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

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Running head: Effects of ocean acidification and algal contact on coral recruitment

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 algal contact (*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 algal 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 algal presence for settlement on the CCA substrate, such that low pH exacerbated the negative effects of *S. zonale*. Post-settlement growth for two weeks was unaffected by either algal presence or low pH on either substrate. While our results demonstrate that algal contact likely remains as 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 are being increasingly reported, broadly attributable to the negative effects of algal proliferation, disease, increased seawater temperatures, and ocean acidification (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 et al. 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, 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* has 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, Rasher et al. 2011, Paul 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 space 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 are known to 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, also known to 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 (ocean acidification) 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 ocean acidification 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 document increased mortality and tissue loss of adult corals under the combined stressors of algal contact and ocean acidification (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 ocean acidification (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 *Porites astreoides*. This particular species of brooding coral was selected because of its abundance across Florida 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.

Methods

Experimental design

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

Fragments of CCA (*Hydrolithon boergesensii*) were collected from the lower Florida Keys (Big Pine Ledges, 24° 33.213' N, 81° 22.665' W) on April 17, 2015, and attached to glass slides (75 mm x 25 mm) with underwater epoxy (All-Fix). Terracotta tiles (4.5 x 4.5 x 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 April 17, 2015. Both settlement substrates were collected from habitats similar to the collection site of the coral larvae (Lower Florida Keys, ~ 5-6m 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 two replicate CCA slides and one 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 (Fv/Fm) with a PAM fluorometer. No mortality was detected and all yield measurements were within a healthy range (0.6 - 0.8).

We used twelve, independent 37L tanks to create two seawater pH treatments, ambient pH (8.10_{NBS}, n=6) and reduced pH (7.85_{NBS}, n=6). The two 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 220W Aqua Medic T5 HO light fixtures that replicated broad spectrum irradiance (PAR= 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 LPH powerhead. Additional water flow in each tank was provided by vigorous airstone bubbling (with ambient air). Each tank was initially filled with filtered seawater (< 10µm) collected from an offshore oceanic location near Fort Pierce, FL. 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

144 the experiment, weekly water changes were conducted within each tank (50% volume). CO_2
145 concentrations were manipulated via a coupled pH stat system (Aqua Medic, Germany), which
146 monitored individual tank pH using separate electrodes. Low pH tanks were periodically bubbled
147 with 100% gaseous CO_2 as determined by a series of computer-controlled magnetic solenoids.
148 Measurements of pH (National Bureau of Standards scale) within each individual tank were
149 taken 3-4 times per week with an Orion Ross combination electrode to ensure proper calibration
150 and setpoints of the pH stat system. Salinity was recorded simultaneously with pH, and was
151 measured with a YSI Pro20. Weekly water samples were collected to measure total alkalinity
152 (TA) via open-cell potentiometric titration (Mettler Toledo DL15). Certified reference material
153 (Dickson standards, Scripps Institution of Oceanography) was used to ensure the accuracy of TA
154 measurements. All carbonate parameters within each tank were calculated with CO2SYS, using
155 measured parameters of pH, TA, temperature, and salinity, with the carbonate dissociation
156 constants of Mehrbach et al. (1973), as refit by Dickson & Millero (1987). Mean pCO_2 levels
157 were calculated as 517 µatm and 1024 µatm for the ambient pH and low pH treatments,
158 respectively. These values approximate current and future (year 2100) CO_2 forecasts, yet we note
159 that pCO_2 within the ambient tanks was slightly above reported values across the lower Florida
160 Keys (~372 µatm from Manzello et al. 2012).
161 *Larval collection*
162 In May 2015, 50 colonies of *P. astreoides* were collected at approximately 6m depth
163 from Wonderland Reef (24° 33.62' N, 81° 30.08' W) in the lower Florida Keys and transported in
164 coolers to Mote Marine Laboratory, Summerland Key, FL. Colonies were placed in outdoor
165 raceways with running seawater. Larvae were collected following the methods described in
166 Kuffner et al. 2006. Colonies were placed in separate 3L bowls, which were tilted so that

167 released larvae spilled over the handle into separate plastic tri-pour beakers fitted with a 180 μm
168 mesh bottom. Water levels within each beaker remained constant so that released larvae were
169 retained until the following morning when larvae were pooled and transported to the
170 Smithsonian Marine Station. Approximately 1800 larvae were used for this experiment.
171 For the settlement assays, each of the three preconditioned substrates in each OA tank
172 were placed into separate clear acrylic cylinders (10.2 cm diameter, 12.7 cm long) containing 50
173 *P. astreoides* larvae, and then returned to their respective tanks. All chambers were affixed with
174 180 μm mesh sidewalls on either end to ensure adequate water flow during settlement (Kuffner
175 et al. 2006). Given that each tank had 2 replicate CCA slides, the algae or plastic mimic from one
176 of these slides was removed prior to placement in the chamber to examine the direct versus
177 indirect effects of algal presence on coral settlement. This treatment was imposed for the CCA
178 because of prior work highlighting the importance of CCA associated microbes on larval
179 settlement (Webster et al. 2013, Sneed et al. 2014). Thus, each OA tank contained 3 settlement
180 chambers: (1) CCA slide with attached algae or mimic (2) CCA slide with removed algae or
181 mimic and (3) biofilmed tile with attached algae or mimic (Table 1). Larvae were allowed to
182 settle for 96 h, after which all slides and tiles were scored for number of metamorphosed settlers.
183 Percent survival was calculated as (recruits + swimmers / 50) x 100 in each chamber. Total
184 settlement was calculated as (recruits / 50) x 100 in each chamber. After scoring, all algae and
185 mimics were reattached to their respective CCA slides and tiles, except for the slides that had the
186 algal treatment removed prior to settlement. All CCA slides and tiles were placed back into their
187 respective OA tanks for an additional 2 weeks and re-scored for post-settlement survival and
188 growth. Percent survival was calculated as (the number of colonies surviving / the number of

