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An Investigation into the Physiological Impacts of Ocean Acidification on Recruits of the Temperate Coral, Oculina arbuscula

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AN INVESTIGATION INTO THE PHYSIOLOGICAL IMPACTS OF OCEAN ACIDIFICATION ON RECRUITS OF THE TEMPERATE CORAL, *OCULINA ARBUSCULA.*

by

BRIANNE VARNERIN

(Under the Direction of Daniel Gleason)

ABSTRACT

Ocean acidification is well-researched with respect to adult scleractinian corals, however information on whether adults and recruits of the same species respond similarly to this environmental stress is lacking. I investigated the responses to increased pCO_2 of recruits of the temperate coral, *Oculina arbuscula*, whose adults are known to withstand high levels of pCO_2 with no depression in calcification (up to 1000 ppm CO_2). I addressed the hypothesis that *O. arbuscula* recruit health is not affected by increased pCO_2 by exposing small colonies (5-12mm diameter) to 475, 711, and 1270 ppm CO_2 for 75 days. Calcification rates were monitored throughout the experiment, while mortality, respiration rates, photosynthetic rates, zooxanthella densities, and soluble protein were determined at the end. As predicted, higher pCO_2 did not impact survival, zooxanthella densities, or soluble protein. In contrast, both calcification rates and photosynthesis:respiration (P:R) ratios tended to be lower at higher pCO_2. These results suggest that there is a size-dependent response to pCO_2 within *O. arbuscula*, with recruits being unable to keep up with the increased energetic cost of calcification that occurs at higher pCO_2. With the mean pCO_2 increasing approximately 2.4% each year in the South Atlantic Bight (SAB), within the next 30 years *O. arbuscula* recruits are predicted to experience seasonal depressions in calcification rate driven by the overlying natural fluctuations in oceanic pCO_2, and within 50 years recruits are anticipated to exhibit year-round depressions in calcification rate.

INDEX WORDS: Ocean acidification, Physiology, Recruitment, Calcification, Temperate corals

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DEDICATION

I would like to dedicate this to my parents, Bruce and Debbie Varnerin, and my sisters, Nicole and Jessica, for their never-ending support through my endeavors to become a scientist. And to my roommate, Rachel, for helping me get through three of the hardest but most rewarding years of my life—We did it!

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CHAPTER 1

LITERATURE REVIEW

Ocean Carbonate Chemistry

The world's oceans moderate future climate change by absorbing large portions of the anthropogenically-derived carbon dioxide (CO_2) emitted into the atmosphere through the burning of fossil fuels (Revelle 1957, Sabine et al. 2004, Orr et al. 2005). It is estimated that a third of anthropogenic CO_2 emissions over the past two centuries is currently stored in the ocean, with the majority stored at depths <500 m in the North Atlantic Ocean (Feely et al. 2004, Sabine et al. 2004). CO_2 reacts with seawater resulting in an increase in hydrogen ion concentration [H^+] in the ocean (Feely et al. 2004, Hofmann et al. 2010). This relationship is given by the equation:

$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3(aq) \leftrightarrow H^+(aq) + HCO_3^-(aq) \quad (1)$$

According to the Intergovernmental Panel on Climate Change (IPCC) 1992 scenario (IS92a), this process will decrease the pH of oceanic surface waters by 0.14 to 0.35 pH units by the year 2100 (Metz et al. 2007).

Decreased seawater pH has the potential to affect all marine life, but the most sensitive are calcifying species, such as corals, bryozoans, shelled mollusks, pteropods, and coccolithophores, that rely on the presence of calcium and carbonate (CO_3^{2-}) to form their skeletons (Orr et al. 2005, Hofmann et al. 2008, Hofmann et al. 2010). In seawater, CO_2 reacts with available carbonate, which further acidifies the water and leads to a depletion of carbonate. This relationship is given by the equation:

$$CO_2(aq) + H_2O(aq) + CO_3^{2-} \leftrightarrow 2HCO_3^- \quad (2)$$

Carbonate is in limited supply in oceanic waters in the absence of added CO_2, so the further conversion of usable carbonate to unusable bicarbonate (HCO_2^-) that results from CO_2 emissions

has the potential to impede calcification in many species. Calcification is the accumulation of calcium in a tissue, usually the skeleton. For marine calcifiers, calcification occurs through a chemical reaction involving carbonate:

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \quad (3)$$

Through this reaction marine calcifiers, such as scleractinian corals, can grow in length and girth. The ability of marine organisms to calcify is dependent on the saturation of aragonite or calcite (Ω), which is given by the equation:

$$\Omega_{(\text{aragonite or calcite})} = [Ca^{2+}][CO_3^{2-}]/K^*_{sp} \quad (4)$$

where K^*_{sp} is the solubility product at the *in situ* conditions of temperature, salinity and pressure (Zeebe & Wolf-Gladrow 2001). Based on kinetic and thermodynamic principles, calcification is favored when $\Omega_{(\text{aragonite or calcite})}>1$, and dissolution when $\Omega_{(\text{aragonite or calcite})}<1$. The saturation of aragonite or calcite is positively correlated with pH, thus allowing pH to be used as an indicator of the calcium carbonate saturation state. Generally, the aragonite saturation state (Ω_a) decreases with increasing depth and latitude due to higher hydrostatic pressure, lower temperatures, and build-up of CO_2 from the lack of air-sea gas exchange (Jiang et al. 2015).

Recent studies have found temporal and spatial variability in coastal pH and Ω_a at fine geographic scales (Feely et al. 2008, Jiang et al. 2010, Wanninkhof et al. 2015). For example, in the Southern California Bight there is seasonal upwelling of cold water rich in CO_2 and dissolved inorganic carbon, but undersaturated with respect to aragonite (Feely *et al.* 2008). Similar fluctuations have been found in the South Atlantic Bight (SAB; Cape Hatteras, NC to Cape Canaveral, FL), but unlike the Southern California Bight these fluctuations are not controlled by seasonal upwelling (Xue et al. 2016). It is predicted that with a 2°C increase in sea-surface temperature and an increase in the atmospheric pCO_2 to 800 ppm, the Ω_a of the SAB will fall

considerably by the year 2100, as coastal waters are an important sink for anthropogenic CO_2 (Jiang et al. 2010).

This evidence of a periodic influx of CO_2 laden water in the SAB was found through the Ocean Monitoring Program at Gray's Reef National Marine Sanctuary (GRNMS), a marine protected area located approximately 15 NM east of Sapelo Island, GA, USA. pCO_2 at the surface and bottom (~19m deep) have been monitored at GRNMS since 2006. These data show regular temporal oscillations in pCO_2, along with a linear increase over time (Xue et al. 2016, Fig. 1). The highest concentrations of pCO_2, comparable to future predicted averages, are seen in the summer months, whereas the lowest are seen in the winter. Xue et al. (2016) evaluated the first two years of this long-term data set for the major processes which drive the fluctuations and found that temperature does have a part in driving the system, but river inputs, especially during the wet seasons, and biological respiration and production also had important influences on pCO_2.

Impact of Ocean Acidification on Corals

One of the earliest ocean acidification reviews to mention coral vulnerability to increasing seawater pCO_2 noted a positive correlation between Ω_a and coral presence (Orr et al. 2005). This conclusion suggested that as the pCO_2 of the oceans increases, corals could become scarcer. Over the past two decades, researchers have experimentally induced future ocean CO_2 conditions in the lab to quantify the exact response of corals to ocean acidification. Researchers warned of the detrimental effects of acidifying oceans, projecting dismal futures for coral reef ecosystems (Hoegh-Guldberg et al. 2007, Anthony et al. 2008, Doney et al. 2009, Hofmann et al. 2010). Many studies started with tropical reef-building corals to assess the future state of coral reefs. One early study found *Acropora cervicornis* to have significantly depressed calcficiation

rate under high pCO_2 conditions (Renegar & Riegl 2005). This research was followed by work on species such as *Stylophora pistillata, Porites* sp.(Krief et al. 2010), *Porites lutea* (Ohde & Hossain 2004), and *Acropora eurystoma* (Schneider & Erez 2006), that also found significant decreases in calcification under increased pCO_2. However, as the list of coral species investigated continued to expand the story became more complex.

One of the first studies to detect tolerance to pCO_2 in a coral species investigated impacts on a temperate coral native to the western Atlantic: *Oculina arbuscula* (Ries et al. 2010). This study found that *O. arbuscula* did not show decreased calcification when exposed to pCO_2 predicted for 100 years in the future. Expanding on these findings, a research lab in Moorea has done extensive research to demonstrate the species-specificity of ocean acidification. Specifically, Comeau *et al.* (2014) compared the calcification of eight coral species when exposed to high pCO_2. They classified each coral based on their morphology (mounding/branching), skeleton (perforate/imperforate), and calcification (fast/slow). Comeau *et al.* (2014) used those classifications to draw the conclusion that branching, imperforate, and slow basal calcifiers are more resistant to pCO_2 than other corals. Other recent studies have found that neither *Pocillopora acuta* (Wall et al. 2017) nor *Acropora digitifera* (Takahashi & Kurihara 2013) show significant depression of calcification when exposed to pCO_2 levels expected in the year 2100. Both corals are branching, imperforate, slow basal calcifiers which strengthens the conclusions drawn by Comeau *et al.* (2014). The bottom line is that while some species may be in peril, others will persist. However, if the slow basal calcifiers are the ones to persist, as Comeau *et al.* (2014) suggested, recolonization and establishment will be a slow process.

Calcification is often measured in studies gauging the impact of pCO_2 on corals because it is the process that combines Ca^{2+} and CO_3^{2-} in the basal layer of the tissue to create new

skeletal growth (Cai et al. 2016), and is integral to the success of a coral colony. However, understanding the impacts of pCO_2 increases on coral extend well-beyond the process of calcification. Another measure of coral physiology commonly used in ocean acidification experiments is respiration rate (Edmunds 2012, Strahl et al. 2015). Respiration rate is an indicator of metabolic activity, and can be used to determine the energetic contribution of the coral's symbiotic dinoflagellate, *Symbiodinium sp*., to the holobiont. These intracellular algal symbionts, commonly called zooxanthellae, provide coral with energy through photosynthesis. Light and dark respiration are used to calculate the photosynthesis to respiration (P:R) ratio, which estimates the net energy flow between the coral and zooxanthellae. P:R is formally defined as the ratio of gross zooxanthellae photosynthesis to coral respiration, corrected for coral biomass (McCloskey et al. 1978). Based on this definition, a coral colony is considered to be autotrophic if $P:R>1$ and heterotrophic if $P:R<1$. A $P:R>1$ implies the coral's energy needs are exceeded by the zooxanthellar production through photosynthesis. While an autotrophic coral's respiration may increase under stress, the zooxanthellar photosynthesis could be high enough to meet the increased energy demand. This complex relationship makes the presence of zooxanthellae imperative for the survival and growth of autotrophic corals.

Despite their importance to coral health, loss of the intracellular symbionts can occur when conditions become unfavorable. This "bleaching" response is most commonly seen with thermal stress (Warner et al. 1996, Jones et al. 1998, Baker 2001), but has been recorded in response to other stressors such as ocean acidification (Anthony et al. 2008), low salinity (Kerswell & Jones 2003), and low food availability (Matterson 2012). While zooxanthellae do have a narrow pH tolerance, bleaching with respect to ocean acidification has only been recorded once (Anthony et al. 2008). The rarity of bleaching in ocean acidification experiments is most

likely due to the physiological regulation of intracellular pH (Cai et al. 2016). This regulation is believed to be through passive CO_2 diffusion and maintaining low dissolved inorganic carbon in calcifying fluids, however, the authors acknowledge that additional studies are needed to elucidate a firm coral calcification mechanism.

