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Indirect Effects of Ocean Warming and Acidification on the Realized Recruitment of Agaricia agaricites

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Thesis of
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M.S. Marine Biology

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“Indirect Effects of Ocean Warming and Acidification on the Realized Recruitment of *Agaricia agaricites*”

By:
Allan Anderson

Submitted to the Faculty of Halmos College of Natural Sciences and Oceanography in partial fulfillment of the requirements for the degree of Master of Science with a specialty in Marine Biology

Nova Southeastern University

December 2018
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Abstract

Over the past few decades, coral cover has declined worldwide due to overfishing, disease, and storms, and these effects have been exacerbated by ocean warming and acidification. Corals are extremely susceptible to these changes because they are already living close to their thermal and aragonite saturation thresholds. Ocean warming and acidification (OAW) may also impact coral survival and growth by impacting their settlement cues. Coral larvae use crustose coralline algae (CCA) and their associated biofilms as cues for settlement, i.e., habitat selection. Settlement cues can also be negatively affected by increased water temperature and acidity. It was hypothesized that the impacts of OAW on settlement substrate can further threaten coral persistence by altering/inhibiting larval settlement and potentially decreasing the post-settlement survival and growth of coral recruits. In this study, we 1) assessed the effect of substrate quality (substrate conditioned in ambient or OAW conditions) on settlement of *A. agaricites* larvae, 2) determined the effect of substrate quality on post-settlement survival and growth of *A. agaricites* recruits, and 3) determined the effect of ocean warming and acidification on the post-settlement survival and growth of *A. agaricites* recruits.

Aragonite settlement tiles were placed offshore for one month to accrue CCA and associated biofilms, and were then conditioned in either ambient (29°C, 8.2 pH) or predicted future oceanic conditions (31°C, 7.9 pH) conditions for 7 – 10 days. *Agaricia agaricites* larvae were then introduced to the settlement tiles, and their settlement percentage was calculated. Once a week for 12 weeks after larval settlement, the size, survival, and pigmentation of *A. agaricites* recruits was recorded. Larvae settled marginally more on optimally conditioned tiles than on tiles previously exposed to OAW conditions (p=0.053). The survival of coral recruits in OAW conditions was greatly reduced, their growth was very limited, and they became paler over time. When reared in ambient conditions, recruits on OAW treated substrate initially displayed higher survival rates than recruits on ambient treated substrate. After 3 weeks in ambient conditions, however, survival rates were similar for recruits on ambient and OAW treated substrate; their growth curves were very similar, and coral recruits became more pigmented over time. Ocean warming and acidification conditions not only directly impacted the growth, survival, and pigmentation of *A. agaricites* recruits, but it also indirectly affected larval settlement by likely altering microbial composition in bacterial biofilms on the settlement tiles. These results indicate that future conditions of ocean warming and acidification can be deleterious for *A. agaricites*, particularly after settlement. If the early life stages of scleractinian corals are negatively affected by OAW conditions, successful recruitment throughout the Caribbean and Florida Reef Tract could decrease. As a result, recovery from disturbances could be hindered, thus compromising the sustainability of many coral species and other marine ecosystems that depend on coral reefs for protection, habitat, and food.

**Keywords:** Coral, larvae, settlement, metamorphosis, survival, mortality, growth, pigmentation, coral recruitment, crustose coralline algae, CCA, microbiome
Introduction

Coral reefs account for approximately 0.2% of the ocean floor, yet they provide habitat for an estimated 25% of all marine organisms, making them one of the most diverse habitats on the planet (Spalding et al., 2001; Roberts et al., 2002). The foundation of coral reef ecosystems are scleractinian corals, commonly called “stony corals” (Stanley, 1981). These corals accrete CaCO$_3$ to build their skeletons and experience relatively slow growth rates when compared to non-calcifying marine organisms (Stanley, 2003). Reefs formed by stony corals provide flood and erosion protection for approximately 71,000 km of coastline, and reduce the expected damages from storms by an estimated $4 billion USD globally every year (Beck et al., 2018) by breaking up high-energy waves and reducing wave height from severe weather events (Bryant et al., 1999; Barbier et al., 2011; Beck et al., 2018). Many coastal economies depend on recreation and tourism opportunities presented by coral reefs and their adjacent ecosystems, with global coral reef tourism calculated to be worth approximately $35.8 billion USD (Spalding et al., 2017). On a global scale, fishing on coral reefs contributes to a $152 billion USD fisheries industry, with 90.9 million tons of fish caught in 2016 (The State of World Fisheries and Aquaculture, 2018). For these reasons, the persistence of scleractinian corals is not only crucial to the overall health of coral reef ecosystems, but also to economies all over the world.