originally settled coral spat) x 100. Growth was calculated as (the total number of new coral polyps 2 weeks after settlement/ the number of surviving coral spat) x100.

Statistical analysis

Tank chemistry was analyzed by comparing the 95% confidence intervals of measured and calculated seawater parameters. For the settlement assays, the treatments of pH (8.10 vs 7.85) and algae (*S. zonale* vs plastic mimic) were applied in a factorial design at the tank level (n=3, four treatments randomly distributed across 12 tanks). Within each tank, an additional factor was imposed during the settlement assays for the two CCA slides, whereby one slide had the conditioning algae or mimic present, and the other slide had the conditioning algae 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 restrict these analyses to CCA slides that had the algae or mimic removed prior to settlement, and therefore did not have algae attached during the 2 week 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 algae/mimics remained attached during post

212 settlement growth, and data were analyzed with a 2-way ANOVA with pH and algae as fixed
213 factors. Data were arcsine-square root transformed and passed all tests for normality and
214 homoscedasticity, as checked with a Shapiro-Wilk and Levene's test, respectively.

215 **Results**

216 Comparisons of the 95% confidence intervals reveal that pH, pCO_2, DIC and $\Omega_{aragonite}$
217 were significantly distinct between the ambient pH and low pH tanks during the course of the
218 conditioning, settlement and growth phases of the experiment (Table 2). Temperature, salinity,
219 and total alkalinity did not differ between pH treatments.

220 *CCA slides*

221 Total survival (all recruits + swimmers) for the assays with the CCA slides was not
222 affected by pH (p=0.702), but was significantly reduced by the presence of *S. zonale* (p=0.004,
223 Fig 1, Table 3). Within tanks, total survival was not affected by algal / mimic presence during the
224 settlement assays (contact: p=0.1); however, there was a weak interaction between the factors of
225 algae and contact (p=0.052, Table 3), suggesting an effect of *S. zonale* removal on survival for
226 the CCA slides conditioned with algae, yet no effect of mimic removal on survival for CCA
227 slides conditioned with the mimic. Thus, only the presence of *S. zonale* during conditioning and
228 settlement reduced larval survival.

229 For total settlement (all recruits), there was a significant interaction between pH and
230 algae (p=0.035, Fig. 2, Table 3). Post hoc analysis (adjusted for multiple comparisons, Holm-
231 Sidak) revealed that at ambient pH, there was no effect of algae on total settlement (p=0.306),
232 however at low pH, there was a significant reduction in total settlement with *S. zonale* presence
233 (p=0.037). When examining the within-subjects effect of contact during settlement, there was a
234 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 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 algae / 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 ($p=0.835$).

Discussion

Ocean acidification 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 fail 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 explore how abiotic-biotic interactions can influence reef recruitment, recovery and resilience under

258 future climate scenarios. Our work demonstrates that while algal presence plays a major role in
259 reducing larval survival and settlement, in certain cases, ocean acidification may exacerbate
260 these negative effects, further inhibiting rates of reef recruitment. Thus, from a management
261 perspective, efforts that strive to reduce the proliferation of algae that are detrimental to corals
262 (e.g. *S. zonale*) should take on increased importance as oceanic CO_2 concentrations continue to
263 rise.

264 Across both settlement substrates (CCA slides and biofilmed tiles), the presence of
265 *Stypopodium zonale* reduced rates of larval survival and settlement. Coral larvae can respond to a
266 broad range of stimuli, both abiotic and biotic, that may either positively or negatively influence
267 rates of recruitment (Ritson-Williams et al. 2009). Our findings support prior conclusions
268 highlighting the negative effects of certain algal groups on coral recruitment, such as
269 cyanobacteria (Kuffner et al. 2006, Kuffner & Paul 2004), *Dictyota* spp. (Paul et al. 2011, Olsen
270 et al. 2014, 2015), *Padina* sp. (Birrell et al. 2008), *Ulva fasciata* (Vermeij et al. 2009) and
271 *Lobophora variegata* (Diaz-Pulido et al. 2010). Allelopathy is one suggested mechanism behind
272 these effects, as many algal groups can produce a variety of both waterborne and lipophilic
273 compounds that can be toxic to corals across a range of life history stages (Paul et al. 2011,
274 Rasher & Hay 2010, Rasher et al. 2011, Ritson-Williams et al. 2016). As opposed to direct
275 allelopathy, algal presence may also impose indirect effects on corals by shifting the composition
276 of either the coral microbiome (Smith et al. 2006, Zaneveld et al. 2016), or the associated
277 microbes on preferred settlement substrates (Vermeij et al. 2009). In the current study, by
278 preconditioning the CCA with algae, and then conducting the settlement assays with both the
279 algae remaining or removed, we tested for latent effects that algal contact might have imposed on
280 CCA surface properties or microbial assemblages. We document that the effects of *S. zonale*

were most prominent when the algae 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. *Stypopodium 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 secondary 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.