Finally, tissue biomass can be used to glean information about coral health. The biomass of tissue may be impacted by ocean acidification as a result of altered metabolic demands or resource allocation. Several studies have investigated soluble protein, with all but one showing trends of suppressed soluble protein under high pCO_2 (Krief et al. 2010, Horwitz & Fine 2014, Strahl et al. 2016, Wall et al. 2017). These findings suggest that ocean acidification can have multiple effects on coral physiology, from calcification to respiration to protein content.

Biology of Oculina arbuscula *and their recruits*

The SAB off the coast of Georgia, U.S.A. is characterized by expanses of sand interspersed with live-bottom reefs formed on rocky outcroppings. These ledges offer up to 2 m of vertical relief from the surrounding sand, are composed of sandstone and relic scallop shell ridges, and support a diverse assemblage of sponge, ascidian, bryozoan, coral, crustacean, anemone, and polychaete species (Kendall et al. 2005, Ruzicka & Gleason 2009, Freeman & Gleason 2010, Poirson 2014). Among the invertebrates colonizing these rocky outcrops is *Oculina arbuscula*, the most structurally complex, branching scleractinian coral found in the SAB.

While adults of this species reach a maximum diameter of only 0.5 m, their bushy form provides habitat for small invertebrate and fish species (Miller 1995). Based on long-term research conducted on the diversity and abundance of benthic organisms on temperate live-bottom reefs off coastal Georgia, *O. arbuscula* occupies up to 30% of the exposed rocky

substrate (Matterson 2012). Unlike tropical scleractinian corals, *O. arbuscula*'s symbiosis with zooxanthellae is facultative; therefore, individuals can vary widely in zooxanthella density (Szmant-Froelich & Pilson 1984, Schuhmacher & Zibrowius 1985, Miller 1995). Azooxanthellate colonies are common where light levels are low, suggesting that the symbiosis ends when it is no longer advantageous for both constituents (Miller 1995, Matterson 2012). Azooxenthellate colonies are also common among newly-settled recruits, as *O. arbuscula* do not have maternal transmission of zooxanthellae and need to acquire their symbionts from the water column (Babcock & Heyward 1986, Richmond & Hunter 1990).

It is well documented that juvenile mortality is high in sessile marine invertebrates. Factors such as competition, predation, disease, and sedimentation are responsible for mortality rates of up to 90% in young benthic invertebrates due to their small size and high surface area to volume ratio (Goodbody 1963, Sebens 1983, Young & Chia 1984, Keough 1986, Davis 1987, Stoner 1990, Hurlbut 1991, Worcester 1994, Osman & Whitlatch 2004, Doropoulos et al. 2016). Recruits can mitigate mortality from these factors through selective placement on the substrata, i.e. seeking out crevices hidden from predators or areas that have high herbivory (Doropoulos et al. 2016). It is important to note that the authors did find trade-offs between factors such as growth, predation, and competition, and if a new stressor were to be introduced into the system, such as increased pCO_2, these choices would be impacted.

Recently, more effort has been focused on the response of coral recruits to ocean acidification, and decreased Ω_a has been found to negatively impact the biomineralization of *Porites astreoides* (Albright & Langdon 2011, de Putron et al. 2011), *Favia fragum* (Cohen et al. 2009, de Putron et al. 2011), *Acropora millepora* (Doropoulos et al. 2012) and *Acropora spicifera* (Foster et al. 2016) larvae upon settlement. Additionally, these studies have shown that

OA has the potential to not just affect biomineralization of coral recruits, but also depress metabolism (de Putron et al. 2011) and reduce settlement rates (Albright & Langdon 2011) when exposed to increased pCO_2. Depressed metabolic and calcification rates inhibit the growth of coral recruits causing them to be at smaller and more vulnerable sizes longer.

While studies investigating the effects of ocean acidification on coral recruits have become more common in recent years, there are still few species where more than one life stage have been addressed. In fact, there has only been one study which compared the skeletal mineralogy of both coral recruits and adult skeletons (Clode et al. 2011). This study found that recruit skeletons were composed of mainly aragonite, consistent with those of adults and concluded that recruits likely respond similarly to adults when it comes to increasing pCO_2 (Clode et al. 2011). In this study, I sought to decrease this gap of knowledge by investigating the physiological responses of *O. arbuscula* recruits to increased pCO_2, and comparing those responses to the already known responses of the adult life-stage. These data are useful for determining if there is a differential response between recruits and adults, which has implications for predicting the stability of the species in the future.

CHAPTER 2

PHYSIOLOGICAL IMPACTS OF OCEAN ACIDIFICATION ON RECRUITS OF THE TEMPERATE CORAL, *OCULINA ARBUSCULA*

INTRODUCTION

Over the past two centuries the world's oceans have absorbed ~28% of CO_2 emissions, and will continue to do so until its holding capacity is reached (Sabine et al. 2004). The average open ocean pH is currently 8.1, but with the additional CO_2 in seawater this value is projected to fall to 7.8 by the year 2100 (Metz et al. 2007, Hofmann et al. 2010). Excess CO_2 in the water reacts with carbonate, one of the essential building blocks of skeleton for calcifying marine organisms, converting it to bicarbonate and rendering it unusable to calcifying organisms. Additionally, CO_2 creates a more acidic environment, which causes dissolution of calcium carbonate skeletons in high concentrations.

One group of organisms that may be particularly vulnerable to ocean acidification is scleractinian corals, due to their production of aragonite skeleton (Oliver 1980, Cuif et al. 2003, Stolarski 2003). Early investigations into the effects of pCO_2 on corals suggested that as pCO_2 increases and the aragonite saturation state (Ω_a) decreases, the abundance of all coral species will decline (Orr et al. 2005, Hoegh-Guldberg et al. 2007, Hofmann et al. 2010). The explanation for this response was grounded in the inability of corals to cope with aragonite-poor environments, thus causing the corals to fall into net dissolution. However, as more investigations into the direct impacts of acidification on coral calcification were completed, it became clear that the response was species specific (Edmunds et al. 2012, Comeau et al. 2013, Comeau et al. 2014). For example, species such as *Porites rus* and *Stylophora pistillata* (Krief et al. 2010) have reduced calcification rates under high pCO_2, while others, including massive *Porites* spp. (Edmunds et al. 2012) and *Oculina arbuscula* (Ries et al. 2010) see no change. These species-

specific responses are believed to be due to differences in ecologically-relevant taxonomic 'functional groups', such as basal calcification rate, gross morphology, and skeletal porosity. Slow growing, imperforate, branching corals are the most robust to ocean acidification, while fast calcifiers are the most vulnerable (Comeau et al. 2014).

To date most studies have focused on the adult stage, rather than the vulnerable larval and recruit phases. It is well documented that juvenile mortality in sessile marine invertebrates is high, up to 90% (Goodbody 1963, Keough & Downes 1982, Young & Chia 1984, Stoner 1990, Worcester 1994, Doropoulos et al. 2016). This mortality rate can be due to biotic factors such as predation, competition, and disease (Goodbody 1963, Young & Chia 1984, Doropoulos et al. 2016), and abiotic factors such as sedimentation (Young & Chia 1984, Gleason et al. in press). Several studies found that the biomineralization of coral recruits was affected by increased pCO_2 (Cohen et al. 2009, Albright & Langdon 2011, de Putron et al. 2011, Foster et al. 2016) and one linked depressed calcification with an increase in predation (Doropoulos et al. 2012). Rapid increase in size is imperative to the survival of coral recruits, and changes in biomineralization at an early life stage has the possibility to hamper that success.

A coral species of interest to Georgia coastal managers is *Oculina arbuscula*, the only habitat forming scleractinian off the coast of Georgia. This coral inhabits rocky ledges and artificial surfaces throughout the eastern US. At Gray's Reef National Marine Sanctuary (GRNMS), located 17 NM off the coast of Sapelo Island, GA, *O. arbuscula* covers ~30% of the available ledge habitat (Gleason, unpub. data). The facultatively symbiotic *O. arbuscula* is known to be a relatively adaptable species, tolerating a broad range of temperature (4-30°C), light (1-100%) (Miller 1995), and, recently, pCO_2 (400-900 ppm) (Ries et al. 2010). In 2010, Ries *et al.* found that adult calcification rates were only affected when exposed to a CO_2

saturation state that favors dissolution of $CaCO_3$ skeletons. This robustness with respect to pCO_2 is of particular importance for *O. arbuscula* due to seasonal fluctuations in pCO_2, where concentrations reach predicted near-future averages (600-700 ppm) in the summer months (Fig. 1).

While *O. arbuscula* adults may be unaffected by the future acidification, it is unknown as to how the larval and recruit stages will respond. To obtain a better understanding of the ability of *O. arbuscula* to persist under current and future pCO_2 conditions, I investigated the responses of recruits to ocean acidification. With seawater off the Georgia coast reaching near-future pCO_2 levels annually and recruitment rates appearing to be high (Gleason et al. in press), I hypothesized that *O. arbuscula* recruits possess physiological mechanisms to withstand the effects of increased pCO_2. I addressed this hypothesis by exposing *O. arbuscula* recruits to current and near-future pCO_2 levels in the lab for 75 days while monitoring physiological parameters related to coral health. I predicted that the physiological parameters would be similar across pCO_2 treatments if this hypothesis were true.

MATERIALS AND METHODS

Coral Collection and Experimental Design

Coral samples were collected from exposed surfaces of three artificial reefs off the coast of Georgia, as *O. arbuscula* is known to recruit in highest densities on artificial surfaces (Gleason et al. in press). The first two sites are the main and stern decks of the "SS Addie Bagley Daniels" (31°36.207 N, 80°47.750 W and 31°36.260 N, 80°47.680 W, respectively), 17 m below sea level. The third is the vessel "Jane Yarn" (31°36114 N, 80°47.725 W), 18 m below sea level. In May 2017, a total of 144 recruits, defined in this study as individuals with a diameter between 5 and 10 mm, were collected from the exposed surfaces of the three artificial reefs and

transported to Georgia Southern University in well aerated sea water. As *O. arbuscula* recruits may not assimilate zooxanthellae immediately upon settlement, coral color was not considered in collection, meaning that both colonies with high and low densities of zooxanthellae (i.e. brown to white) were collected. After a two-day temperature acclimation period to ~25°C, recruits were sorted first by size and then by color to be epoxied in pairs on pre-labeled acrylic squares. Total surface area of each coral pair was kept approximately equal among squares, and less pigmented corals were randomly dispersed among the squares to reduce bias related to differences in zooxanthellae density.

For the 75-day experiment, 72 coral pairs were divided equally among nine aquaria. Each aquarium was filled with artificial seawater (Instant Ocean, 35 ppt). The total alkalinity (TA) was adjusted to ~2300 µM $CaCO_3$ using 10% hydrochloric acid mixed in a large carboy over 36 hours. The pH of the seawater was checked before adding it to the aquaria. Each aquarium was outfitted with a power filter, one airstone, a small circulation pump (60 gph), a 19” LED light and cover (Novia, 13W), and a PVC pedestal to bring the recruits as close to the light source as possible. The aquaria were subjected to a 12 hr light:12 hr dark cycle. Individuals were fed *Artemia* sp. nauplii *ad libitum* twice a week in small containers within each aquarium. After feedings, partial water changes (~15%) were done, and a soft toothbrush was used to clean the plexiglass squares.

The Apex Aquarium Control System (Neptune, Inc., Fig. 2) was used to control temperature and pH. Each aquarium contained a pH and temperature probe, logging data every 5 minutes. Temperature was regulated in each aquarium using a submersible heater (Aqueon Products). pH (used as a proxy for pCO_2) was maintained in each aquarium by bubbling pure carbon dioxide for 5 seconds at a time when the pH rose above the set point. As pH probes do

not measure total pH, the pH was also determined spectrophotometrically once a week using *m*-cresol purple (Riccoh Inc.)(Dickson et al. 2007). The spectrophotometric pH was used to adjust the value recorded by the probes to total pH.

