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CORALS AND OCEAN ACIDIFICATION: INSIGHTS ON REEF COMMUNITY DEVELOPMENT AND CORAL CALCIFICATION IN AN ACIDIFIED OCEAN

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ABSTRACT

CORALS AND OCEAN ACIDIFICATION: INSIGHTS ON REEF COMMUNITY DEVELOPMENT AND CORAL CALCIFICATION IN AN ACIDIFIED OCEAN

ELIZABETH DERSE CROOK

As the surface ocean equilibrates with rising atmospheric pCO_2, the pH of surface seawater is decreasing with potentially negative impacts to coral calcification and coral reef ecosystems. This dissertation is composed of 4 individual studies that explore the impacts of ocean acidification on community reef development, coral calcification rates, and the acclimatization potential of corals to decreasing seawater pH. This is accomplished through *in-situ* field investigations on a tropical coral reef and laboratory experiments on temperate solitary corals.

In Chapters II-IV, I present findings from field investigations at Puerto Morelos, Mexico concerning the impact of *in-situ* declines in saturation state (Ω_{arag}) on a reef community. Chapter II is a survey of the impact of saturation state on coral species richness, abundance, and colony size. I observe that while corals are often found in under-saturated waters, species richness, number of individuals, and colony size all decrease with decreasing saturation state. The study concludes that impacts of ocean acidification vary widely by species and geographic distribution, but that overall coral coverage will decline significantly in the 21st century.

Chapter III explores the calcification rates of *Porites astreoides* corals in low and under-saturated waters and compares them to rates of colonies growing in control zones nearby. I conclude that decreases in saturation state are associated with significant declines in coral calcification, driven mainly by decreasing density of the skeletal material. Additionally, decreasing saturation state was associated with significant increases in the rate of bioerosion by boring organisms.

In Chapter IV, I address how ocean acidification may impact a reef ecosystem through a year-long recruitment experiment. I deploy limestone tiles in both low saturation and control zones and recover them at 3, 6, and 14 month intervals. Tiles in low saturation zones have up to 70% less coverage of calcifying organisms, coincident with an increase in fleshy algal coverage. Crustose and upright coralline algae are up to 90% less abundant on low saturation tiles after 14 months, despite their ability to establish on the tiles. These findings indicate that calcifying organisms, while physiologically tolerant of low saturation, are outcompeted by fleshy algae under ocean acidification conditions.

In Chapter V, I explore laboratory experiments on a temperate scleractinian coral, *Balanophyllia elegans,* to address how decreasing pH and level of nutrition impact coral calcification. In these experiments, I manipulate pCO_2 (410, 770, and 1220 μatm) and feeding frequency (3 days vs. 21 days) in a closed seawater system to address the energetic requirements of calcification in corals without the aid of the symbiotic dinoflagellate, zooxanthellae. Planulation rates were affected by food level but not pCO_2, while juvenile mortality was highest under high pCO_2 (1220 μatm) and

low food (21 day intervals). While net calcification was positive even at 1220 µatm (~3 times current atmospheric pCO_2), overall calcification declined by ~25-45%, and skeletal density declined by ~35-45% as pCO_2 increased from 410 to 1220 µatm. Aragonite crystal morphology changed at high pCO_2, becoming significantly shorter but not wider at 1220 µatm.

Combined, these chapters suggest that the response of organisms to ocean acidification will be highly species-specific, complex, and will depend on multiple factors, such as community interactions and feeding amount. There is, however, overwhelming evidence suggesting that coral calcification and reef accretion will decline significantly over the 21st century.

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I. Introduction

The increasing use of fossil fuels since the industrial revolution, combined with the effects of deforestation, has led to an overall increase in atmospheric carbon dioxide by nearly 40% over pre-industrial levels (Solomon *et al*. 2007, Doney *et al*., 2009). Currently at 400 ppmv, this number is expected to rise to between 800-1200 ppmv by the year 2100 (Caldeira and Wickett, 2005; IPCC, 2014). This rate of increase is at least an order of magnitude faster than has occurred for many millions of years (Doney and Schimel 2007), and approximately 30% of anthropogenic CO_2 is sequestered by the oceans (Sabine and Feely 2007, Sabine *et al*. 2004). Consequently, rising atmospheric pCO_2 increases aqueous pCO_2 concentrations and subsequently lowers the pH of the water (Caldeira and Wickett 2003, 2005; Feely *et al*. 2004, 2009; Orr *et al*. 2005). The reduction in ocean pH changes the calcium carbonate ($CaCO_3$) saturation state and alters seawater chemical speciation and biogeochemical cycles of many other elements and compounds. Popularly known as "ocean acidification," these changing balances in water chemistry are likely to impact many aspects of marine ecosystems, and are projected to be one of the most important environmental concerns of our time (Doney *et al*., 2009, Kleypas *et al*., 2006, Hoegh-Guldberg *et al*., 2007).

The primary goals of my research were to: 1) understand how changing ocean pH impacts the calcification of tropical and temperate scleractinian corals, 2) determine

the acclimation potential of reef-building corals to ocean acidification, and 3) analyze the community response of tropical coral reefs to ocean acidification. I addressed these concerns through a series of *in-situ* field investigations at Puerto Morelos, Mexico, and laboratory experiments at the University of California, Santa Cruz Long Marine Laboratory.

I.1. Ocean Acidification: The CO_2 Problem

Altered oceanic pH and resulting carbonate chemistry are described by the equations:

$$CO_{2\,(atmos)} \leftrightarrow CO_{2(aq)}$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

$$H^+ + CO_3^{2-} \leftrightarrow HCO_3^-$$

$$CO_2 + H_2O + CO_3^{2-} \leftrightarrow 2HCO_3^- \qquad \text{(Eq.1)}$$

Atmospheric CO_2 is incorporated into the surface ocean and equilibrates within a year. In seawater, aqueous CO_2 reacts with water to form carbonic acid, which further dissociates into bicarbonate ions by releasing hydrogen ions (H^+). Protons produced by this process react with carbonate to form more bicarbonate. Accumulation of H^+ lowers the pH of the water and decreases the concentration of carbonate ions which are critical for calcification in marine organisms. Currently, the surface ocean has an average pH of approximately 8.1; however, it is estimated that over the next 100 years this value will decrease by ~0.4 pH units (Orr *et al*., 2005; Caldeira and Wickett, 2005). This reduction in pH will be driven by an increase of 150% in H^+

concentration, combined with a decrease in CO_3^{2-} ion concentration of 50% (Doney *et al*., 2009). A decline in surface CO_3^{2-} concentration is expected to have major impacts on numerous calcifying organisms (e.g. plankton, corals, urchins, coralline algae, and countless other marine invertebrates). Specifically, the rate at which $CaCO_3$ can precipitate is strongly influenced by its saturation state (Ω). The saturation state of $CaCO_3$ in seawater is described by the equation:

$$\Omega = [Ca^{2+}]\,[CO_3^{2-}]/\,K'_{sp} \qquad \text{(Eq. 2)}$$

K'_{sp} is the apparent solubility product, which depends on temperature, salinity, pressure, and whether the mineral deposited is calcite or aragonite (aragonite is 50% more soluble than calcite) (Doney *et al*., 2009). Because Ca^{2+} has a long residence time in the oceans and the concentration is relatively constant (10.3mmol/kg) over anthropogenic timescales, as $[CO_3^{2-}]$ decreases, the saturation state will decline and is expected to hinder organisms' abilities to form new shell or skeleton. Indeed, reduced calcification rates have been observed following acidification in a variety of calcareous organisms, although the response is varied and highly species specific (Kroeker *et al*., 2012).

It is predicted that corals, particularly tropical reef-building species, will be particularly sensitive to declining pH (Hoegh-Guldberg *et al*., 2007). Coral reef ecosystems are biologically diverse and vital to the world economy by providing essential ecosystem services to people across the globe. Ocean acidification is a pervasive threat to these critical marine habitats, the loss of which could be

detrimental to fisheries, biochemical research, building materials, coastal protection, and tourism.

Much of this dissertation is a response to the urgent need for *in-situ* field investigations on the impacts of ocean acidification on marine organisms, with a particular emphasis on calcifying corals. While laboratory studies are useful in describing species-specific impacts, most laboratory investigations cover a single species, are of limited duration, and only concern a single stage of life history. *In-situ* investigations, therefore, are particularly insightful because they can span entire ecosystems, include all stages of development, and include multiple generations of organisms.

In Chapters II-IV, I explore the responses of tropical corals and coral reef communities to acidification through a series of *in-situ* field investigations, and address the acclimation potential of individual species. In Chapter II, I describe a field survey at Puerto Morelos, in a back-reef lagoon that experiences localized drops in pH and aragonite saturation state at sites of submarine groundwater discharge (locally referred to as "ojos"). Using these "ojos" as indicators of future responses to acidification, I suggest that while certain species appear to be relatively tolerant of low pH conditions, corals are unlikely to acclimate to future acidification.

Chapter III is a follow-up study on *Porites astreoides*, one of only three coral species living at the ojo sites. I take cores from corals at the ojos and compare their skeletal density, extension, and calcification rates to corals growing in ambient conditions. Using these cores and a linear regression model, I project a drop in calcification of up to 15% by the year 2065.

Chapter IV addresses how development of new reef communities is impacted by a reduction in saturation state, and how community dynamics alter the response of individuals. In this investigation, recruitment substrates were deployed for up to one year at the ojos to determine the impact of acidification on a benthic back-reef community. Fleshy algae outcompete coralline algae under low saturation conditions, and I project declines in calcification of up to 80% in extreme acidification conditions.

I.2. Coral Biomineralization

To understand why corals may be particularly impacted by ocean acidification, it is important to review the basic mechanics behind coral calcification. Due to several kinetic barriers that prevent spontaneous formation of calcite or aragonite in seawater, corals nucleate and grow $CaCO_3$ crystals in highly regulated compartments that are isolated from the external fluid (Cohen and Holcomb, 2009). The coral polyp is composed of two distinct layers, the endoderm and ectoderm, and the part of the ectoderm that sits atop the coral skeleton is known as the calicoblastic layer. While it

is unclear exactly what role the calicoblastic layer plays in calcification, it is commonly believed that these ectodermal cells, as well as the underlying organic matrix, control the biomineralization process (Cohen and McConnaughey, 2003). Calcification occurs via the following equation:

$$Ca^{2+} + CO_2 + H_2O \leftrightarrow CaCO_3 + 2H^+ \qquad \text{(Eq. 3)}$$

Responses of calcifying corals to ocean acidification depend on species-specific energy allocations for calcification. Corals expend energy to remove protons from their calcifying compartments, the extracellular medium between the calicoblastic layer and the skeleton below (Al Horani *et al*., 2003; Cohen and McConnaughey, 2003). Removing protons from this closed compartment facilitates calcification by increasing the pH and aragonite saturation state (Ω_{arag}) in the calcifying fluid. Lower saturation in the external seawater requires corals to expend more energy to remove excess protons (Ries, 2011; McCulloch *et al*., 2012).

There are two possible models for biomineralization under ocean acidification conditions (Cohen and Holcomb, 2009, Gagnon *et al*., 2013) that will be discussed in this dissertation. In the first, called the "finite alkalinity model," corals expend energy to expel a fixed number of protons. In the case of ocean acidification, a decrease in saturation state in the seawater will lead to a decrease in the final pH of the calcifying fluid. With the "finite" model, negative effects in calcification would be seen with relatively small downward shifts in seawater saturation. In the second scenario, called the "pH control model," corals pump protons until a target pH is reached in the

calcifying fluid, regardless of the pH of the external seawater. In the case of ocean acidification, the pH control model implies that the coral will expend more and more energy to pump excess protons out of the calcifying space. That is, the energy budget of the coral is flexible and enables the coral to maintain calcification despite acidification, given an increase in energetic resources. Chapters III and V specifically explore the energy budget of tropical and temperate scleractinian species, and discuss coral acclimation and adaptation potential in ocean acidification conditions.

In Chapter III, I use the coral cores as evidence to suggest that *Porites astreoides* can not maintain calcification rates in acidification conditions, despite ample nutritional sources. In Chapter V, I explore the response of temperate, solitary scleractinian corals that lack photosynthetic symbionts (*Balanophyllia elegans*) to ocean acidification. I used these corals as a model organism for investigating the energy requirements of calcification in an 8-month laboratory experiment that varied food quantity and measured its impacts on calcification during acidification. I conclude that feeding rate had a greater impact on calcification than pCO_2, although declines in calcification of up to 45% were still observed when atmospheric pCO_2 tripled.

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II. Calcifying Coral Abundance Near Low pH Springs: Implications for Future Ocean Acidification

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Summary

Rising atmospheric CO_2 and its equilibration with surface ocean seawater is lowering both the pH and carbonate saturation state (Ω) of the oceans. Numerous calcifying organisms, including reef-building corals, may be severely impacted by declining aragonite and calcite saturation, but the fate of coral reef ecosystems in response to ocean acidification remains largely unexplored. Naturally low saturation (Ω~0.5) low pH (6.70-7.30) groundwater has been discharging for millennia at localized submarine springs (called "ojos") at Puerto Morelos, México near the Mesoamerican Reef. This ecosystem provides insights into potential long term responses of coral ecosystems to low saturation conditions. In-situ chemical and biological data indicate that both coral species richness and coral colony size decline with increasing proximity to low-saturation, low-pH waters at the ojo centers. Only three scleractinian coral species (*Porites astreoides*, *Porites divaricata*, and *Siderastrea radians*) occur in undersaturated waters at all ojos examined. Because these three species are rarely major contributors to Caribbean reef framework, these data may indicate that today's more complex frame-building species may be replaced by smaller, possibly patchy, colonies of only a few species along the Mesoamerican Barrier Reef. The growth of these scleractinian coral species at undersaturated conditions illustrates that the response to ocean acidification is likely to vary across species and environments; thus, our data emphasizes the need to better understand the mechanisms of calcification to more accurately predict future impacts of ocean acidification.

II.1. Introduction

Atmospheric CO_2 is currently on the rise and its equilibration with surface seawater is expected to reduce the pH of the surface oceans by approximately 0.4 pH units by year 2100 (Caldeira and Wicket 2005; Orr et al. 2005; Doney et al. 2009). Numerous calcifying organisms, including reef-building corals, may be severely impacted by this reduction in pH, which will lower the aragonite and calcite saturation state (Ω) and make skeletal and shell formation for many organisms more difficult (Fine and Tchernov 2007; Anthony et al. 2008; Doney et al. 2009). Recent field studies in the Mediterranean and Papua New Guinea have demonstrated strong impacts of low pH related to volcanic CO_2 vents on both individual organisms and community structure (Hall-Spencer et al. 2008; Cigliano et al. 2010; Dias et al. 2010; Rodolfo-Metalpa et al. 2010; Fabricius et al. 2011). However, additional field studies under different natural conditions are necessary to ascertain a wider range of potential long term impacts of ocean acidification on communities and ecosystem processes (Doney et al. 2009; Riebesell et al. 2010).

Along the eastern coast of the Yucatán Peninsula, México (Fig. II.1) nearshore springs, referred to locally as "ojos," discharge naturally low-pH, low carbonate saturation groundwater ($\Omega = [Ca^{2+}][CO_3^{2-}]/K'_{sp}$). These highly localized springs are a natural feature of the karst terrain, have been continuously discharging water for millennia (Beddows et al. 2002), and have been the focus of many studies since the early 1990's (Tussenbroek 1995; Ruiz-Renteria et al. 1998; Carruthers et al. 2005).

Discharge from these "ojos" is markedly more acidic (pH = 6.70-7.30 total scale) and less saturated (Ω_{arag} = 0.30-0.97 at ojo centers) than the surrounding ocean water (Ω_{arag}=3.60), and they occur in close proximity to one of the Caribbean's largest coral reef ecosystems (the Mesoamerican Barrier Reef). Thus, the ojos of the Yucatán Peninsula provide a natural laboratory for examining the long-term impacts of low saturation waters on specific organisms and the coastal ecosystem. Specifically, much can be learned from this site about the response of calcifying organisms exposed to reduced saturation states over time scales much longer than the life span of individual organisms. The conditions creating low pH seawater at the ojos differ from those of the ocean acidification scenario: specifically, the discharging water at the ojos is derived from high CO_2 concentrations associated with brackish water that has interacted with soil and limestone, and is thus characterized by low pH, high Ca^{2+} (salinity normalized), high dissolved inorganic carbon (DIC), and high total alkalinity (TA). However, the organisms residing at the ojos have been exposed to low-pH and low aragonite saturation as is predicted for future ocean acidification (Table II.1), and these environments provide an opportunity to study *in situ* community impacts on corals exposed to low carbonate saturation conditions and often extreme drops in pH for extended time intervals.

At least thirteen ojos lie approximately 500 m offshore within the National Maritime Park at Puerto Morelos, in a shallow lagoon approximately 5 m deep (Fig. II.1). The ojos range from 10 m long "fractures" to small circular depressions (seeps) only a few

centimeters across (Fig. II.2). Based on monitoring over two and a half years (April 2008 to September 2010), discharge from the ojos is continuous with a combined discharge flux (estimated using excess ^{224}Ra measurements) reaching as high as ~800,000 m^3 h^{-1} (Derse et al. 2008). Discrete water samples taken during three different sampling events (June 2009, November 2009, and September 2010) indicate that the pH (and other water chemical characteristics) at each ojo center varied on tidal time scales. However, the water discharged at the ojo center remained undersaturated during all sampling events (Table II.1). Continuous pH monitoring over two months using a SeapHOx sensor supports this conclusion (Hofmann et al. 2011). The relationship between water chemistry and benthic biota (identity, density, size,) was investigated at 10 ojos dominated by rocky substrates and characterized by similar temperature, salinity, light, and pH conditions over the three different sampling events. We report the calculated saturation states (Pierrot et al. 2006) based on measured DIC, TA, and nutrient concentrations for each discrete sample using CO_2Sys. For samples with higher than expected Ca^{2+} concentrations, conservative aragonite saturation values were calculated based on measured Ca^{2+} concentrations (Table II.1).

II.2. Materials and Methods

The area of influence of the low pH waters was determined by direct measurement of physiochemical parameters either in-situ (temperature, salinity, pH) or from discrete water samples (DIC, TA, salinity and nutrients). In the case of circular seeps (see Fig

2.3e), water samples were collected at 0.25 m intervals along transects placed at right angles and intersecting over the center of each ojo: transects were at least 4 m from the ojo center in all directions. In the case of fractures (see Fig II.3a, II.3d), samples were taken along one long transect (up to 10 m) following the fracture line, and samples were also collected at 5 or more cross-transect lines perpendicular to the main fracture. Divers collected the water in syringes that were immediately transported to a waiting boat for filtration, poisoning (for DIC and TA), and storage using standard operating procedures outlined by Dickson et al. (2007).

Ecological surveys commenced after the water was sampled to reduce the risk of contamination or mechanical mixing by divers. Data from benthic biota (identity, density, size) around each ojo were scored in contiguous 0.25 x 0.25 m quadrats along the transect lines as described above (Fig. II.4). Particular care was taken to record coral size and position within each transect.

DIC samples were analyzed in triplicates on a model 5011 CO_2 Coulometer (UIC, Inc), and care was taken to ensure that the samples were not exposed to the atmosphere prior to analysis (measurement error of ± 3μM). The TA samples were run using an automated, open cell, potentiometric titration procedure (measurement error ± 2μM). Certified CO_2 reference material (Batch 90) from the Andrew Dickson lab at UC San Diego was used to ascertain the quality of results obtained. Nutrient (NO_3^-, NO_2^-, NH_4^+, Si and PO_4^{-3}) analyses were run on a flow injection autoanalyzer

(FIA, Lachat Instruments Model QuickChem 8000) using standard procedures. Ca^{2+} concentrations were determined via ICP-OES (Perkin Elmer Optima 4300) using standard dilution and internal spikes. The carbonate system (carbonate saturation) was calculated from the measured parameters (DIC, TA, pH, temperature, salinity, Ca, and nutrients) using the program CO_2Sys (see Table II.1).