Because adult corals are sessile, gene flow between populations, colonization of new habitats, and replenishment of disturbed reefs is accomplished by planktonic larvae (produced through sexual reproduction) that recruit to distant or adjacent reefs. The exchange of larvae between reefs increases gene flow between populations and creates a more diverse genetic pool, which can reduce inbreeding, aid in reef recovery from disturbances, and increase resilience to pressures that threaten corals (Slarkin, 1985; Clobert et al., 2001). Currently, the greatest threats to scleractinian corals and coral reefs on a global scale are ocean warming and acidification (Glynn, 1984). During recruitment, the main settlement and metamorphosis cues for the larvae of scleractinian corals are produced by multiple species of CCA and their associated bacterial biofilms commonly found on suitable reef substrate (Heyward and Negri, 1999; Negri and Heyward, 2000; Hadfield and Paul, 2001; Negri et al., 2001; Ritson-Williams et al., 2016). When coral
larvae settle onto a substrate to begin their sessile lives, they are named “settlers”; successful settlement and metamorphosis into a coral polyp is termed “recruitment” (Levin, 2006). “Realized recruitment” extends the concept of recruitment to include the survival and growth of the newly settled corals into their juvenile stage (Levitan et al., 2004; Underwood et al., 2009). Scleractinian recruitment is crucial to the overall health of coral reef ecosystems by diversifying genetic pools and replenishing damaged reefs after disturbances (Slarkin, 1985; Clobert et al., 2001). The ability to quickly replenish populations after disturbances is essential for the persistence of corals, and this process may be hindered or altered by predicted future climate change scenarios of ocean warming and acidification.

The Intergovernmental Panel on Climate Change (IPCC) predicts ocean temperatures will rise by at least 2°C and pH will decrease by 0.3 units by the year 2100 (IPCC, 2014; IPCC, 2018). Ocean warming is caused by an increase in anthropogenic greenhouse gas emissions, such as carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) (Meinshausen et al., 2011). These gases trap solar heat inside of Earth’s atmosphere, causing air temperatures, and subsequently ocean temperatures, to rise (IPCC, 2014). Approximately 30% of atmospheric CO$_2$ is absorbed by the oceans (Sabine et al., 2004; Canadell et al., 2007), making them one of the largest global CO$_2$ sinks (Maier-Reimer and Hasselmann, 1987). Over the last few decades, atmospheric CO$_2$ levels have increased dramatically from around 300 ppm in 2000 (Berendse et al., 2001) to 408.40 ppm in October 2018 (Ed Dlugokencky and Pieter Tans, NOAA/ESRL [www.esrl.noaa.gov/gmd/ccgg/trends]). This abrupt increase in atmospheric CO$_2$ has led to a greater uptake of CO$_2$ by our oceans where a series of chemical reactions take place to create carbonic acid. Carbonic acid forms bicarbonate ions and reduces the abundance of carbonate ions (Brewer, 1997; Hoegh-Guldberg et al., 2007; Doney et al., 2009; Moya et al., 2012) that are available for corals use to build their skeletons.

Under ocean warming and acidification conditions, adult corals experience greater mortality (Glynn, 1984), slower growth (Anthony et al., 2008), release less gametes (Grottoli et al., 2006), less larvae are exchanged between reefs (Chua et al., 2013; Figueiredo et al., 2014), and coral juveniles have greater difficulty producing a skeleton (Moya et al., 2012). Corals have a symbiotic relationship with photosynthetic
dinoflagellate algae (Muscatine and Porter, 1977), which provide the coral host with energy that can be used to catalyze calcification (Trench, 1979; Muscatine, 1990). When corals experience stress, such as warm conditions and/or increased irradiance, they “bleach”, i.e., they expulse their algal symbionts (Glynn, 1984). When these symbionts are released, the coral polyps become pale and appear to be bleached, hence the term (Glynn, 1993). Once bleaching occurs, calcification and tissue growth of the coral decrease or cease completely. Recent bleaching events have decreased coral cover by an estimated 70-90% worldwide (Wilkinson and Hodgson, 1999; Eakin et al., 2009; Hughes et al., 2018; Manzello et al., 2018) with estimates of 50-70% coral cover declines throughout the Great Barrier Reef (Hughes et al., 2017). Consequently, these bleaching events have had negative impacts on the structure of reef fish and crustacean communities (Graham et al., 2007; Baker et al., 2008; Komyakova et al., 2013).