After a two-week acclimation period at ambient pH (~8.0), the pH of the six experimental aquaria were brought down to their respective set point, either 7.6 (high pCO_2) or 7.8 (moderate pCO_2), over a 24-hour period. The three control aquaria were maintained at a pH of 8.0. These treatments were chosen to signify the current average pH (8.0), the current low pH (7.8), and a possible future low pH (7.6) experienced by *O. arbuscula* on the reef. TA was measured at the end of the experiment from preserved water samples according to SOP 3b (Dickson et al. 2007). Samples from each aquarium were taken three times a week at the same time each day and fixed with mercury (II) chloride, then analyzed off-site at the end of the experiment using an automatic seawater titrator (Apollo SciTech). pCO_2 and aragonite saturation state were determined using the CO2SYS program, with temperature, pressure, salinity, TA, and pH measurements as inputs (Dickson et al. 2007).

Coral Physiological Measures

The calcification rate of each coral pair was calculated as the percent-change in buoyant weight over time. Buoyant weight was measured initially and every 15 days to the nearest 0.1 mg using an electronic balance (Sartorius R200D). Each coral pair was weighed in a wide-mouth mason jar in their original seawater to avoid the stress of rapid change in water chemistry. Salinity (35 ppt) and temperature (25.5°C) were measured and adjusted prior to weighing to maintain the same seawater density throughout the experiment. The plexiglass plates were suspended approximately 4 cm below the surface of the water on a wire hook that was attached to the underhook of the balance. After being weighed each coral colony was visually inspected,

and any complete mortality was recorded. Mortality was defined in this study as a whole colony having little to no tissue cover, with no response to touch.

Respiration measurements were initiated on day 76 and were completed over 3 days. Two coral pairs were randomly chosen from each aquarium to complete respiration measurements. Both light and dark respiration were measured on each coral pair, with light measurements beginning one hour after the lights turned on and dark measurements beginning two hours after the lights turned off. The start times were chosen to minimize residual effects of light history (Edmunds 2012). Each coral pair was placed in a plexiglass respiration chamber hooked up to a recirculation pump (total volume= 547 ml) and allowed 15 minutes of acclimation. The dissolved oxygen was recorded at the start of the thirty-minute measurement period (Oakton DO6+), and every three minutes thereafter. Once completed, the dissolved oxygen of the seawater in the empty chamber was recorded every minute for ten minutes to document background respiration rates. The light and dark respiration rates were then corrected based on the background levels to obtain net rates of photosynthesis and respiration (McCloskey et al. 1978).

All 72 coral pairs were placed in a -20°C freezer at the end of the experiment. To standardize other measurements, surface area of each coral pair was quantified as the mean weight for aluminum foil that was carefully molded to the tissue surface three consecutive times (Marsh 1970). Thirty, 1 cm^2 pieces of aluminum foil were weighed and the mean of these measures was used to convert individual aluminum foil weights to surface area. Each recruit was subsequently crushed and homogenized with a mortar and pestle in DI water. The slurry was centrifuged at ~5000 rpm in a 50 mL tube for 7 minutes, and the supernatant was decanted, lyophilized to isolate the tissue, and stored at -20°C. The pellet was resuspended in 30 mL DI water, and divided equally between two centrifuge tubes. One of these tubes was used to

quantify chlorophyll and soluble protein concentrations, and the other for estimates of zooxanthella densities.

Measures of zooxanthella density proceeded by initially decalcifying samples at 4ºC using 5 ml of 10% HCl for 24 hours, or until no bubbles were produced upon the addition of more acid. Fully decalcified samples were centrifuged for 7 minutes at ~5000 rpm, and the pellet was resuspended in 15 mL DI water. Samples were homogenized with a tissue grinder and stored at -20°C until they were analyzed for zooxanthella densities on a hemocytometer (n=3 replicates per aliquot), expressed as cells cm^{-2} of coral surface area.

In many tropical scleractinian corals individual zooxanthella cells can undergo seasonal fluctuations in chlorophyll concentrations (Fitt et al. 2000). To determine if differences in chlorophyll concentrations among recruits was due to the number of zooxanthellae or the chlorophyll concentration within zooxanthella cells, chlorophyll *a* concentration analysis was carried out. The aliquots set aside for zooxanthellae counts were centrifuged for 7 minutes at ~5,000 rpm, decanting the supernatant, and performing two 24-hour chlorophyll *a* extractions on the pellet in the dark at 4°C using 20 ml 100% HPLC grade acetone. Following the second extraction, the two supernatants were combined and the absorbance at 630 and 663 nm was determined on a spectrophotometer (Shimadzu UV2600). Concentrations of chlorophyll *a*, expressed as $\mu g\ cm^{-2}$ of coral surface area, were calculated using the equations in Jefferey and Humphrey (1975). The number of extractions required to remove the chlorophyll was determined by carrying out consecutive 20 mL extractions over a 3 day period. Approximately 97% of the chlorophyll was extracted in the first two days, while the third extraction resulted in absorbance values that were at the sensitivity limits of the spectrophotometer.

The pellet remaining after extraction of chlorophyll and the corresponding lyophilized tissue were combined and processed for soluble protein. Soluble protein is a common proxy for biomass used in scleractinian corals, to achieve an index of stress. (Krief et al. 2010, Strahl et al. 2015, Wall et al. 2017). Both the pellet and the lyophilized tissue were resuspended in 3 ml 1N NaOH, vortexed for 30 seconds, and incubated in a 90°C water bath for 1 hr to dissolve protein. Upon dissolution, samples were centrifuged for 7 minutes and the supernatants were decanted, combining the supernatants of the pellet and the corresponding lyophilized tissue. A portion of the combined samples were then diluted with diH_2O to a concentration of 0.05 N NaOH in 1.5 ml microcentrifuge tubes. Protein concentrations were estimated using the Bradford technique (Bradford 1976), whereby 160 µl of each sample was combined with 40 µl diluted dye reagent (Bio Rad Inc.) in a 96-well plate and incubated for 10 min at 25°C. Protein standards in the range of 0-85 µg/ml were also prepared using bovine gamma globulin (Bio Rad Inc.) and incubated as above. Blanks were prepared by combining 160 µl of 0.05 N sodium hydroxide and 40 µl of dye reagent. Absorbance was measured at 595 nm and converted to soluble protein concentration based on the standard curve created with bovine gamma globulin.

Statistical Analyses

TA, pH, and temperature were analyzed on 14 of the 75 days, approximately once a week. These three seawater parameters were then used to calculate pCO_2 for each date, thus, any change in one of the three parameters would cause a shift in pCO_2.

Temperature, pH, TA, pCO_2 and buoyant weight were measured over time while calcification, respiration, photosynthesis, P:R, zooxanthellae density, and protein concentrations were quantified at the end of the experiment. All variables were analyzed for normality and equality of variance using the Shapiro Wilk W and Levene tests, respectively. Data that failed to

meet statistical assumptions were either log +1 or square root transformed to meet normality assumptions and reduce heterogeneity of variance. To test for differences in temperature, pH, TA, and pCO_2 among treatments over time, I used a repeated measures ANOVA. To test for differences in calcification, chlorophyll *a* concentration, zooxanthellae density, and soluble protein within and among treatments, I used a one-way nested ANOVA. Regression analysis was employed to investigate the relationship between calcification and zooxanthellae density, and also the relationship between chlorophyll *a* concentration and zooxanthellae density. Mortality was quantified as the number of individual colonies dead in each aquarium and differences in mortality among treatments were evaluated with a chi square test of independence.

As the within treatment sample sizes for respiration, photosynthesis, and P:R were insufficient for nesting (n = 2), coral pairs were treated as independent replicates and the means of respiration, photosynthesis, and P:R among treatments were compared with a one-way ANOVA.

RESULTS

Good separation of pH was maintained between treatments (Fig. 3a). As expected, pH and pCO_2 were significantly different among treatments, while temperature and TA were not (Table 1). TA was also similar among aquaria within treatments, but pH, pCO_2, and temperature were all significantly different within treatments (Table 1). The latter result is likely an artifact of the large sample sizes greatly reducing the variance (Table 2). Temperature and pH remained constant over time however, TA, and as a result pCO_2, both declined significantly over time (Table 1, Fig. 3). Further evaluation revealed that this decline was due to a change in the TA of the last batch of salt mix that was used for the experiment. However, while the TA level does fall, it was still well-within the range needed for coral calcification.

Coral Physiological Measures

Corals appeared healthy throughout the experiment, as evidenced by extended polyps and tentacles throughout the day, consistent with individuals observed on reefs off shore. Several aquaria experienced a cyanobacteria bloom during the experiment, but the bloom did not seem to affect the health of *O. arbuscula* recruits as the tentacles remained extended, partial mortality did not increase, and calcification rates were unchanged. Some partial recruit mortality occurred in all treatments and aquaria, but this was not extensive with only one or two polyps dying in each aquarium.

Complete mortality of coral recruits in each treatment was low, with the highest instance being 4 out of 16 colonies in one aquarium. Two coral pairs in the highest pCO_2 treatment did not survive the experiment, while any remaining mortality affected only one colony in a pair. Total mortality was only 7.6% across all treatments and independent of pCO_2 treatment (Fig. 4, $\chi^2 = 2.478$, $p = 0.2897$).

All coral pairs exhibited positive calcification rates over the 75-day experiment (Fig. 4). Overall, no significant differences in calcification rates were detected among aquaria within treatments or between treatments, however, there was a trend ($p = 0.05$) for lower calcification rates with higher pCO_2 exposure (Table 3, Fig. 5). The highest pCO_2 of 1261 ppm depressed calcification rates by ~20% when compared to the lowest pCO_2 of 475 ppm (Fig. 5). When calcification rates for coral pairs exposed to different pCO_2 treatments showed a consistent pattern of divergence throughout the 75 day experiment (Fig. 6). These differences in calcification rates were not attributable to dissimilarities in zooxanthella densities because there was no significant relationship between these two variables (Fig. 7, $R^2 = 0.0383$, $p = 0.1021$).

At the end of the 75-day experiment, zooxanthellae densities in recruit pairs ranged from 0 to 3026 cm^{-2}. No significant differences in zooxanthellae density were detected within or among treatments (Table 3, Fig. 10). As individual zooxanthella cells can vary their chlorophyll concentrations, the relationship between chlorophyll *a* concentration and zooxanthellae density was investigated (Fitt et al. 2001). Chlorophyll *a* concentrations are dependent on zooxanthella densities, however the relationship is not strong (Fig. 9, $R^2 = 0.1785$, $p = 0.0003$) and chlorophyll *a* concentrations did not differ significantly within or among treatments (Table 3, Fig. 8). Likewise, soluble protein concentrations varied widely (14.68 to 116.39 µg cm^{-2}), with no significant differences either within or among treatments (Table 3, Fig. 11).

Respiration and photosynthesis were quantified at the end of the experiment for two coral pairs per aquarium, six per pCO_2 treatment. Respiration and photosynthesis were both similar among treatments (Table 4, Fig. 12). To evaluate the ability of the zooxanthellae to meet the metabolic needs of the coral recruits the ratio between photosynthesis and respiration was calculated for each coral pair. This P:R ratio ranged from 0.37 to 2.62, with mean P:R depressed 60% by high pCO_2 relative to the ambient treatment (Fig. 12). Significant differences in P:R were detected between CO_2 treatments (Table 4). A Tukey-Kramer a posteriori test showed that P:R in the 1261 ppm treatment was significantly lower than in the 475 ppm treatment ($p < 0.05$), while the 711 ppm treatment was not significantly different from either the high or low pCO_2 treatments (Fig. 12).