Total coral area was calculated based on observed measurements from the field and reported as area coverage per 25 cm^2. Due to differences in the area influenced by the ojo waters between sites (e.g. discharge flux was different at each ojo as was the area impacted by the discharging water), statistical analyses were conducted for data grouped based on the calculated saturation state. Samples were grouped by "supersaturation" ($\Omega > 2.5$), "low saturation" ($1 < \Omega < 2.5$), and "undersaturation" ($\Omega < 1$) at each site.

II.3. Results

At all 10 ojos sampled, the center of each discharge point (Fig. II.3) was undersaturated with respect to aragonite (i.e. $\Omega_{arag}<1$), and low saturation conditions (i.e. $\Omega_{arag} < 2.5$) were seen close to the discharge area. Saturation values increased rapidly with distance from the ojo center (Fig. II.3). While waters generally reached saturation ($\Omega_{arag} = 1$) within 0.5 m of the center of discharge, saturation values below 2.5 were observed up to 2 m from the ojo (ambient Ω in the lagoon was 3.60; see Fig. II.3). Salinities at the ojo center were always lower than ambient due to the brackish

discharge, but not lower than 25. Despite these low salinities, Ca^{2+} concentrations were generally somewhat higher than expected from simple dilution of seawater to the measured salinities, because Ca^{2+} was added to the groundwater from to the dissolution of limestone (Fig. II.5). DIC and TA concentrations were highest at the center of discharge and decreased with distance from the discharge site. TA and DIC were correlated (R^2=0.86) (Fig. II.6). Dissolved nutrient (nitrate, ammonium, phosphate, silica) concentrations ranged from approximately 2 to 10 times ambient (Derse et al. 2008).

Calcifying organisms such as corals, coralline algae, and calcifying macroalgae were often present in under-saturated or low saturation waters close to the ojos. Here we focus on calcifying corals, which were present where $\Omega_{arag} < 2.5$ at all 10 ojos sampled. Only three scleractinian species (*Porites astreoides*, *Porites divaricata*, and *Siderastrea radians*) and one hydrozoan "fire coral" (*Millepora alcicornis*) were observed where $\Omega_{arag} < 2.5$. All of these species have aragonitic skeletons. Another six scleractinian coral species (*Diploria, Montipora, Montastraea, Agaricia, Porites,* and *Favia*) were present near the ojos, but only where the water saturation was above 2.5 ($\Omega_{arag} > 2.5$). These distributions may be evidence that certain calcifying coral species are more tolerant of low saturation waters than other species, with potential implications for differential survival under future CO_2 projections. A goodness-of-fit analysis indicates that the number of species (i.e., species richness) increased

significantly as saturation values increased with distance from the ojo centers ($p < 0.0001$; Fig. II.7).

To determine whether saturation state affected the sizes of coral colonies living near the ojos, we compared colony sizes (measured as the plane area) of the three scleractinian species in under-saturated ($\Omega_{arag} < 1$) plus low saturation ($1 < \Omega_{arag} < 2.5$) waters with sizes of the same species at control sites in super-saturated waters where $\Omega_{arag} > 2.5$ (close to, but outside the influence of discharge). The size of *Siderastrea radians* colonies did not differ significantly between the saturation levels; however, *Porites astreoides* colonies in under-saturated and low saturation waters near ojos were significantly smaller than *Porites astreoides* colonies in super-saturated water (ANOVA, $p = 0.05$; Fig. II.7). We then compared the sizes of all colonies present where $\Omega<2.5$ to the sizes of all coral species found in supersaturated waters, and found that the colonies in less saturated waters were also significantly smaller (ANOVA, $p = 0.03$; Fig. II.7). We note that this last comparison provides only a qualitative analysis, as the comparison is across species with different morphologies. Combined, our data suggests that as saturation levels approached maximum ambient values, both the number of species present and the average size of individual colonies of *Porites astreoides* increased significantly and that in general the coral colonies tend to be larger away from the ojo.

When abundances (number of colonies) of *Porites astreoides* and *Siderastrea radians* in low saturation ($\Omega_{arag} < 2.5$) and supersaturated ($\Omega_{arag} > 2.5$) waters were compared, the densities of these individual species did not vary with saturation state. However, the number of coral colonies per unit area (number of colonies per 0.25 cm^2) of all species combined was significantly greater away from the ojos (goodness-of-fit; $p < 0.001$; Fig. II.7). This is attributed to the increase in species number as supersaturation was reached.

II.4. Discussion

Ecological surveys at the ojo sites indicate that certain scleractinian coral species can grow in undersaturated conditions. Thus, these species may be more tolerant of low pH and low aragonite saturation conditions, and hence more resistant than other species when exposed to changing oceanic pH and carbonate saturation. However, the number of species that can survive at these low saturation conditions is limited compared to the species richness of the surrounding area. These findings are generally consistent with those of Fabricius et al. (2011) from CO_2 vents in Papua New Guinea, where the diversity and abundance of structurally complex corals was reduced threefold at low pH, yet *Porites* corals were still found at pH below 7.7 and aragonite saturation of 2.9. At Puerto Morelos, the conditions are more extreme as the water is often under-saturated or has much lower saturation values than 2.9, yet *Porites astreoides* and *Siderastrea radians* corals are still abundant. Therefore, the Puerto Morelos site demonstrates that certain coral species may tolerate extreme

acidification events and still maintain their ability to calcify. While we did not measure calcification rates during his study, we note that the ojos are part of a complex and elaborate underground conduit system that has developed over millennia in this karstic terrain. The seepage at these sites has therefore been continuous for an extended period of time compared to the average age of coral colonies. Thus, the corals settled, calcified, and the colonies grew within the plume of low pH groundwater discharge. This is different than the more ephemeral volcanic vent sites, and therefore the ojos represent areas where the ecosystem had ample time to adapt and evolve exposed to low pH conditions.

Although the coral species found at the ojo sites (*S. radians*, *P. astreoides*, and *P. divaricata*) all occur on reef structures, they are rarely major contributors to the framework of the Meso-American Barrier Reef: thus, while their presence is encouraging when considering the future of these specific scleractinian species, there are severe implications for the future of reef ecosystems and the many organisms that rely on structurally complex corals to build the reef framework. Specifically, our data suggests that as seawater saturation nears 2.5, today's larger, dominant, framework-building corals of the Meso-American Reef (e.g., *Acropora, Montastraea*) may be replaced by smaller, patchily distributed colonies of only a few species.

While only a limited number of coral species live in areas exposed to the low pH groundwater, their presence in under-saturated waters raises interesting questions.

Physiological and/or genetic adaptations allowing corals and other calcifying organisms to persist under low saturation, low pH conditions are largely unknown and are currently under investigation. One suggestion is that the energy allocated to calcification in such conditions may come at the expense of other metabolic activities and result in lower growth rates (Atkinson et al. 1995; Jokiel et al. 2008; Cohen and Holcomb 2009); another idea is that high nutrient concentrations could provide energetic resources that may offset deleterious effects of high CO_2 (Jokiel et al. 2008; Cohen and Holcomb 2009). Laboratory experiments with *Astrangia, Occulina, Porites, Montipora,* and *Favia sp*. show that while calcification rates were reduced under low saturation ($\Omega \sim 1.5$) conditions, the addition of inorganic nutrients or food under the same low saturation conditions enabled the corals to maintain 75-100% of their calcification rates (Langdon and Atkinson 2005; Ries et al. 2009; Cohen and Holcomb 2009; Holcomb et al. 2009). These ideas are consistent with our field observations that certain coral species may survive in undersaturated waters when nutrient concentrations are high (ojo waters had 2-10 times higher nutrient concentrations than surrounding water). The interplay of nutrient availability, low saturation conditions, and calcification in corals should be further investigated under natural conditions as this may be important for predicting future coral distribution and survival.

Another potential explanation for the survival of some coral species in the immediate vicinity of the ojos is that daily or seasonal fluctuations in discharge may periodically

expose the corals to ambient waters with high saturation levels. Our samples were taken on three field trips over a 15-month period and were monitored continuously over two months, and pH levels were nearly always low at ojo sites where the three tolerant scleractinian species were present (Hofmann et al. 2011). However, it is possible that the groundwater discharge fluxes do vary over time scales we have not captured, and that these corals experience intermittent relief from low pH, low aragonite saturation waters. Semi-permanent sensors installed over a whole year will enable us to determine the consistency of saturation levels around the corals. If fluctuations in discharge do impact saturation conditions, we will be able to estimate possible response thresholds for the coral species living nearest to the ojo centers (e.g., minimum duration or fraction of time spent in supersaturated conditions required for corals to survive).

In natural environments it is not possible to entirely exclude the impact of other variables, and for the ojos specifically the impact of lower salinity, on the observed coral distribution. However, previous studies suggest that many species are able to withstand osmotic stress with limited harmful effects when exposed to lower than ambient salinities (Coles and Jokiel, 1978; Hoegh-Guldberg and Smith, 1989; Xiubau et al. 2009). In fact, Coles and Jokiel (1978) showed that salinities as low as 25 in themselves were insufficient to have any negative impacts on *Montipora* sp., a coral found in abundance along the Puerto Morelos coast. Here, we only present data from ojos with salinities consistently above 25. As *Montipora* and many other corals were

commonly observed at the control sites, yet not within the low saturation zones, this suggests that salinity itself is insufficient to explain the observed distribution patterns. While the impact of multiple stressors (i.e. high temperature, light, and/or sedimentation) can compound the salinity factor and cause negative responses across species (Coles and Jokiel; Hoegh-Guldberg and Smith; Lirman and Manzello, 2009; Xiubau et al.), these discharge sites actually experience lower than ambient temperatures while light and sedimentation levels remain the same at the ojos and at the control sites. Therefore, by only including data from ojos that have salinities consistently higher than 25, we have attempted to control for, if not entirely negate, the impact of salinity on coral distribution found at Puerto Morelos.

This work illustrates that while the effects of ocean acidification on coral reefs and other calcifying organisms may be severe, the impacts will differ considerably across various species and ecosystems. It is possible, therefore, that the ocean acidification scenario will result in an ecosystem shift along the Mesoamerican Barrier Reef, in which today's frame-building colonies are replaced by more tolerant species such as *Porites* and *Siderastrea*. The decrease in species richness observed when $\Omega_{arag} < 2.5$ indicates that an acidified ocean may change the composition and species diversity of reefs, which has the potential to impact the ecosystem services they provide. This work gives a first insight as to the highly adaptive nature of certain reef species; however, it also calls for the future need to increase protection in areas that might

serve as ecological refuges for corals that may have adapted to survival in low pH low saturation waters.

II.5. Acknowledgements

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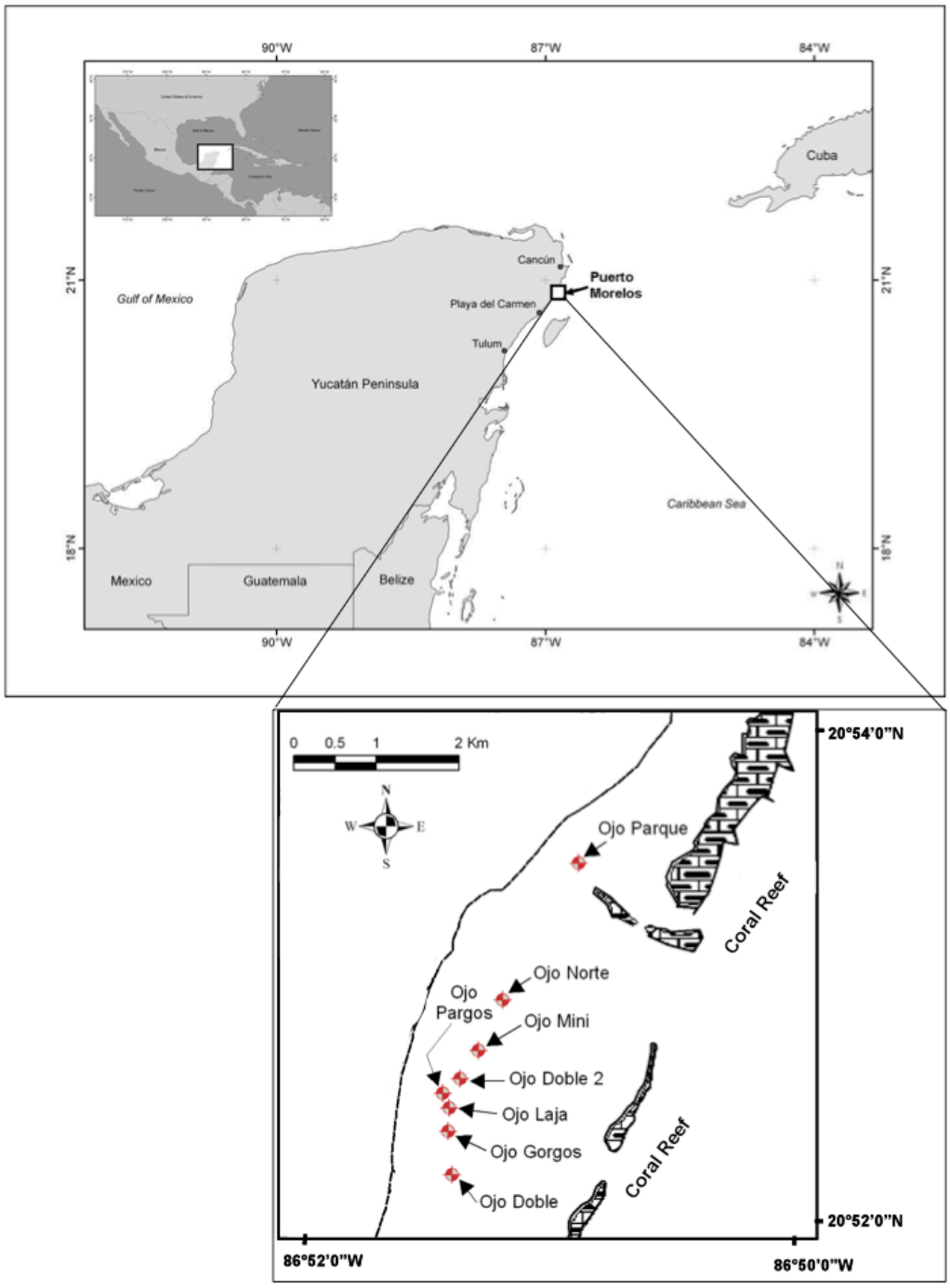


Figure II.1. Location map of Puerto Morelos, Mexico. The submarine springs, referred to locally as "ojos," exist approximately 500 m offshore in shallow (~5 m) lagoon waters.

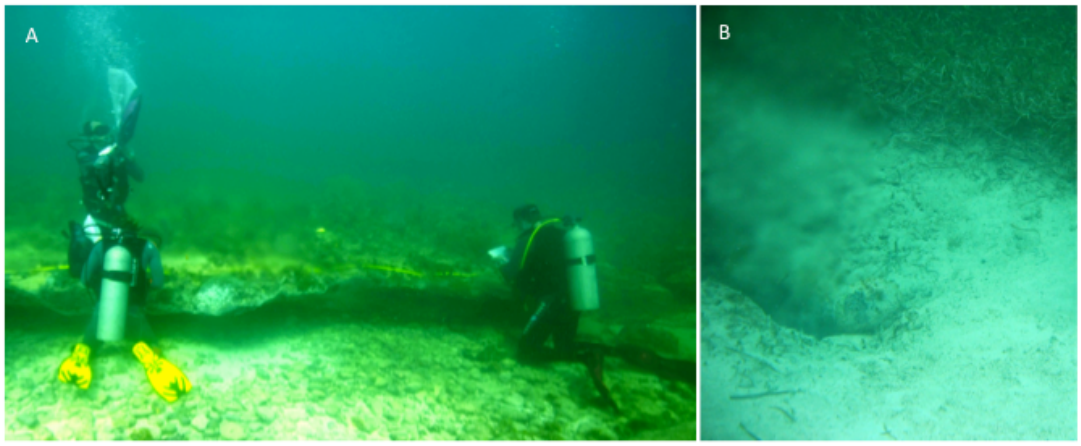
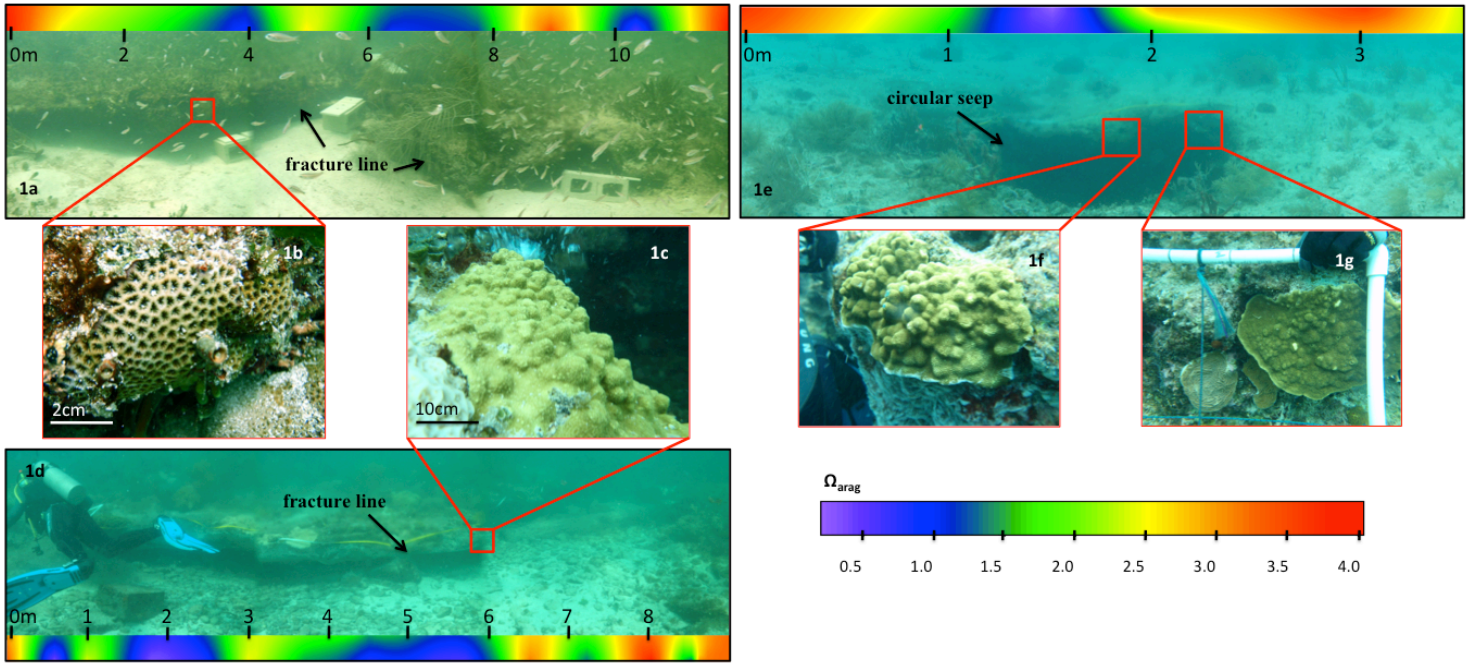


Figure II.2. Two examples of ojo sites. (A) depicts a "fracture" approximately 8m long, directly beneath a rocky shelf which overhangs the spring. (B) shows a circular hole in a sandy bed from which groundwater seeps. Note the blurred image, due to salinity differences in the water. For the purposes of this study, sandy beds and seeps were omitted due to lack of rocky substrate.

