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Patterns of larval settlement preferences and post-settlement survival for seven Caribbean corals

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ABSTRACT: Caribbean coral reefs continue to decline in coral cover; however, recruitment is a natural process that could increase coral abundance. Benthic habitats that increase coral recruitment are a key factor for coral persistence, but very little is known about habitat selectivity for larvae of most species of corals. The larval settlement preferences and post-settlement survival of 3 brooding and 4 broadcast spawning coral species were compared in this study. The crustose coralline algae *Titanoderma prototypum* and *Hydrolithon boergesenii* facilitated larval settlement more than the biofilm control for the broadcast spawning corals but not for the majority of the brooding corals. In paired choice experiments, the larvae of all 7 corals preferred *T. prototypum* over *Paragoniolithon solubile,* and 6 of them preferred *H. boergesenii* over *Pa. solubile*, the exception being larvae of *Porites astreoides.* All corals equally preferred *T. prototypum* and *H. boergesenii*, except *Pseudodiploria strigosa*, which preferred *T. prototypum,* and *Acropora palmata,* which preferred *H. boergesenii*. Some recruits from the 3 brooding corals survived longer than 1 yr in the field, but of the 4 spawning corals, only *P. strigosa* had 2 recruits that survived >1 yr. Corals that spawned their gametes had increased settlement in the presence of a few species of coralline algae, but corals that brooded their larvae settled on biofilms and had much greater post-settlement survival, suggesting that the recruitment of brooding corals will dominate on reefs without facilitating species of crustose coralline algae.

KEY WORDS: Facilitation · Coral recruitment · Resilience · Larval ecology · Crustose coralline algae

INTRODUCTION

Supply-side ecology can drive population dynamics in marine habitats (Gaines & Roughgarden 1985, Lewin 1986), and the importance of larval dispersal and connectivity for marine populations has received increased attention (Levin 2006, Cowen & Sponaugle 2009, Jones et al. 2009). Benthic organisms are often critical associative cues for settling marine larvae (Pawlik 1992, Hadfield & Paul 2001). Facilitation is recognized as an important process that can drive the patterns of diversity of an ecosystem (Bruno et al. 2003) and is known to be an important mechanism for maintaining adult corals on reefs (Stachowicz 2001). Facilitation is also known to play a role in increasing coral larval settlement (Raimondi & Morse 2000). The facilitation of coral recruitment is an important process for maintaining these diverse ecosystems and ensuring that corals will persist in the future (Ritson-Williams et al. 2009).

Coral larval ecology has focused on relatively few species of corals, and variation in settlement ecology and post-settlement survival is rarely tested among different coral species. Brooding corals with internal fertilization are hypothesized to persist in extreme and variable habitats (Szmant 1986, Edinger & Risk 1995). The brooding corals *Agaricia agaricites* and *Porites astreoides* are common throughout the Carib-

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bean Basin, and new recruits of these species are frequently observed in recruitment studies in that region (Arnold et al. 2010, Arnold & Steneck 2011, Green & Edmunds 2011). Corals with a spawning life history are rarely found in recruitment studies in the Caribbean (Vermeij 2006, Arnold & Steneck 2011, Green & Edmunds 2011, Vermeij et al. 2011) but can dominate recruitment in the Indo-Pacific (Wallace 1985, Harrison & Wallace 1990). As coral reefs continue to be degraded by multiple stressors, a better understanding of the benthic organisms that could facilitate or inhibit coral recruitment is necessary to manage reefs for recovery and resilience (Mumby & Steneck 2008, Ritson-Williams et al. 2009).

Crustose coralline algae (CCA) are considered important facilitators for coral settlement and survival. Coralline algae induced settlement in agariciid corals in the Caribbean (Morse et al. 1988, Raimondi & Morse 2000) and were assumed to be required for their settlement (Morse & Morse 1991). Two species of CCA, *Titanoderma prototypum* and *Hydrolithon boergesenii*, consistently induce higher rates of settlement in *Acropora* spp. in the Pacific and Caribbean (Harrington et al. 2004, Ritson-Williams et al. 2010, 2014). The facilitation of coral settlement by *T. prototypum* has been confirmed in field studies using recruitment tiles (Arnold et al. 2010, Price 2010, Arnold & Steneck 2011). Other species of CCA can also induce high rates of settlement, but it is clear that not all species of CCA facilitate settlement for the spawning corals tested (Ritson-Williams et al. 2014).

Some studies show that CCA is not required for coral larval settlement; biofilms can also be important settlement substrata for the larvae of some coral species (Negri et al. 2001, Webster et al. 2004, Tran & Hadfield 2011, Sneed et al. 2014). Experiments in Guam and Hawaii showed that different coral species had different preferences for biofilm versus CCA (Golbuu & Richmond 2007, Tran & Hadfield 2012). The literature shows that both biofilms and CCA can induce coral larval settlement, but due to the variety of methods used and coral species tested, it is difficult to parse trends in coral recruitment ecology from species-specific traits. To test larger trends in larval settlement, the same methods need to be used to elucidate patterns of settlement preferences among larvae of different coral species.

Surprisingly few studies have compared coral reproductive strategies to determine their relative impact on patterns of recruitment and species persistence (Vermeij et al. 2007, and for a geologic perspective, see Edinger & Risk 1995). To better understand the processes that drive patterns of recruitment among coral species with different life-history strategies, coral settlement and post-settlement survival were tested using 3 species of brooding and 4 species of broadcast spawning corals. These experiments test whether all coral species settle at the same rates in response to the same ecologically relevant substrata and track post-settlement survival in the field. We document different strategies in settlement ecology and post-settlement survival among coral species with brooding and spawning life-history strategies.

MATERIALS AND METHODS

Study site and species

All of our studies were conducted at the Smithsonian Institution's Carrie Bow Cay Field Station in Belize. Ten to 40 coral colonies of *Agaricia agaricites*, *Favia fragum* and *Porites astreoides* were collected and maintained in the laboratory with flowing seawater pumped directly from the back reef for 2 to 7 d and then returned to their sites of collection. Larvae released at night were collected using a transfer pipette the next morning and subsequently used for experiments (Table 1). All brooded larvae were used 1 d after release, except for 4 d old larvae of *P. astreoides* used in the choice experiments in April 2008.