DISCUSSION

This study explored how *O. arbuscula* recruits respond physiologically to several concentrations of dissolved CO_2, to determine if recruit function and survival is jeopardized under high pCO_2. Based on their existence in the naturally fluctuating pCO_2 environment of the

SAB, I hypothesized that *O. arbuscula* recruits possess physiological mechanisms to withstand the effects of increased pCO_2 and predicted that all seven physiological parameters measured would be similar among treatments. *Oculina arbuscula* recruits subjected to three pCO_2 levels in the laboratory for 75 days demonstrated a trend for depressed calcification with increasing pCO_2 and a negative relationship between P:R and pCO_2, while mortality, respiration, photosynthesis, zooxanthellae density, chlorophyll *a*, and soluble protein were all similar among treatments. These results demonstrate that the health of *O. arbuscula* recruits is affected by ocean acidification, but only with respect to calcification rate and P:R ratio.

This study found that higher pCO_2 causes a significant reduction in P:R, with the mean P:R under 1 in the highest pCO_2 treatment. While neither photosynthesis nor respiration alone were found to be significantly different among pCO_2 treatments, these parameters covaried in a manner that resulted in the inverse relationship between P:R ratio and pCO_2. This result was interesting because it was not consistent with previous studies on corals. A meta-analysis of eleven studies revealed that increased pCO_2 had no discernable effect on photosynthesis (Kroeker et al. 2013), and further evidence showed that the response of coral respiration to pCO_2 was equivocal. For example, there were no effects of increased pCO_2 on dark respiration of *Acropora eurystoma* (Schneider & Erez 2006) and *A. formosa*, while there was a decrease in dark respiration for massive *Porites* spp. (Edmunds 2012), *A. millepora* (Kaniewska et al. 2012), and larvae of *P. astreoides* (Albright & Langdon 2011).

A P:R<1 signifies the zooxanthellae are unable to meet the metabolic needs of the coral (McCloskey et al. 1978). This situation is commonly seen in tropical corals bleached due to temperature stress, and can result in the death of the colony if the stress is not abated (Fitt et al. 2000, Baker et al. 2008, Lesser 2011). However, P:R<1 is also seen in corals which are

azooxanthellate or in facultatively symbiotic coral species occurring in environmental conditions unfavorable to the zooxanthellae (Miller 1995). Facultative symbiosis means that the coral can occur naturally without zooxanthellae as an energy source, relying on heterotrophy (Szmant-Froelich & Pilson 1984, Schuhmacher & Zibrowius 1985, Miller 1995). Facultative symbiosis may explain the large variation in zooxanthella density (0-3000 cells/cm^2) among the recruits in the study in addition to the drastic differences in zooxanthella density observed in tropical corals (10^6 cells/cm^2, Fitt et al. 2000) relative to those of *O. arbuscula* (10^3 cells/cm^2, present study). While *O. arbuscula* is able to survive for long periods of time with a P:R<1, this condition has been shown to negatively impact calcification rates. Miller (1995) conducted extensive laboratory and field studies demonstrating that colonies of *O. arbuscula* possessing lower zooxanthella densities (assumed to have lower P:R ratios) exhibit significantly lower calcification rates. As I found calcification rate to be independent of zooxanthellae density, these two studies seem to be in direct contradiction. There is the possibility that the added pCO_2 stress is overwhelming the effect of zooxanthella density on calcification, however, further investigation is needed to parse out the effects.

Calcification rates for adult *O. arbuscula* were not affected by pCO_2 concentrations similar to those used here, with mean changes in weight per day across the three treatments of 0.185-0.197 % (Ries et al. 2010). These calcifications rates are similar to those documented for the recruits exposed to 475 and 710 ppm CO_2, but not for the recruits exposed to 1261 ppm CO_2. These results suggest that the response of *O. arbuscula* to increasing pCO_2 is size-dependent when it comes to calcification rate. This finding is similar to that of Edmunds and Burgess (2016), who found that adult *Pocillopora verrucosa* show depressed calcification rates with decreased colony size when exposed to increased pCO_2. While all *P. verrucosa* individuals in the

Edmunds and Burgess (2016) study were taken from adult colonies, their results provide further evidence of a size-dependent calcification response to higher pCO_2.

While the trend of decreased calcification rate with increased pCO_2 I found was not significant, this was likely due to the small sample size (n=3 aquaria). Analyses for the calcification measurements confirmed that the power was only 0.57 and retrospective power analyses indicated that doubling the sample size would have increased the power to an acceptable range (>0.8), reducing the probability of a type II error. Additionally, given the calcification trajectories in all three treatments, the available evidence suggested that if the experiment had been run for a longer period of time the skeletal weights of the recruits would have continued to diverge (Fig. 6). As environmental monitoring has shown, *O. arbuscula* recruits in the SAB are seasonally exposed to increased pCO_2 for 90 or more days each year (Xue et al. 2016, Fig. 1), thus it would be ecologically relevant to increase the exposure time to 90+ days in future experiments.

The increase in skeletal weight observed over time in all three treatments provided no evidence of an ability of *O. arbuscula* to acclimate to higher pCO_2 over 75 days. The skeletal weight curve of the highest pCO_2 treatment never converges with the control treatment. This result shows that *O. arbuscula* recruits exhibit a chronic depression in calcification with no apparent acclimation, which could be classified as disrupted negative feedback (Romero 2004). The acute response elicited by *O. arbuscula* recruits in the increased pCO_2 became the disrupted baseline, causing the recruits to continue to have lower calcification rates. Calcification is a highly regulated process, with the coral maintaining a high pH in the calcification fluid right above the skeleton through H^+-pumping (Cai et al. 2016). Under elevated pCO_2 conditions, H^+ removal is increasingly difficult, which increases the energetic cost of calcification (Cai et al.

2016). The increased energetic cost of calcification coupled with the size difference between recruits and adults may explain why adult *O. arbuscula* are able to calcify at normal levels under increased pCO_2 (Ries et al. 2010) while recruits are not. The explanation for this size-dependent response rests in the idea that increasing pCO_2 exerts a cost and larger colonies can share the ost over a larger surface area and greater number of polyps (Edmunds & Burgess 2016). Future research should explore this size-dependent response in *O. arbuscula* and identify the "pCO_2-size-escape threshold".

The size-dependent differences in calcification rates observed in recruit (this study) versus adult (Ries et al. 2010) *O. arbuscula* contrasts with the findings of Clode *et al.* (2010), who concluded that recruit and adult stages of all scleractinian coral species should respond similarly to acidifying oceans. The conclusions of Clode *et al.* (2010) were based solely on the similarity in skeletal mineralogy between recruits of *Acropora millepora* and the general composition of adult scleractinian coral skeletons. In contrast, studies on recruits of other scleractinian species with respect to increased pCO_2 found depressed calcification (Cohen et al. 2009, Albright & Langdon 2011, de Putron et al. 2011, Doropoulos et al. 2012), several also finding skeletal deformities (Foster et al. 2016) and decreased settlement rates (Albright & Langdon 2011, Allen et al. 2017).

These depressions in calcification seen in coral recruits, while sublethal in isolation, have been shown to increase the chance of predation up to ~60% depending on the fish species (Doropoulos et al. 2012). The higher depredation rates observed under increased pCO_2 can be attributed to the smaller diameter of the recruit and the weaker skeletal structure due to the depressed calcification (Doropoulos et al. 2012). The conclusion that grazer predation on corals is enhanced in high pCO_2 conditions follows the principles of size-escape theory (Paine 1976,

Gosselin & Qian 1997), and the longer *O. arbuscula* recruits remain below the size threshold, the greater the chance they have to be depredated. Consequently, *O. arbuscula* recruits in the SAB (South Atlantic Bight) which settle from May-August will have a greater chance of being depredated by predators such as the urchins *Arbacia punctulata* and *Lytechinus variegatus*, and several species of generalist fishes (e.g., *Halichoeres bivittatus* and *Serranus subligarius*) (Gleason et al. in press).

While predation is one contributing factor of mortality at Gray's Reef, two other prevalent factors which coral recruits must overcome to survive to the next life-stage are sedimentation and competition. It is hypothesized that sedimentation is the largest contributor to recruit mortality (Gleason et al. in press), as a negative relationship between survival of *O, arbuscula* recruits <40mm in diameter and sedimentation rates was detected (Divine 2011). Once the coral recruits have an upright, branching morphology, they are less likely to suffer mortality than recruits that are encrusted (Divine 2011). For *O. arbuscula* recruits under increased pCO_2, their depressed calcification rates will leave them vulnerable to the threat of sedimentation longer than if they were able to grow at a normal pace. Lastly, coral recruits must compete with other sessile invertebrates for space, making rapid growth imperative to survival (Keough & Downes 1982, Branch 1984, Gosselin & Qian 1997). Thus, decreased calcification and P:R ratio will result in *O. arbuscula* recruits being outcompeted by their neighbors.

Competition, sedimentation, and predation will all have a greater negative impact on the survival and growth of *O. arbuscula* recruits in the presence of increased pCO_2. In the SAB, seasonal fluctuations result in summer pCO_2 as high as 600 ppm, which is close to ocean averages predicted for 50-100 years in the future (Metz et al. 2007). This means that in the summer months *O. arbuscula* recruits likely exhibit reduced calcification rates and remain at a

smaller size for a longer period of time. This impact on the calcification rate of these coral recruits will lead to more mortality not from the pCO_2 itself, but from competition, sedimentation, predation, and other such factors that impact all sessile invertebrate recruits (Gosselin & Qian 1997, Doropoulos et al. 2016).

Most tropical corals show seasonal reproduction and recruitment (reviewed in Gleason and Hofmann 2011), however, *O. arbuscula* recruits at low levels throughout the year (Gleason et al. in press). Year-round recruitment means that larvae will be settling during the more acidic summer months and growing at a depressed rate until the pCO_2 begins to decrease around September. Recruits which settle as the pCO_2 is changing, around October and November, will have the advantage of the most time spent outside of the increased pCO_2 stress. Interestingly, we already see a spike in recruitment during those months (Gleason et al. in press), which further suggests that these months are the optimal time for recruitment.

In the SAB, seasonal fluctuations in pCO_2 present an interesting system in which all organisms, including *O. arbuscula*, need to cope for months at a time with pCO_2 levels not predicted to occur for 50-100 years in the future. My results suggest that *O. arbuscula* recruits in the SAB will be susceptible to depressions in calcification rate and autotrophic energy availability as the average pCO_2 continues to increase. With the average pCO_2 increasing 2.4% each year, if nothing changes it is possible that in 30 years the pCO_2 will reach levels >1200 ppm in the summer, depressing the calcification rate of recruits during those months. Not much further into the future *O. arbuscula* may face year-round depressions in calcification rate. While currently the populations of *O. arbuscula* are not seeing detrimental levels of pCO_2, they very well could be in the near future. As the average ocean pCO_2 increases, *O. arbuscula* recruits will have depressed calcification in the summer and we may see increased recruit mortality which

could lead to a reduction in abundance. However, based on the findings of Ries et al. (2010), that adults do not have depressed calcification at these high future levels of pCO_2, we know that adults will continue to survive in the foreseeable future, and those coral recruits that surpass the pCO_2-size-escape threshold will as well.

TABLES AND FIGURES

Table 1. Repeated measures ANOVA results of time and pCO_2 treatment for four water quality variables. N=3 aquaria for each of the three pCO_2 treatments: 475, 711, and 1261 ppm pCO_2.