Figure II.3. Saturation state distribution along select transects in the vicinity of three different ojos with pictures of corals situated around the ojo centers. The saturation state (Ω_{arag}) is shown as a contour image (plotted in Surfer®), and the colors represent the range of saturation levels from blue (low saturation) to red (supersaturation). The contour image gives a visual representation of what the saturation state may look like along a sample transect line. Distance along the transect line is noted in the contour image in meters. The coral images give examples of where the corals were found along these transect lines. **(a)** and **(d)** are examples of large "fractures". (**e)** is a large circular "seep". Calcifying coral species including *Siderastrea radians* **(b)**, and *Porites astreoides* **(c, f)**, were found in undersaturated and low saturation water at all 10 ojos sampled. Six additional species, including *Agaricia* (**g)** lived in supersaturated waters where $\Omega_{arag} > 2.5$.


0m
2
4
6
8
10
fracture line
1a
1b
2cm
1c
10cm
1d
fracture line
0m
1
2
3
4
5
6
7
8
0m
1
2
3
circular seep
1e
1f
1g
Ω_{arag}
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

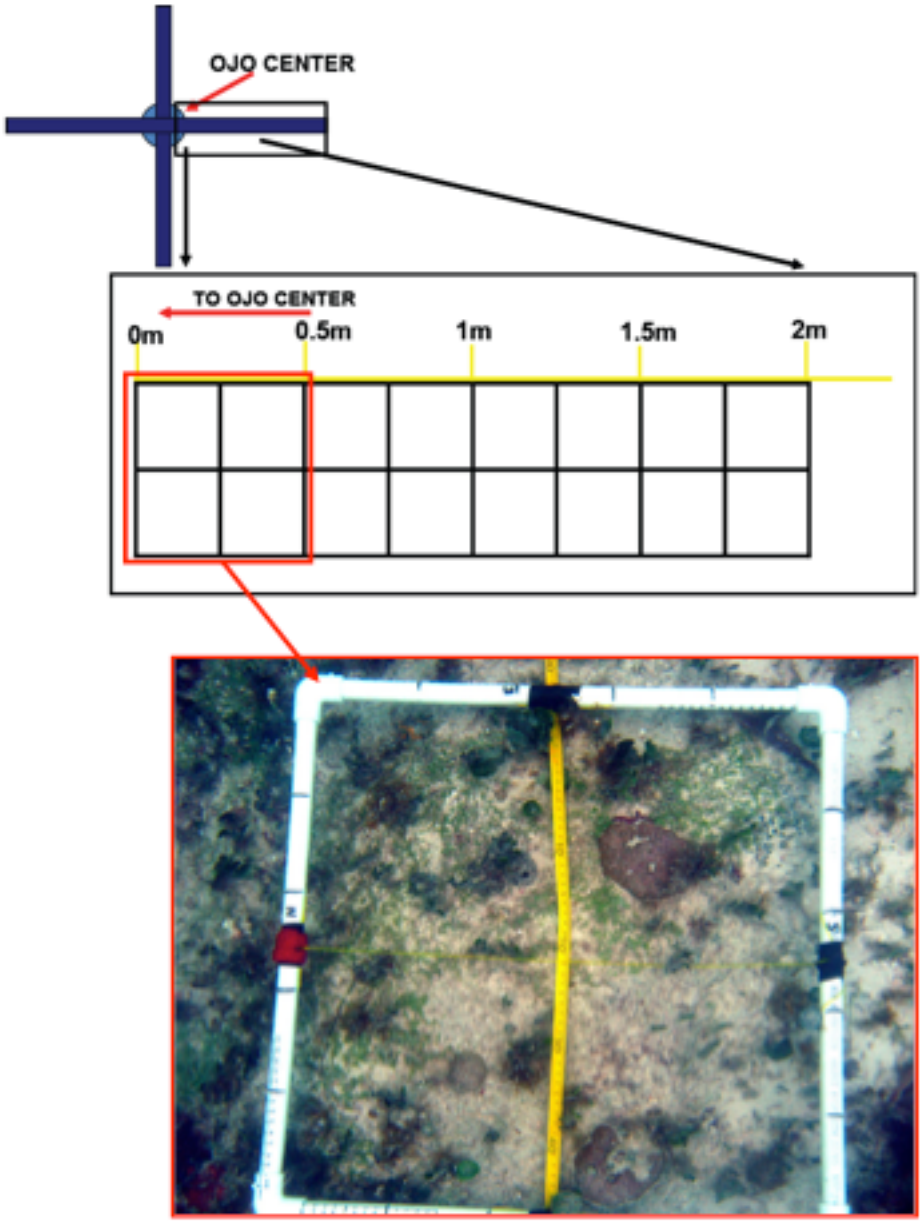


Figure II.4. Diagram of an underwater transect. At least two transects (up to 9 m long) were completed at each ojo, and in the case of larger "fractures" up to 5 cross-transects were made. Water was sampled every 0.25 m along each transect line. To sample the biota (identity, density, size, location), 0.50 x 0.50 m grids were broken into 4 quadrats of 0.25 x 0.25 m. Corals were directly measured on site, and extensive underwater photographs were taken.

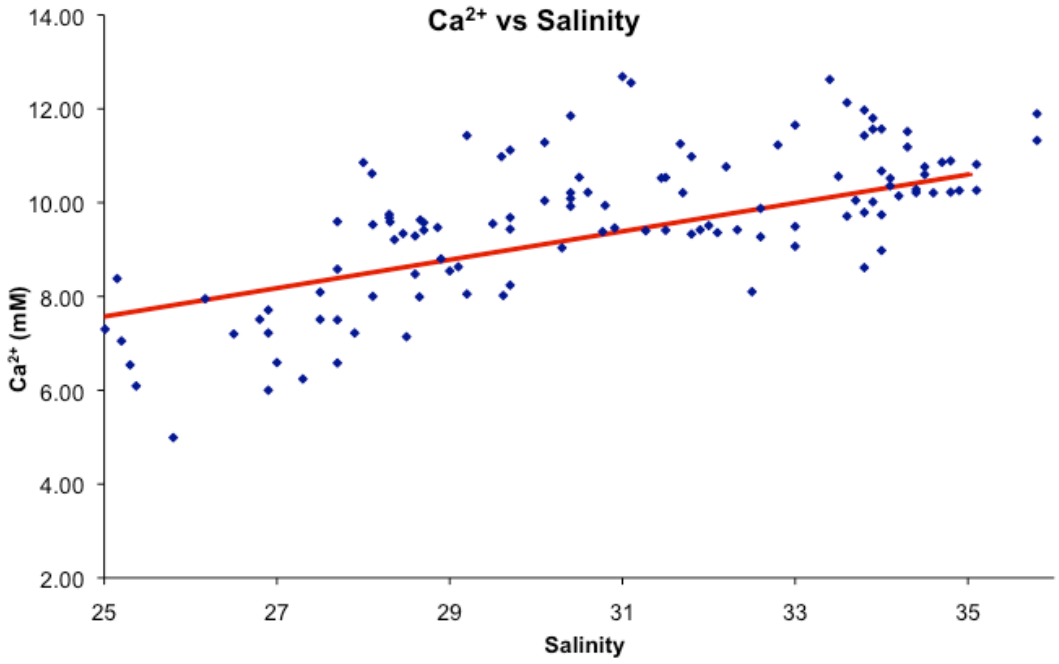


Figure II.5. Ca^{2+} concentrations as a function of salinity (R2=0.53). For certain ojo waters, Ca^{2+} concentrations were higher than expected based on a linear mixing model between freshwater of zero salinity and zero Ca^{2+} and ambient seawater (linear mixing line shown in red). This is a result of the dissolution of limestone in groundwater. In the case of high Ca^{2+} concentrations, the correction factor [Ca^{2+} observed] / [Ca^{2+}expected] was used in the CO_2-Sys program to calculate saturation values. If lower than expected values were found, a correction factor was not used (i.e., conservative saturation values are reported in both cases).

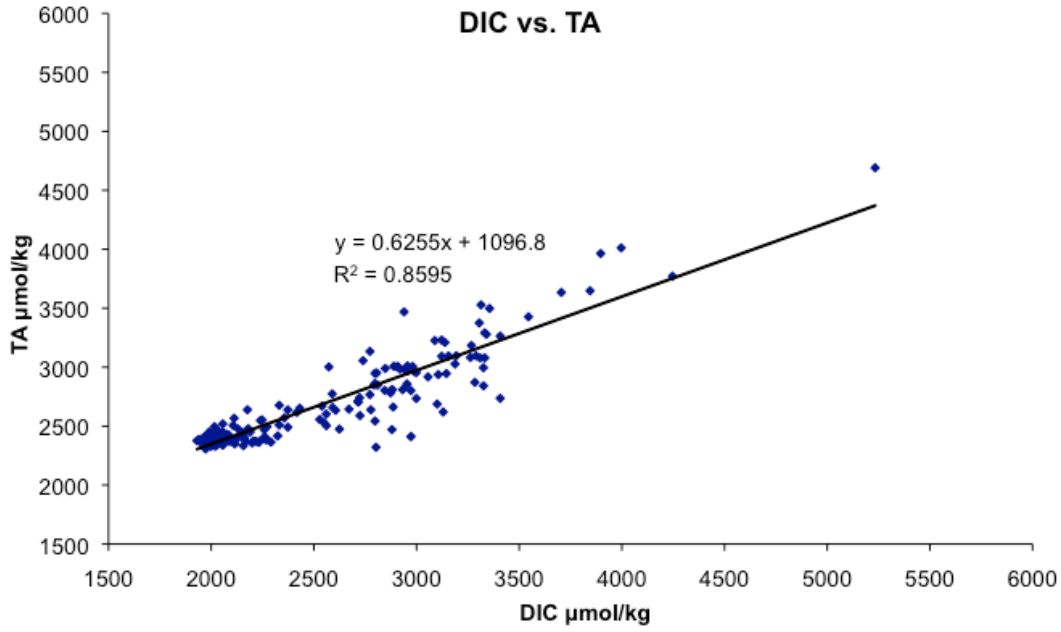


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Figure II.7. Number of species, total coral area, and number of individuals as a function of saturation state. **(a)** Number of species as a function of saturation state. No "other hard corals" (*Diploria, Montipora, Montastrea, Agaricia, Porites,* and *Favia sp*.) were found in low saturation or undersaturated ($\Omega<2.5$) waters. **(b)** Coral size as a function of saturation state for the different species. Average values for each group are reported, and error bars indicate standard error. Corals were grouped into 3 classes based on the saturation of the water in which they were observed: $\Omega<1$ (n=31); $1<\Omega<2.5$ (n=72); or $\Omega>2.5$ (n=172). *Porites divericata* colonies were omitted due to the rarity of their occurrence in both undersaturated and supersaturated water. Although comparing coral colony size for assemblages composed of different species is complicated by species-related morphology and growth rates, we also report the average size of all species found in supersaturated waters to provide qualitative information regarding coral growth outside of the springs. **(c)** Number of coral colonies per unit area as a function of saturation state. Results are normalized to per unit area ($0.25 m^2$) to account for differences in area sampled between groups.

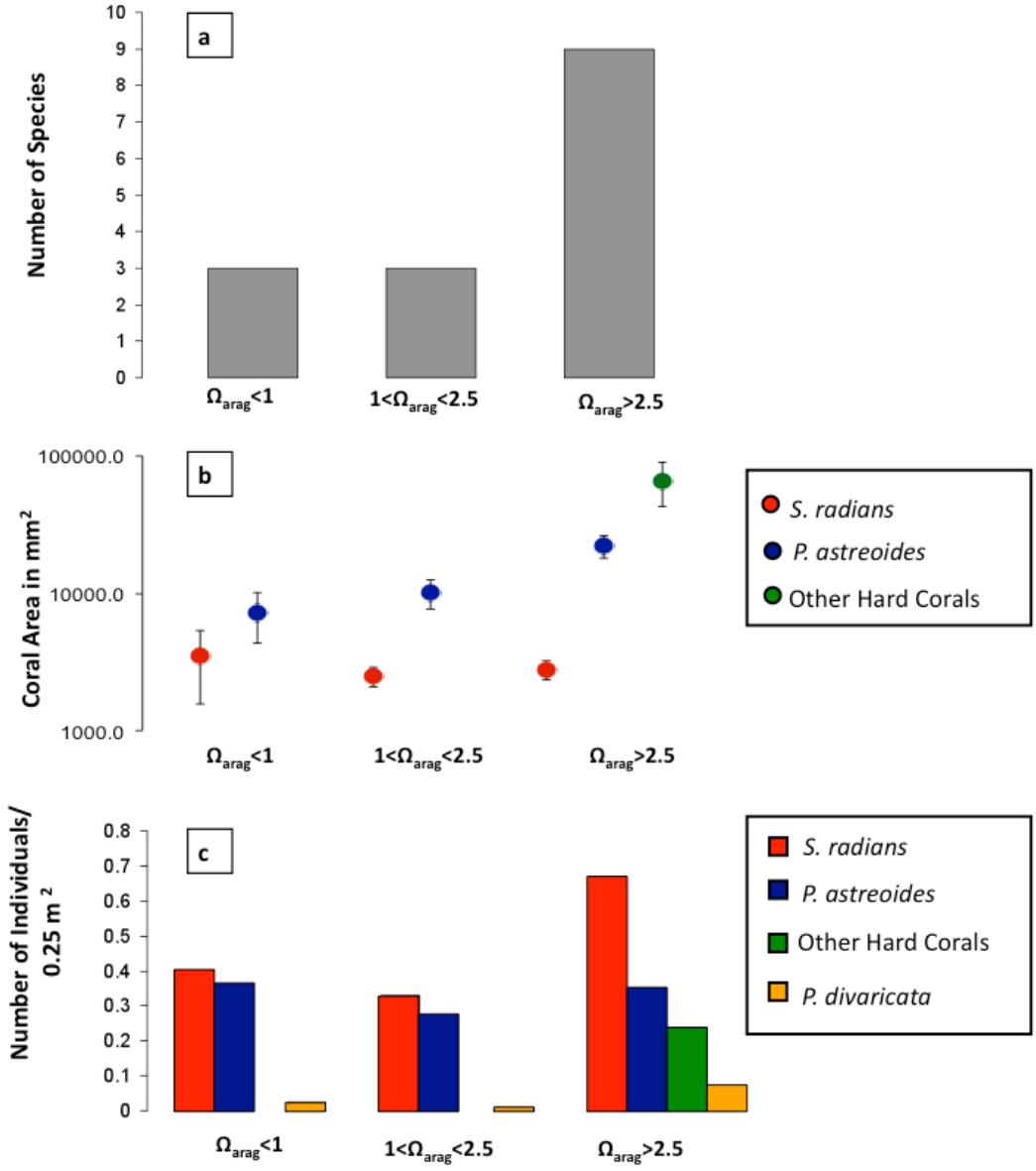
a
Number of Species
10
9
8
7
6
5
4
3
2
1
0
Ωarag<1
1<Ωarag<2.5
Ωarag>2.5
b
Coral Area in mm²
100000.0
10000.0
1000.0
Ωarag<1
1<Ωarag<2.5
Ωarag>2.5
S. radians
P. astreoides
Other Hard Corals
c
Number of Individuals/ 0.25 m ²
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ωarag<1
1<Ωarag<2.5
Ωarag>2.5
S. radians
P. astreoides
Other Hard Corals
P. divaricata

Table II.1 Chemical measurements and calculations at centers of ojo discharge. Summary of chemical parameters measured over three sampling excursions (June 2009, November 2009, and September 2010) at the 10 ojos.. Saturation values were calculated using the program CO_2Sys, unless noted by (*), which indicates that measured calcium concentrations were higher than expected due to limestone dissolution. In the case of (*), a more conservative estimate of Ω is reported that accounts for these higher Ca^{2+} concentrations. Natural variability in water characteristics measured at each site that was sampled more than once during the sampling trips was approximately: TIC (7%), TA (6%), Salinity (6%) and Temperature (4%). Natural variability in calculated pH (total scale) and saturation levels using CO_2Sys at sites sampled multiple times was pH (2%) and Ω (20%).

Date and site	DIC ± 3 µmol kg^{-1}	TA ± 2 µmol kg^{-1}	Salinity	Temp °C	pH	Ω_{cal}	Ω_{arag}
Control	2,099	2,403	35.5	31.6	8.03	5.35	3.60
June 2009							
Ojo Gorgos	3,545	3,427	28.6	27.2	7.13	1.15	0.75
Ojo Norte	3,122	3,113	28.7	27.0	7.23	1.28	0.84
Ojo Laja	3,314	3,228	25.8	28.1	7.20	1.22	0.79
Ojo Mini	4,246	3,771	25.9	27.2	6.74	0.50	0.32
Ojo H-10 Fractura	2,773	2,740	30.5	29.2	7.24	1.30	0.86
Ojo Fractura	3,996	3,864	26.7	30.4	7.10	1.45	0.96
November 2009							
Ojo Gorgos (1)	3,340	3,279	26.2	28.3	7.25	1.41	0.91
Ojo Gorgos (2)	3,466	3,264	25.4	25.8	7.01	0.81*	0.54*
Ojo Laja	3,473	3,392	26.9	28.3	7.21	1.35	0.88
Ojo Pargos (1)	3,156	3,095	24.7	24.9	7.29	1.32	0.87
Ojo Pargos (2)	3,178	3,109	25.2	25.2	7.26	1.28	0.82
Ojo Mini	5,233	4,691	25.3	25.6	6.79	0.65*	0.41*
August 2010							
Ojo Gorgos (1)	3,194	3,097	29.6	27.2	7.14	1.38*	0.90*
Ojo Gorgos (2)	3,326	2,996	29.6	27.2	6.77	0.75*	0.49*
Ojo Norte	3,326	3,142	29.2	27.0	6.61	0.77	0.50
Ojo Laja	3,187	3,027	28.7	27.5	7.02	0.91*	0.6*
Ojo Pargos (1)	3,332	3,079	28.7	27.5	6.88	0.70*	0.46*
Ojo H-10 Fractura	3,407	2,736	29.4	27.9	7.09	0.81*	0.54*
Ojo de Agua	3,145	2,946	29.7	27.2	6.94	0.84*	0.55*

III. Reduced calcification and lack of acclimatization by coral colonies growing in areas of persistent natural acidification

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Summary

As the surface ocean equilibrates with rising atmospheric CO_2, the pH of surface seawater is decreasing with potentially negative impacts on coral calcification. A critical question is whether corals will be able to adapt or acclimate to these changes in seawater chemistry. We use high precision CT scanning of skeletal cores of *Porites astreoides*, an important Caribbean reef-building coral, to show that calcification rates decrease significantly along a natural gradient in pH and aragonite saturation (Ω_{arag}). This decrease is accompanied by an increase in skeletal erosion and predation by boring organisms. The degree of sensitivity to reduced Ω_{arag} measured on our field corals is consistent with that exhibited by the same species in laboratory CO_2 manipulation experiments. We conclude that the *Porites* corals at our field site were not able to acclimatize enough to prevent the impacts of local ocean acidification on their skeletal growth and development, despite spending their entire lifespan in low pH, low Ω_{arag} seawater.

III.1. Introduction

Scleractinian corals, whose $CaCO_3$ skeletons provide the structural framework of coral reef ecosystems, are subject to numerous direct and indirect stressors and are facing steep global decline (Hoegh-Guldberg et al., 2007; Hughes et al., 2003; Carpenter et al., 2008). As the ocean absorbs anthropogenic CO_2, surface ocean pH and the availability of carbonate ions to corals and other reef calcifiers are decreasing (Hoegh-Guldberg et al., 2007; Hughes et al., 2003; Carpenter et al., 2008; Barbier et al., 2008; Fabry et al., 2011). Global climate models predict a drop of 0.3 pH units, from 8.1 to 7.8 by the end of the 21st century (Caldeira and Wickett, 2005; Orr et al., 2005; Doney et al., 2009), resulting in a 50% reduction in carbonate ion concentration (Feely et al., 2009). Consequently, it is predicted that ocean acidification will result in a wide spread reduction in coral calcification by the year 2065 (Cao and Caldeira, 2008), causing large-scale reef degradation and loss (Langdon and Atkinson, 2005).