Ocean acidification 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

304 the alga *Lobophora papenfussii* (Diaz-Pulido et al. 2011). The mechanisms behind the altered
305 effects of algal presence at low pH remain unclear, however, it has been suggested that high CO_2
306 may increase the production of carbon-based allelochemicals that might serve to inhibit larval
307 settlement (Diaz-Pulido et al. 2011, Del Monaco et al. 2017). However, other studies with *P.*
308 *astreoides* larvae have not found significant interactions between OA and other algal groups
309 (*Dictyota* spp.) (Olsen et al. 2015), suggesting that these effects may depend upon the specific
310 alga under consideration.

311 Ocean acidification, in isolation, had no effect on larval survivorship or settlement,
312 similar to the conclusions of prior studies (Albright et al. 2008, Chua et al. 2013). These findings
313 suggest that this specific life-history stage may be relatively unaffected directly by CO_2.
314 However, we recognize contrasting results from other studies utilizing different species.
315 Doropoulos & Diaz-Pulido (2013) found that high CO_2 directly reduced the settlement of the
316 coral *Acropora selago* on three species of CCA (*Porolithon onkodes*, *Sporolithon sp.*,
317 *Titanoderma sp.*), whereas Webster et al. (2013) showed that OA exposure (6 weeks) can alter
318 the associated microbial communities and biochemistry of CCA (*Hydrolithon onkodes*), thereby
319 indirectly reducing coral (*Acropora millepora* and *A. tenuis*) settlement and metamorphosis.
320 Similar OA-induced disruptions to larval-algal settlement interactions have been further
321 documented with *A. millepora* and *Titanoderma* spp. (Doropoulos et al. 2012). Albright &
322 Langdon (2011) documented that OA reduces the relative abundance of CCA on substrate
323 surfaces, leading to significant declines in rates of coral settlement. These distinctions highlight
324 that the effects of OA on coral settlement may be more nuanced than previously thought,
325 depending upon the particular coral and settlement substrate under consideration. Post-settlement
326 growth was also not strongly affected by OA in our experiment, however, 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), document OA-induced declines in juvenile *P. astreoides* growth over the course of several months. In our experiment, while we do note a trend of decreased growth on the biofilmed tiles exposed to low pH (Fig. 6), growth was only assessed for 2 weeks 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, ocean acidification 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 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.S., and V.J.P. This is contribution no. xx of the Smithsonian Marine Station at Fort Pierce.

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Table 1. Details of experimental design. Settlement substrates (CCA / biofilmed tiles), algae, and coral larvae were collected April - May 2015 from the lower Florida Keys. Left column designates the pH and algal treatments for each tank. Center column designates the type and number of settlement substrates 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, one settlement assay was conducted with the algae/mimic present (a - examining direct contact effects) and a second assay was conducted with the algae/mimic removed following the conditioning period (b - examining indirect effects from conditioning).

Treatments (Tank pH , Attached algae)	Substrate conditioning (12 d)		Larval Settlement Assays (96 h)	
Ambient pH , plastic mimic (n=3)	Each tank received:	a) CCA slide w/ plastic mimic b) CCA slide w/ plastic mimic c) Biofilmed tile w/ plastic mimic	Settlement assay with:	a) CCA slide w/ plastic mimic present b) CCA slide w/ plastic mimic removed c) Biofilmed tile w/ plastic mimic present
Ambient pH , *S. zonale* (n=3)	Each tank received:	a) CCA slide w/ *S. zonale* b) CCA slide w/ *S. zonale* c) Biofilmed tile w/ *S. zonale*	Settlement assay with:	a) CCA slide w/ *S. zonale* present b) CCA slide w/ *S. zonale* removed c) Biofilmed tile w/ *S. zonale* present
Reduced pH , plastic mimic (n=3)	Each tank received:	a) CCA slide w/ plastic mimic b) CCA slide w/ plastic mimic c) Biofilmed tile w/ plastic mimic	Settlement assay with:	a) CCA slide w/ plastic mimic present b) CCA slide w/ plastic mimic removed c) Biofilmed tile w/ plastic mimic present
Reduced pH , *S. zonale* (n=3)	Each tank received:	a) CCA slide w/ *S. zonale* b) CCA slide w/ *S. zonale* c) Biofilmed tile w/ *S. zonale*	Settlement assay with:	a) CCA slide w/ *S. zonale* present b) CCA slide w/ *S. zonale* removed c) Biofilmed tile w/ *S. zonale* present

Table 2. Seawater carbonate chemistry (means and bracketed 95% CI) across the ambient and low pH tanks. Temperature, pH_{NBS}, and salinity represent discrete measurements (n=20) from within each tank during the experiment. Total alkalinity (TA) was measured weekly within each tank (n=5).