Variable	F	DF	p
Temperature			
Among Treatments	0.05	2,6	0.96
Within Treatments	2379.26	6,131	<0.0001*
Day	0.86	1,131	0.36
Among*Day	0.23	2,131	0.79
pH			
Among Treatments	266.29	2,6	<0.0001*
Within Treatments	6.46	6,131	<0.0001*
Day	14.26	1,131	0.0002*
Among* Day	0.91	2,131	0.4049
Total Alkalinity			
Among Treatments	1.11	2,6	0.39
Within Treatments	1.76	6,131	0.11
Day	111.95	1,131	<0.0001*
Among* Day	1.31	2,131	0.27
pCO2			
Among Treatments	307.96	2,6	<0.0001*
Within Treatments	10.75	6,131	<0.0001*
Day	108.59	1,131	<0.0001*
Among* Day	0.68	2,131	0.51

Table 2. Summary statistics (mean±SE) of water quality parameters for three pCO_2 treatments over the 75-day experimental period. Three aquaria are nested within each treatment for each variable.

	pCO_2 Treatment		
	1261 ppm	710 ppm	475 ppm
Temperature	25.59±0.003	25.74±0.003	25.98±0.003
	25.89±0.003	26.01±0.003	25.67±0.003
	25.81±0.003	25.65±0.003	25.63±0.003
Total pH	7.59±0.01	7.79±0.01	7.95±0.01
	7.55±0.01	7.78±0.01	7.97±0.01
	7.56±0.01	7.83±0.01	7.98±0.01
Total Alkalinity	2148.57±43.06	20.82.27±44.47	2124.30±43.06
	2196.21±43.06	2142.63±43.06	2069.89±43.06
	2127.05±43.06	2103.92±43.06	2178.96±43.06
pCO_2	1206.77±17.02	700.27±17.58	462.98±17.02
	1350.66±17.02	729.61±17.02	459.41±17.02
	1249.84±17.02	701.23±17.02	502.21±17.02

Table 3. Nested ANOVA results of pCO_2 treatment for physiological measures of *O. arbuscula* recruit pairs. N=8 within each aquarium, and n=3 aquaria for each of the three pCO_2 treatments: 475, 711, and 1261 ppm pCO_2.

Variable	F	DF	p
Calcification			
Among	4.85	2,6	0.05
Within	0.61	6,62	0.72
Chlorophyll *a* Concentration			
Among	0.51	2,6	0.62
Within	0.78	6,60	0.59
Zooxanthellae Density			
Among	1.5	2,6	0.3
Within	1.04	6,60	0.41
Soluble Protein			
Among	0.28	2,6	0.76
Within	0.79	6,60	0.58

Table 4. One-way ANOVA for respiration and photosynthesis measures of *O. arbuscula* recruit pairs maintained at three pCO_2 levels: 475, 711, and 1261 ppm pCO_2. Two coral pairs were analyzed from each aquarium, but each pair was treated as an independent replicate for data analysis. Thus, n=6 for all treatments.

Variable	F	DF	p
Respiration Rates	0.54	2,15	0.59
Photosynthetic Rates	0.69	2,15	0.52
Photosynthesis:Respiration	5.01	2,15	0.022*

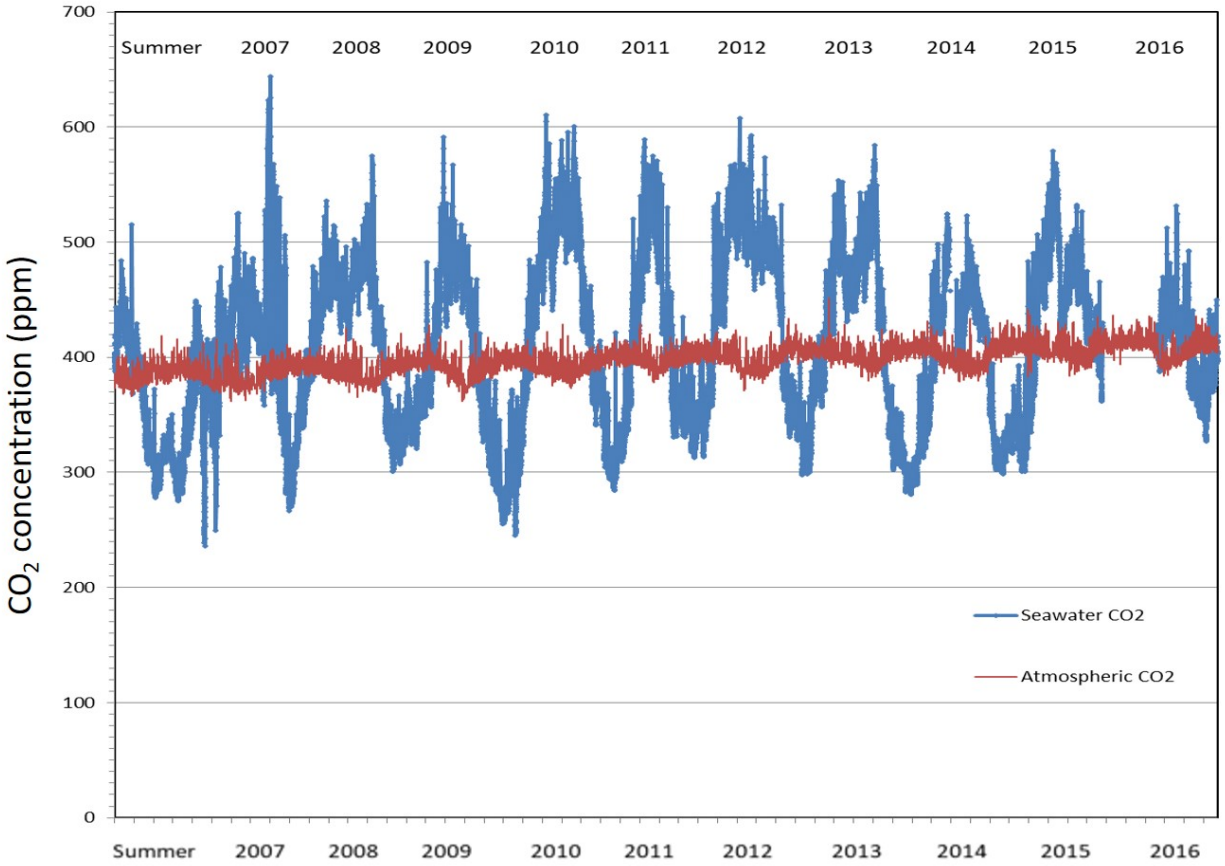


Fig. 1. Seawater and air pCO_2 at Gray's Reef National Marine Sanctuary from 2006-2016. Seawater measurements are in blue, and air are in red. There is a linear increase in seawater pCO_2 apparent, along with the seasonal oscillations. Data were obtained from Dr. Scott Noakes, as part of an international CO_2 monitoring program.

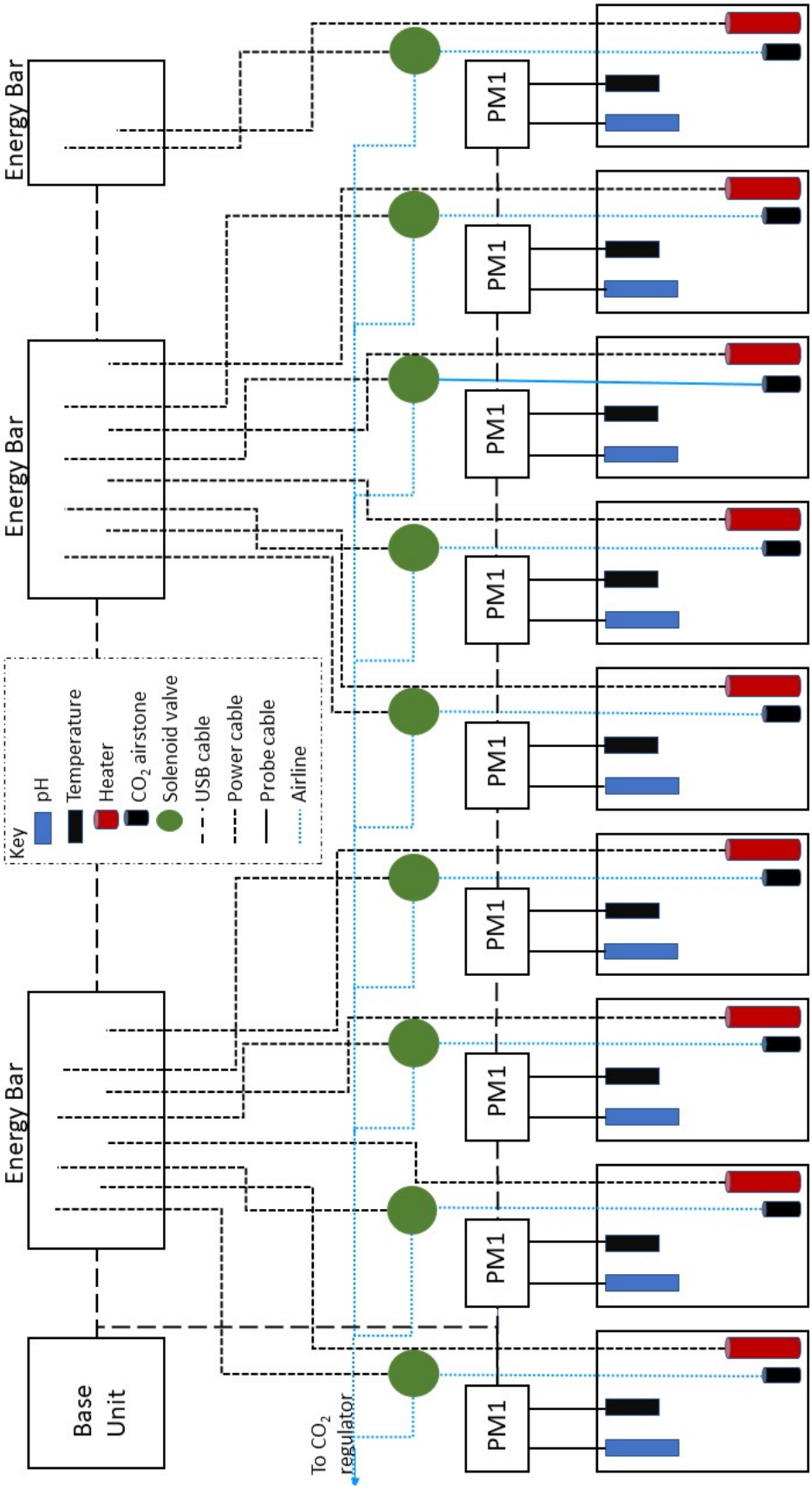

Energy Bar
Energy Bar
Energy Bar
Base Unit
PM1
PM1
PM1
PM1
PM1
PM1
PM1
PM1
Key
pH
Temperature
Heater
CO_2 airstone
Solenoid valve
USB cable
Power cable
Probe cable
Airline
To CO_2 regulator

Fig. 2. Schematic of the aquarium control system set-up. The nine aquaria are at the bottom of the page, under the PM1 modules which record the pH and temperature. The base unit is connected to the internet via wifi and accessed through an external computer. The base unit, energy bars, PM1 modules, and probes were all supplied by Neptune, the solenoid valves were manufactured by Milwaukee Instruments, and the heaters were manufactured by Aqueon.