The spawning corals *Pseudodiploria* (previously *Diploria*) *strigosa* and *Orbicella* (previously *Montastraea*) *faveolata* are massive, habitat-forming species found commonly throughout the Caribbean. Corals were monitored *in situ* at night, and when spawning occurred, the gamete bundles from 4 to 10 colonies were collected with nylon nets and subsequently fertilized and maintained in the laboratory following methods described by Ritson-Williams et al. (2010, 2014). Larvae of *P. strigosa* were raised in flowing seawater, and larvae of *O. faveolata* were raised in standing seawater (4 l) that was exchanged 1 to 2 times daily. Any dead embryos or debris were removed with a pipette or forceps, and larval containers were exchanged and cleaned with freshwater every 2 d. Larvae of *P. strigosa* were used 4 d after fertilization in the choice experiments and 5 d after fertilization in the no-choice experiments. Larvae of *O. faveolata* were used 6 d after fertilization for all experiments. To increase the breadth of our comparison among species, we have included patterns of settlement for *Acropora cervicornis* and *A. palmata*. The settlement ecology of these 2 species was tested

Table 1. Dates of larval release (the night of fertilization for the spawners) and experiments conducted for each coral species (*Agaricia agaricites*, *Favia fragum*, *Porites astreoides*, *Pseudodiploria strigosa*, *Orbicella faveolata*). Reproductive (Rep.) mode describes corals that have internal fertilization and brood their larvae (B) or corals that broadcast-spawn their gametes and have external fertilization (S). 'X' represents the experiment that was conducted from that larval collection. Letters in the post-settlement survival column correspond to the experiments in Fig. 8; A is the spawner comparison, B is the spawner versus brooder comparison, C is the brooder comparison. Values in parentheses equal n

Species	Rep. mode	Larval release date	Settlement No-choice	Settlement Choice	Post-settlement survival
Ag. agaricites	B	7–9 Aug 2010			B (12)
		3–29 Jun 2011	X (3)	X (9–11)	C (18)
		21–26 Apr 2012	X (5)		
F. fragum	B	14 Aug 2008		X (12)	
		12 Oct 2008	X (12)		
		22–24 Jul 2010			B (12)
		9–14 Jun 2011			C (19)
P. astreoides	B	6 Apr 2008		X (11–12)	
		4–9 Jun 2011		X (9)	C (12)
		19 Apr 2012	X (15)		
P. strigosa	S	12 Aug 2009	X (15)	X (15)	A (8)
O. faveolata	S	16 Aug 2006		X (12)	
		19 Sep 2011	X (15)	X (12)	
A. cervicornis	S	19 Aug 2008	[a](15)	[a](12)	
		7 Aug 2009			A (8)
		31 Jul 2010			B (20)
A. palmata	S	18 Aug 2008	[a](15)	[a](12)	
		7 Aug 2009			A (8)
		30 Jul 2010			B (20)

[a]Data for these experiments were reanalyzed from data with 5 d old larvae published by Ritson-Williams et al. (2010)

with the same methods presented here, and the original settlement data for these species were published by Ritson-Williams et al. (2010). To compare among a wider breadth of species, we have conducted new analyses of these data and present novel results and figures in this study.

It took 6 yr to test the larval ecology described in this study because different coral species spawn at different seasons, and in some years, the adults did not release enough larvae to perform replicated experiments. We always used all of the larvae available, and whenever possible, we tested the larval ecology of each species within a year, but for *Ag. agaricites*, we had to combine data from multiple years due to consistent low fecundity (Table 1).

Four species of CCA were used to test the settlement behavior of these coral larvae: *Hydrolithon boergesenii*, *Paragoniolithon solubile*, *Porolithon pachydermum* and *Titanoderma prototypum.* These species were identified using microscopic morphological characteristics described by Ritson-Williams et al. (2014).

Larval settlement experiments

No-choice

Larval settlement was assessed in no-choice experiments measuring settlement and metamorphosis in the presence of 1 species of CCA in a 60 mm petri dish. All of the replicate dishes contained 10 or 20 larvae (kept consistent for every dish within an experiment) of 1 coral species with 0.2 or 0.45 µm filtered seawater (FSW) to reduce the abundance of bacteria in seawater during the experiments. As a control for a biofilmed substratum without CCA we collected a small fragment of dead *A. palmata* skeleton from the same habitat as the CCA species. All of the CCA fragments and the biofilmed dead *A. palmata* skeleton were cut into 1 cm^2 (top surface area) fragments with diagonal pliers to ensure that the potential settlement area was similar among the treatments. Settlement is defined as the behavioral attachment to the substratum and metamorphosis as the physiological and morphological transition from a planula larva to a benthic polyp. Settlement can be a reversible process (Richmond 1985), and metamorphosis can occur in the water column without settlement (Vermeij 2009), so only larvae that had both settled and metamorphosed were counted after 24 h in each replicate dish. Proportions were calculated by dividing the number of new recruits by the total number of live larvae in the dish (larvae that were still swimming plus those that had settled and metamorphosed). We included a FSW treatment (a negative control) to show that there was little or no spontaneous or gregarious settlement and metamorphosis. The FSW treatment was not included in the statistical analysis because our hypothesis was that coral species have different rates of larval settlement and metamorphosis on different ecologically relevant substrata.

In the no-choice experiments, larvae could settle on 3 potential settlement substrata within each dish including the top surface of CCA, the clean rock on the underside of the fragment, and the dish itself.

The dishes with the dead *A. palmata* skeleton also had 3 potential settlement substrata: the top biofilmed surface, the clean rock on the underside of the fragment and the dish itself. For the dead *A. palmata* skeleton, the upper surface that was naturally biofilmed was compared to the top surface of CCA because these surfaces are representative of ecologically relevant benthic substrata found in the field. Each dish contained 1 species of CCA (or the biofilm on the dead *A. palmata* skeleton) and was considered an individual replicate. The same individual piece of CCA or dead *A. palmata* skeleton was never used in more than 1 dish to ensure independence among the replicate dishes. For each experiment, the number of replicate dishes varied due to the availability of larvae, and the level of replication is detailed in Table 1. None of the data were normally distributed or had homogenous variances, so a 1-way ANOVA with rank transformed data was used to analyze the total settlement (settlement and metamorphosis of the living larvae on the sum of all 3 settlement substrata within a dish), and an ANOVA (with rank transformed data) was conducted to compare the settlement and metamorphosis on the top surface of the CCA (and the corresponding biofilm on the dead *A. palmata* skeleton). The alpha value was adjusted to $p \leq 0.01$ (Bonferoni adjusted for multiple comparisons) to determine if the means among treatments were significantly different, and Tukey's HSD post-hoc test was used to determine significant groupings.