Treatment	Temperature (^{o}C)	Salinity	pH_{NBS}	pCO_2 (µatm)	DIC (µmol kg^{-1} SW)	Ω_{arag}	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)

Table 3. *Porites astreoides* settlement on CCA slides. ANOVA results for the effects of pH, algae, and algal contact 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 x 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 x pH	1	0.020	0.245	0.634
Contact x Algae	1	0.418	5.177	0.052
Contact x Algae x 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 x 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 x pH	1	0.001	0.003	0.959
Contact x Algae	**1**	**0.304**	**50.345**	**<0.001**
Contact x Algae x 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 x Algae	1	0.008	0.098	0.762
Error	8	0.082		

Table 4. *Porites astreoides* settlement on biofilmed tiles. ANOVA results for the effects of pH and algae on total survival, total settlement, settlement on tile top, and post-settlement growth. Significant results are highlighted 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 x 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 x Algae	1	0.013	0.966	0.354
Error	8	0.014		
Settlement Tile Top				
pH	1	0.004	0.149	0.710
Algae	**1**	**0.249**	**9.275**	**0.016**
pH x 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 x Algae	1	0.118	0.495	0.502
Error	8	0.238		

Figure legend

Figure 1. Survival of *Porites astreoides* in chambers with CCA. Total survival (mean ± 1 SE) of larvae after 96 hours in the experimental chambers with CCA-mounted on slides. Paired bars indicate chambers that either had the algae / mimic present (gray) or removed (open) during the 96 hours 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*).

Figure 2. Settlement of *Porites astreoides* in chambers with CCA. Total settlement (mean ± 1 SE) of larvae on CCA-mounted slides after 96 hours in the experimental chambers. Paired bars indicate chambers that either had the conditioning algae/mimic present (gray) or removed (open) during the 96 hours 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*).

Figure 3. *Porites astreoides* recruits on CCA. Post-settlement survival (top panel, mean ± 1 SE) and post-settlement growth (bottom panel, means ± SE) of recruits on CCA slides after 2 weeks under treatment conditions. Note that these data are from slides that were preconditioned with either *S. zonale* or the mimic, yet had them removed during the settlement and growth phase. Statistical results are shown for the factors of pH and algae.

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

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

Figure 6. *Porites astreoides* recruits on biofilmed tiles. Post-settlement survival (top panel, mean ± 1 SE) and post-settlement growth (bottom panel, mean ± 1 SE) of recruits on the biofilmed tiles after 2 weeks under treatment conditions.

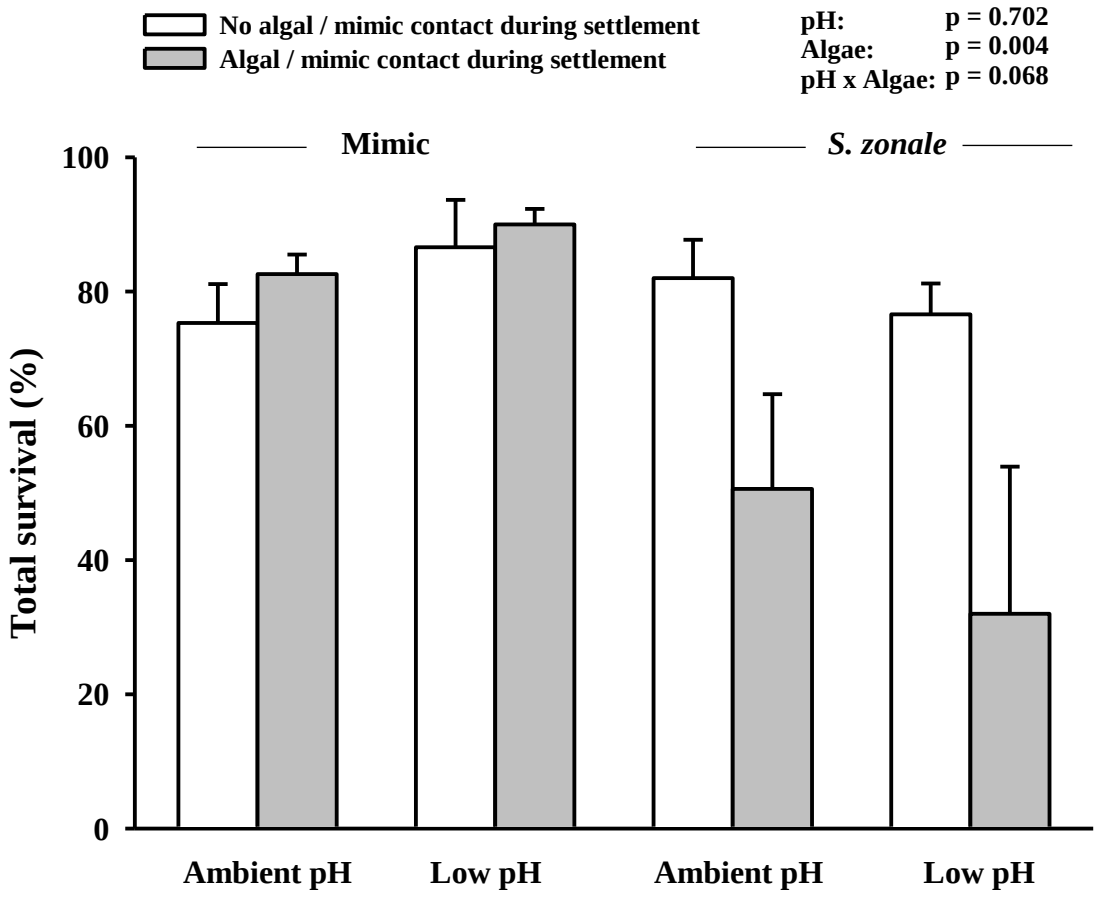

No algal / mimic contact during settlement
Algal / mimic contact during settlement
pH: p = 0.702
Algae: p = 0.004
pH x Algae: p = 0.068
Mimic
S. zonale
Total survival (%)
100
80
60
40
20
0
Ambient pH
Low pH
Ambient pH
Low pH