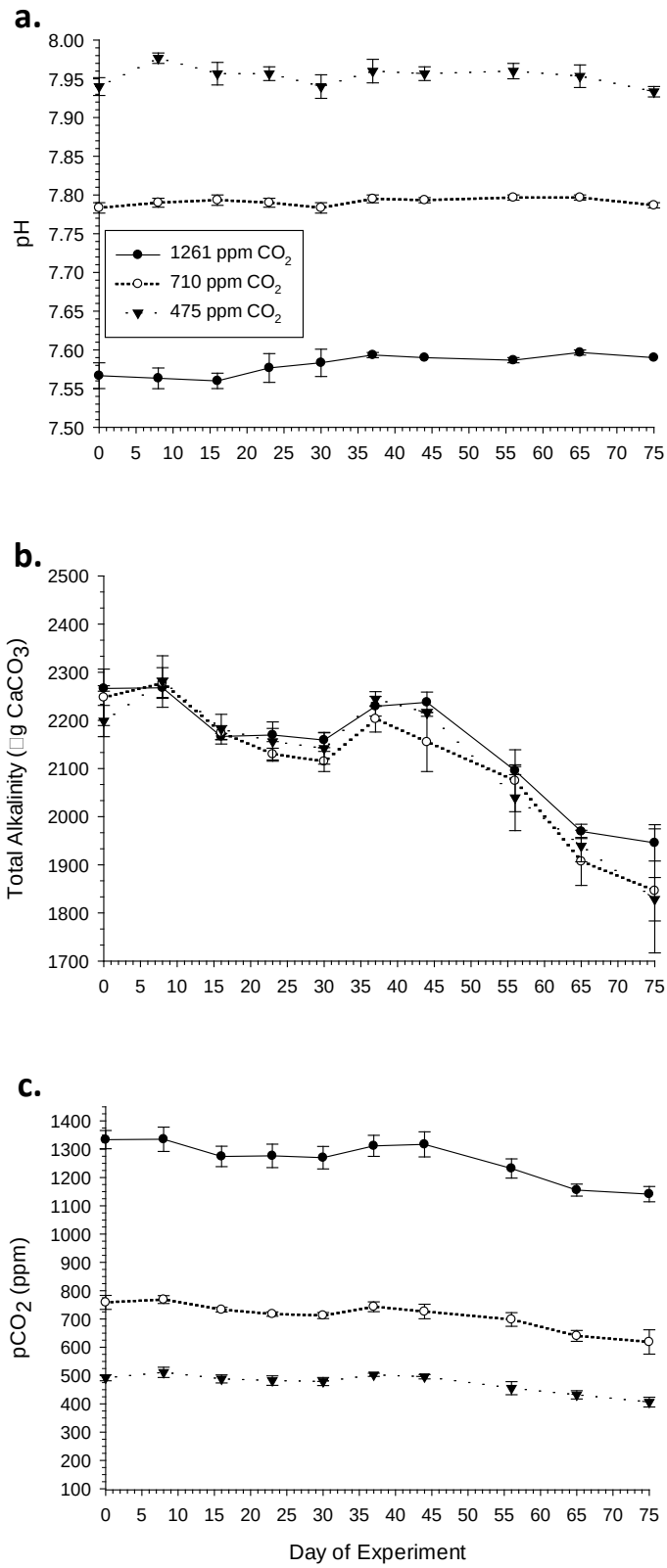

a.
pH
1261 ppm CO2
710 ppm CO2
475 ppm CO2
b.
Total Alkalinity (□g CaCO3)
c.
pCO2 (ppm)
Day of Experiment

Fig. 3. Mean (±SE) of three water chemistry variables, a) total pH, b) TA, and c) pCO_2 of ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 treatments over the 75-day experimental period. The values graphed are approximately every week, chosen from the 14 days of TA data analyzed. A decline in TA over the last 35 days, illustrated in graph (b), was due to changes in the chemical composition of the salt mix used.

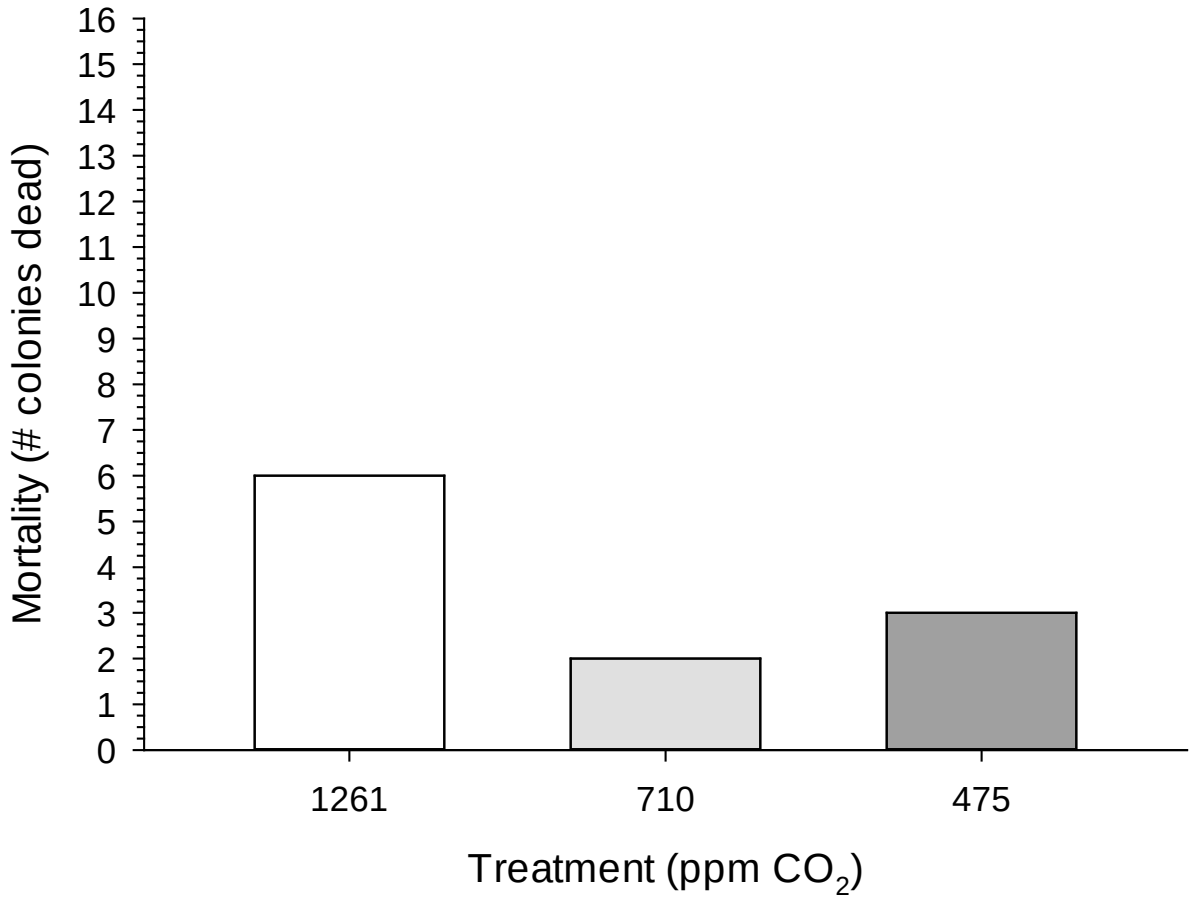


Fig. 4. Mortality of *O. arbuscula* recruits at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 treatments over 75 days. There were no differences between treatments. N = 48 in all treatments, with each colony treated as an independent replicate.

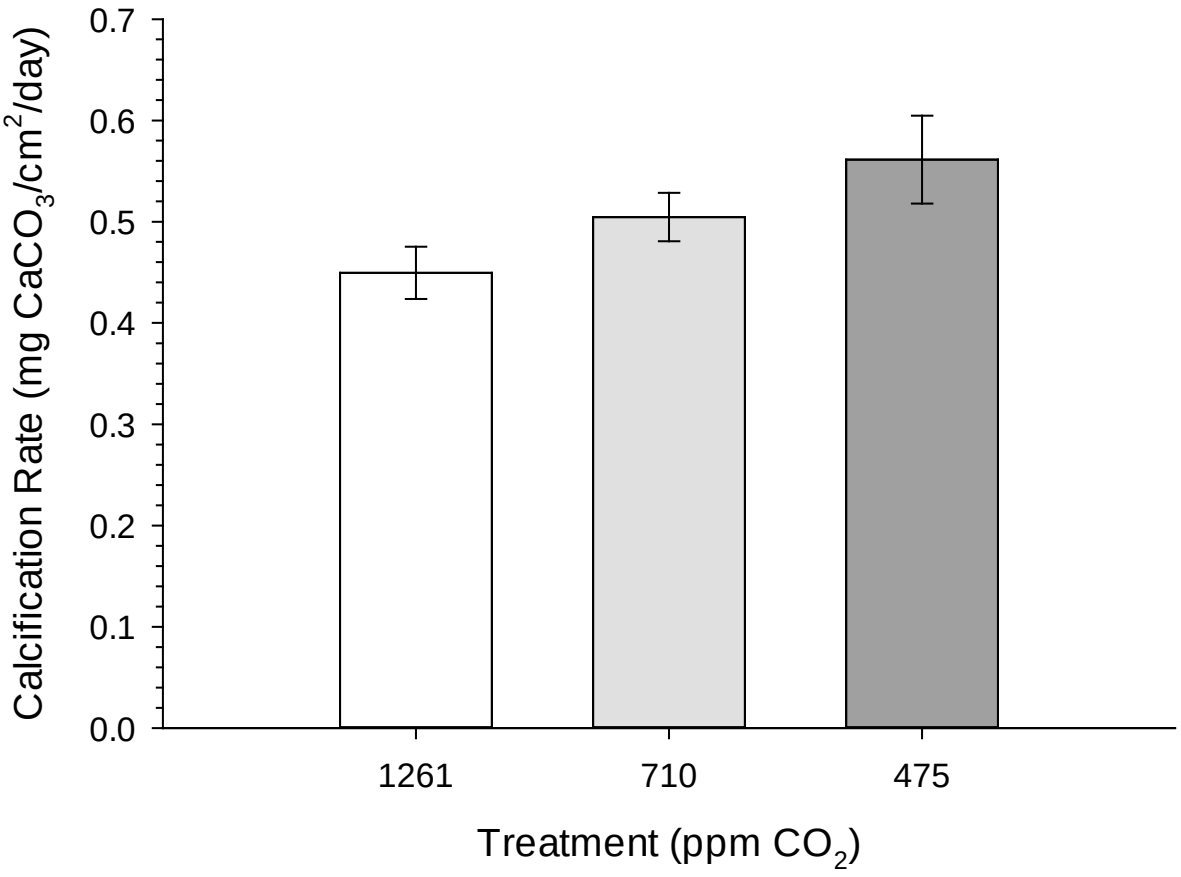


Fig. 5. Mean (±SE) calcification rates for *O. arbuscula* recruit pairs maintained at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 for 75 days. Calcification rates were not significantly different from each other, but there was a trend for depressed calcification with increased pCO_2. N=3 aquaria for each treatment, with 8 coral pairs in each aquarium. Aquaria within treatments were not significantly different from each other.

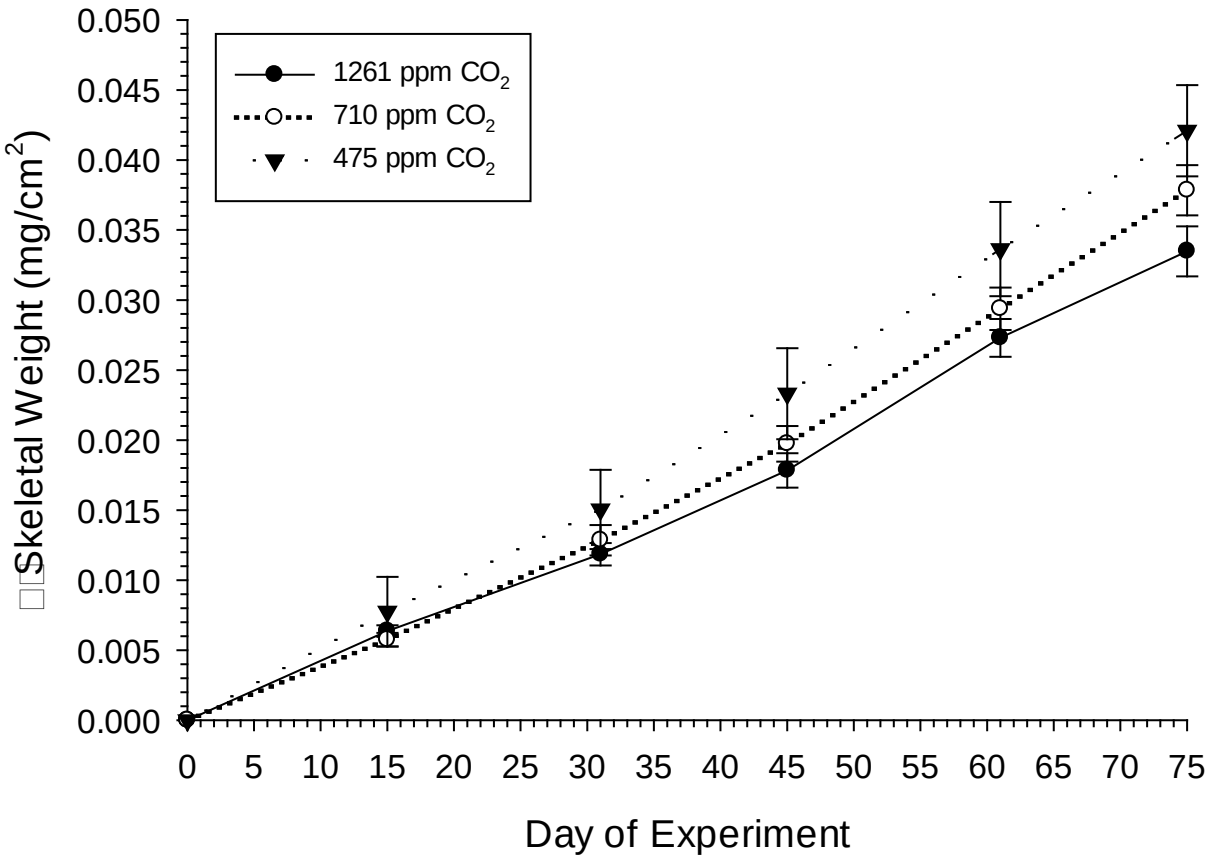


Fig. 6. Mean (±SE) calcification of O. arbuscula recruit pairs at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 treatments over a 75-day period. N=3 aquaria for all treatments.