Comparison among coral species

Multiple studies show that both biofilms and CCA can induce coral larval settlement. Further analyses of our data were conducted to test the hypothesis that some CCA species facilitated or inhibited larval settlement compared to an ecologically relevant natural biofilmed surface (the top surface of a dead *A. palmata* skeleton). Within a coral species, Dunnett's test was used to determine if the larval settlement on each CCA surface was significantly different than settlement on the biofilmed surface. To display these data, the mean proportion of larvae that had settled on the surface of each CCA species was divided by the mean proportion of settlers on the biofilmed surface of the dead *A. palmata* skeleton. A value significantly greater than 1 shows that the CCA facilitates settlement more than a biofilmed surface, and a value significantly less than 1 indicates the CCA inhibits settlement relative to a biofilm. Normalized settlement data for *A. palmata* and *A. cervicornis* were calculated from original data (5 d old larvae for both species) published by Ritson-Williams et al. (2010). This is a novel analysis of the *Acropora* spp. data and has not been previously published. The data for settlement and metamorphosis directly on the biofilm surface was normally distributed and therefore was statistically compared between spawners and brooders using a *t*-test (with arcsine square-root transformed data) using each coral species as a replicate, for spawners (n = 4: *A. cervicornis, A. palmata, O. faveolata* and *P. strigosa*) and brooders (n = 3: *Ag. agaricites, F. fragum* and *P. astreoides*).

Paired choice

In the paired choice experiments, there were 5 potential settlement substrata, the surface of each CCA (2), the rock on the underside of each CCA (2), and the dish. These data were normally distributed and had homogenous variances, so a 1-way ANOVA tested whether the proportion settlement and metamorphosis (arcsine square-root transformed) on any of these substrata were different ($p \leq 0.01$, Bonferoni adjusted for multiple comparisons), which was followed by Tukey's HSD post-hoc test. For each coral species, the proportion of settlement on the surface of one CCA was compared to the settlement on the surface of the other CCA from the same dish using a paired *t*-test. The choice settlement data for *A. palmata* and *A. cervicornis* presented here were newly calculated from original data published by Ritson-Williams et al. (2010).

Post-settlement survival experiments

Experimental set-up

Post-settlement survival is a critical life-history stage for successful recruitment, and the 3 experiments described below were designed to test the hypothesis that different species of coral recruits have different survival in the field. All post-settlement survival experiments were conducted on a reef 200 m south of Carrie Bow Cay at 3 m depth. This reef was within 75 m of where the gametes of the spawning corals were collected. The details of the date of acquisition of new recruits are listed in Table 1. Larvae were settled onto 1 cm^2 CCA chips and then maintained in flowing seawater for 1 to 6 d in the laboratory. The number of coral recruits per chip was standardized by removing recruits as necessary before

the chips were attached to 10 × 10 × 1 cm terracotta tiles. CCA chips were imbedded in Splash Zone epoxy (Z-Spar) 1 cm from the edge of a tile so that the CCA (top) part of the chip with the coral recruits was exposed to the seawater. Twelve hours after CCA attachment to the tile, all chips were visually inspected and photographed to ensure the CCA were securely attached and none of the coral recruits had died during the manipulations. Each tile was attached to the reef with a stainless steel bolt that was screwed into a plastic insert that had previously been drilled into the benthos (methods following Arnold et al. 2010) with the CCA chips on the underside of the tiles (Ritson-Williams et al. 2010). All of the tiles were haphazardly arranged along the reef at 3 m depth. At regular time intervals (described for each experiment below), the tiles were brought to the laboratory, assessed for recruit survival with a dissecting microscope and then returned to the reef later the same day. New recruitment was excluded by ensuring that each recruit that was counted matched the original recruit location photographed at the beginning of the experiment. While a variety of marine organisms were observed recruiting to the tiles over time, we assume that interactions of coral recruits with new organisms on the tile are a natural process.

Spawning species

In the experiment comparing recruit survival of spawning corals, 3 chips of *H. boergesenii* were attached to each tile with 6 recruits of either *A. cervicornis*, *A. palmata* or *P. strigosa* on each chip of CCA. Eight replicate tiles were placed on the reef on 18 August 2009 and were assessed for survivorship after 2, 11, 22 and 32 mo. Each tile was a replicate, so means were calculated from the proportion survivorship on each tile. The data for each species were not normally distributed, so they were rank transformed and compared at each time point using 1-way ANOVA followed by Tukey's HSD post-hoc test.

Spawning versus brooding species

For the spawners vs. brooders experiment, there was 1 chip of *H. boergesenii* attached to each tile with 3 recruits of 1 coral species on each chip. There were 12 replicate tiles each for *Ag. agaricites* and *F. fragum* and 20 replicate tiles for *A. cervicornis* and *A. palmata*. These tiles were placed on the reef on 10 August 2010 (except *F. fragum* tiles, which were placed on the reef on 1 August 2010 because these larvae were released 10 d before the others) and were assessed for survivorship after 25 d and 3, 10 and 20 mo. Again, each tile was a replicate, so means were calculated from the proportion survivorship on each tile. The data were never normally distributed, so they were rank transformed and compared using 1-way ANOVA followed by Tukey's HSD post-hoc test.

Brooding species

The third experiment was a comparison of post-settlement survival of brooding coral species on *H. boergesenii* and *T. prototypum*. Each tile had 1 recruit of a coral species on a chip of *H. boergesenii* and a recruit on the chip of *T. prototypum* so that survival on these 2 species of CCA could be directly compared. There were 18 replicate tiles of *Ag. agaricites*, 12 of *P. astreoides* and 19 of *F. fragum*. These tiles were placed on the reef on 13 June 2011 and were assessed after 25 d, and 3 and 10 mo. Because these data were counts, the recruit survival data were analyzed with chi-squared tests at each time point to test for differences in survivorship among the coral species. The survival of each coral species on *H. boergesenii* was compared to survival on *T. prototypum* by Fisher's exact test.

RESULTS

Larval settlements

No-choice experiments

The brooder *Agaricia agaricites* had rates of settlement and metamorphosis among the treatments between 26 and 57%, and there was no difference in the total amount of settlement in response to different substrata (Fig. 1a; $p = 0.089$, $F = 2.20$). Settlement and metamorphosis on the top surface was not different among the biofilmed *Acropora palmata* skeleton, *Hydrolithon boergesenii* and *Titanoderma prototypum* (Fig. 1b; $p = 0.001$, $F = 5.57$), but *T. prototypum* had greater settlement on its surface compared to *Porolithon pachydermum* and *Paragoniolithon solubile* (Tukey's post-hoc test).