The predicted response of coral reef calcification to decreasing aragonite saturation state is based primarily on model calculations of future Ω_{arag} (Caldeira and Wicket, 2003, 2005; Orr et al., 2005; Feely et al., 2009) and the observed response of coral calcification to low Ω_{arag} in short-term laboratory-based or mesocosm carbonate chemistry manipulation experiments (Langdon and Atkinson, 2005; Gattuso et al., 1998; Leclercq et al., 2002; Jokiel et al., 2008; Schneider et al., 2008). Additionally, field-based observations of net coral reef ecosystem calcification responses to changes in Ω_{arag} state *in situ* also suggest declines in calcification (Pandolfi et al.,

2011; Fabricius et al., 2011; Cooper et al., 2008; De'ath et al., 2009; Tanzil et al., 2009). However, key questions remain regarding the acclimation and adaptation potential of coral calcification to ocean acidification. Acclimatization, or the potential for an organism to adjust to changes in an environment via physical modifications, is distinguished from adaptation, or permanent evolutionary modifications made by an organism in response to repeated stressors. Specifically, an outstanding question is whether corals will be able to acclimate or adapt in order to maintain sufficient rates of calcification to sustain the reef structure (Pandolfi et al., 2011a,b; Hoegh-Guldberg et al., 2011). To address these questions, field-based studies where corals have been naturally exposed to chronic low pH conditions for extended periods could provide important new insights. In this study, we quantify calcification rates of the common Atlantic coral, *Porites astreoides*, growing in an environment of low pH and Ω_{arag} along the Caribbean coast of the Yucatan Peninsula, Mexico for time scales long enough for acclimation. We compare annual calcification rates of these corals with corals of the same species living in close proximity (less than 10 meters away) under ambient pH and Ω_{arag} conditions. Results from short-term laboratory CO_2 manipulation experiments with the same species provide an empirical framework within which to interpret the field data, enabling us to determine whether these corals, which have been exposed to low Ω_{arag} for their entire life span, have acclimated to ocean acidification.

The karstic region of the Yucatan Peninsula is an area where low pH groundwater and seawater have been interacting since the last deglaciation ~18,000 years ago (Medina-Elizalde and Rohling, 2012). Due to the high porosity of the limestone bedrock, there is no surficial runoff; rather, rainfall rapidly infiltrates the water table and is drained through a series of interconnected caves and fractures directly to the coast (Beddows et al., 2007) at highly localized submarine springs in close proximity to the Mesoamerican Barrier Reef. Before the water is discharged, extensive mixing with seawater occurs within the aquifer. As a result, water with low pH, high DIC, high alkalinity, low Ω_{arag} and near oceanic salinities is discharged at submarine springs (Beddows et al., 2007; Crook et al., 2011; Inoue et al., 2012; Table III.1 and Figure III.1). Light, temperature and sedimentation conditions are similar between the springs and control sites although nutrient levels are higher at the springs (Crook et al., 2011).

Importantly, the discharge at these springs has been continuous for millennia: thus, the coral colonies at these sites settled, calcified, and grew into mature colonies within the plume of low-pH groundwater discharge. The Yucatan springs represent areas where the ecosystem has had ample time to acclimate to low-pH conditions. Previous work off the coast of Puerto Morelos, Quintana Roo, Mexico, demonstrates that these springs, despite their low Ω_{arag} water, are host to corals and other benthic calcifiers, although coral diversity and coral cover are reduced close to the springs, likely driven by the chronically low Ω_{arag} conditions (Crook et al.). Monitoring over a

three-year period indicates that the pH of the discharging water fluctuates considerably on multiple time scales, but the water at the center of the springs remains under-saturated for a majority of the time (Crook et al., Hofmann et al., 2011). To compliment previous findings at Puerto Morelos, we measured calcification rates of the corals found in close proximity to four springs characterized by low saturation and near oceanic salinities (>30 ~93% of the time, Figure III.1) and compared them to similar colonies found nearby in ambient seawater conditions.

III.2. Materials and Methods

The skeletal coral cores were removed from each colony with a submersible hydraulic drill, and care was taken to fill the drilled holes with cement plugs to promote tissue growth over the scar. After drying in a 50º C oven for five days, the cores were scanned with a Siemens Volume Zoom Spiral Computerized Tomography (CT) Scanner at 1-2mm resolution (Figure III.2). The 3D imaging capabilities of the CT scanner and software allow precise measurement of annual growth bands, and a more accurate identification of the vertical growth axis than conventional x-ray techniques (De'ath et al., 2009; Cantin et al., 2010). The density (g cm^{-3}) of each of the cores was determined using the scanned greyscale images and a conversion to apparent absolute density using hydroxyapatite standards (Cantin et al.). Annual linear extension rates (cm yr^{-1}) were obtained by precise measurement of the distance between high density bands representing annual accumulation to 0.5mm accuracy (Figure III.2). Annual

calcification rates were calculated as the product of density and linear extension (Cantin et al.).

The scanned images were also used to determine the extent of boring (% volume bored) in each of the cores. Because the scanned CT images can be rotated in 3D and visualized in multiple layers, precise measurements of length, width, and depth of each bore hole can be made. The bored volume was calculated and a ratio to total coral volume was determined. To determine tissue thickness, dried cores were spliced in half and imaged using a Nikon SMZ1500 Stereo microscope and SPOT imaging software. Nine measurements of tissue thickness were made per sample for statistical analysis.

Chemical analyses of the water samples were completed following Crook et al., 2011. From these components, the pH and saturation state was calculated using the program CO_2 Sys (Pierrot et al., 2006). Saturation values represent site specific averages determined from this sampling and data reported in Crook et al., 2011 and Hofmann et al., 2011.

III.3. Results

Skeletal samples from 14 *Porites astreoides* colonies were obtained from the vicinity of four springs at Puerto Morelos: 7 within the impact of the discharge (Ω_{arag} <2.0), and 7 from areas with ambient seawater conditions (Ω_{arag} >3.5) in close proximity to

the springs (less than ten meters away). Cores were removed using a handheld drill fitted with a 1" round diamond tipped coring bit. Dried, intact cores were scanned using a Siemens Volume Zoom Spiral Computerized Tomography (CT) scanner at the Woods Hole Oceanographic Institution (Saenger et al. 2009; Cantin et al., 2010) together with a set of hydroxyapatite standards with known densities, to enable precise quantification of annual linear extension rates (cm yr^{-1}), density (g cm^{-3}), and calcification (g cm^{-2} yr^{-1}) (Figure III.2). The 3-D images produced from the CT scans were also used to assess the extent of erosion in each core. After CT scanning, each core was sliced in half using a high precision wet saw fitted with a diamond wafer blade. Tissue thickness, a measure of the volume of coral soft tissue occupying the skeleton, was measured on each core half using a Nikon SMZ1500 Stereo microscope and SPOT imaging software. We define tissue thickness as the distance between the last (most recently accreted) dissepiment and the tip of the calical walls. At the time of coring, *in-situ* temperature and pH were measured and water samples were taken for dissolved inorganic carbon (DIC), total alkalinity (TA), calcium and nutrients concentrations and salinity. Chemical measurements taken at the time of sampling, as well as during previous sampling events (Crook et al., 2011; Hofmann et al., 2011), indicate that all coral skeletal cores taken directly from the springs were residing in under-saturated ($\Omega_{arag} < 1$) or mildly supersaturated water ($1 < \Omega_{arag} < 2$). The remaining cores were removed from corals residing in ambient seawater coinciding with saturation states greater than 3.5.

Linear extension rates were not statistically different between corals in low pH water (Ω_{arag} <2) and corals in the ambient seawater (Ω_{arag} >3.5) (p=0.33, Figure III.3a); however, a trend towards lower extension rates for the corals in under-saturated waters is observed (Ω_{arag} < 1) (Figure III.3b). When divided into three saturation groups, average annual extension for each group was 0.19 cm yr^{-1} ± 0.07 (Ω_{arag} < 1), 0.29 cm yr^{-1} ± 0.09 (1 < Ω_{arag} < 2), and 0.30 cm yr^{-1} ± 0.12 (Ω_{arag} >3.5). The linear extension decline of 38% between ambient Ω_{arag} and under-saturation may indicate a threshold response (Figure III.3b), although the trend between ambient and under-saturated extension rates is not significant and more samples are required to test this hypothesis (ANOVA, F(2,11)=1.90, p=0.19).

Conversely, a statistically significant drop in skeletal density occurred between corals growing in ambient conditions (Ω_{arag} > 3.5) and corals growing in low Ω_{arag} and Ω_{arag} under-saturated waters (Ω_{arag} < 2) (ANOVA, F(2,11)=18.618, p=0.0003, Figure III.3c). Average skeletal density dropped approximately 31% from Ω_{arag} > 3.5 to Ω_{arag} < 2. However, a Tukey HSD post-hoc test reveals that the low (1 < Ω_{arag} < 2) and under-saturated (Ω_{arag} < 1) groups both differ significantly from the control (p=0.001) but not from each other (p=0.36, Figure III.3d). Rather, the sharp decline in density occurred between 2.0 < Ω_{arag} < 3.5. Linear regression to see how Ω_{arag} predicts density shows a slope of 0.18, which is highly significantly different from zero (p<0.001), and suggests that each 1 unit decrease in Ω_{arag} is associated with a 0.18 unit decrease in density.

Annual calcification is the amount of calcium carbonate produced by each colony per year and is calculated as the product of annual extension and density. Annual calcification of *P. astreoides* declined significantly between the control ($\Omega_{arag} > 3.5$) and low saturation colonies ($\Omega_{arag} <1$; $1< \Omega_{arag} <2$) (ANOVA, $F(2,11)=5.623$, $p=0.02$, Fig. III.3e). Further analysis reveals that the average calcification for the under-saturated group (0.16 g $cm^{-2}yr^{-1}$) differs significantly from the control colonies (0.42 g cm^{-2} yr^{-1}, $p=0.02$) but not from the low saturation group (0.30 g cm^{-2} yr^{-1}, $p=0.08$, Figure III.3d). Linear regression to see how omega predicts annual calcification shows a slope of 0.10, which is highly significantly different from zero ($p=0.006$), and suggests that each 1 unit decrease in Ω_{arag} is associated with a 0.10 unit decrease in calcification. This translates to an approximate 46% decline in calcification from ambient conditions by the time $\Omega_{arag}=2$, and up to a 68% decline in calcification between ambient and $\Omega_{arag}=1$ (Figure III.3f). As linear extension rates did not vary significantly between low Ω_{arag} and ambient Ω_{arag} waters, the calculated decrease in calcification is driven primarily by the decrease in skeletal density with decreasing Ω_{arag}. However, the drop in calcification rate between corals living in low and under-saturated seawater was driven by the combined effect of reduced linear extension and low skeletal density. By the time under-saturation is reached, the paucity of carbonate ions for skeleton building impacts both skeletal growth parameters: upward linear extension and skeletal thickening.

III.4. Discussion

One of the challenges posed by *in situ* field studies is that multiple environmental parameters may co-vary, making it difficult to resolve the influence of Ω_{arag} on calcification from that of other factors, or to assess the extent to which the influence of Ω_{arag} may be modulated by other, co-varying factors. We can address this question by comparing the change in *Porites astreoides* calcification measured at our Yucatan study site with that observed in laboratory CO_2 manipulation experiments. In these experiments only pH and Ω_{arag} vary while other environmental parameters (e.g. temperature, salinity, light) are kept constant, thus allowing us to isolate the effect of ocean acidification on *P. astreoides* calcification from other factors.

By far the majority of laboratory CO_2 manipulation experiments conducted to date show that *P. astreoides* calcification is sensitive to ocean acidification, consistent with our results from the Yucatan. Results from different experiments are consistent, showing a decline of ~40% with a 65% drop in Ω_{arag} (Fig. III.4). At our Yucatan field site, the sensitivity of *P. astreoides* calcification is identical to results obtained in controlled laboratory experiments using the same species from the Atlantic or Caribbean across the same range in Ω_{arag} (de Putron et al., 2011; Albright and Langdon, 2011). The strong agreement between field and experimental data indicates that *P. astreoides* calcification is responding to the natural Ω_{arag} gradient at the Yucatan, and not to other factors.

Global climate models predict that by the year 2100, tropical surface oceans may have a Ω_{arag} of approximately 2.5 (Feely et al., 2009); therefore, our study implies that net $CaCO_3$ production in Atlantic reefs on which *Porites astreoides* is a major reef builder (such as Bermuda, the Virgin Islands or Belize), could decrease significantly within the next century. The greatest decrease in calcification we observed in under-saturated ($\Omega_{arag} < 1$) waters was 68% from present day values. Using our linear regression model, we predict that *Porites* calcification could decrease by approximately 22% from pre-industrial values by the time tropical surface oceans reach $\Omega_{arag} \approx 3.1$ in the year 2065 (Kleypas et al., 1999). If atmospheric CO_2 concentrations triple ($\Omega_{arag} \sim 2$) a loss of approximately 46% could result. This estimate is in line with field, mesocosm, and laboratory studies that indicate a decline in calcification between 13% and 22% (de Putron et al., 2011; 31, Leclercq et al., 2000; Cohen and Holcomb, 2009; Ries et al., 2009; Holcomb et al., 2009). When combined with the negative impacts of other stressors, including rising sea surface temperatures that cause mass bleaching (Hughes et al., 2003), pollution and over fishing, ocean acidification is likely to deal a significant blow to the health of Atlantic coral reefs within the next few decades (Hoegh-Guldberg et al., 2007).

While calcification is clearly decreasing with decreasing Ω_{arag} *P. astreoides* at Puerto Morelos are maintaining net calcification even in under-saturated seawater. These findings are similar to those of Rodolfo-Metalpa *et al*. (2011) at Ischia, Italy, in which gross calcification occurred in transplanted subtidal calcifiers even in waters with a

pH below 7.4 (Rodolfo-Metalpa et al., 2011). Additionally, other recent studies on *Porites* spp. indicate that some corals show limited, if any, negative responses to increased pCO_2 (Edmunds et al, 2012; Comeau et al., 2013). Physiological mechanisms that enable certain coral species to calcify under extreme levels of acidification have been suggested previously (Cohen and Holcomb, 2009). It is possible, for instance, that in response to the harsher environment encountered at the springs, the corals in low or under-saturated waters utilize more energy to maintain their linear extension rates, but at the cost of skeletal density. This "stretch modulation" has been observed in massive *Montastrea* colonies in the Gulf of Mexico (Carricart-Ganivet and Merino, 2001; Carricart-Ganivet, 2004). These observations fit well with our data, in which density decreases while linear extension rates are maintained. It has also been suggested that tissue thickness may be linked to linear extension, with thicker tissues leading to higher extension rates (Barnes and Lough, 1992; Lough and Barnes, 2000), allowing the corals to overcome stress (i.e. thicker tissues are indicative of more stressful environments). No significant differences in tissue thickness between the ambient corals ($\Omega_{arag} > 3.5$) and the corals residing close to the springs ($\Omega_{arag} < 1.0$; $1 < \Omega_{arag} < 2$) were found ($p=0.36$, Figure III.3g). It is interesting to note, however, that for $1.0 < \Omega_{arag} < 2.0$, a (non-significant) trend is seen where tissue thickness increases slightly, from an average of 3.2 mm to 3.9 mm (ANOVA $F(2,10)=1.921$, $p=0.19$). This may indicate that the low saturation corals are working harder to maintain their rates of linear extension. Indeed, the extension data suggests that no significant differences are found between the low saturation and

ambient corals, although a (non-significant) decrease in extension is observed for the corals growing in under-saturation conditions). Combined, these lines of evidence are indicative of a threshold response seen when waters reach saturation levels of approximately 1.0. Above this saturation index, *P. astreoides* appear to maintain rates of linear extension by increasing their tissue thickness and compensating for decreases in pH. However, once undersaturation is reached, the energy requirements of extension appear too great for tissue thickness alone to maintain, and a significant reduction in calcification rates is observed.

While our data suggests that certain corals may be able to maintain their linear extension under the ocean acidification conditions expected by the year 2100, when considering the impact of density on bioerosion the situation is disheartening. The extent of erosion and predation by boring organisms was found to be significantly greater in corals where $\Omega_{arag} < 2.0$ ($p=0.01$, Figure III.5). In the vicinity of the discharge, total volume eroded was 78% greater than at ambient conditions. The observed increase in total volume eroded at low saturation ($\Omega_{arag} < 2$), which is likely caused by the lower carbonate density, indicates that future acidification events may not only decrease calcification rates, but reduce coral coverage via boring organisms and mechanical erosion. For instance, it has been shown that parrotfish preferentially remove carbonates from lower density substrates (Bruggemann et al., 1996), and the low structural integrity caused by a reduction in density could leave reefs more

vulnerable to wave action leading to a weaker framework and the further degradation of coral reefs.

Notably, our study indicates that despite their life-long exposure to low saturation waters, *Porites astreoides* coral colonies at Puerto Morelos calcify at lower rates than conspecifics residing in ambient waters. These lower calcification rates are similar to those observed in short term exposure experiments (de Putron et al., 2011; Albright and Langdon, 2011; Anthony et al., 2008) (Figure III.4), which suggests the corals have not acclimatized to a degree that would enable the corals to maintain ambient calcification rates. Moreover, while some coral species are able to survive and grow in extreme conditions of under-saturation, a decrease in skeletal density combined with an increase in susceptibility to bioerrosion may indicate a weakening of the reef framework in the future and subsequent degradation of the complex coral reef ecosystem.

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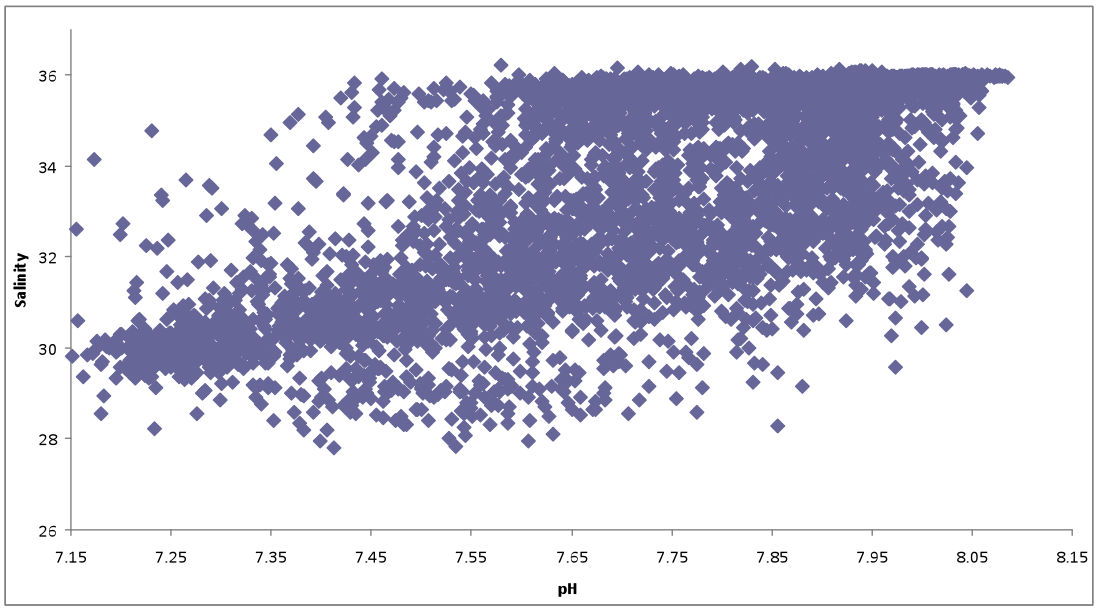


Figure III.1. Salinity and pH over time as measured by an autonomous sensor. Salinity and pH were measured at 15 minute time intervals for a period of 3 months (August-October 2010) for a total of over 5500 data points at a single spring. Salinity is plotted against pH (**a**), and grouped according to the number of data points occurring in a given salinity range (**b**). As depicted, 93% of data points fall above a salinity of 30, and salinity never drops below 27 at the center of discharge. The lower salinity conditions are during low tide in the rainy season and the conditions do not prevail for more than a one hour.