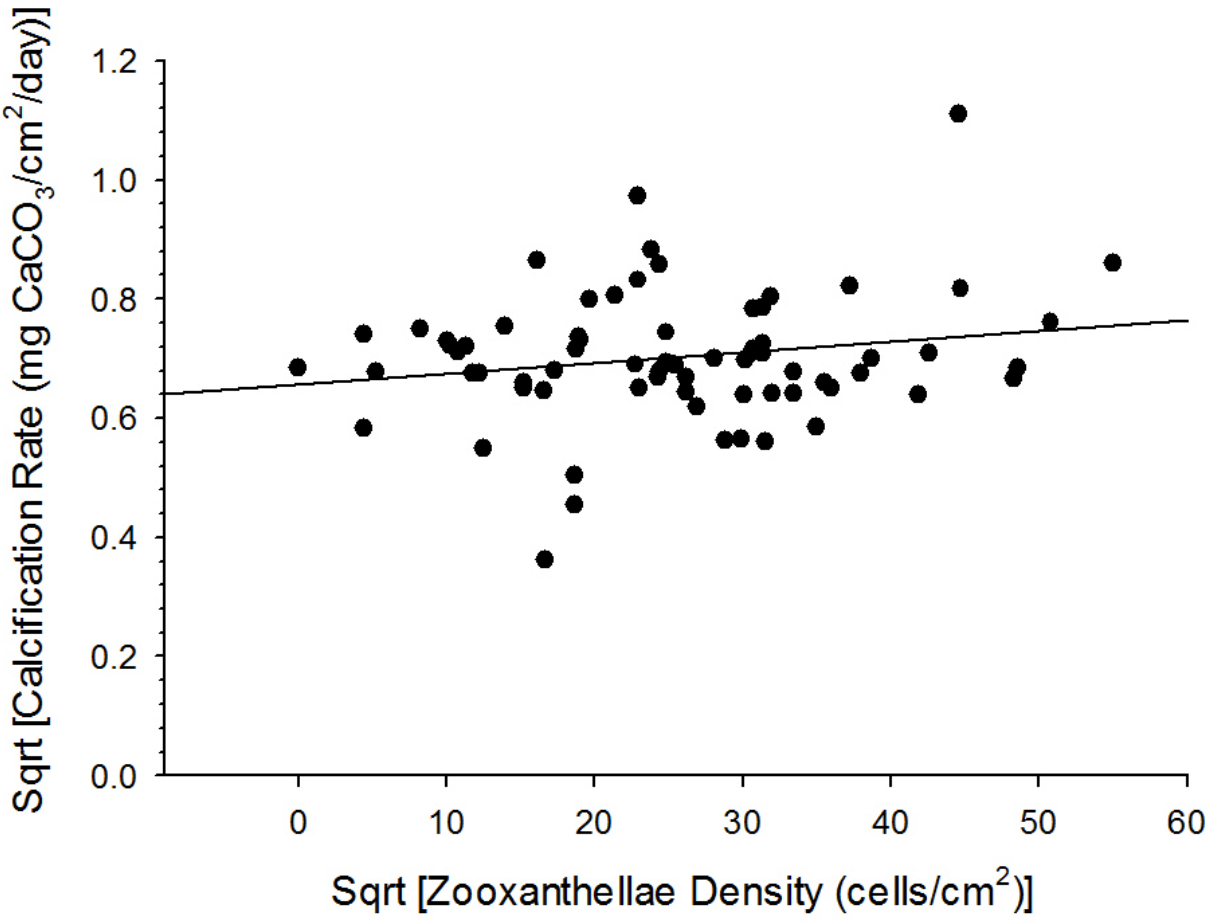


Fig. 7. The relationship between zooxanthella density and recruit calcification rate. Calcification rate was independent of zooxanthella densities (n=71; y=0.66+0.0018x; R^2=0.038; p=0.1021).

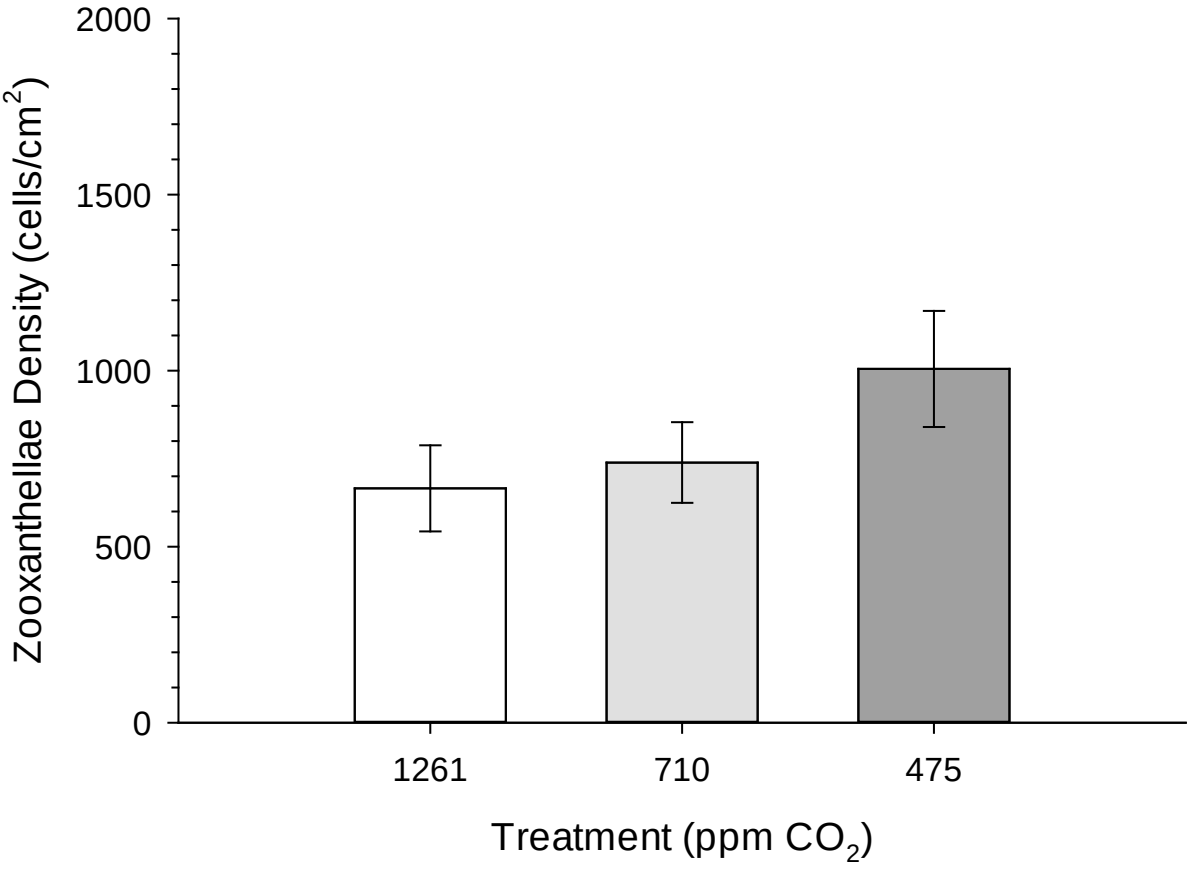


Fig. 8. Mean (±SE) zooxanthella density of *O. arbuscula* recruit pairs at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 for 75 days. There were no significant differences between treatments. N=3 aquaria for each treatment, with 8 coral pairs in each aquarium. Aquaria within treatments were not significantly different from each other.

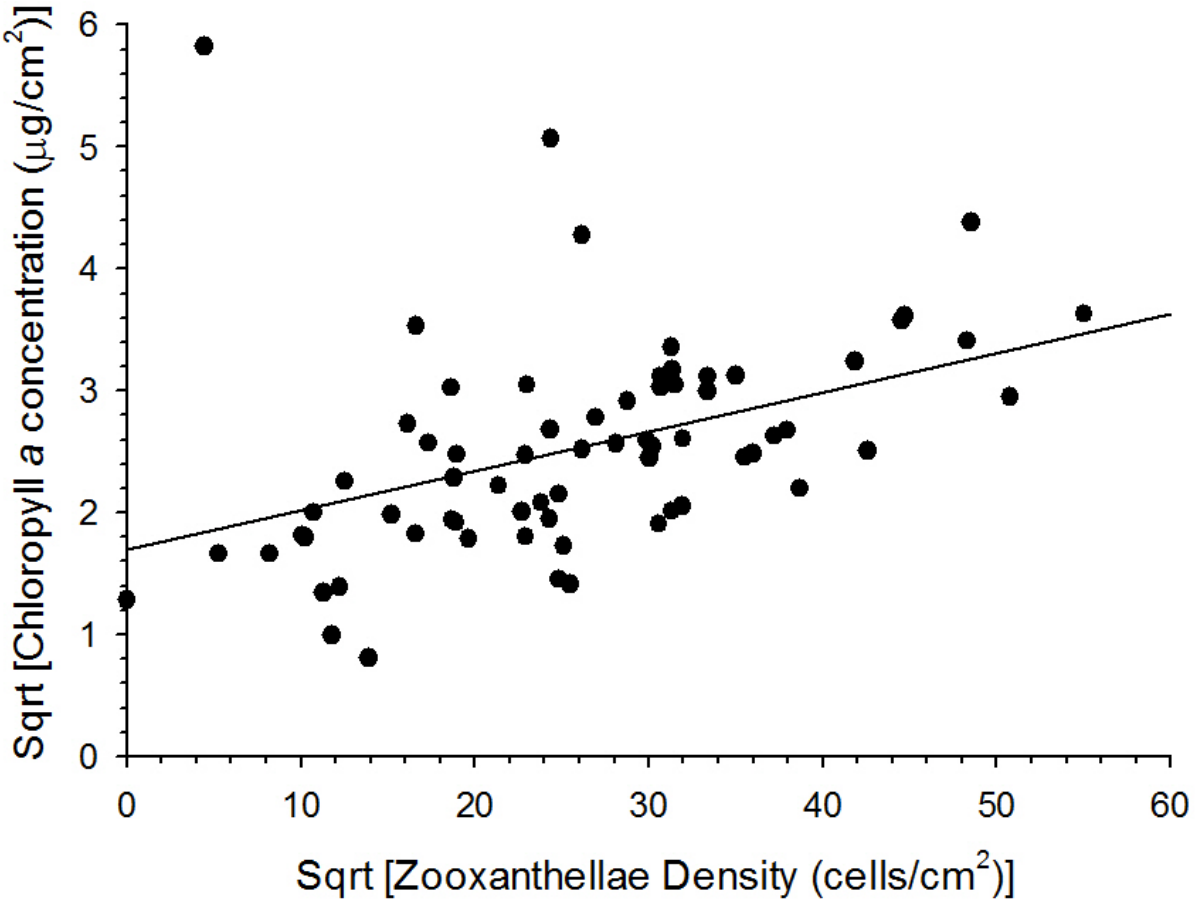


Fig. 9. The relationship between zooxanthella density and recruit chlorophyll *a* concentrations. Concentrations of chlorophyll scaled to coral surface area were dependent to zooxanthella densities, with a weak relationship (n=69; y=1.70+0.0323x; R^2=0.1785; p=0.0003).