Total settlement of the brooder *Favia fragum* ranged from 4 to 33% among the treatments and was greater in the biofilmed dead *A. palmata* skeleton and *T. prototypum* treatments compared to *P. pachydermum* and *Pa. solubile* (Fig. 2a; $p = 0.001$, $F = 5.33$,

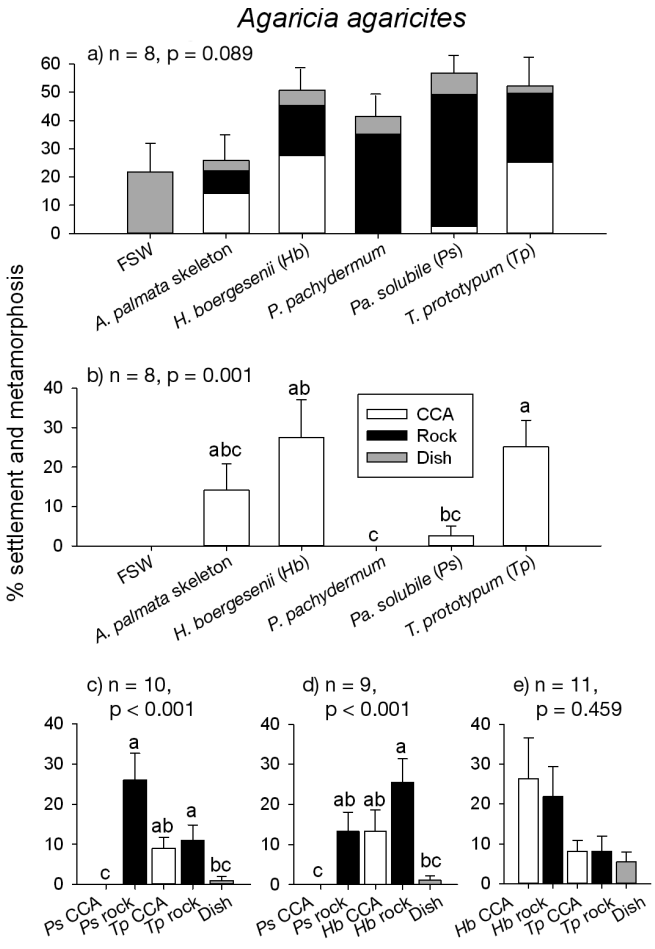

Fig. 1. Larval settlement patterns of *Agaricia agaricites*. Bars are untransformed means; error bars are +1 SE. Results are from (a,b) the no-choice experiment (a = total settlement and b = settlement on the top surface only) and (c–e) paired choice experiments. Different colored bars refer to different potential settlement substrata within a treatment as is indicated in the key. Different letters above the bars indicate significantly different means as determined by Tukey's post-hoc test. Filtered seawater (FSW) was excluded from all analyses. CCA: crustose coralline algae

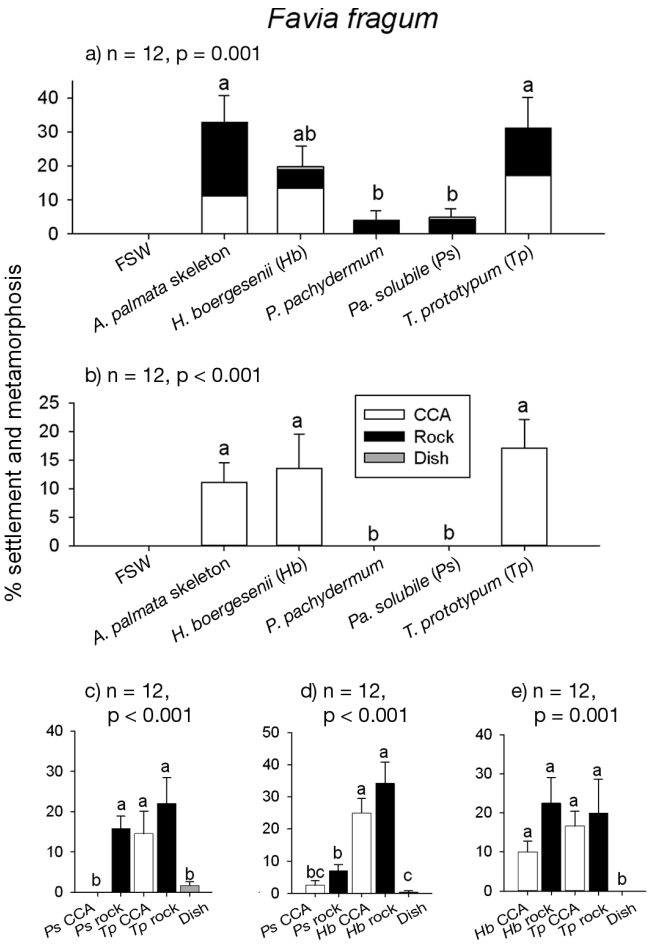

Fig. 2. Larval settlement patterns of *Favia fragum*. See Fig. 1 for more details

Tukey's post-hoc test). On the top surfaces, the biofilm, *H. boergesenii* and *T. prototypum* had more settlement and metamorphosis than the other CCA species (Fig. 2b; $p < 0.001$, $F = 8.17$).

The brooding coral *Porites astreoides* had 90% total settlement and metamorphosis in the *T. prototypum* treatment, which was significantly greater than *P. pachydermum* (74%) and the biofilmed dead *A. palmata* skeleton (55%, Fig. 3a; $p = 0.005$, $F = 4.13$). On the CCA surface, the amount of settlement and metamorphosis was the same on *H. boergesenii* and *T. prototypum*, both of which supported greater settlement than *P. pachydermum* (Fig. 3b; $p < 0.001$, $F = 12.26$). *P. astreoides* had higher settlement on the CCA surface of *H. boergesenii* than on all other CCA species except *T. prototypum*.