Figure 1

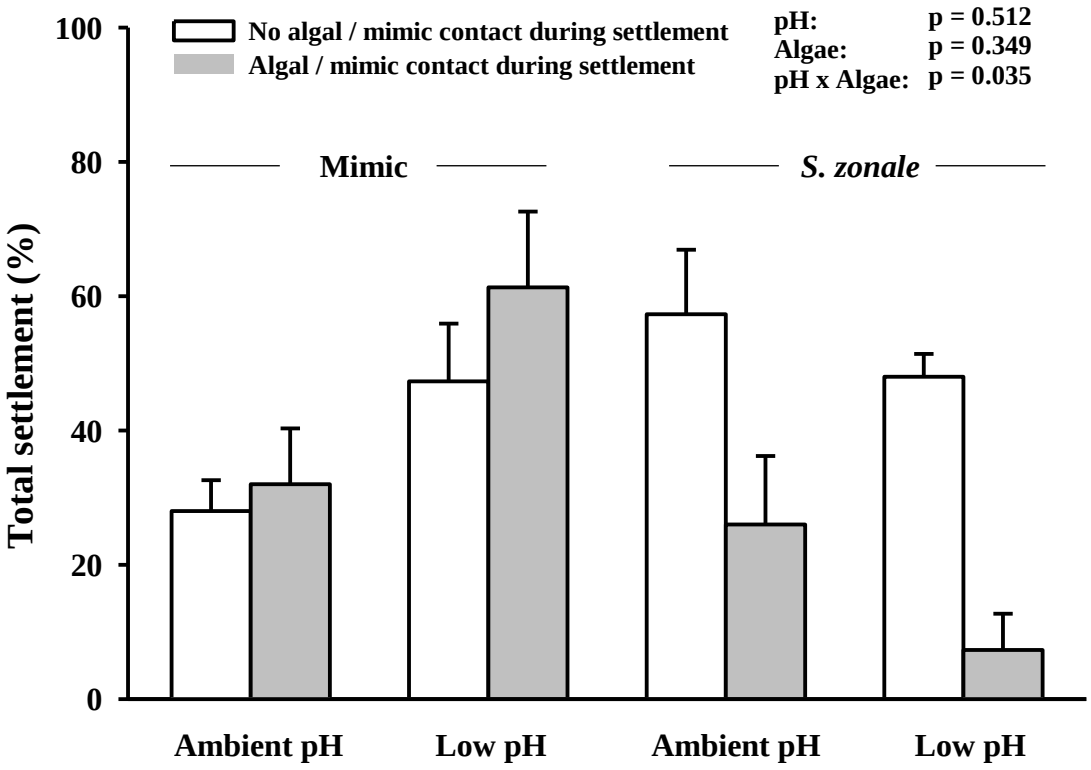
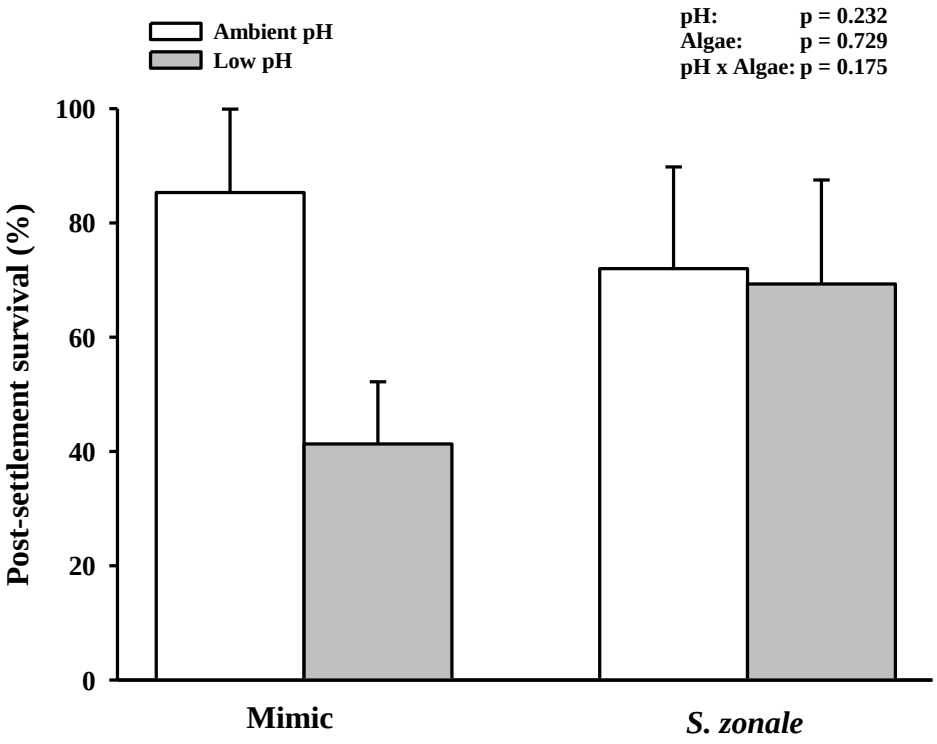