Increased water temperatures are also known to reduce coral fecundity (Grottoli et al., 2014) and hasten coral larval development (Heyward et al., 2010; Figueiredo et al., 2014). The predicted decrease in coral cover, fecundity, and increased larval mortality and hastening of larval development is projected to reduce coral dispersal distances, connectivity, and recruitment. Acidic water conditions have also been shown to negatively affect the deposition of coral skeletons (Moya et al., 2012) and increase rates of bleaching for scleractinian corals (Anthony et al., 2008). The reduced abundance of carbonate ions hinders the deposition of a CaCO₃ skeleton for adults (Hoegh-Guldberg et al., 2007) and juveniles (Moya et al., 2012). Therefore, the exposure to ocean warming and acidification conditions after settlement and metamorphosis is likely to undermine the survival and growth of coral recruits (newly settled coral juveniles). Aside from direct impacts of warming and acidification on the coral recruits, corals may also experience indirect impacts on larval settlement, as the quality and health of the substrate may be affected, including settlement and metamorphosis cues.

Potential impacts on coral settlement cues and habitat may further contribute to the decrease in recruitment. Corals settle in response to the presence of crustose coralline algae (CCA) and its associated bacterial biofilms that are present on the reef (Negri and Heyward, 2000; Price, 2010; Sneed et al., 2014; Ritson-Williams et al., 2016). Both the CCA and their associated bacterial biofilms have been shown to be negatively impacted
by ocean warming and acidification (Doropoulos et al., 2012; Webster et al., 2013; Fabricius et al., 2015; Scherner et al., 2016). Crustose coralline algae tissue mortality rates have been found to increase by 15% under warming conditions, and bacterial community composition can shift completely (Witt et al., 2011; Diaz-Pulido et al., 2012; Tebben et al., 2015). If the available substrate for larval settlement is suboptimal, coral larvae may delay settlement in the pursuit of a more desirable substrate or cease settlement altogether (Graham et al., 2013). A negative impact of ocean warming and acidification on the settlement substrate of corals is thus likely to decrease coral settlement and lead to a poorer substrate selection (Marshall and Keough, 2003).

The lack of optimal settlement cues can lead corals to settle on suboptimal substrate, potentially jeopardizing post-settlement survival and growth, and ultimately realized recruitment. Previous studies in bryozoans and ascidians with short larval stages found that a poor choice of settlement habitat can significantly decrease recruitment success (Wendt, 1998; Maldonado and Young, 1999; Burgess et al., 2009), and consequently reduce connectivity between populations. Impacts on species with longer larval durations, such as scleractinian corals, which can remain planktonic for over 100 days (Montastrea magnistellata, Goniastrea aspera; Graham et al., 2008), have never been assessed. Crustose coralline algae and associated biofilms have been shown to be negatively affected by ocean warming and acidification conditions (Doropoulos et al., 2012; Webster et al., 2013; Fabricius et al., 2015; Scherner et al., 2016), which potentially opens up surface area for other species of turf algae to recruit and grow. When in contact with macroalgae, corals experience slower growth (Tanner, 1995; Lirman, 2001) and, when reared in ocean acidification conditions, higher mortality (Diaz-Pulido et al., 2011). Therefore, settling on suboptimal substrate could subject newly settled recruits to increased algal competition that could potentially decrease their survival and growth. To fully understand the impacts of ocean warming and acidification on the realized recruitment of scleractinian corals, the impacts of climate affected settlement cues and habitat on larval settlement and post-settlement survival and growth need to be evaluated.
Objectives

The objectives of this study were to determine how *Agaricia agaricites* larval settlement and post-settlement survival and growth will be affected if their settlement cues (CCA and bacterial biofilms) and settlement habitat are impacted by ocean warming and acidification. Changes in post-settlement survival and growth could potentially decrease realized recruitment, and consequently connectivity, of *A. agaricites* populations. The objectives of this study were to:

1) Assess the effect of substrate quality, specifically impacts of ocean acidification and warming (OAW), on settlement cues and on the settlement of *A. agaricites* larvae;
2) Determine the effect of substrate quality (i.e., previously exposed to OAW) on post-settlement survival and growth rate of *A. agaricites* recruits;
3) Determine the effect of ocean warming and acidification on the post-settlement survival and growth of *A. agaricites* recruits.