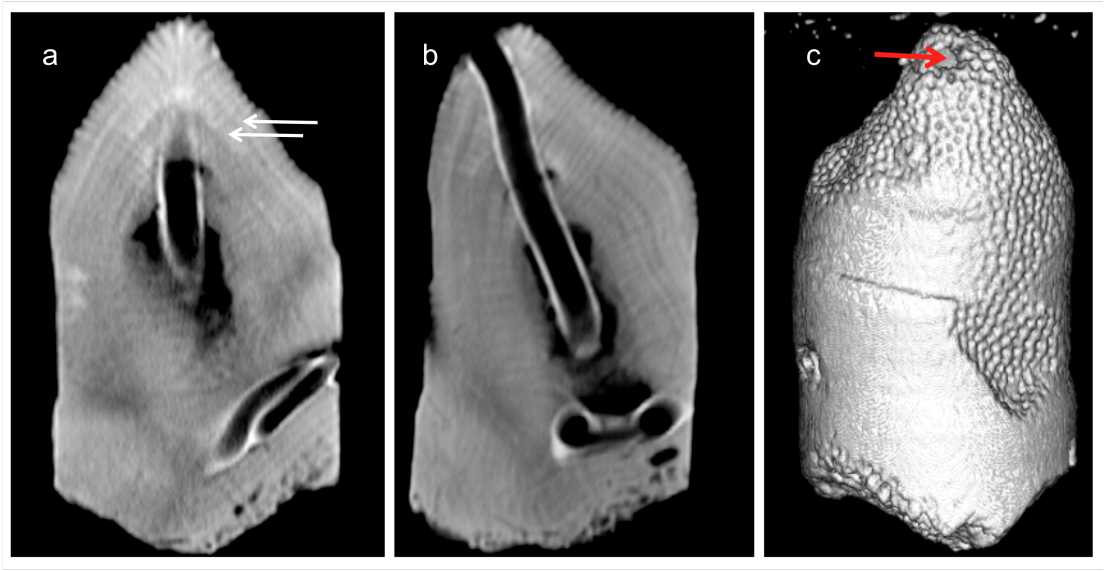
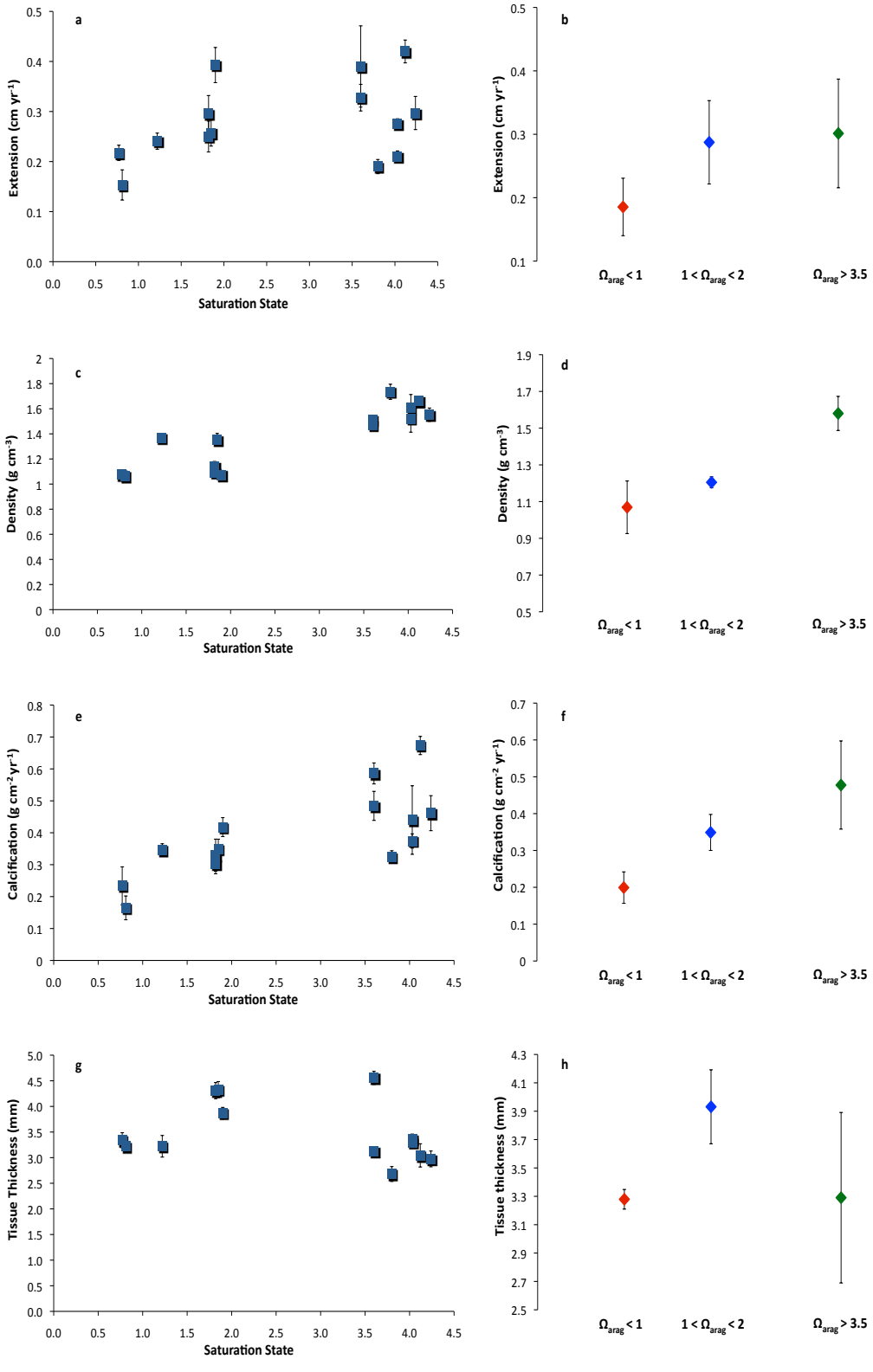


Figure III.2. CT scanned images of a core. Comparisons of linear extension (**a**) can be made to 0.5mm accuracy by measuring the distance between high density bands (white arrows), and density can be determined at a given point via the use of hydroxyapatite standards. CT scanning allows for the rotation of the images (**b**) to reveal additional features in the core, including the exact dimensions of boring and erosion. The reconstructed core (**c**) reveals only a small bore hole (red arrow) that actually runs the length of the core.

Figure III.3

Linear extension, density, calcification, and tissue thickness for all cores. Linear extension (**a,b**), density (**c,d**), calcification (**e,f**) and tissue thickness (**g,h**) as a function of saturation state for all data (**a,c,e,g**), and grouped by saturation state (**b,d,f,h**). In (**a,c,e,g**), error bars represent standard error and in (**b,d,f,h**), error bars depict standard deviation. No significant differences in extension (cm yr^{-1}) are seen with decreasing saturation state ($p=0.33$). However, a trend is noted in which extension rate drops as undersaturation is reached. Regression analysis indicates density (g cm^{-3}) decreases significantly with decreasing saturation ($p<0.001$), by up to 31% from ambient. Calcification (g cm^{-2} yr^{-1}) decreases by up to 68% by the time undersaturated waters are reached ($p=0.006$). Tissue thickness does not vary significantly with saturation (g); however, a trend is seen where tissue thickness increases slightly where $1 < \Omega < 2$ (h).


a
Extension (cm yr⁻¹)
Saturation State
b
Extension (cm yr⁻¹)
Ωarag < 1
1 < Ωarag < 2
Ωarag > 3.5
c
Density (g cm⁻³)
Saturation State
d
Density (g cm⁻³)
Ωarag < 1
1 < Ωarag < 2
Ωarag > 3.5
e
Calcification (g cm⁻² yr⁻¹)
Saturation State
f
Calcification (g cm⁻² yr⁻¹)
Ωarag < 1
1 < Ωarag < 2
Ωarag > 3.5
g
Tissue Thickness (mm)
Saturation State
h
Tissue thickness (mm)
Ωarag < 1
1 < Ωarag < 2
Ωarag > 3.5

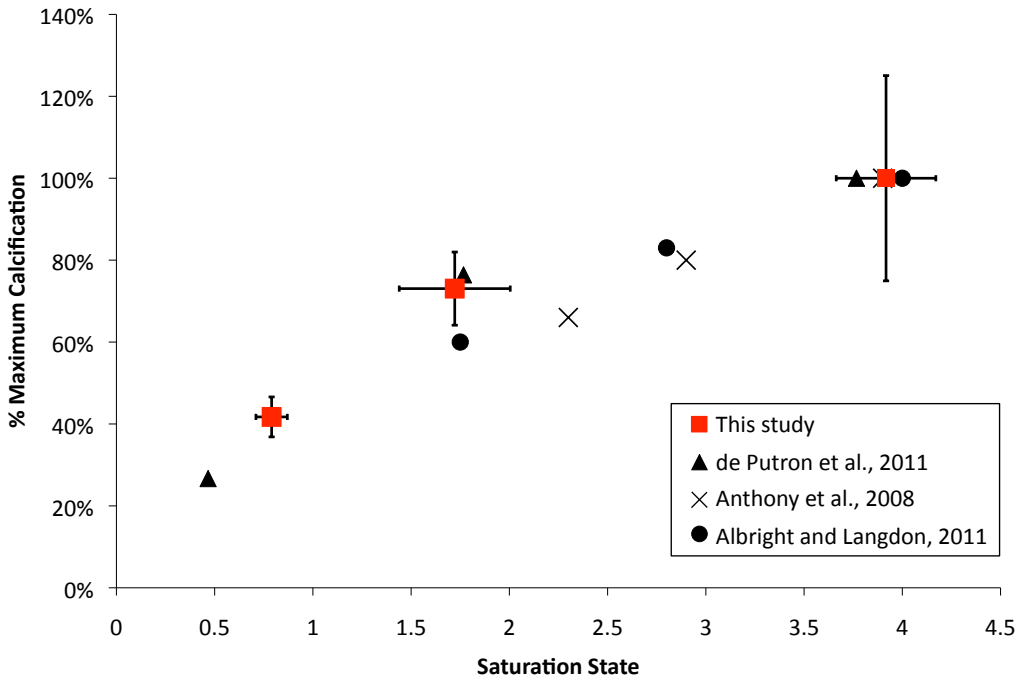


Figure III.4. Calcification of *Porites astreoides* in acidification conditions. Impact of Ω_{arag} on *Porites astreoides* calcification in this study (red squares) plotted against laboratory studies of *Porites* spp. (de Putron et al., 2011; Anthony et al., 2008; Albright and Langdon, 2011), and *P. astreoides* in particular (de Putron et al., 2011; Anthony et al., 2008). Calcification at a given saturation was calculated as a percent of the maximum observed calcification rate for each study. Error bars (this study) depict ±SD.

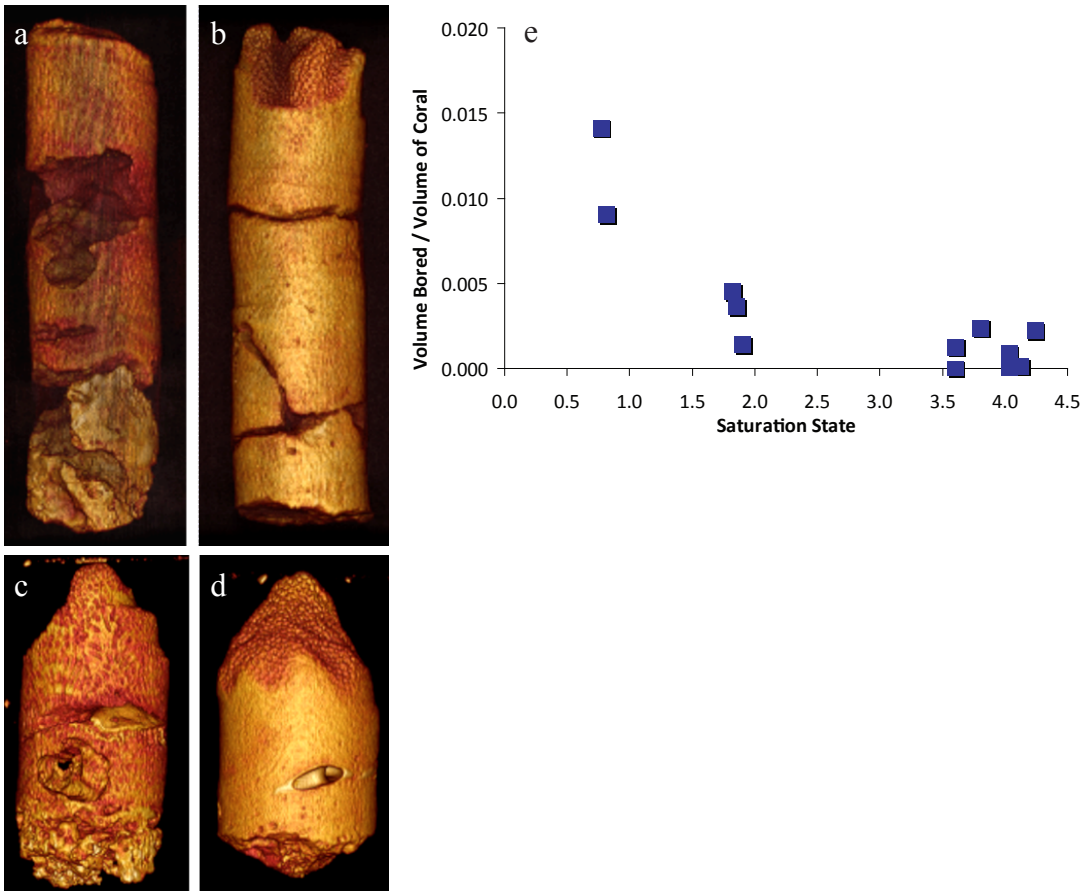


Figure III.5. Impact of Ω_{arag} on erosion and predation.The extent of erosion and predation by boring organisms was determined using the scanned images (shown as reconstructed cores of four corals). (a) and (b) are of equal size (15cm) as are (c) and (d) (5.5cm). The two cores from the center of a spring (a,c) are shown alongside their counterparts from ambient waters (b,d). The volume bored (normalized to the size of the core) increased by 78% with decreasing saturation state (e, P=0.01).

Table III.1. Variability of pH and saturation state by site, from discrete water samples. Water samples for measurement of DIC and TA were obtained for each coral core at the time of sampling (March 2011). In-situ temperature and salinity were obtained with a hand-held YSI-63 (YSI, Inc.). For the discrete measurements, pH and Ω_{arag} were calculated in CO_2 Sys (Pierre et al., 2006). Ca^{2+} values for each site were also obtained in the event that high Ca^{2+} concentrations required correction factors in the calculation of Ω_{arag} (due to high Ca^{2+} in the limestone bedrock, e.g. Crook *et al*., 2011); however, Ca^{2+} concentrations did not vary from ambient ocean values. "Center" implies the core was obtained within the area of influence of the discharging water: 7 "center" and 7 "control" cores were obtained.

Site / Coral ID	Salinity	Temp (°C)	DIC ±6 µmol kg^{-1}	TA ±7 µmol kg^{-1}	pH	Ω_{arag}
Ojo A/ Center 01	33.8	28.7	2559	2601	7.41	1.22
Ojo A/ Center 02	33.9	27.8	2409	2533	7.63	1.85
Ojo A/ Center 03	34.2	28.2	2483	2609	7.63	1.90
Ojo B/ Center 04	32.6	27.3	2492	2518	7.30	0.77
Ojo B/ Center 05	33.1	27.6	2900	2997	7.56	1.82
Ojo B/ Center 06	31.1	27.4	2904	2999	7.57	1.82
Ojo C/ Center 07	32.7	27.5	3169	3096	7.20	0.81
Ojo A/ Control 01	35.1	29.2	2052	2399	8.04	4.03
Ojo A/ Control 02	35.3	29.4	2056	2404	8.04	4.03
Ojo A/ Control 03	35.3	29.4	2050	2406	8.05	4.12
Ojo B/ Control 04	34.8	28.8	2069	2398	8.02	3.83
Ojo B/ Control 06	35.4	28.8	2083	2392	8.00	3.60
Ojo B/ Control 07	35.3	28.2	2076	2387	8.00	3.60
Ojo C/ Control 05	34.9	28.6	2020	2388	8.09	4.24

IV. Recruitment and succession in a tropical benthic community in response to *in-situ* ocean acidification

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Summary

Ocean acidification is a pervasive threat to coral reef ecosystems, and our understanding of the ecological processes driving patterns in tropical benthic community development in conditions of acidification is limited. We deployed limestone recruitment tiles in low aragonite saturation (Ω_{arag}) waters during an *in-situ* field experiment at Puerto Morelos, Mexico, and compared them to tiles placed in control zones over a 14-month investigation. The early stages of succession showed relatively little difference in coverage of calcifying organisms between the low Ω_{arag} and control zones. However, after 14 months of development, tiles from the low Ω_{arag} zones had up to 70% less cover of calcifying organisms coincident with 42% more fleshy algae than the controls. The percent cover of biofilm and turf algae was also significantly greater in the low Ω_{arag} zones, while the number of key grazing taxa remained constant. We hypothesize that fleshy algae have a competitive edge over the primary calcified space holders, coralline algae, and that acidification leads to altered competitive dynamics between various taxa. We suggest that as acidification impacts reefs in the future, there will be a shift in community assemblages away from upright and crustose coralline algae toward more fleshy algae and turf, established in the early stages of succession.

IV.1. Introduction

Declining surface ocean pH (ocean acidification) is a global environmental issue likely to be deleterious for a wide range of marine organisms (Orr et al., 2005; Fabry et al., 2008; Doney et al., 2009). Coral reef systems are expected to be particularly susceptible to ocean acidification and will likely see significant declines in calcification over the 21st century due to declining aragonite saturation state (Ω_{arag}) (Hoegh-Guldberg et al., 2007; De'ath et al., 2009; Pandolfi et al., 2011). Laboratory studies have described responses of many individual species to acidification (Anthony et al., 2008; Jokiel et al., 2008; de Putron et al., 2011), but ecosystem responses to acidification are complex (Ries et al., 2009; Kroeker et al., 2010). Field studies are essential for understanding how complex assemblages of species may respond to declining pH (Hall-Spencer et al., 2008; Manzello et al., 2010; Fabricius et al., 2011; Price et al., 2012; Kroeker et al., 2011, 2012; Crook et al., 2011).