No algal / mimic contact during settlement
Algal / mimic contact during settlement
pH: p = 0.512
Algae: p = 0.349
pH x Algae: p = 0.035
Mimic
S. zonale
Total settlement (%)
100
80
60
40
20
0
Ambient pH
Low pH
Ambient pH
Low pH

Figure 2


Ambient pH
Low pH
pH: p = 0.232
Algae: p = 0.729
pH x Algae: p = 0.175
Post-settlement survival (%)
100
80
60
40
20
0
Mimic
S. zonale

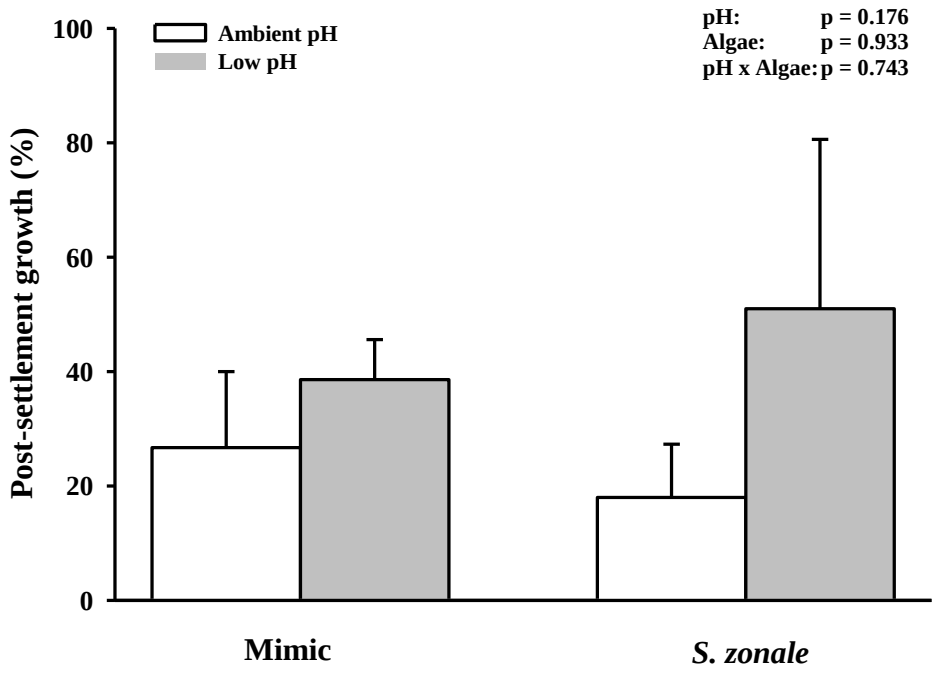

Ambient pH
Low pH
pH: p = 0.176
Algae: p = 0.933
pH x Algae: p = 0.743
Post-settlement growth (%)
100
80
60
40
20
0
Mimic
S. zonale