Methods

Study Species

*Agaricia agaricites*, also classified under *Undaria agaricites* (Figure 1), is a common, hermaphroditic, brooding scleractinian coral inhabiting the Florida Reef Tract with a peak abundance at 5 – 35 m depth (Van Moorsel, 1983; Helmuth and Sebens, 1993; Chiappone and Sullivan, 1996). Adult colonies can reach up to 60 cm in diameter and are commonly platey or encrusting, with colony morphology being heavily dependent on water flow rates (Helmuth and Sebens, 1993). Planulation begins during the late spring months and extends into late summer/early fall, and occurs several days before and after the new moon between 2300 and 0700 h (Van Moorsel, 1983). *Agaricia agaricites* larvae are fully competent within a couple hours after release (Van Moorsel, 1983).
Coral Collection

On May 24, 2018 (5 days prior to full moon of May 29) twenty sexually mature (15 – 20 cm in diameter) colonies of A. agaricites were collected off the coast of Broward County, Florida via SCUBA using hammer and chisel. The adult colonies were bubble-wrapped, placed in coolers with seawater, and carefully transported to Nova Southeastern University’s Guy Harvey Oceanographic Center. Adult colonies were kept together in 1500 L recirculating outdoor aquaria under current summer conditions (25.5˚C, pH 8.2). These conditions were maintained using heaters, a chiller, biological filtration, and a protein skimmer. Temperature and salinity were monitored daily and water quality (ammonia, nitrites, nitrates, phosphate, alkalinity and pH) was monitored weekly.

Larval Release

Each evening at 1800 h, the colonies were moved into individual plastic mixing bowls with handles and placed in the larval collection apparatus (Figure 2a). Water entered the apparatus at the tank’s flow inputs and exited via a single outflow equipped with a ball valve. Six, eight-port drip manifolds with flow control (Orbit DripMaster 8-Port Manifold) were attached at the top of each PVC arm to supply constant water flow to each bowl containing a coral colony. Flow rate was controlled with the ball valve attached at the end of the outflow pipe and the individual flow control on the drip manifolds to ensure adequate turnover rate to keep temperature and water quality optimal. When the water level reached the top of the bowl, water flowed over the handle.
and carried the larvae into a 100µm sieve placed at the bottom of the handle (Figure 2b). Each morning, larvae would be collected from the sieve, and the number of larvae released by each colony was recorded.

Figure 2a (Left): Larval collection apparatus showing the tubing leading into each bowl (Photo Credit: Rachael Stevenson). Figure 2b (Right): Close up of the water running over the handle of the bowl into the collection sieve below (Photo Credit: Rachael Stevenson).
Larval Settlement Experiment

To assess the effect of habitat quality on the settlement of *A. agaricites* larvae, we measured settlement success in two treatments: settlement on ambient treated substrate and settlement on OAW treated. To produce ambient and OAW treated substrate, 500 aragonite tiles were deployed (3.2 cm diameter, 0.8 cm thick) near the coral collection sites for one month prior to experimentation in order to be conditioned, i.e., colonized by bacterial biofilms and CCA naturally found in *A. agaricites* habitats. The conditioned tiles were brought back to Nova Southeastern University’s Guy Harvey Oceanographic Center before larval release. Ambient treated settlement tiles were kept in recirculating tanks under ambient conditions (29°C, 8.2 pH) for 7 – 10 days, and OAW treated settlement tiles were kept in end-of-century conditions predicted by the Intergovernmental Panel on Climate Change (31°C, 7.9 pH; IPCC, 2014) for 7 – 10 days. These conditions were maintained using heaters, pH controllers (American Marine Pinpoint pH Controller connected to a CO₂ tank and solenoid), and LED lights to mimic natural irradiance levels on the reef. Settlement success in each of the treatments was assessed using 25 replicates of 10 larvae that were introduced to either ambient or OAW treated substrate. Each replicate contained 10 larvae in a 200 mL glass jar filled with filtered seawater (kept at ambient conditions, 29°C, 8.2 pH, using a water bath). After 48 h, each tile was removed from the glass jar and scored for settlement under an Olympus™ dissecting microscope. Settlement on both the glass jar and the settlement tile were recorded.

