Vol. 577: 67–77, 2017
https://doi.org/10.3354/meps12249

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 18

Effects of ocean acidification and contact with the brown alga *Stypopodium zonale* on the settlement and early survival of the coral *Porites astreoides*

Justin E. Campbell[1,*], Jennifer M. Sneed[1], Lane Johnston[1,2], Valerie J. Paul[1]

[1]Smithsonian Marine Station, Ft. Pierce, FL 34949, USA
[2]School of Marine Science and Policy, University of Delaware, Lewes, DE 19958, USA

ABSTRACT: To evaluate the effects of ocean acidification (OA) and algal presence on the early life-history stages of corals, we conducted an aquarium study that examined the isolated and combined effects of reduced pH (pH 8.10 vs. 7.85) and contact with the alga *Stypopodium zonale* on the survival, settlement, and post-settlement growth of larvae from the brooding coral *Porites astreoides*. Two settlement substrates, biofilmed tiles and the crustose coralline alga (CCA) *Hydrolithon boergesenii*, were initially incubated for 12 d in separate tanks under a factorial combination of low pH and *S. zonale* contact, and then subjected to a series of settlement assays. Across both substrate types, *S. zonale* presence significantly reduced coral settlement. Low pH imposed relatively minor effects; however, there was a significant interaction between pH and *S. zonale* presence for settlement on the CCA substrate, such that low pH exacerbated the negative effects of *S. zonale*. Post-settlement growth for 2 wk was unaffected by either *S. zonale* or low pH on either substrate. While our results demonstrate that algal contact likely remains a dominant threat to larval survival and settlement, in certain cases, OA may amplify the negative effects of algal presence, highlighting the need to consider multiple factors in studies aimed at assessing the future health of coral reef ecosystems.

KEY WORDS: Coral–algal interactions · Climate change · Reef recruitment · *Porites astreoides* · *Stypopodium zonale* · *Hydrolithon boergesenii*

INTRODUCTION

Coral reefs currently face a multitude of local, regional, and global stressors (Pandolfi et al. 2003). Over the past several decades, declines in live coral cover have been increasingly reported, broadly attributable to the negative effects of algal proliferation, disease, increased seawater temperatures, and ocean acidification (OA) (Hoegh-Guldberg et al. 2007). In particular, reefs in the Caribbean have degraded, with live coral cover declining by nearly 80% in recent decades (Gardner et al. 2003, Jackson et al. 2014). While the deterioration of reefs has prompted research into the individual causes of degradation, we lack an understanding of the potentially interactive effects of multiple stressors on coral reef health. As prior work has addressed the effects of co-occurring abiotic stressors (Castillo et al. 2014, Comeau et al. 2014, Okazaki et al. 2017), few studies have specifically examined the combined effects of localized biotic (algal proliferation) and abiotic stressors (climate change) on coral reef health and functioning (Diaz-Pulido et al. 2011, Olsen et al. 2014, Ritson-Williams et al. 2016, Del Monaco et al. 2017).

Algae are becoming increasingly dominant across many reefs throughout the Caribbean (McClanahan & Muthiga 1998, Gardner et al. 2003). The primary drivers of these trends are variable and complex, likely involving a combination of multiple factors such as proximity to urban development, coastal pollution,

*Corresponding author: campbellju@si.edu

and the loss of key grazers by either overfishing or disease (McCook 1999, Burkepile & Hay 2006, Lessios 2016). The 1980s die-off of the sea urchin *Diadema antillarum* coincided with a marked increase in algal abundance across many reefs (Mumby & Steneck 2008, Lessios 2016), threatening the future health and resilience of Caribbean reefs. These long-term shifts from coral- to algal-dominated states are commonly referred to as phase-shifts (Hughes 1994). Algae can exert a number of negative effects on adult coral functioning, via a variety of mechanisms ranging from physical shading and/or abrasion (Box & Mumby 2007), to allelopathic chemical interactions (Rasher & Hay 2010, Paul et al. 2011, Rasher et al. 2011), to a disruption of the coral microbiome (Morrow et al. 2013, Zaneveld et al. 2016). It is likely that these coral–algal associations undergird the larger role that spatial competition plays in structuring benthic reef communities (Porter 1974, Jackson & Buss 1975).

In addition to interactions with adult corals, algal effects during other coral life-history stages may also be critically important in the broader context of reef health and for the capacity of reef ecosystems to recover from a variety of disturbances. Coral recruitment is an important process whereby new individuals may be added to a population through the successive life-history stages of larval availability, larval settlement, and post-settlement survival (Ritson-Williams et al. 2009). Algal interactions at any one of these early life-history stages may place hard boundaries on the ability for reefs to repopulate. Certain types of algae negatively influence the settlement and recruitment of coral larvae (Kuffner & Paul 2004, Kuffner et al. 2006, Diaz Pulido et al. 2010, Paul et al. 2011, Dixson et al. 2014), and the survival and growth of juvenile corals (Box & Mumby 2007, Olsen et al. 2014). For instance, the presence of brown algae (*Dictyota* spp.) has been shown to reduce larval survival and recruitment of the common coral *Porites astreoides* (Kuffner et al. 2006, Paul et al. 2011, Olsen et al. 2014). These effects are likely mediated by the production of terpenoid secondary metabolites, which also have a series of effects on adult corals (Rasher et al. 2011) and generalist herbivores (Hay et al. 1987, Paul et al. 2001). While coral–algal interactions, across a variety of life-history stages, have received considerable attention, there remains a relatively poor understanding of how these interactions may be further modified by other global stressors.

Declines in oceanic pH (i.e. OA) represent a prominent and growing threat to coral reefs worldwide. Relative to the preindustrial period, current forecasts predict a near 0.4 pH unit decline by the year 2100 (Caldeira & Wickett 2003, Gattuso et al. 2015). These trends, driven by anthropogenic increases in atmospheric carbon dioxide (CO_2), decrease seawater carbonate ion (CO_3^{2-}) concentrations, thereby impairing the growth and calcification of corals and other marine calcifiers. While the effects of OA alone have been explored for adult corals (Langdon et al. 2000, Leclercq et al. 2000, Marubini et al. 2001, Anthony et al. 2008, Chan & Connolly 2013, Comeau et al. 2013, Castillo et al. 2014, Okazaki et al. 2017), and across early coral life-history stages (Albright et al. 2010, Albright & Langdon 2011, Albright & Mason 2013), little is known about the potential interaction between reduced pH and algal contact on coral health and survival. Some studies have documented increased mortality and tissue loss of adult corals under the combined stressors of algal contact and OA (Diaz-Pulido et al. 2011, Del Monaco et al. 2017). However, the manner by which these pH–algal interactions play out across early coral life-history stages has yet to be fully investigated.