Ocean acidification is predicted to directly impact calcifying organisms by reducing calcification rates. However, a key question for ecosystems is how acidification may impact communities by altering competitive interactions between organisms, resulting in phase shifts [Kroeker et al., 2012; Russell et al., 2010; Connell et al., 2013; Falkenberg et al., 2013). Recently, Kroeker *et al.* at (2012) found that calcareous species were rapidly overgrown by fleshy algae in acidified conditions; that is, competitive interactions between fleshy algae and calcifying species alter community structure in reduced pH environments in the temperate Mediterranean Sea

(Kroeker et al., 2012). Growing evidence suggests that non-calcareous algae appear to benefit in low pH conditions (Kroeker et al, 2010; ,Connell et al., 2011; Porzio et al., 2011) while calcifying organisms are either directly impacted (*i.e*. reduced calcification rates) or outcompeted by fleshy algal species (Fabricius et al., 2011; Price et al., 2012; Kroeker et al., 2012). However, most studies have been observation-based, and our understanding of the processes driving these patterns in community development is limited. Here, we conducted a field experiment to investigate recruitment and early succession near a tropical coral reef to determine how competitive interactions drive ecosystem responses to acidification in a tropical community. We investigate how acidification affects competition and dominance of space among various taxa along a natural gradient in pH on the Mesoamerican Barrier Reef. Our study design is similar to that of Kroeker *et al*., (2012), allowing for comparison between the responses in temperate and tropical systems. We focused on interactions among organisms under lower than ambient Ω_{arag} conditions to determine whether, and how, reduced Ω_{arag} may affect this community.

The Mesoamerican Barrier Reef lies off the east coast of the Yucatan Peninsula. Rainwater rapidly infiltrates the porous karstic limestone of the Yucatan, and then flows towards the ocean through interconnected caves and fractures. Along the flow path, the groundwater mixes extensively with seawater in underground aquifers before discharging into the lagoon between the shore and the offshore reefs at localized submarine springs (known locally as “ojos”) (Beddows et al., 2007).

Although these submarine groundwater springs have near-oceanic salinities and temperatures (Paytan et al., 2014) the water has high dissolved inorganic carbon (C_T), high total alkalinity (A_T), low pH and low Ω_{arag} (Paytan et al.). The ojos are typically at 4-7 m depth, and the chemistry of the water affects the diversity, abundance, and calcification rates of corals that settle and grow at the springs (Crook et al., 2011, 2013).

Understanding the ecological processes leading to the observed differences in diversity and abundance of organisms along these pH-saturation gradients is a critical step for predicting future impacts of acidification on reef environments. We deployed limestone recruitment tiles in low pH-Ω_{arag} waters at the ojo centers and compared them to those concurrently placed in control zones within a few meters of the springs. A subset of tiles were collected on three occasions (3 months, 6 months and 14 months) for analysis of recruitment and community succession. Although the average saturation state at the ojo centers (Ω_{arag} = 1.5) is much lower than most predictions for the late 21st century, this study assesses potential impacts of ocean acidification on developing reef communities that may be particularly relevant if atmospheric CO_2 follows more extreme IPCC scenarios (Solomon et al., 2007) or if local conditions (e.g. river or groundwater inputs, upwelling) exacerbate global acidification.

IV.2. Materials and Methods

The experiment took place at two ojos (Sites A and B), approximately 500 m offshore near Puerto Morelos, Mexico (20.853° N, 86.898° W). The ojos were chosen based on previous monitoring, which suggested their water had consistently low saturation (Ojo A Ω_{arag} = 1.4 ± 0.4, Ojo B Ω_{arag} =1.6 ± 0.4) and relatively high salinities (> 30) in the immediate vicinity of the discharge (Table IV.1) (Crook et al., 2013; Paytan et al., 2014). We used a 2 x 2 factorial design (2 Ω_{arag} levels x 2 ojo sites), which allowed us to compare the response as a function of chemical changes (e,g. different chemistry regimes with low pH-Ω_{arag} and ambient pH-Ω_{arag}) and location (site A and site B). If saturation or pH is an important controlling factor we expect little difference between sites at similar pH-Ω_{arag} and larger differences regardless of site for different pH-Ω_{arag}. To mimic the natural karst substrate, we deployed 40 limestone tiles (15 x 15 cm), acquired from a quarry near Puerto Morelos. Twenty tiles were deployed at each site; 10 in a low saturation zone (Ω_{arag} ~ 1.5, hereafter referred to as "ojo") in the direct vicinity of the spring discharge, and 10 in an ambient zone (Ω_{arag} ~ 3.8, hereafter referred to as "control") about 5 m from the springs. The tiles were bolted to concrete masonry blocks with stainless steel screws through a hole drilled in the center of each tile (Fig. IV.1). We deployed the tiles on 28 August 2010, immediately preceding a coral mass-spawning event. We removed subsets of three randomly selected tiles from each treatment after 3 months (25 November 2010) and 6 months (14 March 2011), and removed the four remaining tiles after 14 months (19 October 2011). Upon removal, the tiles were photographed, fixed in a 4% formalin solution for 48 hours, and then stored in 70% ethanol until analyzed (Fig. IV.2).

We collected discrete water samples for dissolved inorganic carbon (C_T), total alkalinity (A_T), salinity, and nutrients at initial deployment, during each recovery, and at monthly intervals throughout the 14-month deployment (Table IV.1). C_T was measured using a CM5011 Carbon Coulometer (UIC, Inc.) and A_T was measured with an automated, open-cell potentiometric titration procedure. Certified Reference Materials (batch 118) from the laboratory of Dr. Andrew Dickson at Scripps Institution of Oceanography were used to calibrate each instrument. C_T and A_T were used to calculate aragonite saturation state (Ω_{arag}) and pH via CO_2sys software (Pierrot et al., 2006), using CO_2 dissociation constants from Merhbach et al. (1973) refitted by Dickson and Millero (1987). pH is reported in total scale (pH_T). Salinity was measured with a salinometer (Guildline 8410 PortaSal), and nutrients were analyzed on a flow injection autoanalyzer (FIA, Lachat Instruments Model QuickChem 8000). In addition to the discrete samples, pH, temperature, and salinity were monitored using a SeapHOx sensor, which suggest that the discharge at the spring was continuous throughout the experiment (Fig IV.3).

The main focus of this study was to determine how acidification may impact ecosystem level changes, and specifically, to determine functional differences between the communities inside and outside of the springs. We therefore focus on functional groups rather than conducting species level analyses. This approach is consistent with previous investigations (Kroeker et al., 2012) and thus allows

comparison between the Caribbean and Mediterranean sites. Organisms on the tiles were assigned to eleven functional groups (Fig. IV.4). The tiles were divided into 1.5 x 3 cm subplots on the edges and 3 x 3 cm subplots on the face of the tiles for visual estimates of percent cover (Kroeker et al., 2012). Subplot estimates were then summed for total percent cover. The cover of erect fleshy algae forming a canopy over the tile was analyzed first and then removed to estimate the percent cover of encrusting groups. Encrusting foraminifera, molluscs, and polychaetes were counted and measured using a Celestron digital microscope (0.1 mm accuracy).

Community composition, defined as the presence or absence of functional groups, was compared between chemistry regime (ojo or control zones) and sites (Site A or B) for the 14-month tiles on a Bray-Curtis (BC) similarity matrix of presence/absence of functional groups. Community structure, defined as the relative abundance of functional groups, was analyzed on a zero-adjusted BC similarity matrix of square-root transformed total percent cover of functional groups. Permutational Multivariate Analysis of Variance (PERMANOVAs) were used to test variation in community composition and structure, with site and chemistry regime as categorical, fixed factors using 9,999 unrestricted permutations of the transformed data and Type III SS. Non-metric multi-dimensional scaling (nMDS) plots were made to visualize the variability in community structure. NMDS plots are ordinations of the multivariate data, in this case community data, where each point on the ordination represents the community on a single tile. In an nMDS plot, the multivariate data are placed into two-

dimensional space so that the rank differences among the data are preserved, based on the BC dissimilarity matrix. Thus communities that are more dissimilar to one another are farther apart on the plot.

Variation in succession was tested on the community structure among *site* x *saturation* x *time* using PERMANOVA with site (Site A or B), chemistry regime (ojo or control zone), and time (3, 6 or 14 months) as fixed factors. In addition, we tested for differences in the percent cover of select fauna. Due to numerous zero values that violated the assumptions of parametric statistics, we used PERMANOVA to test univariate variables ($\alpha = 0.05$).

All work was conducted at the Puerto Morelos nature reserve. Samples were collected under Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) permit DGOPA.00153.170111.-0051 and were exported with a Convention on International Trade in Endangered Species (CITES) Permit MX52912.

IV.3. Results

Eleven functional groups were common on the tiles (Fig. IV.2, IV.4). Five groups were comprised of calcareous organisms: including erect and crustose coralline algae (CCA), vermetid molluscs, tubicolous polychaetes, and encrusting foraminifera. Six groups were comprised of non-calcareous forms: including red, green and brown erect fleshy algae, turf algae, encrusting fleshy algae, and bacterial biofilm. Because

only 4 individual corals settled (*Siderastrea radians*) on tiles, only at the control sites, they were excluded from the analyses. The tiles from the ojos were generally dominated by fleshy algae, turf, and biofilm, while those in control conditions were dominated by CCA (Fig. IV.4). Erect coralline algae were often entirely absent from ojo tiles.

After 14 months of development, the community structure, defined as the relative abundance of functional groups, was significantly different between ojo and control conditions (PERMANOVA *chemistry*, $F_{1,12} = 14.89$, $p = 0.0001$; Fig. IV.5). The differences in community structure between ojos and controls were driven primarily by higher abundances of CCA and erect calcified algae in the ambient zone, while the ojos had higher abundances of biofilm, erect fleshy algae, and turf algae (Fig. IV.6). After 14 months, there were relatively minor differences between sites A and B ($F_{1,12}=2.96$, $p=0.05$). There were only marginal differences in the relative abundance between sites, with erect red algae being slightly more abundant at Site A and erect green algae and encrusting calcified algae being slightly more abundant at Site B. However, the site variable contributed only minimally to variation, and the impact of the sites was negligible compared to the impact of Ω_{arag} (Fig. IV.5).

In addition to the differences in the relative abundance of functional groups, the composition of the assemblages, defined as the presence or absence of functional groups, also varied between chemistry regimes (PERMANOVA, $F_{1,12}=7.44$,

p=0.002). These differences in community composition were mainly due to the absence of erect and crustose coralline algae (CCA) on some, but not all, of the ojo tiles. In contrast, the community composition did not differ between sites (PERMANOVA *site* $F_{1,12} = 0.24$, $p = 0.76$) after 14 months of development.

Community structure changed through time (PERMANOVA *time* $F_{2,28} = 17.64$, $p = 0.0001$) in both chemistry regimes. Significant differences between chemistry regimes ($F_{1,28} = 18.14$, $p = 0.0001$) and sites ($F_{1,28} = 3.54$, $p = 0.02$) were maintained through time. Within each chemistry regime and site, however, the community structure only differed between 3 months and the following time points, but not between 6 and 14 months.

Foraminifera abundance was significantly affected by both chemistry regime and time (ANOVA *pH x time*, $F_{2,28} = 6.44$, $p = 0.005$). On control tiles, the number of foraminifera increased significantly from 3 to 6 months (paired t-test, $p = 0.01$), and then significantly declined by 14 months ($p = 0.0007$) (Fig. IV.7a). The number of forams was greater on control than ojo tiles at 3 months ($p = 0.04$), but did not differ significantly at 6 months ($p = 0.1$). At 14 months, there were more forams at the ojos than at the controls ($p = 0.03$), stemming from a significant decline in foram abundance at the controls. The number of forams at the ojo centers increased marginally from 3 to 14 months ($p = 0.06$). Conversely, no trends were found in relation to chemistry regime for either polychaete (Fig. IV.7b) or vermetid mollusc

(Fig. IV.7c) abundance, although vermetids increased in number over time (PERMANOVA *time*, $F_{2,28} = 5.42$, $p = 0.009$; Fig. IV.7b,c).

To address the small relative percent cover of fauna present on the tiles, the percent cover estimates of all calcified taxa (calcareous algae and all animals) were grouped for a univariate comparison. The total percent cover of all calcified taxa was greater in the controls than in the ojos at all time periods (Fig. IV.8a). Importantly, the percent cover of all calcified taxa increased significantly among all time periods on the control tiles, but did not increase substantially between 6 and 14 months on the ojo tiles. Rather, the percent cover of all calcified taxa stagnated on the ojo center tiles at 6 months.

An additional univariate comparison was made for aggregate fleshy algal indices (summing erect green, red, and brown algae and turf algae). Total fleshy algal coverage was significantly greater at the ojo centers than at the controls at each time point, and by 14 months was 42% greater at the ojos (Fig IV.8b). Within the control group, the total fleshy algal coverage decreased from 3 to 14 months.

IV.4. Discussion

After 14 months of recruitment and development, there were significant differences in species composition and relative abundance of species present on the ojo tiles compared to the controls. Differences in community structure between ojos and

controls were primarily due to greater percent cover of erect and crustose coralline algae on the control tiles. In our study, both upright and crustose coralline algae (CCA) were found at the ojos in low saturation conditions, although coverage was significantly reduced compared to control sites. CCA appear to recruit early regardless of saturation, suggesting that they may be physiologically tolerant to low saturation waters during early settlement and growth. However, the development of the CCA ceased after 3 months at the ojos, and by 14 months, the ojo tiles had 82% less CCA than controls. This finding is similar to laboratory results of Kuffner *et al.* (2007), who estimated a drop in percent cover of CCA of more than 90% for a similar decrease in saturation (Ω_{arag}=1.5). On average, erect calcified algae had 89% less cover near the ojos and were conspicuously absent from numerous ojo tiles altogether.

This cessation of development in the percent cover of the coralline algae on the ojo tiles coincided with an increase in percent cover of fleshy algae, a trend that was consistent through time and which resulted in 42% greater coverage by fleshy algae at ojo than controls after 14 months of development. While there was variability among the functional groups present on the tiles, such that no statistically significant trends were visible for individual groups of fleshy algae, as a whole, the fleshy algal coverage was greater on ojo tiles compared to controls at each time point. Similar trends were observed by Kroeker *et al.* (2012) at the CO_2 vent site at Ischia, Italy, in which calcareous species did not increase in percent cover after 6 months, which was

attributed to overgrowth by fleshy and turf algae. Our study lends support to the idea that despite being physiologically capable of recruitment, growth and survival in conditions of acidification, competitive interactions between fleshy algae and coralline algae at high pCO_2 result in dramatically reduced percent cover of coralline algae. This competitive advantage of fleshy alga over other taxa was also noted by Connell and Russell (2010), who observed that space occupation by fleshy algae increase at high pCO_2, and Kuffner *et al*. (2007) who showed that overgrowth of turf algae can decrease CCA abundance. In conjunction with these previous findings, our study implies that other anthropogenic stressors that allow fleshy algae to flourish on coral reefs, such as nutrient loading (Lapointe et al., 1997; Fabricius et al., 2005) or overfishing (Bellwood et al., 2004; Mumby et al., 2007), could exacerbate the effects of ocean acidification on CCA development and cover. That is, fleshy algae appear to have a competitive edge over corallines, and additional stressors to coralline communities, or any factor that would give more advantage to the fleshy algae (*i.e.* higher nutrient levels or decreased herbivory) (Lapointe et al., 1997; Thacker et al., 2001; Koop et al., 2001), will likely compound the direct effects of acidification to coralline communities.

Coralline algae are important ecological components of a coral reef, as they cement the reef framework and provide chemical settlement cues and settlement substrate for coral larvae (Morse et al., 1988; Heyward et al., 1999): understanding the response of coralline algae to ocean acidification is therefore of critical importance. A dramatic

decline in coverage under conditions of ocean acidification indicates that both the basic framework of reefs and the recruitment of corals could decrease with decreasing Ω_{arag}. Doropoulos *et al*. (2012) investigated coral recruitment in response to reduced coralline algae abundance at high pCO_2 and found that coral settlement was mediated by settlement cues from CCA's, which were most heavily impacted by saturation state. Their study noted a greater than 45% decline in coral recruitment due to declining CCA and loss of settlement cues. In our study, the experimental substrates were deployed in August (a likely time for coral mass-spawning) and retrieved 14 months later in the hopes of capturing at least one mass-spawning event. Despite this, the number of corals that recruited and settled on the tiles was not sufficient to address how acidification may impact coral recruitment and growth. As the recruitment substrates were placed in a lagoon with low overall coral coverage, this was not unexpected. However, the 14-month control tiles had 4 colonies of *Siderastrea radians*, while no corals were present on the low saturation tiles, a trend that we feel is worth noting. The small juvenile colonies present suggested the corals were recent recruits. As previously noted, corals rely on important settlement cues from coralline algae, and as the low saturation tiles had approximately 80% less CCA than the control tiles, they may have been less hospitable to coral larvae. Our study also suggests that this direct decline in coral recruitment can in part be attributed to the competitive advantage of fleshy algae over CCA in more acidic conditions.

When considering all calcareous species (flora and fauna combined), the difference in

percent cover between control and ojo tiles was similar at all time points, with approximately 70% less cover on the ojo tiles. The percent cover at the controls increased over time; however, in the ojo zones, there was no increase in calcifying cover after 6 months. Unlike the Ischia site, where no calcareous species were found at Ω_{arag}=1.2, low saturation at Puerto Morelos ($\Omega_{arag}\leq$1.5) still had up to 30% cover of calcifying organisms. This could be because the calcareous species present at Puerto Morelos are naturally more resilient to acidification, or because calcification continues to drop as the saturation level decreases reaching negligible levels at Ω_{arag}=1.2. Regardless, this finding has significant implications for future reef development, as the reduction in calcified taxa was immediate and persistent throughout the duration of the deployment.

The observed patterns are also consistent with competition between calcifying taxa. The trends observed for encrusting foraminifera (Fig. IV.6) over the 14-month study suggest that CCA have a decreased ability to compete under acidification conditions. While the abundance of encrusting foraminifera increased from 3-6 months by 40% on the control tiles, at 14 months when CCA became established, the trend reversed and they decreased by 70%. Visual analysis of the tiles revealed that many of the forams were overgrown by CCA between 6 months and 14 months, suggesting CCA out-competed the forams for remaining space. This trend was not seen on the ojo tiles. Instead, the number of forams continued to increase on the ojo center tiles where CCA were not able to establish dominance and were 80% less abundant. As with the

calcifying algae, our results suggest that the calcifying foraminifera were able to grow in the ojo conditions, and that community changes were primarily driven by competition among species, where those that did not compete with CCA occupied more space on the hard substrate.

Differences in community structure between chemistry regimes were also reflected by a higher abundance of biofilm on the low saturation tiles. By 14 months, biofilm cover was significantly higher on the ojo than at the control tiles, a trend that is also consistent with the Kroeker *et al*. (2012) study. Additionally, in our study, there was a significant increase in the amount of biofilm in both zones at 6 months. Biofilms are essential components of marine ecosystems, as they are food for a number of grazers (Hill et al., 1991; Thompson et al., 2004), and more importantly to this study, likely help mediate the settlement and metamorphosis of benthic organisms (Meadows et al., 1963; Thompson et al., 1998; Tebben et al., 2011; Johnson et al., 2013). Biofilms begin to settle on substrates within hours of submersion and are associated with early stages of succession (Meadows et al., 1963; Thompson et al., 2004). The significantly higher percent cover of biofilm on the ojo tiles after 14 months of development could suggest they remained in an earlier stage of development for a longer time compared to the control tiles (as seen at Ischia). The increase in biofilm on both tiles at 6 months of growth can potentially be explained by seasonal variability (the tiles were removed in the dry season), as biofilm abundance has been shown to be inversely related to solar stress (Hill et al., 1991; Thompson et al., 2004). Indeed, the 3 months

preceding the 6-month tile removal (March 2011) had characteristically low precipitation totals, only accounting for approximately 10% of the total rainfall for the year (Fig IV.9).

There were no obvious trends in vermetid mollusc or polychaete abundance by site or zone. In fact, the vermetid mollusc abundance increased significantly by 14 months on the control tiles. This observation of resilience by certain taxa to acidification is mirrored by several studies to date (Jokiel et al., 2008; Kroeker et al., 2012, 2013; Fabricius et al., 2014). However, as these organisms were likely only exposed to low saturation conditions after settlement on the tiles, it is not clear from our study if acidification may impact earlier life history stages. It is possible that these taxa are impacted more in the early larval stages before settlement, and that "carry-over" effects which are expressed only in adults that were exposed to acidification during early stages of growth (Hettinger et al., 2012) are responsible for the discrepancy seen between the populations in this study and those of laboratory experiments.