Post-Settlement Survival and Growth Experiment

To test the effect of substrate quality of the post-settlement survival, growth rate, and pigmentation (indicative of symbiont acquisition) of *A. agaricites* recruits, I reared recruits in 40 L tanks split between three treatments (Figure 3): Treatment 1 reared recruits on ambient treated substrate in ambient conditions (29°C, 8.2 pH). Treatment 2 reared recruits on OAW treated substrate in ambient conditions (29°C, 8.2 pH). Treatment 3 reared recruits on OAW treated substrate in OAW conditions (31°C, 7.9 pH). Tiles were haphazardly distributed to eliminate bias and to ensure there were approximately equal numbers of recruits in each of the three treatments. There were
approximately 150 individuals per treatment split by four replicate tanks for treatments 1 and 2, and two replicate tanks for treatment 3. All tanks were equipped with two submersible pumps (SunSun JP-032), a heater (Aqueon Pro Heater), and LED lights (Hydra Twenty Six HD set at 45 µmol quanta/m².s) to mimic natural irradiance levels in crevices within the reef. Tanks of treatment 3 also had a pH controller (American Marine Pinpoint pH Controller) connected to a CO₂ tank and solenoid that bubbled CO₂ to maintain a pH of 7.9 ± 0.1. Monitored recruits were all facing upward to ensure equal light and food availability. Temperature, salinity, and pH were monitored daily. To control water quality and guarantee adequate alkalinity, 50% water changes were performed every other day and 100% water changes weekly. Corals were fed rotifers twice per week to stimulate tentacle formation and polyp growth. Coral surface area and survival were measured once per week for 12 weeks for each treatment. Beginning at week 3, pigmentation was also assessed once per week by comparing recruit pigmentation with the CoralWatch Coral Health Chart (Figure 4).

**Figure 3:** This figure is a visual representation of the three experimental treatments used in the Post-Settlement Survival and Growth Experiment.
Data Analysis

To assess the effect of substrate quality on *A. agaricites* larval settlement, a one-tailed t-test was used. To determine if there were differences in post-settlement survival between the three treatments, I used a Mantel-Haenszel log-rank test. To test for differences in growth between the three treatments, I first used a generalized linear model to determine if mortality was size dependent. Since mortality was not size dependent, several non-linear models (exponential, power) were fit to the data, regardless of treatment, to determine which fit the data the best. Once this was found, the fitness of the model (where parameters were independent of treatment), was compared to the models fit to each of the treatments (i.e., where parameters were specific for each treatment). To determine if the treatment-dependent models led to a significantly better goodness of fit, these models were compared to the original model (treatment-independent parameters) using a log-likelihood ratio test. To determine if the pigmentation of the corals changed over time within each treatment, a Frequency Analysis with a Contingency Table was used. All data analyses for this experiment were performed in R (v3.5.1).

Results

Larval Release

Overall, 1,224 *A. agaricites* larvae were collected from May 25 to June 1. Adult colonies released larvae after June 1, but they were not collected because enough larvae had been collected to complete the experiment. The majority of larvae were released after

![Figure 4: The CoralWatch Coral Health Chart used to score pigmentation.](image)
the full moon on May 29 (Figure 5), though some larvae were released prior to the full moon. Larvae were released steadily from 2400 h to 0600 h with no peak time of release. After larvae were collected, they were then placed into settlement treatments or were mass settled.