We examined the isolated and combined effects of OA (replicated via CO_2 addition) and algal contact (with the brown alga *Stypopodium zonale*) on the early life-history stages of the common Caribbean coral *P. astreoides*. This particular species of brooding coral was selected because of its abundance across Florida (USA) reefs and ease of larval collection. The alga *S. zonale* was primarily selected because of its potential to bloom on certain reefs during periods of warm temperatures and high irradiance (Lirman & Biber 2000). Based upon prior studies with adult corals (Diaz-Pulido et al. 2011), we hypothesized that low pH would exacerbate the negative effects of algal contact on larval survivorship and settlement of *P. astreoides*.

MATERIALS AND METHODS

Experimental design

Two different substrates, biofilmed terracotta tiles (Sunshine Pavers®) and live fragments of the crustose coralline alga (CCA) *Hydrolithon boergesenii*, were used for the settlement assays. These substrates were initially conditioned for 12 d in experimental tanks that factorially manipulated OA (ambient vs. low pH) and algal contact (plastic mimic vs. live *Stypopodium zonale*). After this conditioning period, *Porites astreoides* larvae were collected and subjected to settlement assays using both substrates within the various tank treatments.

Fragments of the CCA *H. boergesenii* were collected from the lower Florida Keys (Big Pine Ledges, 24° 33.213′ N, 81° 22.665′ W) on 17 April 2015, and attached to glass slides (75 × 25 mm) with underwater epoxy (All-Fix). Terracotta tiles (4.5 × 4.5 × 1 cm) that had been previously deployed (for 19 d) on a patch reef east of Looe Key Reef (24° 34.130′ N, 81° 22.868′ W) for biofilm development were also collected on 17 April 2015. Both settlement substrates were collected from habitats similar to the collection site of the coral larvae (Lower Florida Keys, ~5–6 m depth). CCA slides and biofilmed tiles were transported to the Smithsonian Marine Station and kept under flowing seawater until use (11 d).

To account for the potential influence of OA and algal presence on substrate suitability, all tiles and CCA fragments were conditioned for a period of 12 d under the experimental treatments prior to the settlement assays. This conditioning allowed the CCA and *S. zonale* to become acclimated to the tanks, and also provided a period for the treatments to potentially influence substrate surface properties, as prior work has demonstrated that OA can have indirect effects on coral settlement via alterations in microbial assemblages (Webster et al. 2013). Using cable ties, we attached either live *S. zonale* or a plastic aquarium plant (to control for shading and abrasion) to the upper surface of the CCA slides and biofilmed tiles. We did not include settlement substrates with nothing attached during the conditioning phase, as our primary interest was to examine any inhibitory effects of algal presence on substrate suitability beyond structural shading or abrasion. Once attached to the appropriate algal treatment, substrates were placed into tanks assigned to the following treatments: (1) substrates with mimics at ambient pH; (2) substrates with mimics at low pH; (3) substrates with *S. zonale* at ambient pH; (4) substrates with *S. zonale* at low pH (Table 1). Each tank received 2 replicate CCA slides and 1 biofilmed tile, for an experiment total of 24 CCA slides and 12 tiles. CCA health was assessed before and after the conditioning period via measurements of maximum quantum yield (F_v/F_m) with a pulse amplitude modulated (PAM) fluorometer. No mortality was detected, and all yield measurements were within a healthy range (0.6–0.8).

We used 12 independent 37 l tanks to create 2 seawater pH treatments, i.e. ambient pH (8.10_{NBS}, n = 6) and reduced pH (7.85_{NBS}, n = 6). The 2 levels of algal treatment, mimic vs. *S. zonale*, were created for each pH treatment to yield a factorial design (n = 3 for each treatment combination). All tanks were housed indoors, and lighting was provided by a series of 220 W Aqua Medic T5 HO light fixtures that replicated broad-spectrum irradiance (photosynthetically

Table 1. Experimental design. Settlement substrates (crustose coralline alga, CCA; or biofilmed tiles), *Stypopodium zonale*, and larvae of the coral *Porites astreoides* were collected in April and May 2015 from the lower Florida Keys (USA). Left column designates the pH and algal treatments for each tank (n = 3). Center column designates the type of settlement substrate placed into each tank for 12 d of conditioning. Right column designates the 96 h settlement assays conducted in each tank. For the CCA substrate, 1 settlement assay was conducted with *S. zonale*/mimic present (1: examining direct contact effects) and a second assay was conducted with *S. zonale*/mimic removed following the conditioning period (2: examining indirect effects from conditioning)

Treatments (tank pH, attached algae)	Substrate conditioning (12 d)	Larval settlement assays (96 h)
Ambient pH, plastic mimic	Each tank received: (1) CCA slide with plastic mimic (2) CCA slide with plastic mimic (3) Biofilmed tile with plastic mimic	Settlement assay with: (1) CCA slide with plastic mimic present (2) CCA slide with plastic mimic removed (3) Biofilmed tile with plastic mimic present
Ambient pH, *S. zonale*	Each tank received: (1) CCA slide with *S. zonale* (2) CCA slide with *S. zonale* (3) Biofilmed tile with *S. zonale*	Settlement assay with: (1) CCA slide with *S. zonale* present (2) CCA slide with *S. zonale* removed (3) Biofilmed tile with *S. zonale* present
Reduced pH, plastic mimic	Each tank received: (1) CCA slide with plastic mimic (2) CCA slide with plastic mimic (3) Biofilmed tile with plastic mimic	Settlement assay with: (1) CCA slide with plastic mimic present (2) CCA slide with plastic mimic removed (3) Biofilmed tile with plastic mimic present
Reduced pH, *S. zonale*	Each tank received: (1) CCA slide with *S. zonale* (2) CCA slide with *S. zonale* (3) Biofilmed tile with *S. zonale*	Settlement assay with: (1) CCA slide with *S. zonale* present (2) CCA slide with *S. zonale* removed (3) Biofilmed tile with *S. zonale* present