The broadcast spawning coral *Pseudodiploria strigosa* had 77 to 79% total settlement and metamorphosis on *H. boergesenii* and *T. prototypum* compared to 55% settlement and metamorphosis on the biofilmed dead *A. palmata* skeleton (Fig. 4a; $p = 0.013$, $F = 3.407$, Tukey's post-hoc), although a majority of the settlement was on the rock under the CCA. The CCA surfaces of both *H. boergesenii* and *T. prototypum* had greater rates of settlement than the other top surfaces, and no settlement occurred on the surface of *P. pachydermum* (Fig. 4b; $p < 0.001$, $F = 21.287$).

Orbicella faveolata, also a spawning coral, had more total settlement in the *H. boergesenii* and *Pa. solubile* treatments compared to the treatment with biofilmed dead *A. palmata* skeleton (Fig. 5a; $p = 0.010$, $F = 3.62$, Tukey's post-hoc test). There was the same amount of settlement and metamorphosis on the top surface of *H. boergesenii* and *T. prototypum*, both

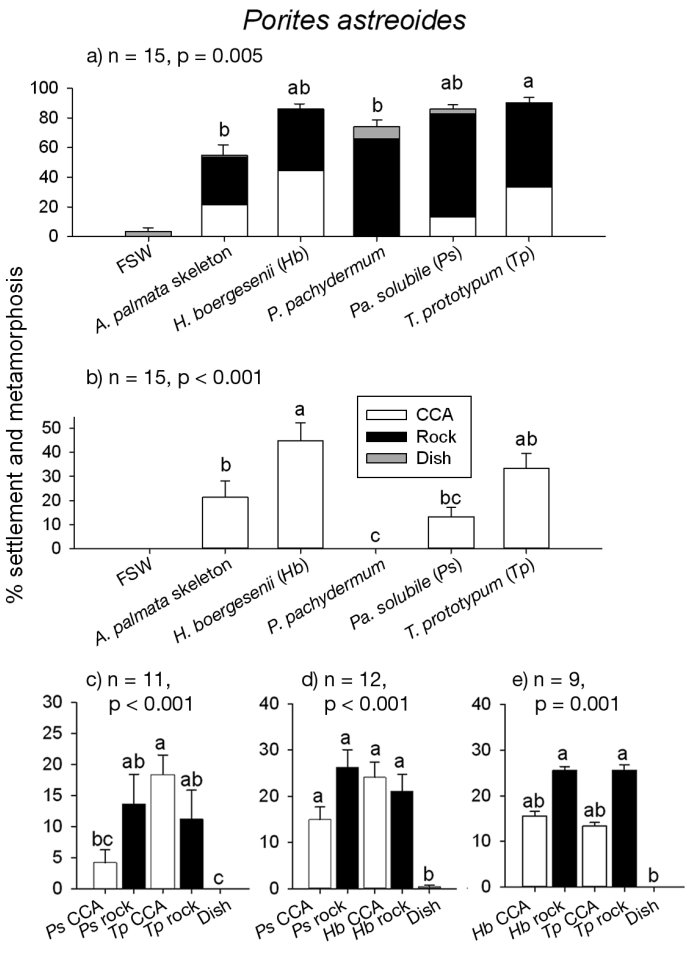

Fig. 3. Larval settlement patterns of *Porites astreoides*. See Fig. 1 for more details

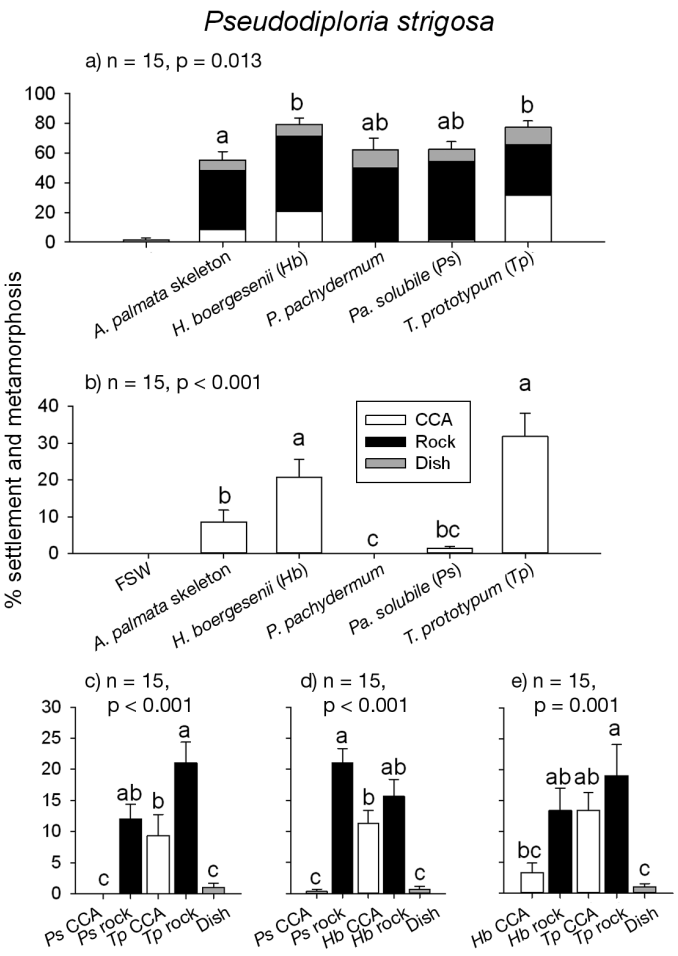

Fig. 4. Larval settlement patterns of *Pseudodiploria strigosa*. See Fig. 1 for more details

of which were greater than on the other 2 species of CCA (Fig 5b; $p < 0.001$, $F = 13.88$, Tukey's post-hoc test). Only settlement on the surface of *T. prototypum* was significantly greater than the biofilm control.

Comparison among coral species

Comparing all of the coral species using normalized settlement for the no-choice experiments shows that both *H. boergesenii* and *T. prototypum* consistently facilitated the settlement and metamorphosis of larvae from the broadcast spawning corals more than the biofilm control (Fig. 6a,b). For the brooding corals, both *H. boergesenii* and *T. prototypum* induced the same amount of larval settlement on their surface as the biofilm control, except for the larvae of *P. astreoides,* which were facilitated by *H. boergesenii* (Fig. 6a). *Pa. solubile* and *P. pachydermum* were settlement inhibitors for *F. fragum* and *P. strigosa,* but only *P. pachydermum* inhibited the settlement of *P. astreoides*. For the other corals, these CCA species induced the same amount of settlement and metamorphosis as the biofilm control (Fig. 6c,d). The biofilm surface induced rates of settlement and metamorphosis between 1 and 8% for the spawning coral species and between 11 and 48% for the brooding species. The mean (±1 SE) percentage of settlement on biofilm for the spawning coral species (3.6 ± 1.8%) was significantly different from the mean percentage of settlement (20.1 ± 11.6%) of the species that brood their larvae (2-tailed *t*-test, $p = 0.04$, $df = 5$).