**Figure 5**: Graphic representation of larval release by adult *A. agaricites* colonies

**Settlement**

There were no significant differences in total settlement (p=0.095, t_{44.922}=1.332; Figure 6a) or settlement on the glass (p=0.17, t_{45.621}=-0.98262; Figure 6b) between treatments. However, there was a marginal statistical difference in settlement on the tile (p=0.05291, t_{47.744}=1.6485; Figure 6c), with settlement on ambient treated tiles (62.78 ± 4.59%) being marginally greater than settlement on OAW treated tiles (52.02 ± 4.64%).
Post-Settlement Survival

There were significant differences in survival between treatments (p<0.001; Figure 7). Corals that settled on OAW treated tiles and were then reared in ambient conditions (Treatment 2) had the best survival, followed by corals that settled on ambient treated tiles that were reared in ambient conditions (Treatment 1). However, this difference seems to be mostly due to differential mortality rates in the first 2 weeks; after that, the mortality rates of the two treatments appear similar (Figure 7). Corals that settled on OAW treated tiles and that were then reared in OAW conditions (Treatment 3) had the worst survival (Figure 7). At the end of the 12 week experiment, only two individuals remained alive in Treatment 3.

Figure 7: Survival rates for A. agaricites recruits throughout the duration of the study.
Post-Settlement Growth

Growth models using treatment-dependent parameters led to a significantly better goodness of fit than the treatment-independent model (p<0.001). There were no significant differences in size at settlement between the treatments (p= 0.3555); individuals in treatment 1 were 2.307 ± 0.074 mm², individuals in treatment 2 were 2.292 ± 0.096 mm², and individuals in treatment 3 were 2.162 ± 0.092 mm². By week 12, individuals in treatment 1 were 9.424 ± 0.506 mm², individuals in treatment 2 were 8.910 ± 0.428 mm², and individuals in treatment 3 were 3.842 ± 0.434 mm². Growth curves of coral recruits reared in ambient conditions (Treatments 1 and 2) appear to be similar regardless of the previous exposure of the tile to OAW or not (Figure 8). Corals reared in OAW conditions displayed lower growth curves (Figure 8).

![Graph](image)

Figure 8: Growth of *A. agaricites* recruits throughout the duration of the study. Circles represent observations, dotted lines represent the line of best fit, and colors represent the treatment.

**Pigmentation**

Polyp pigmentation significantly changed over time for recruits on ambient treated tiles that were reared in ambient conditions (treatment 1; p<0.001; Figure 9) and recruits on OAW treated tiles that were reared in optimal conditions (treatment 2; p<0.001; Figure 9) with individuals becoming darker over time. Polyp color also significantly changed for
recruits on OAW treated tiles that were reared in suboptimal conditions (treatment 3), but with individuals becoming significantly paler over time ($p<0.001$; Figure 9).

Figure 9: Color of *A. agaricites* recruits in treatment 1 (a), treatment 2 (b), and treatment 3 (c) throughout the duration of the study. The pie chart on the left for each treatment shows the distribution of recruit pigmentation at week 3, while the pie chart on the right for each treatment shows the distribution of recruit pigmentation at week 12 of the study.
Discussion

The results of this study show that OAW treated substrate has the ability to alter the settlement location of *A. agaricites* larvae likely due to the alteration of chemical cues that larvae use for the determination of optimal settlement habitat. Post-settlement survival of *A. agaricites* recruits was also influenced by substrate condition, likely caused by algal competition being higher on ambient treated substrate than on OAW treated substrate. Predicted ocean warming and acidification conditions were deleterious to the post-settlement survival and growth of *A. agaricites* recruits, as every recruit bleached and nearly all died by the end of the 12 week study when reared in OAW conditions (31°C, 7.9 pH). This study suggests that *A. agaricites* will not be able to persist under predicted end-of-century conditions of ocean warming and acidification (31°C, pH 7.9), as all phases of recruitment were either directly or indirectly affected by these conditions.