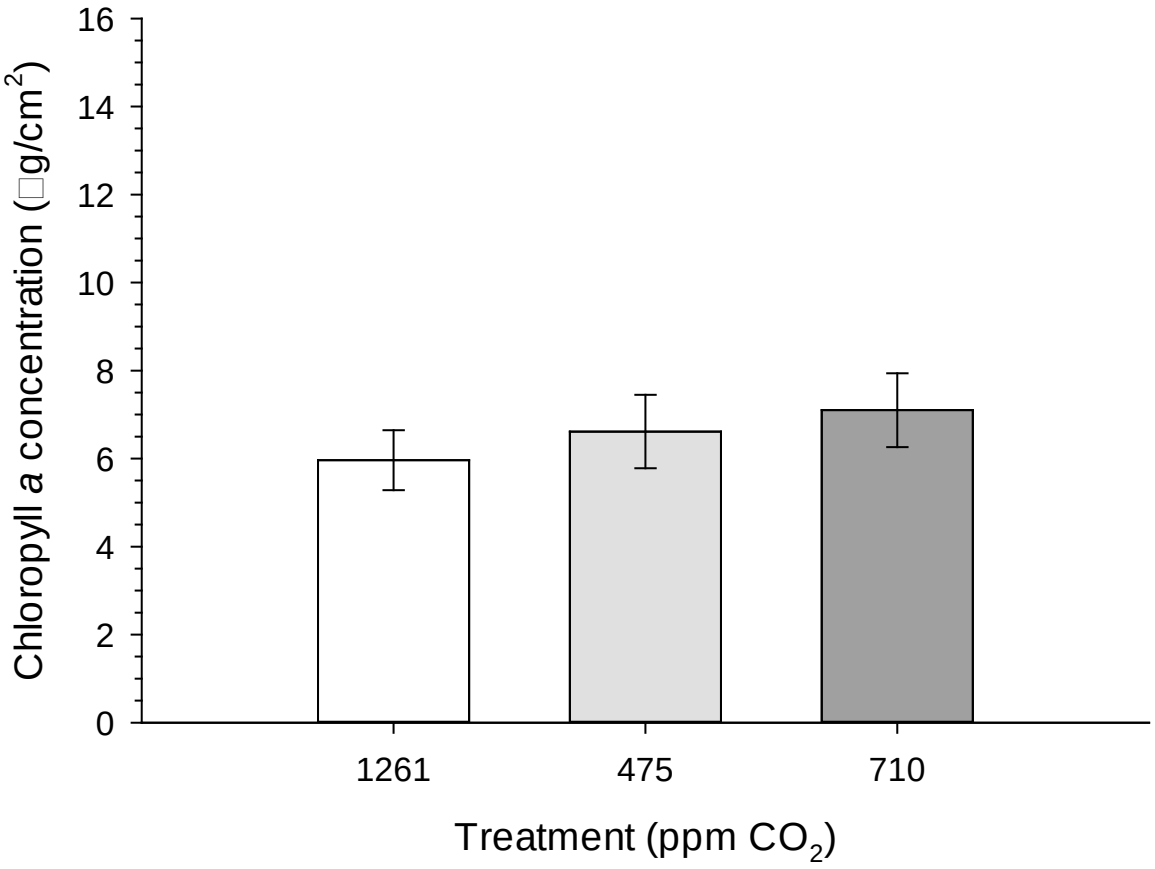


Fig. 10. Mean (±SE) chlorophyll *a* concentration of *O. arbuscula* recruit pairs at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 for 75 days. There were no significant differences between treatments. N=3 aquaria for each treatment, with 8 coral pairs in each aquarium. Aquaria within treatments were not significantly different from each other.

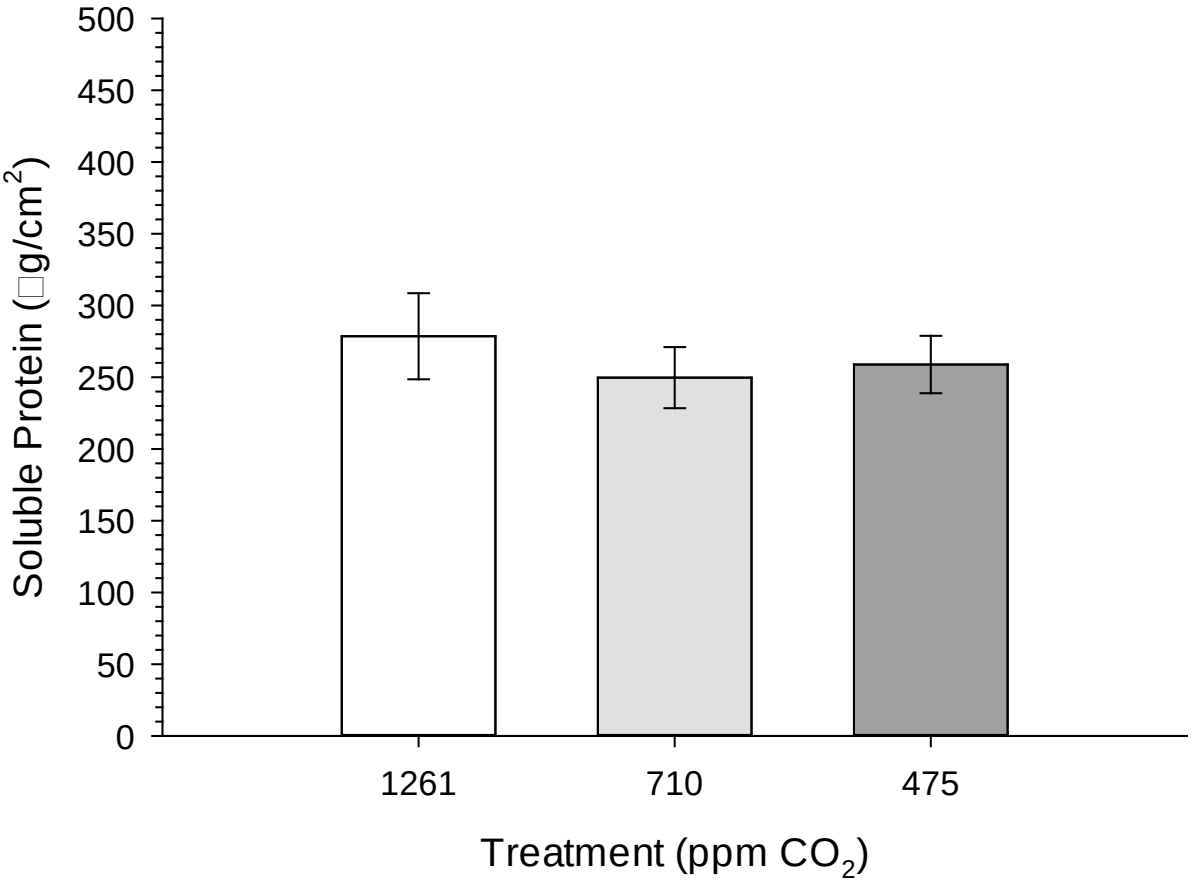
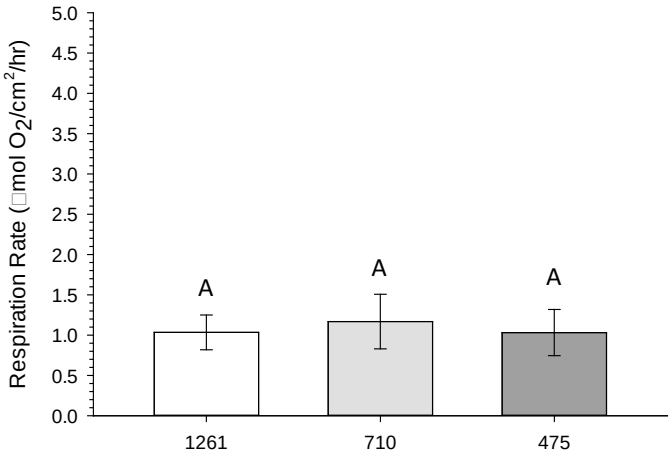
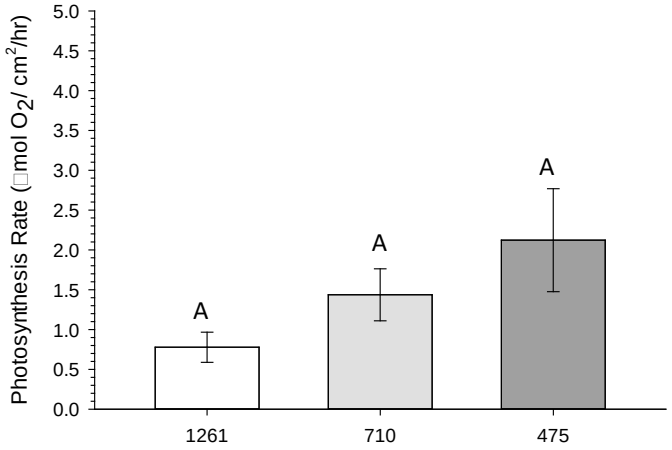


Fig. 11. Mean (±SE) soluble protein of *O. arbuscula* recruit pairs at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 for 75 days. There were no significant differences between treatments. N=3 aquaria for each treatment, with 8 coral pairs in each aquarium. Aquaria within treatments were not significantly different from each other.

a.


Respiration Rate (□mol O2/cm2/hr)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A
A
A
1261
710
475

b.


Photosynthesis Rate (□mol O2/ cm2/hr)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A
A
A
1261
710
475

c.

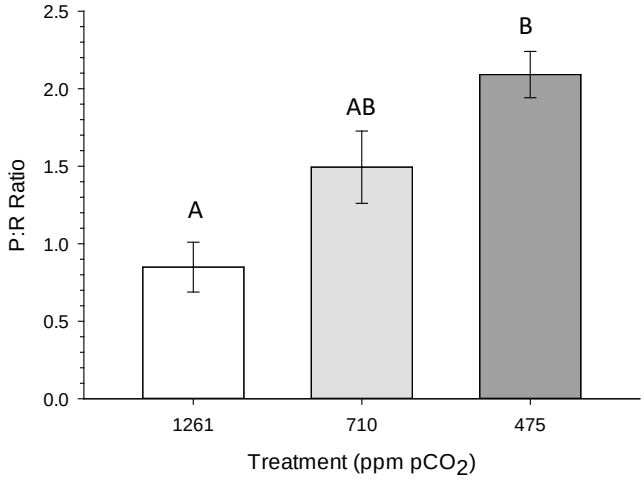

P:R Ratio
2.5
2.0
1.5
1.0
0.5
0.0
A
AB
B
1261
710
475
Treatment (ppm pCO2)

Fig. 12. Mean (±SE) of a) respiration rates, b) photosynthesis rates, and c) photosynthesis:resporation ratios of *O. arbuscula* recruit pairs at ambient (475 ppm), moderate (710 ppm) and high (1261 ppm) pCO_2 at the end of 75 days. Two coral pairs were analyzed per aquarium, but each pair was treated as an independent replicate for data analysis. Thus, n=6 for all treatments. Bars with same letter not significantly different.

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