Paired choice experiments

Patterns of settlement in the paired choice experiments in general were similar to patterns in the no-choice experiments (Figs. 1c–e to 5c–e). There were consistently high rates of settlement and metamorpho-

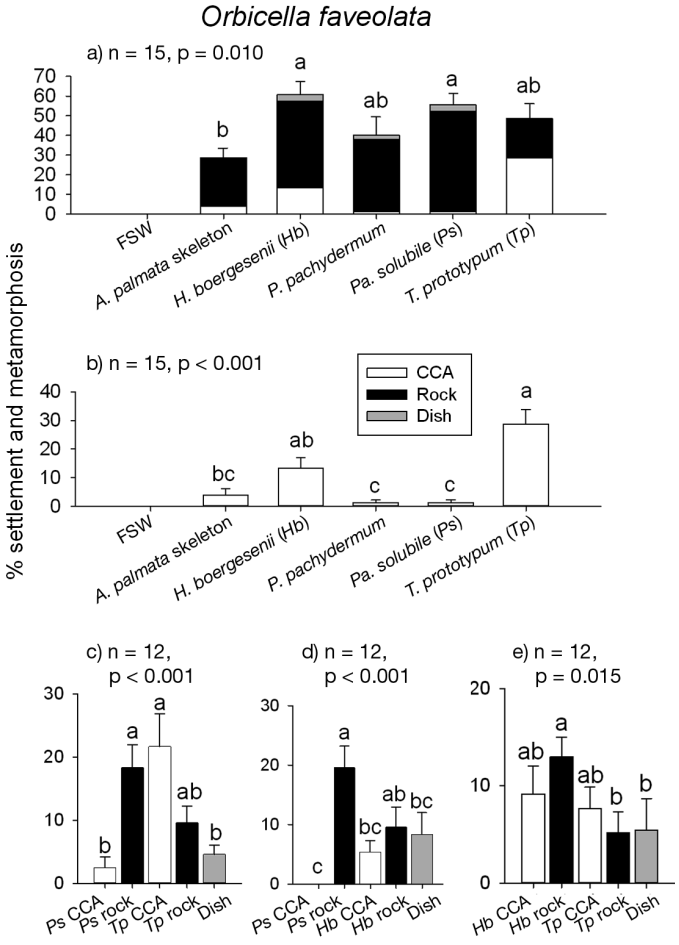

Fig. 5. Larval settlement patterns of *Orbicella faveolata*. See Fig. 1 for more details

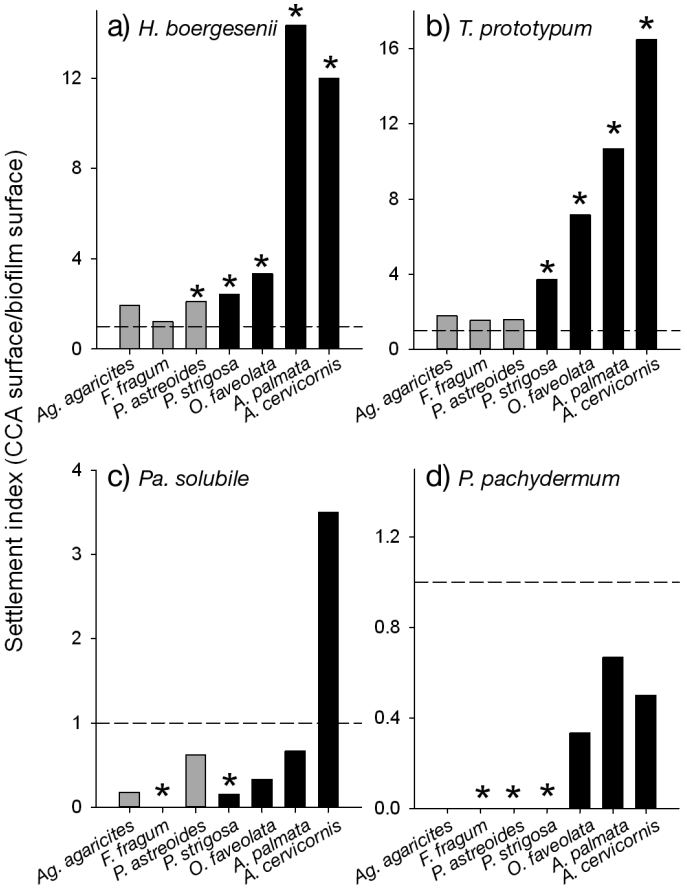

Fig. 6. Normalized settlement of coral larvae (species indicated on the *x*-axis) settled on different CCA species in no-choice experiments: (a) *Hydrolithon boergesenii*, (b) *Titanoderma prototypum*, (c) *Paragoniolithon solubile*, and (d) *Porolithon pachydermum*. Settlement was normalized by dividing the mean proportion of larvae that had settled on the surface of 1 species of CCA by the proportion of larvae settled on the biofilmed dead *A. palmata* skeleton top surface. Values significantly greater than 1 show facilitation, and values significantly < 1 indicate inhibition by that CCA species. *Significant difference of settlement on the CCA species and the biofilmed *A. palmata* coral skeleton determined by Dunnett's test

sis on the clean rock beneath the CCA and generally low rates of settlement on the dish (Figs. 1c–e to 5c–e). When just comparing between the CCA surfaces, *T. prototypum* had more larval settlement than *Pa. solubile* for all 7 coral species tested (Fig. 7a). *H. boergesenii* had more larval settlement than *Pa. solubile* for all 7 corals except for *P. astreoides* (Fig. 7b). When *T. prototypum* was offered with *H. boergesenii*, there was no difference in the proportion of settlement on the CCA surface for most of the coral species (Fig. 7c). Larvae of *P. strigosa* preferred *T. prototypum,* and larvae of *A. palmata* preferred *H. boergesenii* (Fig. 7c).

Post-settlement survival

Spawning species

After 8 wk, the percentage of survival was 33% for *A. palmata*, 10% for *A. cervicornis* and 23% for *P. strigosa* (Fig. 8a). Survival was not significantly different among these 3 species (p = 0.402, F = 0.95). After 11 mo, the only survivors were 3 recruits of *P. strigosa*, 2 of which were still alive (4%) after 22 mo but had died at 32 mo.