Settlement substrate conditioned in predicted future conditions (increase of temperature by 2°C, decrease of pH by 0.3) did not alter the capacity of *A. agaricites* larvae to settle. However, when exposed to OAW treated substrate, larvae settled marginally less than larvae exposed to ambient treated substrate. Though this difference was not statistically significant (p<0.05), it was, however, marginally significant (p=0.053). Coral larvae use chemical cues produced by bacterial biofilms present on common reef substrate for settlement ([Chia and Rice, 1978; Morse and Morse, 1991; Johnson and Sutton, 1994; Morse et al., 1996; Negri and Heyward 2000; Negri et al., 2001]; Harrington et al., 2004; Tebben et al., 2011; Whalan et al., 2012; Sneed et al., 2014). However, these biofilms are extremely sensitive to environmental changes, and bacterial community compositions can shift relatively quickly ([Negri and Heyward, 2000; Witt et al., 2011]). It is likely that introducing settlement substrate to OAW conditions for 7-10 days altered the bacterial community structure within biofilms that produce settlement/metamorphosis cues for coral larvae ([Webster et al., 2011; Witt et al., 2011; Tebben et al., 2015]). I suspect ambient treated substrate did not yield decreased morphogenic compound availability, causing a marginal difference in the settlement location between the two substrate conditions. This behavior may have been triggered out of desperation. If the available substrate condition is suboptimal and morphogenic compounds are absent, larvae have the ability to choose from three options ([Bishop et al., 2011]).
2006): larvae may delay settlement (“Variable Retention Hypothesis”; Marshall and Keough, 2003; Bishop et al., 2006), not settle at all (“Death Before Dishonor Hypothesis”; Pechenik, 1999: Bishop et al., 2006), or settle indiscriminately on any substrate available (“Desperate Larva Hypothesis”; Morse et al., 1988; Pawlik, 1994; Raimondi and Morse, 2000; Bishop et al., 2006). *Agaricia agaricites* larvae are brooded (Chiappone and Sullivan, 1996), therefore it is unlikely that they would delay settlement as these larvae are competent within hours of release (Van Moorsel, 1983). This reproductive mode tailors more towards retainment of larvae close to the parent colony, and yields in local recruitment due to the relatively short larval duration (Sammarco and Andrews, 1989; Carlon and Olson, 1993; Figueiredo et al., 2013). In this study, there were no differences in total settlement between ambient or OAW treated tiles. This means that total settlement success was not altered by substrate condition, therefore the second option does not apply for this particular study. The indiscriminate settlement option, however, is applicable, as larval settlement location was marginally altered by substrate condition. I suspect that settlement was marginally more direct on ambient treated substrate than on OAW treated substrate, caused by the lack of settlement cues on OAW treated substrate. Again, it is important to note that this difference in settlement location was not statistically significant (p<0.05), but it was, however, marginally significant (p=0.053). Substrate in OAW conditions was only treated for 7-10 days, therefore it is possible that this difference in settlement could become much more pronounced under a longer treatment period that would be much likely to occur in nature. A negative impact of ocean warming and acidification on the settlement substrate and habitat of corals is thus likely to decrease larval settlement and lead to poor selection of settlement habitat (Pechenik et al., 1998).

When reared in optimal conditions, survival rates of *A. agaricites* recruits that had settled on OAW treated tiles (which had been exposed to OAW conditions) were surprisingly higher in the first two weeks than on ambient treated tiles, potentially due to reduced competition with OAW-sensitive macroalgae. It is well documented that corals naturally experience high mortality rates during the first few weeks of their sessile life due to being more susceptible to a myriad of factors, including competition with algae (Babcock and Smith, 2000; Kuffner et al., 2006; Penin et al., 2011). In many situations
corals directly compete with macroalgae, and algal growth has been found to be deleterious to the survival of coral recruits (Kuffner et al., 2006). Though the majority of macroalgae were removed from the tiles prior to larval settlement, macroalgae within small crevices and cracks on the surface of the tile could not be removed and remained on the settlement tiles. Though macroalgal growth was not quantified, I suspect that the majority of the remaining macroalgae on OAW treated tiles were killed and the growth of the remaining macroalgae was hindered during the conditioning period in OAW conditions (31°C, 7.9 pH). I also suspect that macroalgal growth and survival were not hindered in ambient conditions (29°C, 8.2 pH). When OAW treated tiles were then exposed to optimal conditions, the biofilms present on both ambient and OAW treated tiles likely became much more similar after the first two weeks, causing algal growth/competition, and subsequently coral mortality rates, to become comparable in both treatments. Therefore, I suspect coral recruits on OAW treated tiles were exposed to less algal competition than recruits on ambient treated tiles, which caused them to have better survival during the first few weeks of the experiment.