active radiation = 200 µmol photons m^{-2} s^{-1}). Each tank consisted of a closed seawater system, whereby the water volume was continuously recirculated by a 473 l h^{-1} powerhead. Additional water flow in each tank was provided by vigorous airstone bubbling (with ambient air). Each tank was initially filled with filtered seawater (<10 µm) collected from an offshore oceanic location near Fort Pierce, Florida, USA. Temperature control was provided by separate water-jacketed heat exchangers attached to each tank and was set and maintained at 28°C by dual-stage digital controllers. Salinity was maintained at 35 by replenishing evaporative losses with deionized water. During the course of the experiment, weekly water changes were conducted within each tank (50% volume). CO_2 concentrations were manipulated via a coupled pH stat system (Aqua Medic), which monitored individual tank pH using separate electrodes. Low pH tanks were periodically bubbled with 100% gaseous CO_2 as determined by a series of computer-controlled magnetic solenoids. Measurements of pH (National Bureau of Standards scale, NBS) within each individual tank were taken 3 to 4 times per week with an Orion Ross combination electrode to ensure proper calibration and setpoints of the pH stat system. Salinity was recorded simultaneously with pH, and was measured with a YSI Pro20. Weekly water samples were collected to measure total alkalinity (TA) via open-cell potentiometric titration (Mettler Toledo DL15). Certified reference material (Dickson standards, Scripps Institution of Oceanography) was used to ensure the accuracy of TA measurements. All carbonate parameters within each tank were calculated with CO2SYS, using measured parameters of pH, TA, temperature, and salinity, with the carbonate dissociation constants of Mehrbach et al. (1973), as refit by Dickson & Millero (1987). Mean pCO_2 levels were calculated as 517 and 1024 µatm for the ambient and low pH treatments, respectively. These values approximate current and future (year 2100) CO_2 forecasts, yet we note that pCO_2 within the ambient tanks was slightly above reported values across the lower Florida Keys (~372 µatm from Manzello et al. 2012).

Larval collection

In May 2015, 50 colonies of *P. astreoides* were collected at approximately 6 m depth from Wonderland Reef (24° 33.62′ N, 81° 30.08′ W) in the lower Florida Keys and transported in coolers to Mote Marine Laboratory, Summerland Key, Florida, USA. Colonies were placed in outdoor raceways with running seawater. Larvae were collected following the methods described by Kuffner et al. (2006). Colonies were placed in separate 3 l bowls, which were tilted so that released larvae spilled over the handle into separate plastic tri-pour beakers fitted with a 180 µm mesh bottom. Water levels within each beaker remained constant so that released larvae were retained until the following morning when larvae were pooled and transported to the Smithsonian Marine Station. Approximately 1800 larvae were used for this experiment.

For the settlement assays, each of the 3 preconditioned substrates in each OA tank were placed into separate clear acrylic cylinders (10.2 cm diameter, 12.7 cm long) containing 50 *P. astreoides* larvae, and then returned to their respective tanks. All chambers were affixed with 180 µm mesh sidewalls on either end to ensure adequate water flow during settlement (Kuffner et al. 2006). Given that each tank had 2 replicate CCA slides, *S. zonale* or the plastic mimic from one of these slides was removed prior to placement in the chamber to examine the direct versus indirect effects of algal presence on coral settlement. This treatment was imposed for the CCA because of prior work highlighting the importance of CCA-associated microbes on larval settlement (Webster et al. 2013, Sneed et al. 2014). Thus, each OA tank contained 3 settlement chambers: (1) CCA slide with attached *S. zonale* or mimic; (2) CCA slide with removed *S. zonale* or mimic; and (3) biofilmed tile with attached *S. zonale* or mimic (Table 1). Larvae were allowed to settle for 96 h, after which all slides and tiles were scored for number of metamorphosed settlers. Percent survival was calculated as (recruits + swimmers/50) × 100 in each chamber. Total settlement was calculated as (recruits/50) × 100 in each chamber. After scoring, all *S. zonale* and mimics were reattached to their respective CCA slides and tiles, except for the slides that had the algal treatment removed prior to settlement. All CCA slides and tiles were placed back into their respective OA tanks for an additional 2 wk and re-scored for post-settlement survival and growth. Percent survival was calculated as (number of colonies surviving/number of originally settled coral spat) × 100. Growth was calculated as (total number of new coral polyps 2 wk after settlement/number of surviving coral spat) × 100.

Statistical analysis

Tank chemistry was analyzed by comparing the 95% confidence intervals of measured and calcu-

Table 2. Seawater (SW) carbonate chemistry (means and bracketed 95% CI) across the ambient and low pH tanks. Temperature, salinity, and pH_{NBS} represent discrete measurements (n = 20) from within each tank during the experiment. Total alkalinity (TA) was measured weekly within each tank (n = 5). DIC: dissolved inorganic carbon; $\Omega_{aragonite}$: aragonite saturation state

Treatment	Temperature (°C)	Salinity	pH_{NBS}	pCO_2 (µatm)	DIC (µmol kg^{-1} SW)	$\Omega_{aragonite}$	TA (µmol kg^{-1} SW)
Ambient pH	28.6 (28.7–28.4)	35.7 (36.0–35.4)	8.10 (8.13–8.06)	517.3 (561.5–473.0)	2013.4 (2034.0–1992.9)	3.25 (3.46–3.04)	2295.6 (2363.1–2228.1)
Low pH	28.7 (28.8–28.5)	35.7 (36.0–35.4)	7.85 (7.88–7.82)	1023.8 (1112.7–935.0)	2148.6 (2162.9–2134.2)	2.07 (2.19–1.94)	2308.8 (2372.7–2244.9)

lated seawater parameters. For the settlement assays, the treatments of pH (8.10 vs. 7.85) and algal presence (*S. zonale* vs. plastic mimic) were applied in a factorial design at the tank level (n = 3; 4 treatments randomly distributed across 12 tanks). Within each tank, an additional factor was imposed during the settlement assays for the 2 CCA slides, whereby one slide had the conditioning *S. zonale* or mimic present, and the other slide had the conditioning *S. zonale* or mimic removed prior to settlement, testing the direct vs. indirect effects of algal conditioning on larval settlement, respectively. The multiple CCA settlement assays within the same tank were not considered independent, thus this additional factor of algal presence during settlement (termed 'algal contact') was statistically treated as a sub-factor within each tank. For the CCA slides, the dependent variables of survival and settlement were analyzed with a mixed-design split-plot ANOVA, with pH and algae as whole-tank between-subjects factors, and algal contact as the sub-tank, within-subjects factor. Due to the strong negative effect of *S. zonale* presence, there were multiple CCA slides with few to no recruits after settlement in this treatment, thus we were unable to incorporate these slides in our analyses of post-settlement survival and growth. Instead, we restricted these analyses to CCA slides that had *S. zonale* or the mimic removed prior to settlement, and therefore did not have algae attached during the 2 wk post-settlement stage. These slides were exposed to algae during the conditioning period, and were used to examine any latent effects of CCA contact with *S. zonale* on recruit survival and growth (2-way ANOVA with pH and algae as fixed factors). For the tiles, all *S. zonale* and mimics remained attached during post-settlement growth, and data were analyzed with a 2-way ANOVA with pH and algae as fixed factors. Data were arcsine-square root transformed and passed all tests for normality and homoscedasticity, as checked with Shapiro-Wilk and Levene's tests, respectively.