While *in-situ* field studies are valuable for investigating how complex assemblages may respond to acidification, multiple environmental parameters may co-vary, making it difficult to resolve the influence of Ω_{arag} on the community assemblages from that of other factors, or to assess the extent to which the influence of Ω_{arag} may be modulated by other, co-varying factors. While temperature and light are comparable between the ojo and control zones in our study, salinity and nutrients

often co-vary with changes in saturation state in the ojos as they are all dependent on the flux of submarine groundwater discharge. It is therefore important to compare our results to those derived from additional field studies where Ω_{arag} conditions are not coupled with groundwater discharge. At the Ischia volcanic vent site, there were no salinity or nutrient changes associated with pH zones, and the similarity of our results with those of Kroeker et al. (2012) (after which this investigation was designed) lends weight to the idea that the carbonate chemistry is the primary driver of change in the communities at the ojos. Specifically, the similarities in community structure and composition with respect to CCA and fleshy algae allow us to isolate the effect of ocean acidification on the assemblages from other factors.

Our study illustrates that while acidification will have significant direct impacts on calcification, the altered competitive interactions between organisms will also impact community assemblages in the future. That is, we expect to see a shift in communities from coralline algal coverage to fleshy algae over time as pCO_2 increases over the 21st century. It is important to note that the tropical benthic calcifying organisms were able to recruit and grow in low Ω_{arag} conditions. However, competition for space as the community developed rather than physiological limitations were leading drivers in the community shifts observed. Thus, our study illustrates the importance of observing the response of entire communities to OA, as interactions between organisms will compound the direct effects of acidification and likely increase reef degradation beyond the estimates derived from species-specific observational studies.

This study thus illustrates the need for conservation and policy decisions that will consider community-wide responses to acidification, particularly with regard to the increased competition between calcifying and fleshy algal species at decreased saturation. For instance, if fleshy algal species are more successful at high nutrient levels, then mitigation strategies that reduce eutrophication of surface waters will prevent fleshy algae from having an even greater competitive edge over calcifying species. As the oceans become more acidic over the 21st century, it will become essential to alleviate human impacts that have the potential to compound competitive interactions between organisms.

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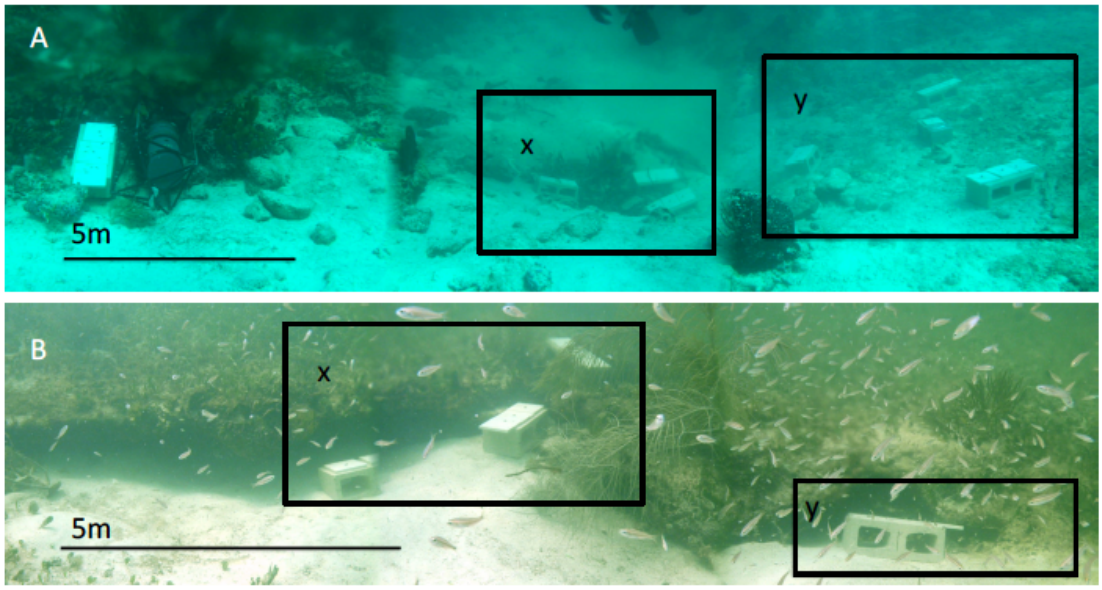


Figure IV.1. The two ojo sites. Ojo A (A) and Ojo B (B) during recruitment substrate deployment (time zero). The low pH-Ω_{arag} zones (x) are within 10m of ambient zones (y) at each site.

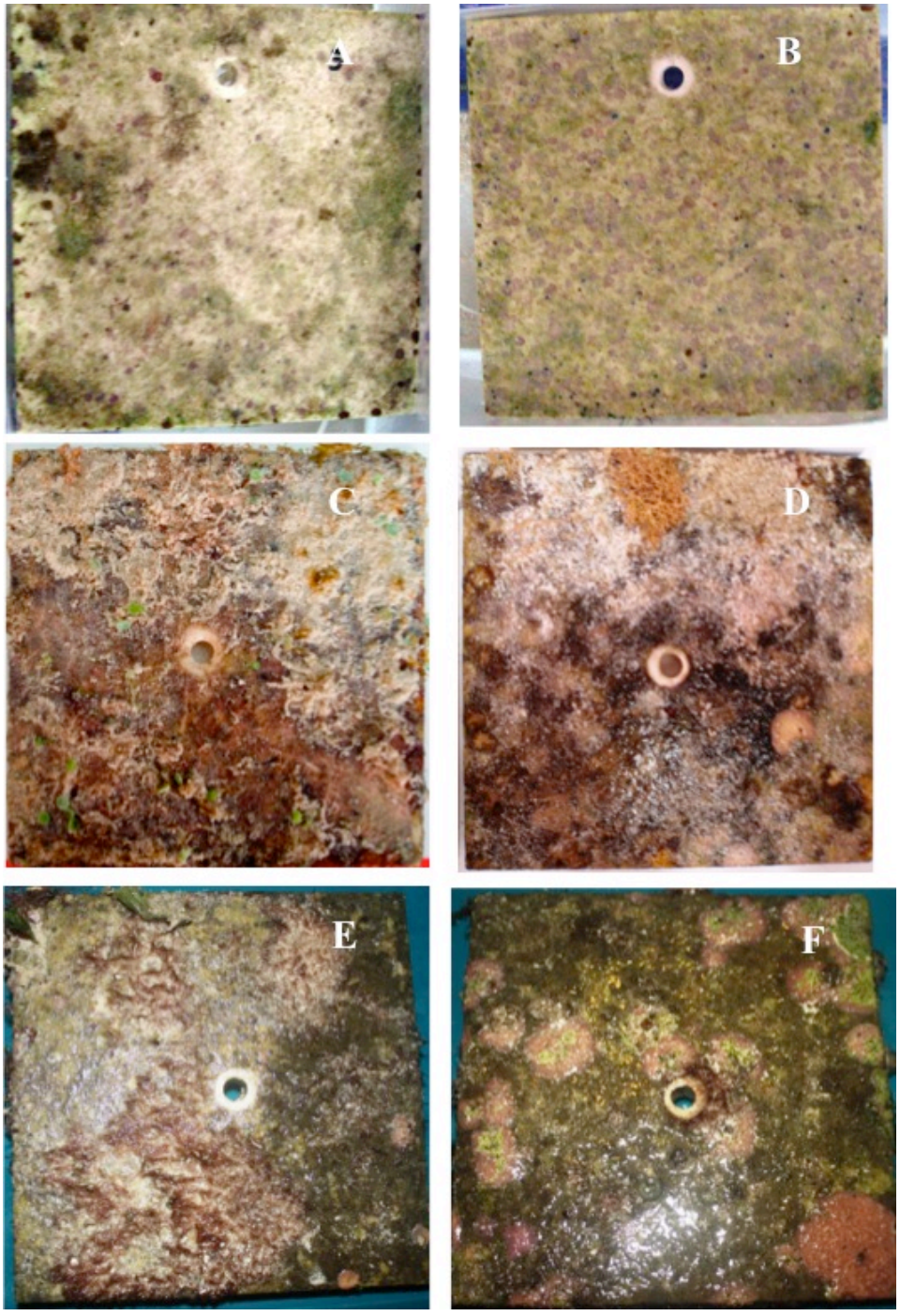


Figure IV.2. Tiles after recovery. Examples of tiles collected at 3 months (A, B), 6 months (C,D), and 14 months (E,F), at ojo centers (A,C,E) and controls (B,D,F).

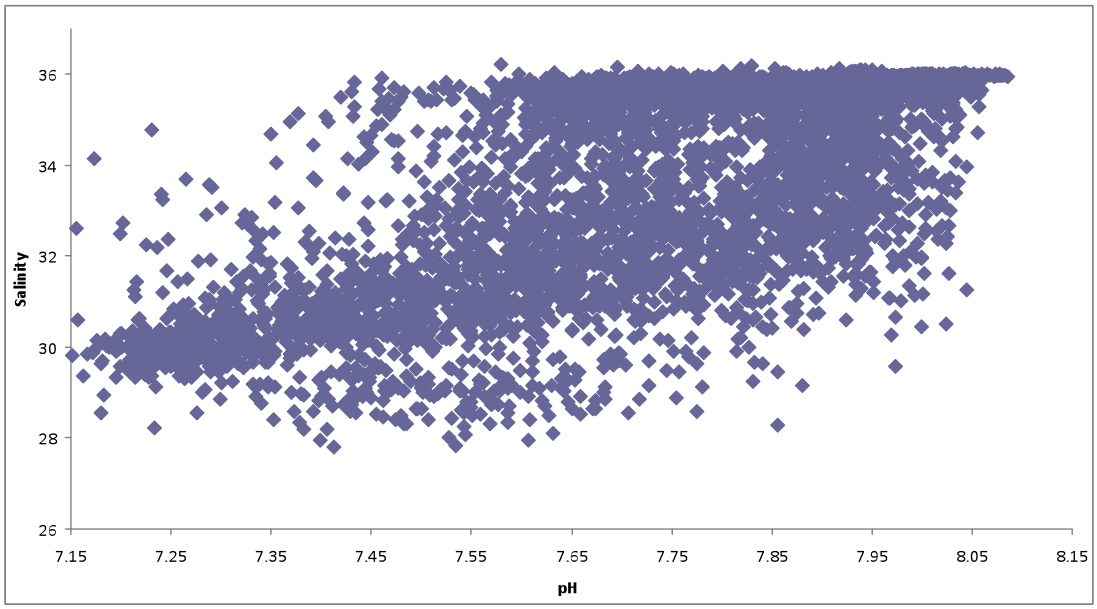


Figure IV.3. Salinity and pH over time as measured by an autonomous sensor. Salinity and pH were measured at 15 minute time intervals for a period of 3 months (August-October 2010) for a total of over 5500 data points at a single spring. Salinity is plotted against pH (**a**), and grouped according to the number of data points occurring in a given salinity range (**b**). As depicted, 93% of data points fall above a salinity of 30, and salinity never drops below 27 at the center of discharge. The lower salinity conditions are during low tide in the rainy season and the conditions do not prevail for more than a one hour.

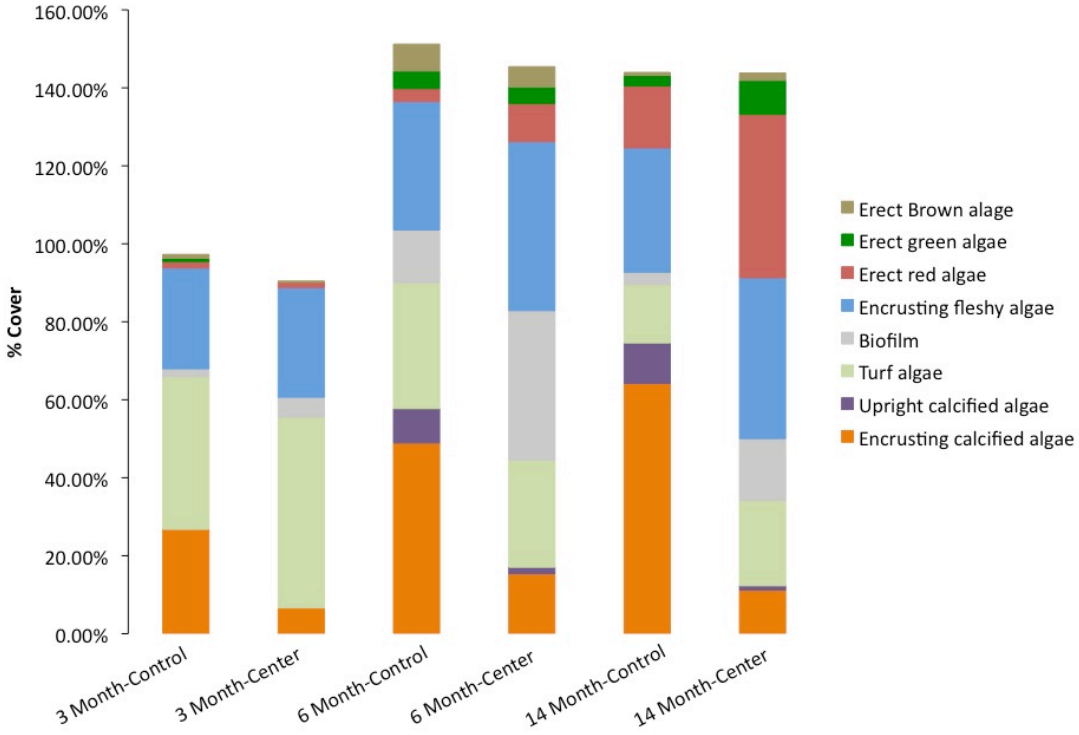


Figure IV.4. Average percent cover by taxonomic group (Sites A+B) at 3, 6 and 14 months. Percent cover can be greater than 100% due to the multiple layers of organisms present on the tiles.

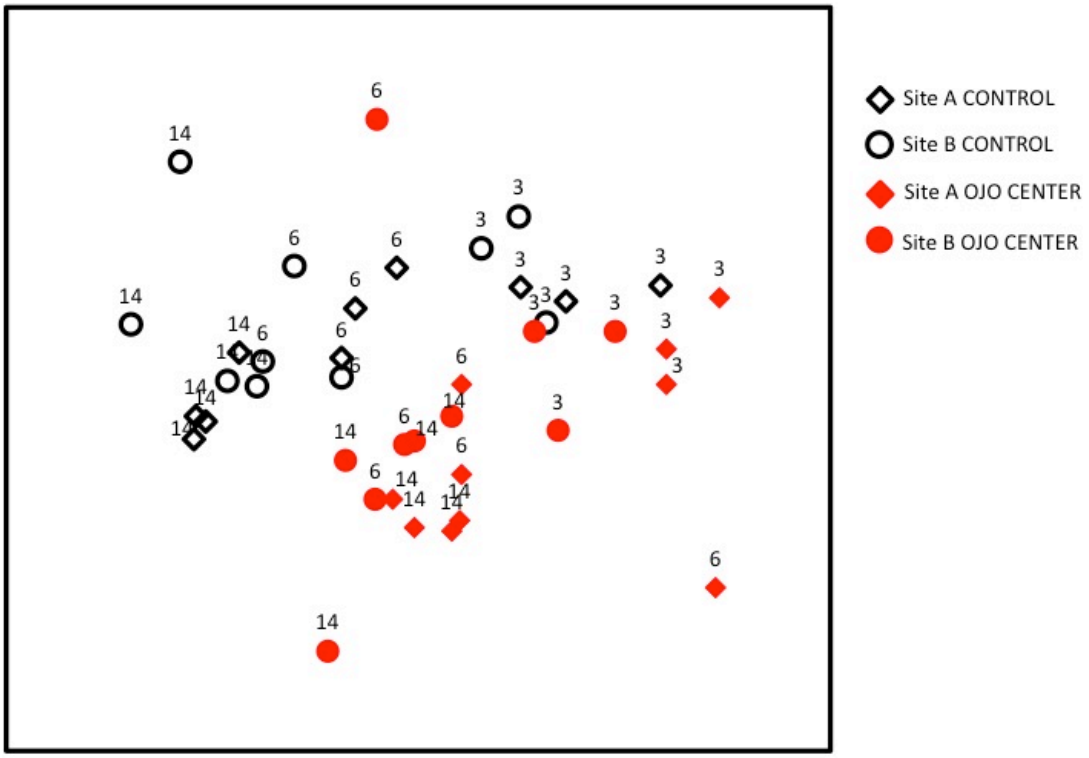


Figure IV.5. Non-metric Multidimensional Scaling (nMDS) for community structure. In an nMDS, distance on the plot is a measure of dissimilarity. Each dot is representative of a single tile, labeled by site (open circles (site A) or closed circles (site B)), time (3, 6, or 14 months) and saturation state (red for ojo centers, black for controls). In an nMDS plot, multivariate data are placed into two-dimensional space so that the rank differences among the data are preserved, based on the BC dissimilarity matrix. Thus communities that are more dissimilar to one another are farther apart on the plot.

Figure IV.6. Average % cover of each taxa over time. Open black symbols represent controls and closed red symbols represent the ojo centers. Diamonds depict Site A and circles depict Site B. Error bars are ± S.D. Note that the vertical (y-axis) scales on each figure are different.