Spawning versus brooding species

In the brooder and spawner comparison, there was a significant difference among the species in their survival after 25 d (p < 0.001, F = 13.19). Recruits of the brooder *F. fragum* had more survival than

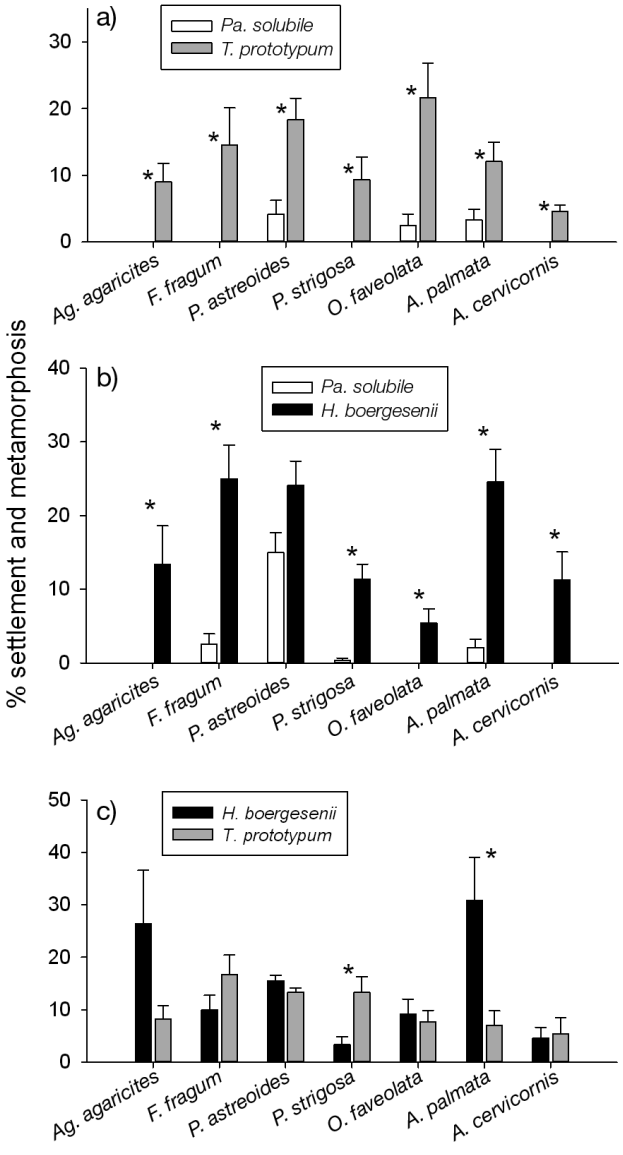

Fig. 7. Percent settlement and metamorphosis of coral larvae tested in paired choice experiments. Bars indicate the mean percent settlement and metamorphosis onto the surface of the CCA species. Error bars are +1 SE. An asterisk (*) indicates significantly different proportion settlement and metamorphosis between the surfaces of the 2 CCA species as determined by a paired *t*-test ($p < 0.05$). (a) *Titanoderma prototypum* with *Paragoniolithon solubile*, (b) *Hydrolithon boergesenii* with *Pa. solubile*, (c) *T. prototypum* with *H. boergesenii*

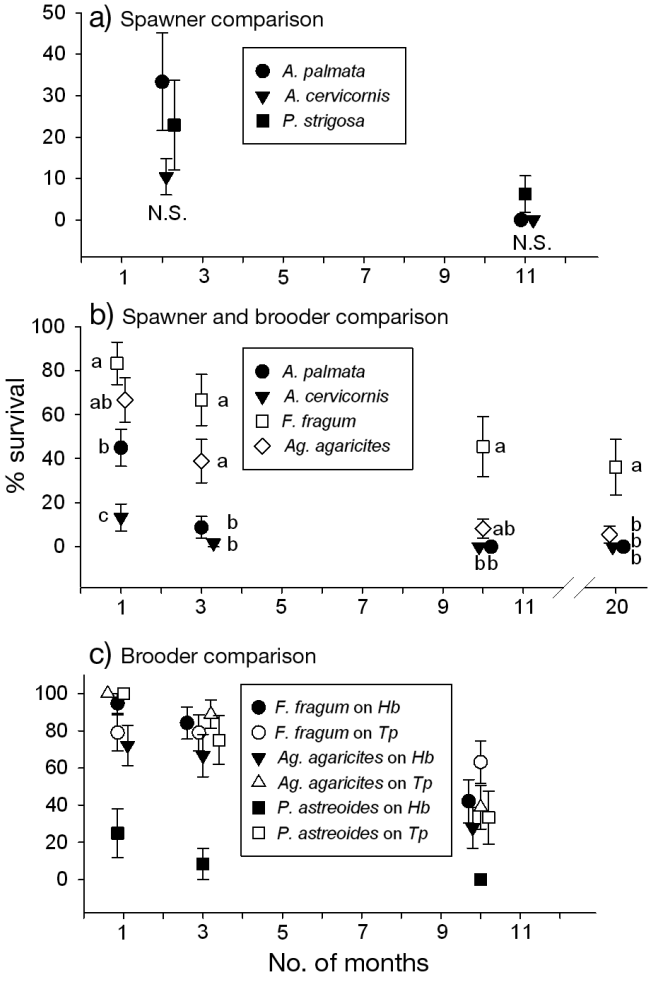

Fig. 8. Post-settlement survival of new recruits on settlement tiles at 3 m depth in the field. Symbols represent the mean percent survival of coral recruits, and error bars are ±1 SE. (a) Spawner comparison, (b) spawner and brooder comparison, (c) brooder comparison of recruits on the CCA species *Titanoderma prototypum* (Tp) and *Hydrolithon boergesenii* (Hb)

the spawners *A. palmata* and *A. cervicornis* (Fig. 8b, Tukey's HSD). After 3 mo in the field, there was significantly greater survival of both brooders *F. fragum* and *Ag. agaricites* compared to *A. palmata* and *A. cervicornis* ($p < 0.001$, $F = 19.69$). After 10 mo, none of the recruits from *Acropora* spp. survived, but *F. fragum* still had 42% survival and *Ag. agaricites* had 8% survival, and at 20 mo, there were 36% and 6% of the recruits surviving, respectively (Fig. 8b).