RESULTS

Comparisons of the 95% confidence intervals reveal that pH, pCO_2, dissolved inorganic carbon, and $\Omega_{aragonite}$ (aragonite saturation state) were significantly distinct between the ambient pH and low pH tanks during the course of the conditioning, settlement, and growth phases of the experiment (Table 2). Temperature, salinity, and TA did not differ between pH treatments.

CCA slides

Total survival (all recruits + swimmers) for the assays with the CCA slides was not affected by pH (p = 0.702), but was significantly reduced by the presence of *Stypopodium zonale* (p = 0.004, Fig. 1, Table 3).

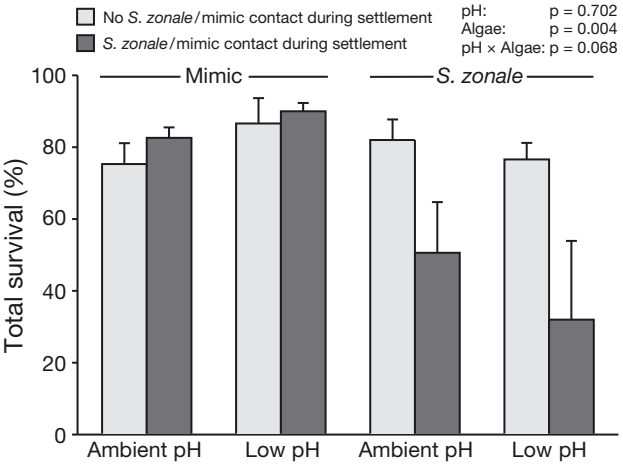

Fig. 1. Survival of *Porites astreoides* larvae in chambers with crustose coralline algae (CCA). Total survival (mean ± 1 SE) of larvae after 96 h in the experimental chambers with CCA mounted on slides. Paired bars indicate chambers that either had the alga *Stypopodium zonale* or a plastic mimic present or removed during the 96 h of settlement. Tank pH treatments are indicated along the *x*-axis. Statistical results are shown for the between-subjects factors of pH (8.10 vs. 7.85) and algae (plastic mimic vs. *S. zonale*)

Table 3. *Porites astreoides* settlement on crustose coralline alga (CCA) slides. ANOVA results for the effects of pH, algae (presence of *Stypopodium zonale* or plastic mimic), and algal contact (see 'Material and methods: Statistical analysis') on total survival, total settlement, and post-settlement growth. Significant results are in **bold**

Dependent variable	df	MS	*F*	p
Total survival - Between subjects				
pH	1	0.007	0.158	0.702
Algae	**1**	**0.687**	**15.558**	**0.004**
pH × Algae	1	0.196	4.429	0.068
Error	8	0.044		
Total survival - Within subjects				
Contact	1	0.279	3.459	0.100
Contact × pH	1	0.020	0.245	0.634
Contact × Algae	1	0.418	5.177	0.052
Contact × Algae × pH	1	0.000	0.006	0.942
Error	8	0.081		
Total settlement - Between subjects				
pH	1	0.016	0.47	0.512
Algae	1	0.034	0.99	0.349
pH × Algae	**1**	**0.22**	**6.464**	**0.035**
Error	8	0.034		
Total settlement - Within subjects				
Contact	**1**	**0.109**	**18.124**	**0.003**
Contact × pH	1	0.001	0.003	0.959
Contact × Algae	**1**	**0.304**	**50.345**	**<0.001**
Contact × Algae × pH	1	0.014	2.323	0.166
Error	8	0.006		
Post-settlement growth				
pH	1	0.185	2.263	0.171
Algae	1	0.001	0.017	0.899
pH × Algae	1	0.008	0.098	0.762
Error	8	0.082		

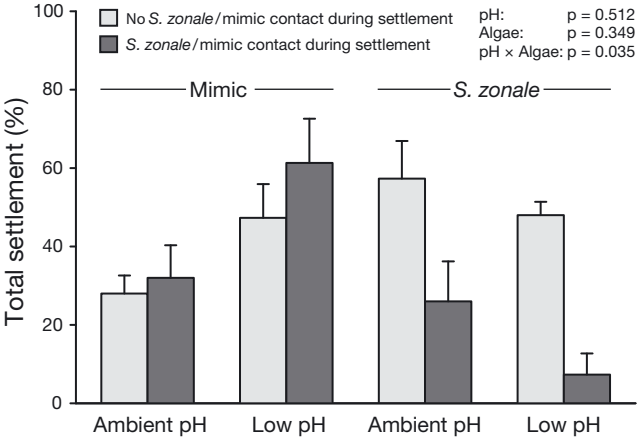

Fig. 2. Settlement of *Porites astreoides* larvae in chambers with crustose coralline algae (CCA). Total settlement (mean ± 1 SE) of larvae on CCA-mounted slides after 96 h in the experimental chambers. Paired bars indicate chambers that either had the conditioning *Stypopodium zonale* or the plastic mimic present or removed during the 96 h of settlement. Tank pH treatments are indicated along the *x*-axis. Statistical results are shown for the between-subjects factors of pH (8.10 vs. 7.85) and algae (plastic mimic vs. *S. zonale*)

Within tanks, total survival was not affected by *S. zonale* or mimic presence during the settlement assays (contact: p = 0.1); however, there was a weak interaction between the factors algae and contact (p = 0.052, Table 3), suggesting an effect of *S. zonale* removal on survival for the CCA slides conditioned with algae, yet no effect of mimic removal on survival for CCA slides conditioned with the mimic. Thus, only the presence of *S. zonale* during conditioning and settlement reduced larval survival.