Figure 3

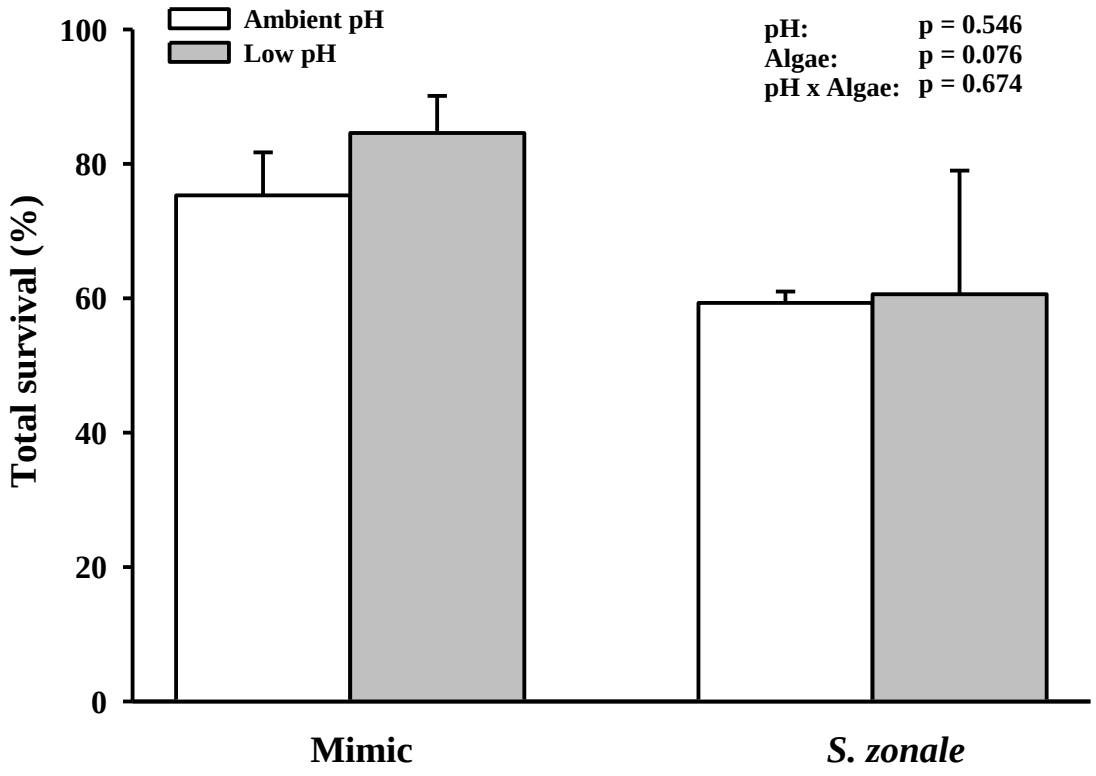
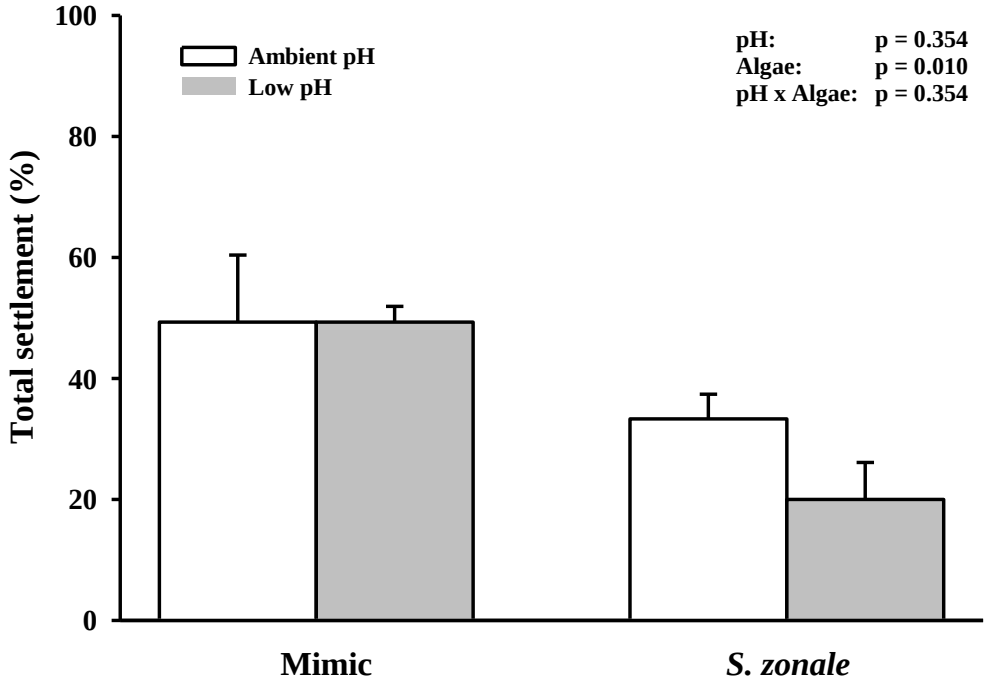

Ambient pH
Low pH
pH: p = 0.546
Algae: p = 0.076
pH x Algae: p = 0.674
Total survival (%)
100
80
60
40
20
0
Mimic
S. zonale

Figure 4


Ambient pH
Low pH
pH: p = 0.354
Algae: p = 0.010
pH x Algae: p = 0.354
Total settlement (%)
100
80
60
40
20
0
Mimic
S. zonale