Brooding species

For the comparison of brooding species (Fig. 8c), survival differed among coral species grown on *H. boergesenii* at each of the time points (chi-squared; 25 d: $p < 0.001$, 3 mo: $p < 0.001$, and 10 mo: $p = 0.0349$). *P. astreoides* had lower survival compared to both *Ag. agaricites* and *F. fragum* at the first 2 time points (chi-squared; $p < 0.05$), but at 10 mo, the survival of *P. astreoides* was not different from *Ag. agaricites* (chi-squared; $p = 0.128$). On *H. boergesenii*,

the survival of *Ag. agaricites* and *F. fragum* was never different at any of the time points (chi-squared; $p > 0.05$). When the recruits were grown on *T. prototypum*, there was a difference in survival of these coral species at 25 d (chi-squared; $p = 0.032$) but no difference at 3 and 10 mo (chi-squared; $p = 0.584$ and $p = 0.186$, respectively).

For each coral species, recruit survival on 2 species of CCA was compared (Fig. 8c). For *Ag. agaricites*, there was less survival on *H. boergesenii* than on *T. prototypum* at 25 d (Fisher's exact test; $p = 0.046$), but the survival was the same at 3 and 10 mo (Fisher's exact test; $p = 0.229$ and $p = 0.725$, respectively). *P. astreoides* recruits had lower survival on *H. boergesenii* than on *T. prototypum* at 25 d (Fisher's exact test; $p < 0.001$) and at 3 mo (Fisher's exact test; $p = 0.003$), but at 10 mo, there was no difference (Fisher's exact test; $p = 0.093$). For *F. fragum*, there was never a difference in the survival of recruits on the 2 CCA species (Fisher's exact test; $p > 0.05$).

DISCUSSION

Our experiments show a dichotomy in settlement patterns among brooding and spawning coral species. The larvae of spawning coral species had a greater preference for the surface of some CCA species relative to biofilmed coral skeleton than the larvae of brooding corals. This is especially important because the CCA species that facilitate settlement can be rare on Caribbean reefs (Ritson-Williams et al. 2014). Both *Hydrolithon boergesenii* and *Titanoderma prototypum* can facilitate larval settlement on their surfaces (Fig. 6), and when given a choice, all 7 of the coral species tested selected 1 of these 2 facilitating species for settlement more than they selected the abundant species *Paragoniolithon solubile* (Fig. 7). CCA were never required for coral larval settlement and metamorphosis (Figs. 1–5), and for most of the brooding corals, there were similar rates of settlement on the CCA surfaces as on the biofilmed surfaces (Figs. 1b, 2b & 3b). We recognize that different coral species have unique larval ecology and expect some exceptions, but in our experimental comparison, there was a significant trend for different rates of settlement and metamorphosis on biofilmed coral skeleton between species that spawn and those that brood their larvae.

Some coral larvae use biofilms as a cue for settlement (Negri et al. 2001, Webster et al. 2004, Golbuu & Richmond 2007, Tran & Hadfield 2011). In this study, the coral species with brooded larvae had significantly greater settlement on a biofilm surface. However, some spawning species also respond to biofilms and isolated bacteria (Negri et al. 2001, Webster et al. 2004, Sneed et al. 2014). *Pseudoalteromonas* spp. are an important component of the biofilms that facilitate coral larval metamorphosis (Negri et al. 2001, Hadfield 2011, Tebben et al. 2011, Sneed et al. 2014). Our experiments show that spawners have higher rates of settlement on some species of CCA, but we do not discount the possibility that facilitating CCA species host a specific biofilm community (Johnson et al. 1991, Negri et al. 2001, Sneed et al. 2015). Dinoflagellate presence in a biofilm might also be important for coral larval settlement (Winkler et al. 2015); however, in these experiments, we cannot distinguish life-history strategy from vertical transfer of symbionts because the larvae of all of our spawning species did not contain *Symbiodinium*.

Both settlement and post-settlement survival affect patterns of coral recruitment, and new recruits of spawning coral species had lower rates of post-settlement survival than recruits from brooding coral species (Fig. 8). Consistently, in our experiments, the brooder *Favia fragum* had the highest survival in the early post-settlement stages. The new recruits of the 2 *Acropora* species had 100% mortality in <1 yr in 2 experiments (Fig. 8) and in a previous experiment at a near-by reef in Belize (Ritson-Williams et al. 2010), which is consistent with data from the Florida Keys (Miller 2014). Post-settlement survival can be an important demographic bottleneck for many corals (Vermeij & Sandin 2008), and studies of coral recruitment to settlement tiles throughout the Caribbean show a consistent paucity of new recruits from the massive, habitat-forming coral species compared to brooded species (Vermeij 2006, Arnold et al. 2010, Arnold & Steneck 2011, Green & Edmunds 2011). Research in Jamaica showed that 3 coral species had declining populations over 16 yr (Hughes & Tanner 2000). The brooders, *Agaricia agaricites* and *L. cucullata*, consistently had new recruits, while the spawner *Orbicella annularis* had only 1 recruit during the 16 yr study. US Virgin Islands populations of *Orbicella* spp. are decreasing because of low recruitment rates and adult mortality (Edmunds & Elahi 2007), although there can be rare localized recruitment of these species (Edmunds et al. 2011). In contrast, the brooding coral *Porites astreoides* had constant rates of recruitment (Green et al. 2008), and this species was predicted to have an increasing population in the US Virgin Islands over the next 100 yr

(Edmunds 2010). In addition, the spawning species *P. strigosa* was predicted to have increasing populations in the next 100 yr. In our study, *P. strigosa* was the only spawning coral that had recruits survive >1 yr. Our research shows an important dichotomy between brooders and broadcast spawners, but we do not suggest that every coral species will fit neatly into a category. However, increased reliance on rare CCA species for settlement and low post-settlement survival probably both contribute to recruitment failure of many coral species in the Caribbean.

Facilitation by CCA plays a variable but important role for successful coral recruitment of diverse coral species on modern reefs. As oceans continue to be changed by global and local threats, corals and coralline algae will be exposed to unprecedented levels, combinations and durations of stress. Already, the habitat-building species of Caribbean *Acropora* have been listed as threatened species (NMFS 2006), and populations in the Florida Keys show no signs of recovery (Williams et al. 2008). If populations of these ecosystem engineers are incapable of recovery after a mortality event because of recruitment failure, there will be a serious reduction of the ecosystem services that these corals provide for thousands of associated species.

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