For total settlement (all recruits), there was a significant interaction between pH and algae (p = 0.035, Fig. 2, Table 3). Post hoc analysis (adjusted for multiple comparisons, Holm-Sidak) revealed that at ambient pH, there was no effect of algae on total settlement (p = 0.306); however, at low pH, there was a significant reduction in total settlement with *S. zonale* presence (p = 0.037). When examining the within-subjects effect of contact during settlement, there was a significant interaction between the factors of algae and contact (p < 0.001, Table 3), and post hoc analysis (Holm-Sidak) revealed an effect of *S. zonale* removal on settlement for the CCA slides conditioned with algae (p < 0.001). There was no significant effect of mimic removal on total settlement for CCA slides conditioned with the mimic (p = 0.08), thus indicating that the effect of algae at low pH was again primarily driven by the presence of *S. zonale* during the settlement assays. Post-settlement survival on the CCA slides was not affected by pH (p = 0.232, Fig. 3, Table 3) or algae (present only during conditioning, p = 0.729). Post-settlement growth on the CCA slides was similarly unaffected by pH (p = 0.176) or algae (present only during conditioning, p = 0.933).

Biofilmed tiles

Total survival was not affected by pH (p = 0.546, Fig. 4, Table 4) or algae (p = 0.076). Total settlement was also not affected by pH (p = 0.354, Fig. 5, Table 4), but was significantly reduced by algae (p = 0.01). Settlement on the upper tile surface (tile top where *S. zonale* or mimic was attached) was not affected by pH (p = 0.710, Fig. 5, Table 4), but was significantly reduced by algal presence (p = 0.016). Post-settlement survival was not affected by pH (p = 0.915, Fig. 6) or algae (p = 0.570). Post-settlement growth was marginally affected by pH (reduced growth at low pH; p = 0.059, Fig. 6) and unaffected by algal presence (p = 0.835).

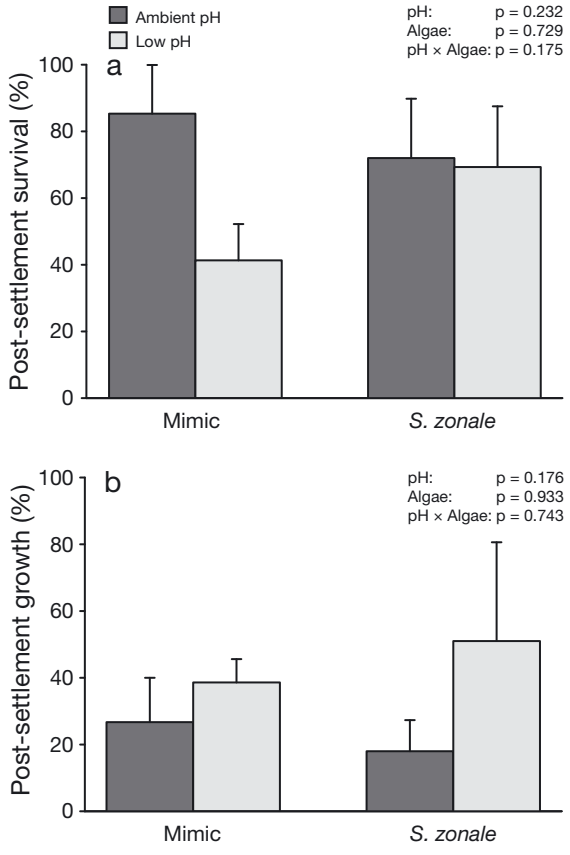

Fig. 3. *Porites astreoides* recruits on crustose coralline algae (CCA). Mean ± 1 SE post-settlement (a) survival and (b) growth of recruits on CCA slides after 2 wk under treatment conditions. Note that these data are from slides that were preconditioned with either *Stypopodium zonale* or the plastic mimic (factor: algae), but had them removed during the settlement and growth phase. Statistical results are shown for the factors of pH and algae

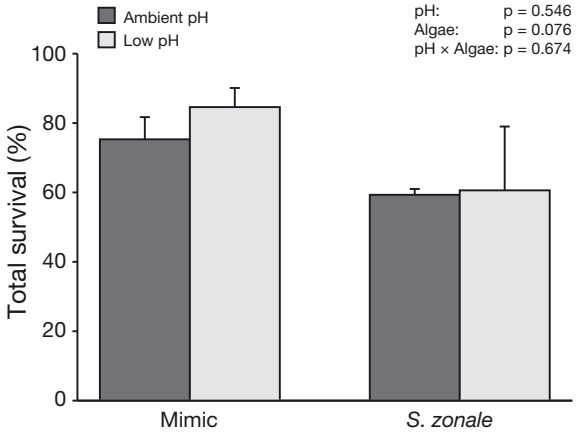

Fig. 4. Survival of *Porites astreoides* larvae in chambers with biofilmed tiles. Total survival (mean ± 1 SE) of larvae after 96 h in the experimental chambers with tiles. Statistical results are shown for the factors of pH and algae

Table 4. *Porites astreoides* settlement on biofilmed tiles. ANOVA results for the effects of pH and algae (presence of *Stypopodium zonale* or plastic mimic) on total survival, total settlement, settlement on tile top, and post-settlement growth. Significant results are in **bold**

Dependent variable	df	MS	*F*	p
Total survival				
pH	1	0.015	0.397	0.546
Algae	1	0.157	4.155	0.076
pH × Algae	1	0.007	0.19	0.674
Error	8	0.038		
Total settlement				
pH	1	0.013	0.966	0.354
Algae	**1**	**0.154**	**11.169**	**0.01**
pH × Algae	1	0.013	0.966	0.354
Error	8	0.014		
Settlement on tile top				
pH	1	0.004	0.149	0.710
Algae	**1**	**0.249**	**9.275**	**0.016**
pH × Algae	1	0.083	3.091	0.117
Error	8	0.027		
Post-settlement growth				
pH	1	1.157	4.857	0.059
Algae	1	0.011	0.046	0.835
pH × Algae	1	0.118	0.495	0.502
Error	8	0.238		

DISCUSSION

OA and algal proliferation represent some of the dominant threats to coral reefs around the world. While both of these factors have been extensively studied, most experiments have only applied these stressors in isolation, and failed to consider potential interactions that might alter the outcomes of single-factor experiments. By examining the combined effects of OA and algal presence on multiple metrics of coral settlement, we explored how abiotic–biotic interactions can influence reef recruitment, recovery, and resilience under future climate scenarios. Our work demonstrates that while algal presence plays a major role in reducing larval survival and settlement, in certain cases, OA may exacerbate these negative effects, further inhibiting rates of reef recruitment. Thus, from a management perspective, efforts that strive to reduce the proliferation of algae that are detrimental to corals (e.g. *Stypopodium zonale*) should take on increased importance as oceanic CO_2 concentrations continue to rise.