When reared under predicted end-of-century OAW conditions (increase in temperature by 2°C, decrease of pH by 0.3), Agaricia agaricites recruits exhibited very high mortality (nearly all recruits were dead after 12 weeks) and diminished growth likely due to the loss of symbionts and energy spent to maintain internal pH (Venn et al., 2013; Georgiou et al., 2015). Agaricia agaricites larvae are pigmented because they are released from the maternal colony already with symbionts (Van Moorsel, 1983; Richmond and Hunter, 1990) and retain this coloration after settlement, pending on environmental conditions being optimal. In this study, coral recruits reared in OAW conditions lost most of their pigmentation within 3 weeks. Because recruits reared in ambient conditions (29°C, 8.2 pH) did not show any signs of bleaching and even became darker over time, the observed bleaching of recruits reared in OAW conditions indicates that the increase in water temperature by 2°C caused a breakdown of the symbiosis between coral and their algal symbionts (Hoegh-Guldberg and Smith, 1989; Glynn and D’Croz, 1990; Iglesias-Prieto et al., 1992; Glynn, 1993; Brown, 1997; Baird et al., 2009; Oakley and Davy, 2018). This loss can be very damaging for corals because the algal symbionts are known to provide up to 100% of the necessary energy requirements to their
host (von Holt and von Holt, 1968; Muscatine and Cernichiari, 1969; Muscatine and Porter, 1977; Muscatine et al., 1981; Battey and Patton, 1984; Muscatine et al., 1984). Without this energy income, corals would have to rely solely on energy obtained through heterotrophic feeding, which at this earlier stage of development is likely insufficient due to the underdevelopment of tentacles. Furthermore, the reduced pH entails that corals need to spend more energy to maintain their internal pH (homeostasis) and calcify (Venn et al., 2013; Georgiou et al., 2015). The 0.3 unit drop in pH causes a depletion of available carbonate ions in the surrounding water (Brewer, 1997; Orr et al., 2005; Hoegh-Guldberg et al., 2007; Doney et al., 2009; Moya et al., 2012;), making it harder for recruits to calcify (Hoegh-Guldberg et al., 2007; Anthony et al., 2008; Brooke and Young, 2009). The reduction in available energy and carbonate ions possibly explains the diminished growth and high mortality rates. Being that successful recruitment is essential for the replenishment of populations, these results suggest that the persistence of A. agaricites through OAW is severely jeopardized.

Corals like Agaricia agaricites and Porites spp. are commonly termed as “weedy” species (Knowlton, 2001; Darling et al., 2012), due to their high fecundity, recruitment success, and relatively higher resistance to warmer conditions and even disease (Van Moorsel, 1983; Richmond and Hunter, 1990; Walton et al., 2018). Adult colonies of A. agaricites have been previously recorded to bleach during heat stress events (Miller et al., 2006; Wagner et al., 2010), but the populations tend to be quickly replenished after disturbances due to the above mentioned high fecundity and recruitment. However, this study suggests that this natural process of recovery, i.e., recruitment, particularly post-settlement survival and growth, is severely affected by OAW. Thus, even species like A. agaricites, which are often perceived as weedy may see their persistence severely compromised under predicted end-of-century conditions of ocean warming and acidification (31°C, pH 7.9). If more sensitive coral reef-building species, such as Montastrea spp., Acropora spp., and Pocillopora spp. (Mendes and Woodley, 2002; Foster et al., 2007; Darling et al., 2012) are similarly affected, then entire coral reef ecosystems could be negatively impacted by the effects of ocean warming and acidification on realized recruitment rates. Reduced realized recruitment rates of corals
throughout the Caribbean and Florida Reef Tracts may compromise the sustainability of all other coral reef organisms that depend on corals for habitat, food, and/or nursery area.

Conclusions

This study shows that predicted ocean warming and acidification (31° C, 7.9 pH) can directly and indirectly impact the persistence of *A. agaricites* populations throughout the Caribbean and Florida Reef Tract. *Agaricia agaricites* recruits were directly affected by these conditions by bleaching and yielding low survival and growth rates during the duration of this study. These conditions indirectly affected *A. agaricites* larval settlement. Specifically, the bacterial communities likely changed leading larvae to settle indiscriminately. Scleractinian corals are extremely vulnerable during their early life stages, as mortality rates are high during the recruitment phase. Any negative impact on the recruitment process could have serious implications on the health of current and future reefs. Replenishing damaged reefs could become all but impossible if realized recruitment rates decrease like the findings of this study suggest. The success of a coral recruit is highly dependent on the condition of the substrate and the composition of the bacterial biofilm. Therefore, future work should include microbiome analysis of bacterial biofilms to assess how the composition of bacterial communities change over time when exposed to predicted future OAW conditions and how coral larvae of other species are impacted by these conditions.

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Literature Cited


