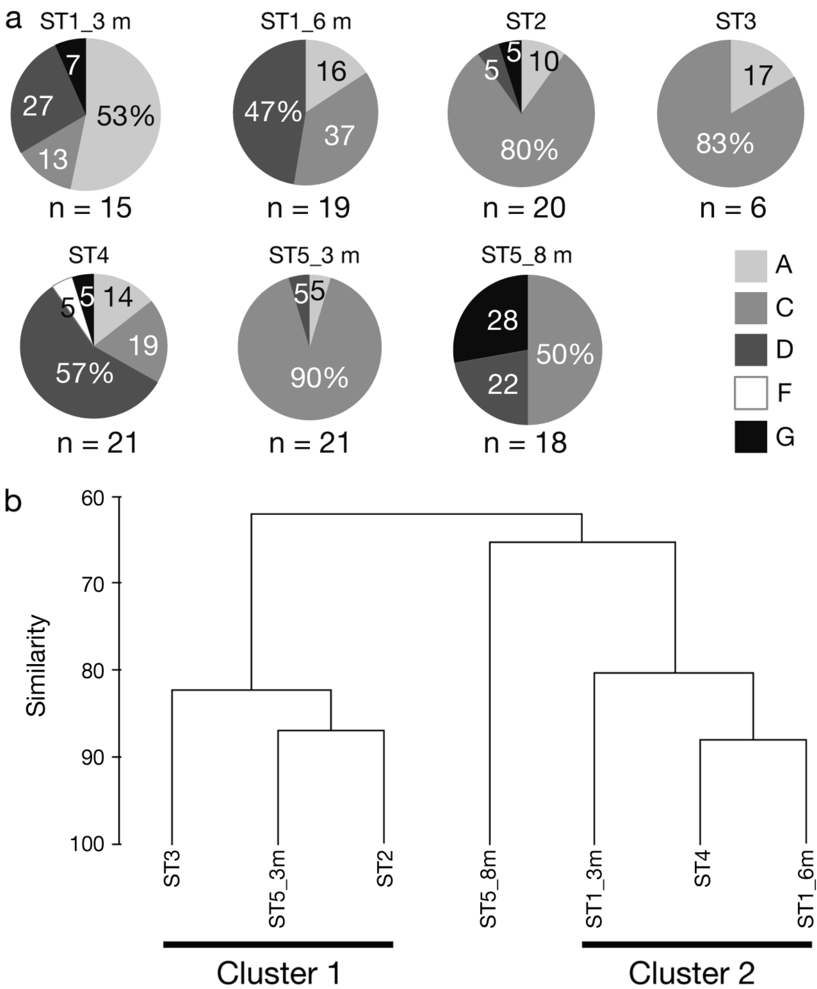

Fig. 9. (a) Genetic clade compositions of symbiotic algae in each sample with percentage values (n = number of clones that could be detected by the cloning procedure, as explained in 'Materials and methods'). (b) A dendrogram based on Bray-Curtis similarities between samples

of our study area. Only Clade C was detected in all samples, whereas there was no Clade A at ST5 (8 m), Clade D at ST3, or Clade G at ST1 (6 m), ST3, and ST5 (3 m) (Fig. 9a). Comparing clade compositions among sites (estimated from the number of cloning copies), each clade could be equally detected at ST1 and ST4, whereas the proportions of Clades A and D were small at ST2, ST3, and ST5. A cluster analysis using similarities between samples showed that 2 clusters were identified by a similarity of >70 (Cluster 1: ST2, ST3, and ST5_3 m; Cluster 2: ST1_3 m, ST1_6 m, and ST4) (Fig. 9b). Only ST5_8 m did not belong to any cluster, probably because Clade G dominated in this sample.

DISCUSSION

We hoped to clarify in this study at what stage settled corals die in places where coral recovery after disturbance is very slow. Here, we tested the working hypothesis that most settled corals died immediately after settlement, although many larvae had once settled in such places. However, there was no significant difference in the survivorship of settled corals among all sites except ST1 for at least 6 mo after settlement in this study. The survivorship at that time was 21% on average across all sites except ST1, which was not low compared with previous studies (10–14% in Suzuki et al. 2011, 2013). This result suggested that settled corals can survive even in areas with low coral cover. In addition, this result suggested that there was little competition with macroalgae on the plates at least during the first 6 mo, because the plates set in sites with low coral cover (i.e. ST2 and ST3) were thickly covered with brown macroalgae. Accordingly, the hypothesis that high mortality of abundant settled corals is responsible for the delay of re-establishment of *Acropora* coral populations was rejected at least in this study area.

The mortality during the first 6 mo at ST1 was significantly higher than at the other sites. This could be caused indirectly by a large amount of natural settlement. In other words, the high settlement density on lattice-shaped plates allowed the spread of some infectious diseases caused mainly by bacteria and resulted in high mortality. Indeed, densities of >0.5 settler cm^{-2} resulted in 80% mortality during 1 mo after settlement in a previous study (Suzuki et al. 2012b). This mass settlement only occurred in 2014, and it is unclear how these settlers survived or died on natural substratum. It is expected that most settled corals were killed by fish grazing on natural reef substratum, whereas some corals that settled in small crevices or gaps on reef substratum might have survived. High mortality due to high density on the lattice plate may have been an artificial factor. Uncovering the post-settlement survivorship on natural reef substratum after such mass settlements is a task for future studies.