Across both settlement substrates (CCA slides and biofilmed tiles), the presence of *S. zonale* reduced rates of larval survival and settlement. Coral larvae can respond to a broad range of stimuli, both abiotic and biotic, that may either positively or negatively

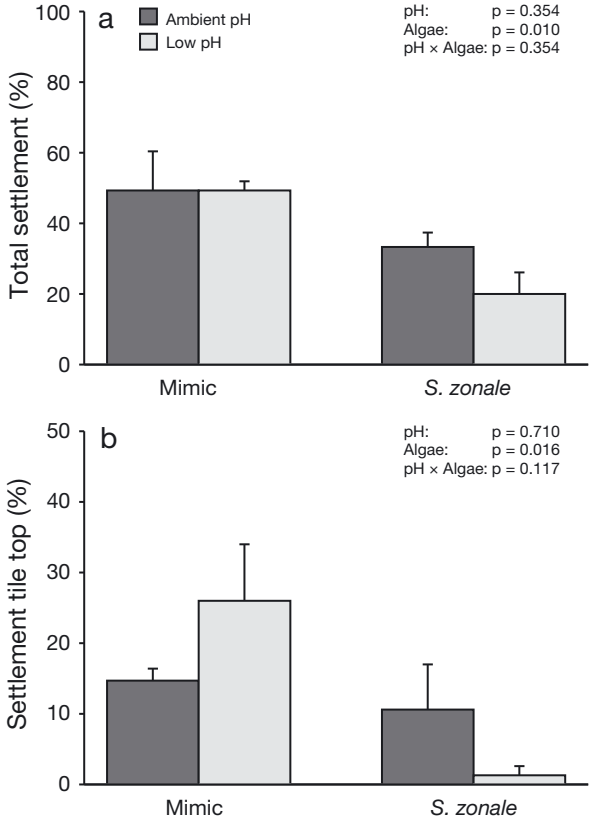

Fig. 5. *Porites astreoides* larvae in chambers with biofilmed tiles. Settlement (mean ± 1 SE) of larvae (a) on all surfaces of the biofilmed tiles and (b) on the upper surfaces of the biofilmed tiles after 96 h in the experimental chambers. Statistical results are shown for the factors of pH and algae

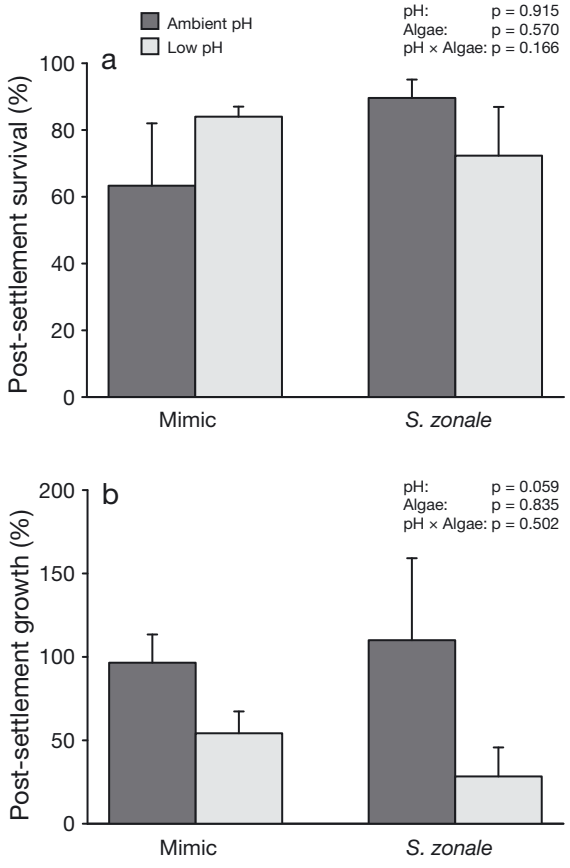

Fig. 6. *Porites astreoides* recruits on biofilmed tiles. Mean ± 1 SE post-settlement (a) survival and (b) growth of recruits on the biofilmed tiles after 2 wk under treatment conditions

influence rates of recruitment (Ritson-Williams et al. 2009). Our findings support prior conclusions highlighting the negative effects of certain algal groups on coral recruitment, such as cyanobacteria (Kuffner & Paul 2004, Kuffner et al. 2006), *Dictyota* spp. (Paul et al. 2011, Olsen et al. 2014, 2015), *Padina* sp. (Birrell et al. 2008), *Ulva fasciata* (Vermeij et al. 2009), and *Lobophora variegata* (Diaz-Pulido et al. 2010). Allelopathy is a suggested mechanism behind these effects, as many algal groups can produce a variety of both waterborne and lipophilic compounds that can be toxic to corals across a range of life history stages (Rasher & Hay 2010, Paul et al. 2011, Rasher et al. 2011, Ritson-Williams et al. 2016). As opposed to direct allelopathy, algal presence may also impose indirect effects on corals by shifting the composition of either the coral microbiome (Smith et al. 2006, Zaneveld et al. 2016) or the associated microbes on preferred settlement substrates (Vermeij et al. 2009). In the present study, by preconditioning the CCA with algae, and then conducting the settlement assays with both the algae remaining or removed, we tested for latent effects that algal contact might have imposed on CCA surface properties or microbial assemblages. We document that the effects of *S. zonale* were most prominent when the alga remained present and in physical contact with the settlement surface, suggesting that algal presence itself was driving reductions in larval survival and settlement, and not shifts in microbial surface properties of the CCA. Furthermore, there was no effect of mimic presence or absence on larval survival or settlement, indicating that the effects of *S. zonale* extended beyond mere space occupation and/or abrasion. As compared to the plastic mimic, larval survival (Fig. 1) and larval settlement (Figs. 2 & 5) were lower for the settlement assays with *S. zonale* present, demonstrating the existence of inhibitory mechanisms which could include hypoxic conditions or allelopathy related to algal presence on the settlement substrate. *S. zonale* is an abundant tropical alga, known to produce several biologically active, terpene-containing compounds (stypoldione, stypotriol, and epistypodiol; Gerwick & Fenical 1981, Wessels et al. 1999). Terpenoid second-

ary metabolites, commonly found in brown algae (Fucales and Dictyotales), can reduce feeding by tropical herbivores (Paul et al. 2001) and cause bleaching in adult corals (Rasher et al. 2011). It is likely that the compounds produced by *S. zonale* are responsible for the effects documented in our experiment. However, studies that specifically test for the activity of isolated compounds on coral larvae are needed to prove their inhibitory function.