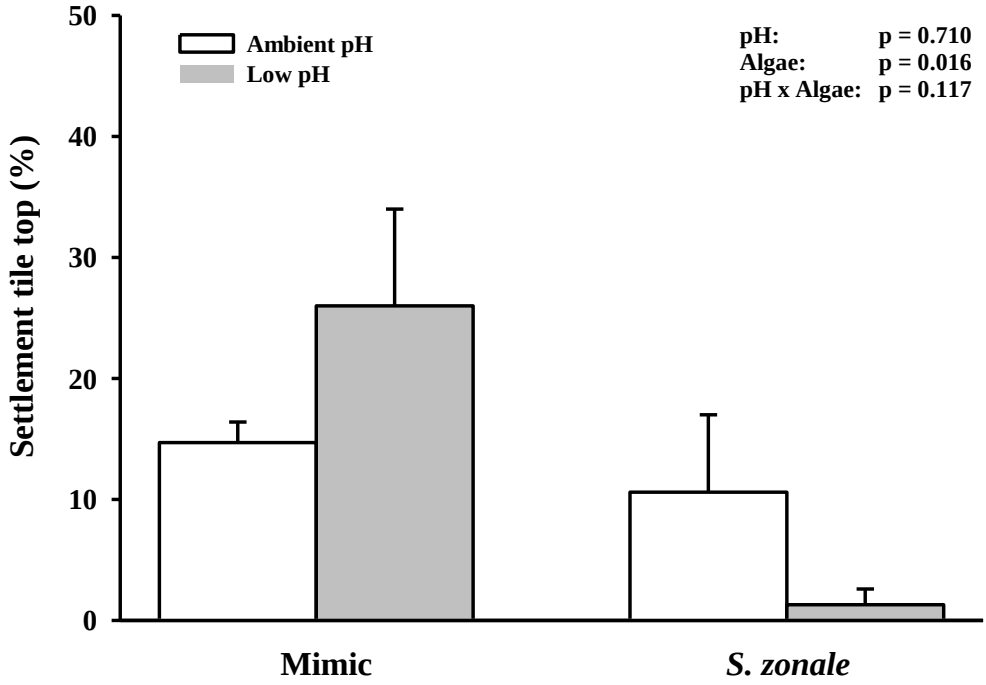
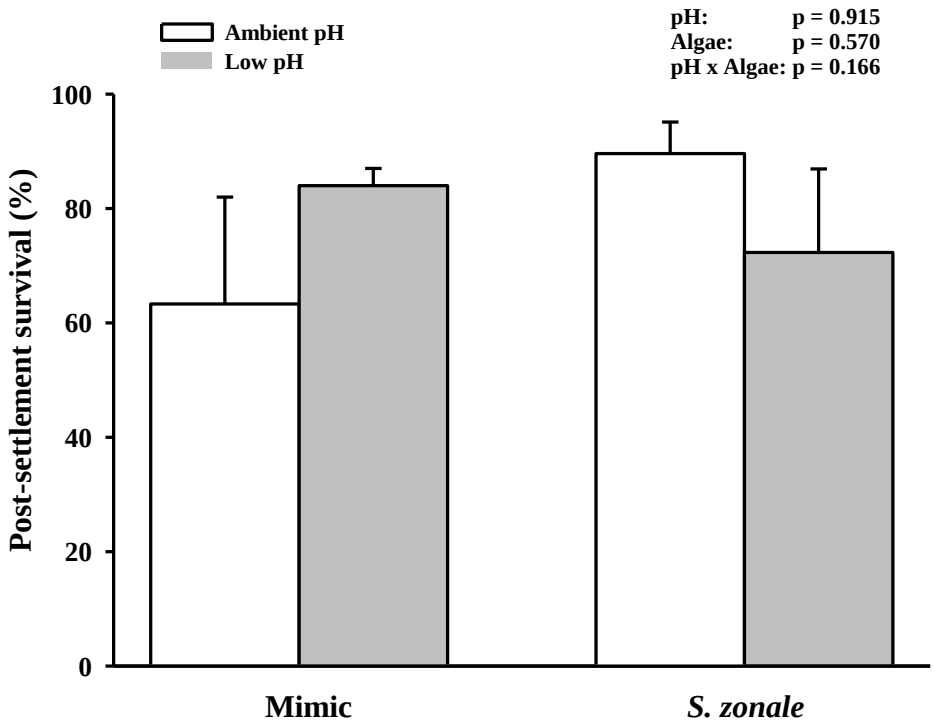

Ambient pH
Low pH
pH: p = 0.710
Algae: p = 0.016
pH x Algae: p = 0.117
Settlement tile top (%)
50
40
30
20
10
0
Mimic
S. zonale

Figure 5


Ambient pH
Low pH
pH: p = 0.915
Algae: p = 0.570
pH x Algae: p = 0.166
Post-settlement survival (%)
100
80
60
40
20
0
Mimic
S. zonale

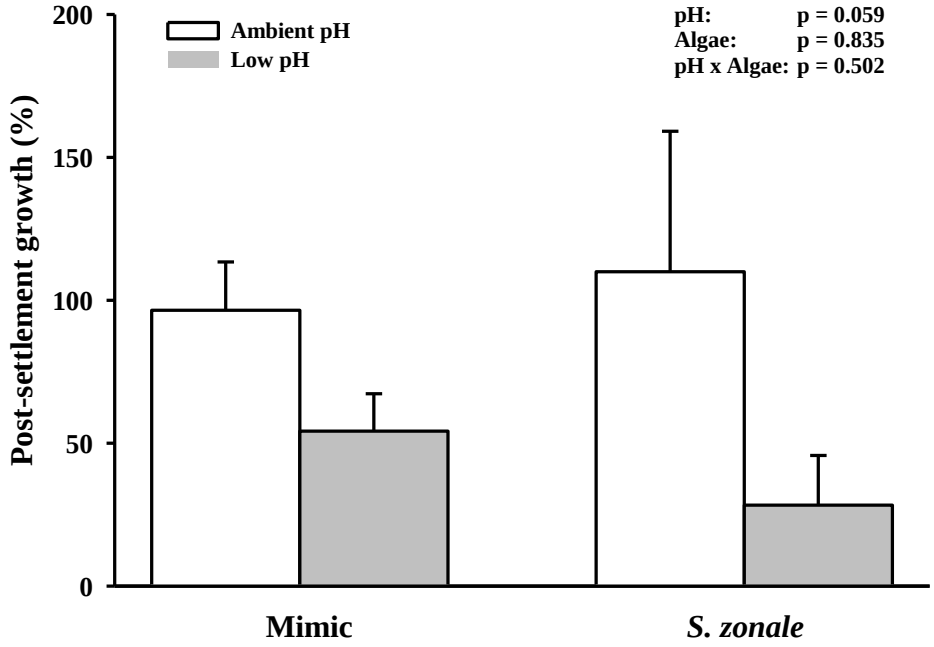

Ambient pH
Low pH
pH: p = 0.059
Algae: p = 0.835
pH x Algae: p = 0.502
Post-settlement growth (%)
200
150
100
50
0
Mimic
S. zonale

Figure 6