Subsequently, the survivorship of settled corals sharply decreased 15 mo after settlement at ST5 and 26 mo after settlement at ST2. Accordingly, the settled corals survived without mass mortality in the short term over 2 yr only at ST3 and ST4. A cause of mortality at ST5 may have been strong southerly waves due to a typhoon, because settled corals with a flat-shaped colony grew into a branching-shaped colony during this period. Branching colonies can grow faster but are easy to break by wave pressure. Mortality at ST2 during the 15 to 26 mo after settlement could have been due to an intensification of competition with macroalgae because many settled corals died in March or April 2016 (the second spring), which overlapped with the growth period of macroalgae. Of the 2 sites with high coral survivorship, ST3 had low adult coral cover for a longer time after a disturbance, whereas ST4 had high adult coral cover. These results suggested that settled corals can survive where few adult corals have been found (Fig. 10).

Lattice-shaped plates were used as the settlement substratum in this study. These plates reduce the influence of fish grazing, which is a major mortality factor on settled corals (Suzuki et al. 2011). If fish grazing is not removed (i.e. the use of flat plates without a cage), the survivorship of settled corals is very low (Suzuki et al. 2009). Some may believe that a very low survivorship of settled corals occurs in a natural reef environment. However, considering that naturally recruited larvae generally avoid fish grazing by settling in small cavities on the reef substratum, the lattice-shaped substratum played much the same role as this coral microhabitat.

Other general mortality factors for settled corals are competition with epibenthic algae such as crustose coralline algae (Harrington et al. 2004), predation by small crustaceans and polychaetes (Robertson 1970), disease (R. Nakamura unpub. data.), and bleaching. There was no bleaching during this experimental period. Although some settled corals may have suffered mortality during this study by the above factors, mortality factors were not identified at any of the sites.

The colony size of settled corals was smaller at ST2 and ST3 (low adult coral cover with high algal cover on the plates) than at ST1 and ST4 (high adult coral cover with low algal cover on the plates). Many macroalgae (mainly *Padina minor* and *Dictyota* spp.) were found on the plates at 2 sites with low coral cover and slow recovery (ST2 and ST3). The amounts of insolation on the settled corals was likely to be low at these sites due to shading by macroalgae. Conversely, few macroalgae were found on the plates at 2 sites with high coral cover (ST1 and ST4), where the amounts of insolation would be high. From these results, it is likely that inhibition of insolation by macroalgae partly caused a reduction in coral growth.

This study also suggested that the genetic clade composition of symbiotic algae in ambient waters was different depending on surrounding coral coverage and species composition. Indeed, clade diversity and the number of cloned copies were high at sites where hard coral cover was high, whereas the number of cloned copies was low and a single clade monopolized sites where hard coral cover was low. Many copies of Clade G, a known symbiont of soft corals, were detected at ST5 where soft coral cover was high (Van Oppen et al. 2005). Importantly, the

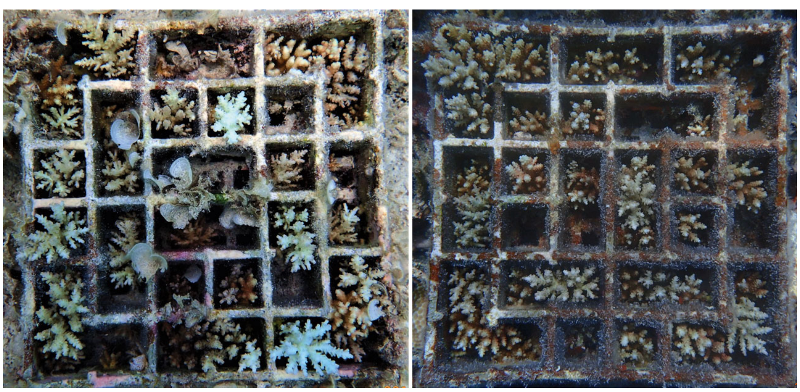

Fig. 10. *Acropora* corals on the plate at ST3 (left) and ST4 (right) 26 mo after settlement

sites with high clade diversity (i.e. high adult coral cover) included some clades that are easy for settled corals to take up (Clades A and D) (Yamashita et al. 2013, 2014). Considering that the post-settlement growth was estimated to be slower at ST2, ST3, and ST5 than at ST1 and ST4, it is possible that a shortage of symbiotic algae in the environmental pool retards the growth of settled corals at sites with low adult coral cover, although further experiments are required to demonstrate this relationship.

In conclusion, the relationship between the post-settlement survivorship of *Acropora* coral juveniles and the surrounding benthic and floral fauna differed not only by site but also by the growth stage of juveniles. We supposed a change of the relationship between post-settlement survivorship and some related environmental factors associated with each growth stage of coral settlers. During the first 6 mo after settlement, because coral juveniles are small and the colony was flat-shaped, shading by macroalgae affected their growth speed, although their survivorship was not threatened. There was competition only for sunlight in this stage. Over the next 6 mo, the coral juveniles formed 3D branches, which resulted in high mortality, especially at sites that were affected by strong typhoon-driven waves. Corals then started to form large colonies from 1 yr after settlement. Macroalgae and corals therefore came into direct contact; hence, competition between them for space intensified due to interference. This scenario is merely a medley of hypotheses at the moment, and future studies should examine the post-settlement survival in each growth stage based on this scenario.

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