OA imposed relatively minor effects on larval survival and settlement; however, we note that in certain cases, OA exacerbated the negative effects of algal presence. When the settlement assays were conducted with the alga or mimic present, *S. zonale* had no effect on total settlement under ambient pH (dark bars, Fig. 2), yet significantly reduced total settlement at low pH. Similar, yet non-significant, trends were further detected for settlement on the upper surfaces of the tile (Fig. 5). Similar results with adult corals have been documented, whereby low pH increased mortality rates of the coral *Acropora intermedia* when in contact with the alga *Lobophora papenfussii* (Diaz-Pulido et al. 2011). The mechanisms behind the altered effects of algal presence at low pH remain unclear, although it has been suggested that high CO_2 may increase the production of carbon-based allelochemicals that might serve to inhibit larval settlement (Diaz-Pulido et al. 2011, Del Monaco et al. 2017). However, other studies with *Porites astreoides* larvae have not found significant interactions between OA and other algal groups (*Dictyota* spp.) (Olsen et al. 2015), suggesting that these effects may depend upon the specific alga under consideration.

OA, in isolation, had no effect on larval survivorship or settlement, similar to the conclusions of prior studies (Albright et al. 2008, Chua et al. 2013). These findings suggest that this specific life-history stage may be relatively unaffected directly by CO_2. However, we recognize contrasting results from other studies utilizing different species. Doropoulos & Diaz-Pulido (2013) found that high CO_2 directly reduced the settlement of the coral *Acropora selago* on 3 species of CCA (*Porolithon onkodes*, *Sporolithon* sp., *Titanoderma* sp.), whereas Webster et al. (2013) showed that OA exposure (6 wk) can alter the associated microbial communities and biochemistry of CCA (*Hydrolithon onkodes*), thereby indirectly reducing coral (*A. millepora* and *A. tenuis*) settlement and metamorphosis. Similar OA-induced disruptions to larval–algal settlement interactions have been further documented with *A. millepora* and *Titanoderma* spp. (Doropoulos et al. 2012). Albright & Langdon (2011) documented that OA reduces the relative abundance of CCA on substrate surfaces, leading to significant declines in rates of coral settlement. These distinctions highlight that the effects of OA on coral settlement may be more nuanced than previously thought, depending upon the particular coral and settlement substrate under consideration. Post-settlement growth was also not strongly affected by OA in our experiment, but caution should be exercised when interpreting these results due to a short experimental duration and relatively low sample size. Albright et al. (2008) and Albright & Langdon (2011) documented OA-induced declines in juvenile *P. astreoides* growth over the course of several months. In our experiment, while we did find a trend of decreased growth on the biofilmed tiles exposed to low pH (Fig. 6), growth was only assessed for 2 wk following the settlement assays, thus OA-induced effects on juvenile coral growth may become apparent after longer time spans.

Our study demonstrates that while physical *S. zonale* presence serves as a dominant factor inhibiting larval survival and settlement, OA does have the potential to magnify the negative effects of algal contact when considering certain settlement metrics (e.g. total settlement in the present study). These effects required *S. zonale* presence during the settlement assays, and are thus likely driven by direct interactions and are not due to shifts in the surface properties of the settlement substrates. Coral recruitment serves as a critical process through which reefs can potentially recover from both regional and global threats. While it is clear that efforts to reduce the abundance of harmful algae will largely benefit coral populations and increase rates of recruitment, we present evidence suggesting that these efforts may become increasingly important as oceanic pH declines. As reefs face a multitude of concurrent threats (OA, elevated temperature, algal proliferation), research that examines the combined effects of these dominant stressors is strongly warranted, and may serve as the only channel to comprehensively understand the future functioning of reef ecosystems.

Acknowledgements. We thank Erich Bartels for assistance with the deployment of settlement tiles and the collection of algae and corals. We also thank Lindsay Spiers for assistance in the lab. This work was conducted under permit no. FKNMS-2015-011 issued by the Florida Keys National Marine Sanctuary and was supported by a 2015 award from the Smithsonian Competitive Grants Program for Science and a Mote Protect Our Reefs Grant 2014-6 awarded to J.E.C., J.M.S., and V.J.P. This is contribution no. 1069 of the Smithsonian Marine Station at Fort Pierce.

LITERATURE CITED

Albright R, Langdon C (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides.* Glob Change Biol 17:2478–2487

Albright R, Mason B (2013) Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. PLOS ONE 8:e56468

Albright R, Mason B, Langdon C (2008) Effect of aragonite saturation state on settlement and post-settlement growth of *Porites astreoides* larvae. Coral Reefs 27:485–490

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata.* Proc Natl Acad Sci USA 107:20400–20404

Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 105:17442–17446

Birrell CL, McCook LJ, Willis BL, Harrington L (2008) Chemical effects of macroalgae on larval settlement of the broadcast spawning coral *Acropora millepora.* Mar Ecol Prog Ser 362:129–137

Box SJ, Mumby PJ (2007) Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. Mar Ecol Prog Ser 342:139–149

Burkepile DE, Hay ME (2006) Herbivore vs. nutrient control of marine primary producers: context-dependent effects. Ecology 87:3128–3139

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Castillo KD, Ries JB, Bruno JF, Westfield IT (2014) The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming. Proc Biol Sci 281:20141856

Chan NCS, Connolly SR (2013) Sensitivity of coral calcification to ocean acidification: a meta-analysis. Glob Change Biol 19:282–290

Chua CM, Leggat W, Moya A, Baird AH (2013) Temperature affects the early life history stages of corals more than near future ocean acidification. Mar Ecol Prog Ser 475: 85–92

Comeau S, Edmunds PJ, Spindel NB, Carpenter RC (2013) The responses of eight coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point. Limnol Oceanogr 58:388–398

Comeau S, Edmunds PJ, Lantz CA, Carpenter RC (2014) Water flow modulates the response of coral reef communities to ocean acidification. Sci Rep 4:6681

Del Monaco C, Hay ME, Gartrell P, Mumby PJ, Diaz-Pulido G (2017) Effects of ocean acidification on the potency of macroalgal allelopathy to a common coral. Sci Rep 7: 41053

Diaz-Pulido G, Harii S, McCook LJ, Hoegh-Guldberg O (2010) The impact of benthic algae on the settlement of a reef-building coral. Coral Reefs 29:203–208

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN (2011) High CO_2 enhances the competitive strength of seaweeds over corals. Ecol Lett 14:156–162

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res 34:1733–1743

Dixson DL, Abrego D, Hay ME (2014) Chemically mediated behavior of recruiting corals and fishes: a tipping point that may limit reef recovery. Science 345:892–897

Doropoulos C, Diaz-Pulido G (2013) High CO_2 reduces the settlement of a spawning coral on three common species of crustose coralline algae. Mar Ecol Prog Ser 475: 93–99

Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecol Lett 15:338–346

Gardner TA, Côté IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958–960

Gattuso JP, Magnan A, Billé R, Cheung WWL and others (2015) Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. Science 349:aac4722

Gerwick WH, Fenical W (1981) Ichthyotoxic and cytotoxic metabolites of the tropical brown alga *Stypopodium zonale* (Lamouroux) Papenfuss. J Org Chem 46:22–27

Hay ME, Fenical W, Gustafson K (1987) Chemical defense against diverse coral reef herbivores. Ecology 68: 1581–1591

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS and others (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Hughes TP (1994) Catastrophes, phase-shifts, and large-scale degradation of a Caribbean coral reef. Science 265: 1547–1551

Jackson JBC, Buss L (1975) Allelopathy and spatial competition among coral reef invertebrates. Proc Natl Acad Sci USA 72:5160–5163

Jackson J, Donovan M, Cramer K, Lam W (2014) Status and trends of Caribbean coral reefs: 1970-2012. Global Coral Reef Monitoring Network, IUCN, Gland

Kuffner IB, Paul VJ (2004) Effects of the benthic cyanobacterium *Lyngbya majuscula* on larval recruitment of the reef corals *Acropora surculosa* and *Pocillopora damicornis.* Coral Reefs 23:455–458

Kuffner IB, Walters LJ, Becerro MA, Paul VJ, Ritson-Williams R, Beach KS (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. Mar Ecol Prog Ser 323:107–117

Langdon C, Takahashi T, Sweeney C, Chipman D and others (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. Global Biogeochem Cycles 14:639–654

Leclercq N, Gattuso JP, Jaubert J (2000) CO_2 partial pressure controls the calcification rate of a coral community. Glob Change Biol 6:329–334

Lessios HA (2016) The great *Diadema antillarum* die-off: 30 years later. Annu Rev Mar Sci 8:267–283

Lirman D, Biber P (2000) Seasonal dynamics of macroalgal communities of the northern Florida reef tract. Bot Mar 43:305–314

Manzello DP, Enochs IC, Melo N, Gledhill DK, Johns EM (2012) Ocean acidification refugia of the Florida Reef Tract. PLOS ONE 7:e41715

Marubini F, Barnett H, Langdon C, Atkinson MJ (2001) Dependence of calcification on light and carbonate ion concentration for the hermatypic coral *Porites compressa.* Mar Ecol Prog Ser 220:153–162

McClanahan TR, Muthiga NA (1998) An ecological shift in a remote coral atoll of Belize over 25 years. Environ Conserv 25:122–130

McCook LJ (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management conse-

quences for the Great Barrier Reef. Coral Reefs 18: 357–367

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907

Morrow KM, Liles MR, Paul VJ, Moss AG, Chadwick NE (2013) Bacterial shifts associated with coral–macroalgal competition in the Caribbean Sea. Mar Ecol Prog Ser 488:103–117

Mumby PJ, Steneck RS (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms. Trends Ecol Evol 23:555–563

Okazaki RR, Towle EK, van Hooidonk R, Mor C and others (2017) Species-specific responses to climate change and community composition determine future calcification rates of Florida Keys reefs. Glob Change Biol 23:1023–1035

Olsen K, Ritson-Williams R, Paul VJ, Ross C (2014) Combined effects of macroalgal presence and elevated temperature on the early life-history stages of a common Caribbean coral. Mar Ecol Prog Ser 509:181–191

Olsen K, Paul VJ, Ross C (2015) Direct effects of elevated temperature, reduced pH, and the presence of macroalgae (*Dictyota* spp.) on larvae of the Caribbean coral *Porites astreoides.* Bull Mar Sci 91:255–270

Pandolfi JM, Bradbury RH, Sala E, Hughes TP and others (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301:955–958

Paul VJ, Cruz-Rivera E, Thacker RW (2001) Chemical mediation of macroalgal herbivore interactions: ecological and evolutionary perspective. Marine Chemical Ecology. CRC Press, Boca Raton, FL

Paul VJ, Kuffner IB, Walters LJ, Ritson-Williams R, Beach KS, Becerro MA (2011) Chemically mediated interactions between macroalgae *Dictyota* spp. and multiple life-history stages of the coral *Porites astreoides.* Mar Ecol Prog Ser 426:161–170

Porter JW (1974) Community structure of coral reefs on opposite sides of the Isthmus of Panama. Science 186: 543–545

Rasher DB, Hay ME (2010) Chemically rich seaweeds poison corals when not controlled by herbivores. Proc Natl Acad Sci USA 107:9683–9688

Rasher DB, Stout EP, Engel S, Kubanek J, Hay ME (2011) Macroalgal terpenes function as allelopathic agents against reef corals. Proc Natl Acad Sci USA 108: 17726–17731

Ritson-Williams R, Arnold S, Fogarty N, Steneck R, Vermeij M, Paul V (2009) New perspectives on ecological mechanisms affecting coral recruitment on reefs. Smithson Contrib Mar Sci 38:437–457

Ritson-Williams R, Ross C, Paul VJ (2016) Elevated temperature and allelopathy impact coral recruitment. PLOS ONE 11:e0166581

Smith JE, Shaw M, Edwards RA, Obura D and others (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecol Lett 9:835–845

Sneed JM, Sharp KH, Ritchie KB, Paul VJ (2014) The chemical cue tetrabromopyrrole from a biofilm bacterium induces settlement of multiple Caribbean corals. Proc Biol Sci 281:20133086

Vermeij MJA, Smith JE, Smith CM, Vega Thurber R, Sandin SA (2009) Survival and settlement success of coral planulae: independent and synergistic effects of macroalgae and microbes. Oecologia 159:325–336

Webster NS, Uthicke S, Botte ES, Flores F, Negri AP (2013) Ocean acidification reduces induction of coral settlement by crustose coralline algae. Glob Change Biol 19: 303–315

Wessels M, Konig GM, Wright AD (1999) A new tyrosine kinase inhibitor from the marine brown alga *Stypopodium zonale.* J Nat Prod 62:927–930

Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE and others (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat Commun 7:1183

Editorial responsibility: Charles Birkeland, Honolulu, Hawaii, USA

Submitted: January 24, 2017; Accepted: July 4, 2017
Proofs received from author(s): August 14, 2017