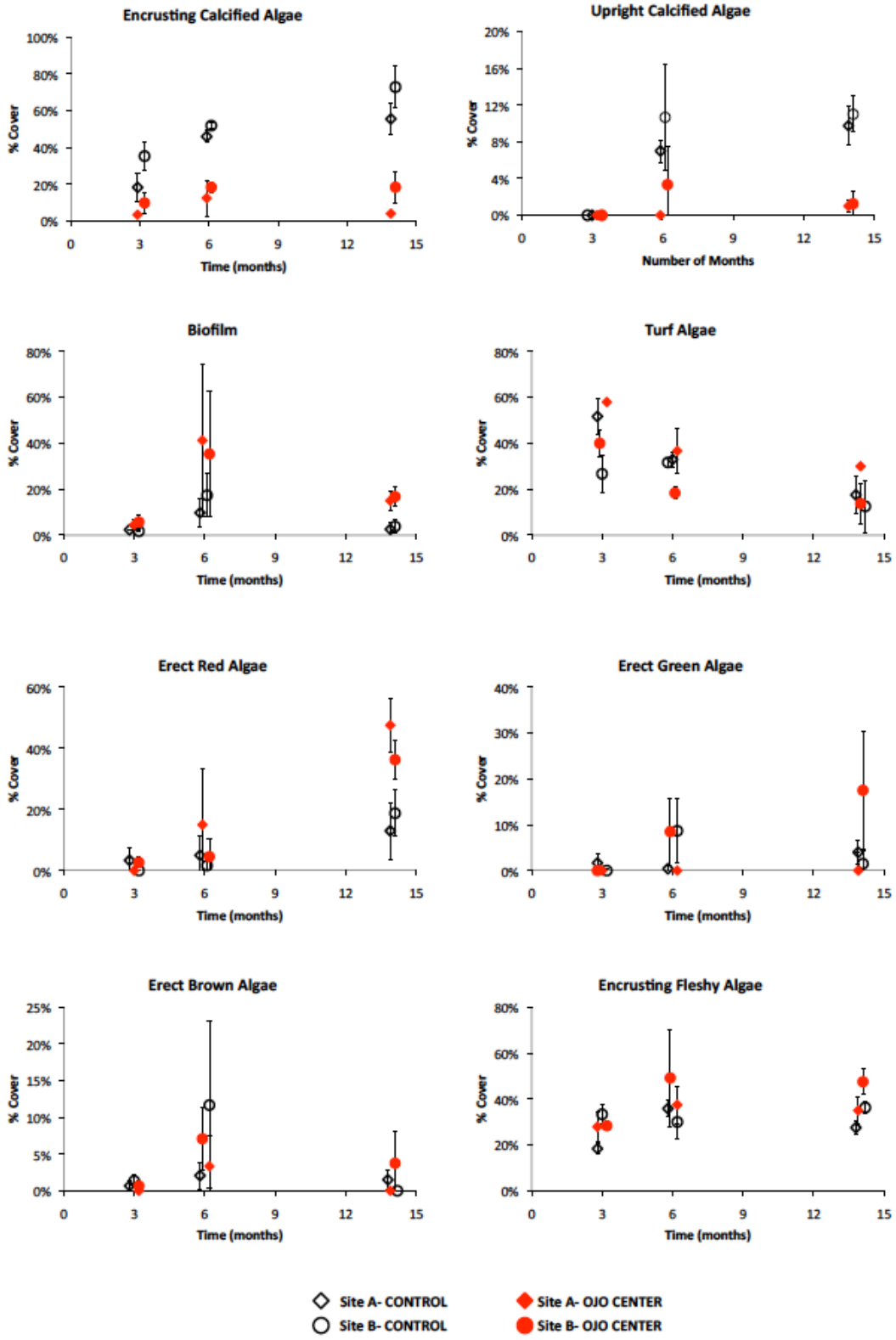
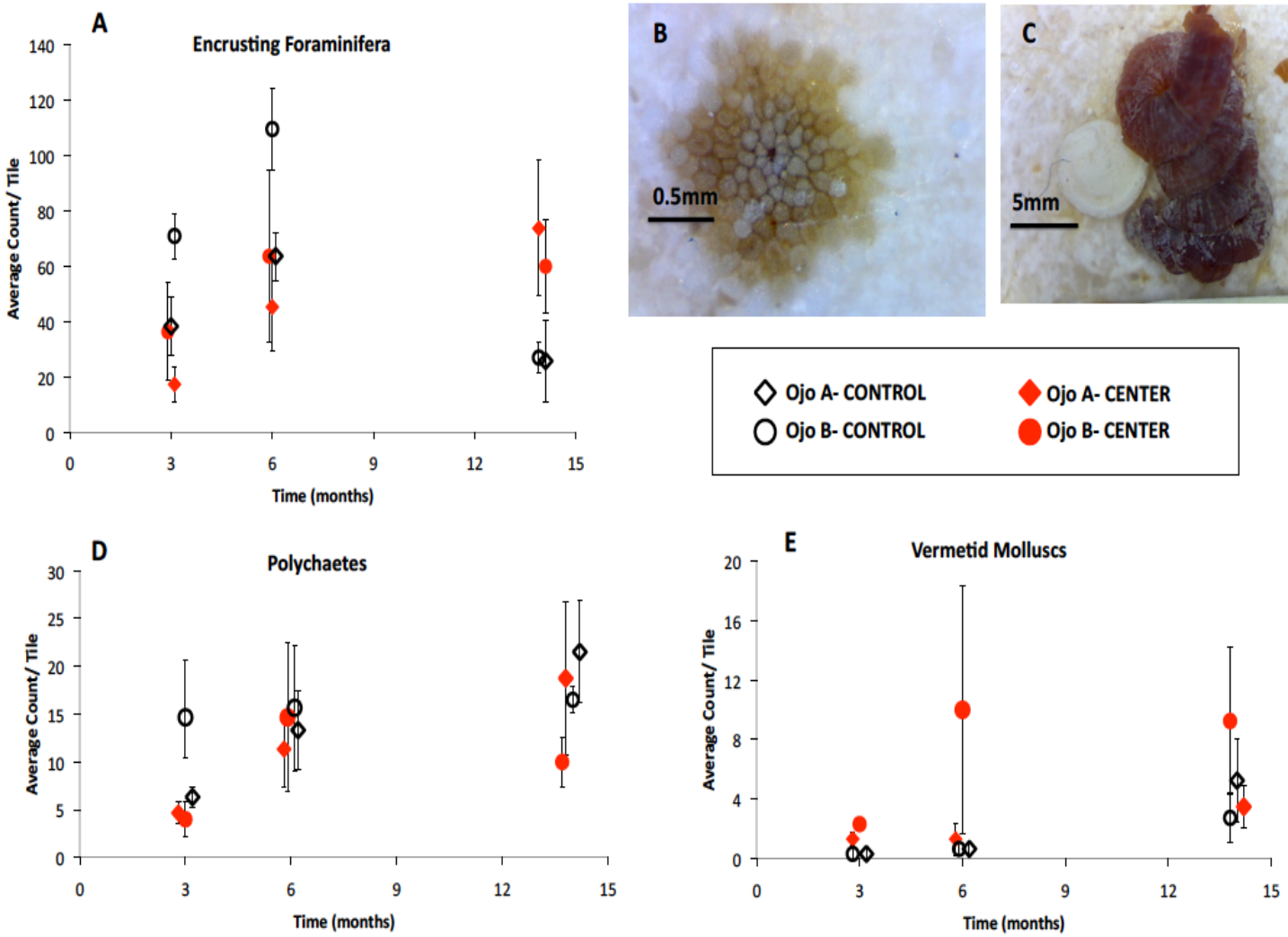

Encrusting Calcified Algae
% Cover
Time (months)
Upright Calcified Algae
% Cover
Number of Months
Biofilm
% Cover
Time (months)
Turf Algae
% Cover
Time (months)
Erect Red Algae
% Cover
Time (months)
Erect Green Algae
% Cover
Time (months)
Erect Brown Algae
% Cover
Time (months)
Encrusting Fleshy Algae
% Cover
Time (months)
Site A- CONTROL
Site B- CONTROL
Site A- OJO CENTER
Site B- OJO CENTER

Figure IV.7. Encrusting foraminifera, polychaete, and vermetid mollusc abundance. (**a,d,e**) Average encrusting foraminifera, polychaete, and vermetid mollusc abundance for each set of tiles by month. Open black symbols represent controls and closed red symbols represent the ojo centers. Diamonds depict Site A and circles depict Site B. Error bars are ± S.D. Note that the vertical (y-axis) scales on each figure are different. Visual examples of encrusting foram (**b**) and vermetid molluscs (**c**) found on the tiles. Images taken with a 0.1mm accuracy digital microscope.


A
Encrusting Foraminifera
Average Count/ Tile
Time (months)
B
0.5mm
C
5mm
Ojo A- CONTROL
Ojo B- CONTROL
Ojo A- CENTER
Ojo B- CENTER
D
Polychaetes
Average Count/ Tile
Time (months)
E
Vermetid Molluscs
Average Count/ Tile
Time (months)

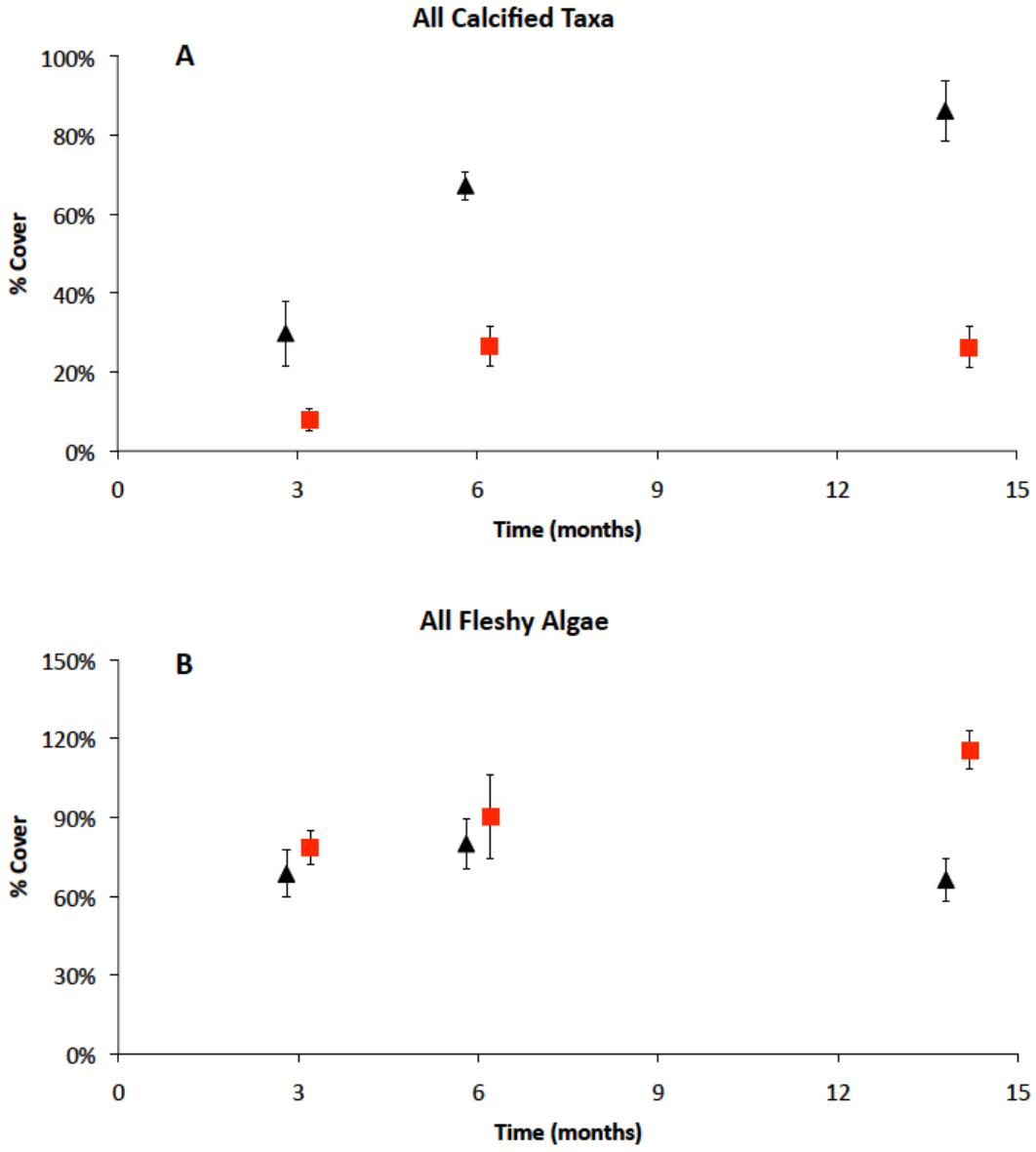


Figure IV.8. Aggregate community indices. Aggregate calcified taxa (A) and combined fleshy algal indices (B) by month. Each point represents combined data for Sites A and B. Black triangles are controls and red squares are ojo centers. Error bars are ± S.D.

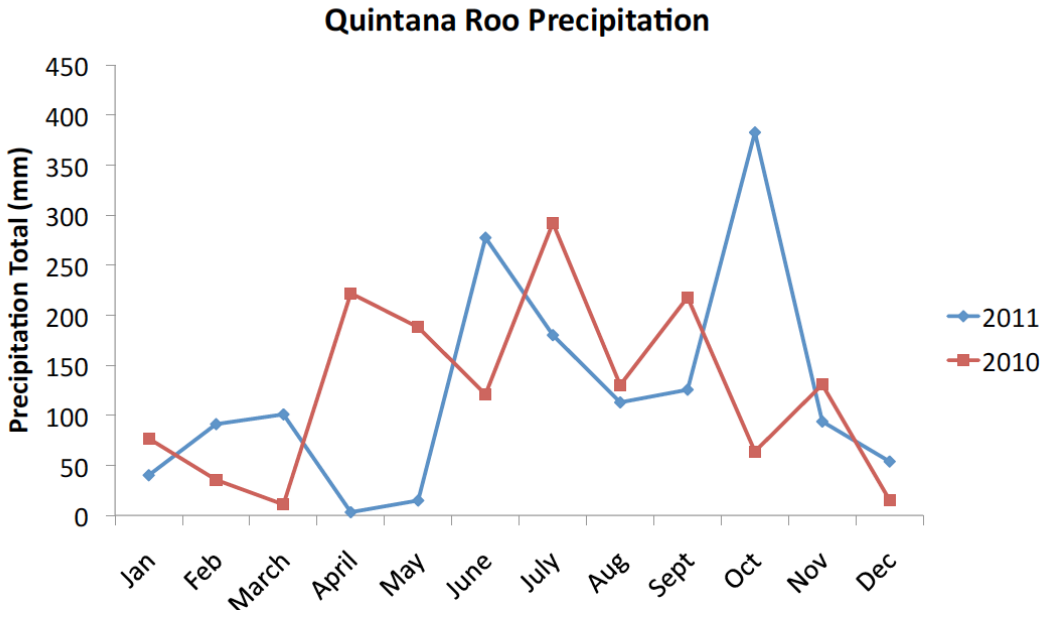


Figure IV.9. Total monthly rainfall (mm) for the Quintana Roo, Mexico region in 2010 and 2011. The study began in August of 2010 and ran until November of 2011. The dry season characteristically runs from November to March. From December 2010 to March 2011, the region received total precipitation of ~147 mm, compared to the yearly total of ~1470 mm. This suggests that the region was characteristically dry with increased solar stress in the months preceding the 6-month tile removal.

	Ojo A	Ojo B	Control A	Control B
N	**20**	**14**	**29**	**26**
A_T **(µmol/kg)**	3065 ± 77	2965 ± 92	2380 ± 34	2396 ± 54
C_T **(µmol/kg)**	3096 ± 83	2882 ± 110	2060 ± 57	2089 ± 70
pH_T	7.44 ± 0.06	7.38 ± 0.05	8.10 ± 0.04	8.09 ± 0.05
Ωarag	1.4 ± 0.4	1.55 ± 0.4	3.9 ± 0.5	3.6 ± 0.6
Temp (°C)	26.7 ± 0.40	28.3 ± 0.2	27.7 ± 1.6	28.0 ± 1.7
Salinity	31.7 ± 0.78	30.5 ± 0.5	33.36 ± 2.2	33.5 ± 2.3

Table IV.1. Water Chemistry at the two ojo sites and controls. Saturation was calculated from discrete water samples for dissolved inorganic carbon (C_T) and total alkalinity (A_T). Values are averages from N=x samples collected over the 14 months deployment.

V. Impacts of food availability and pCO_2 on planulation, juvenile survival, and calcification of the azooxanthellate scleractinian coral *Balanophyllia elegans*

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Summary

Ocean acidification, the assimilation of atmospheric CO_2 by the oceans that decreases the pH and $CaCO_3$ saturation state (Ω) of seawater, is projected to have severe adverse consequences for calcifying organisms. While strong evidence suggests calcification by tropical reef-building corals containing algal symbionts (zooxanthellae) will decline over the next century, likely responses of azooxanthellate corals to ocean acidification are less well understood. Because azooxanthellate corals do not obtain photosynthetic energy from symbionts, they provide a system for studying the direct effects of acidification on energy available for calcification. The solitary azooxanthellate orange cup coral *Balanophyllia elegans* often lives in low pH, upwelled waters along the California coast. In an 8-month factorial experiment, we measured the effects of three pCO_2 treatments (410, 770, and 1220 µatm) and two feeding frequencies (3 d and 21 d intervals) on planulation (larval release) by adult *B. elegans*, and on the survival, skeletal growth, and calcification of newly settled juveniles. Planulation rates were affected by food level but not pCO_2. Juvenile mortality was highest under high pCO_2 (1220 µatm) and low food (21 d intervals). Feeding rate had a greater impact on calcification of *B. elegans* than pCO_2. While net calcification was positive even at 1220 µatm (~3 times current atmospheric pCO_2), overall calcification declined by ~25-45%, and skeletal density declined by ~35-45% as pCO_2 increased from 410 to 1220 µatm. Aragonite crystal morphology changed at high pCO_2, becoming significantly shorter but not wider at 1220 µatm. We conclude

that food abundance is critical for azooxanthellate coral calcification, and that *B. elegans* may be partially protected from adverse consequences of ocean acidification in habitats with abundant heterotrophic food.

V.1. Introduction

As aqueous CO_2 concentrations continue to rise over the next century, pH of oceanic surface waters will decline in the process known as ocean acidification (Caldeira and Wicket, 2003, 2005; Sabine et al., 2004). Growing evidence suggests that calcifying organisms, including reef-building scleractinian corals, will be heavily impacted by a decrease in pH from 8.1 to 7.8 (Orr et al., 2005; Hoegh-Guldberg et al., 2007; Fabry et al., 2008; Doney et al., 2009). In many laboratory and field investigations, calcification rates of tropical corals declined as pH and aragonite saturation state (Ω_{arag}, a measure of ease of $CaCO_3$ formation) decreased (Fine and Tchernov, 2007; Anthony et al., 2008; Jokiel et al., 2008; Krief et al., 2010). Almost all tropical corals have algal symbionts (zooxanthellae) whose photosynthesis contributes to the host's nutrition and increases calcification rates. Most deep and cold-water corals lack zooxanthellae, but their potential responses to ocean acidification are largely unknown; as the saturation state of seawater decreases, their calcification rates are also likely to decline and their geographical distributions may change (Turley et al., 2007; Andersson et al., 2008; Fabry et al., 2009; Maier et al., 2009). Experiments indicate that cold-water species vary in sensitivity to CO_2 manipulation, and some deep-water species can maintain positive net calcification at or below the carbonate saturation horizon, the depth below which $\Omega < 1$ and $CaCO_3$ can dissolve readily (Form and Riebesell, 2012; McCulloch et al., 2012; Maier et al., 2011). Because azooxanthellate corals lack symbionts and rely solely on heterotrophy for energy,

they provide a simplified system for exploring the roles of nutrition (and energy) in coral calcification.

Ocean acidification is predicted to have especially severe impacts in upwelling regions where low saturation waters occur naturally (Feely et al., 2008; Fabry et al., 2009; Hauri et al., 2009). Strong seasonal upwelling along the western North American coast during summer months brings CO_2 rich, low pH water from intermediate depths to the surface, and coastal organisms may be exposed to low and even under-saturated waters for several months of the year (Feely et al., 2008; Hauri et al., 2009). With anticipated increases in surface ocean pCO_2 and shoaling of the carbonate saturation horizon, calcifying organisms living in these coastal waters are likely to experience seasonal increases in the magnitude, duration, and extent of low pH waters (Feely et al., 2008; Hauri et al., 2009). Understanding the impacts of ocean acidification on calcification and on the interplay between calcification and nutritional status is critically important in upwelling regions where many organisms may already be living near their lower thresholds for pH tolerance (Barton et al., 2012) and therefore may be particularly vulnerable to ocean acidification. Several recent studies have described likely negative consequences of future ocean acidification events for several key species in the California coastal upwelling zone (Gaylord et al., 2011; Barton et al., 2012; Hettinger et al., 2012; Timmins-Schiffman et al., 2012) that often experiences seawater pH as low as 7.8 during the summer.

Responses of calcifying corals to ocean acidification depend on species-specific energy allocations for calcification. Corals expend energy to remove protons from their calcifying compartments, the extracellular medium between the coral's basal cell membrane and the skeleton below (Al Horani et al., 2003; Allemand et al., 2004; Cohen and McConnaughey, 2003). Removing protons facilitates calcification by increasing pH and $CaCO_3$ saturation state in the calcifying fluid. In tropical zooxanthellate corals, proton pumping raises the saturation state in the calcifying fluid up to 5-10 times ambient (Al Horani et al., 2003; Cohen and Holcomb, 2009), and deep-water azooxanthellate corals can create even steeper gradients (McCulloch et al., 2012). Lower saturation in the external seawater require corals to expend more energy to remove excess protons (Ries, 2011; McCulloch et al., 2012), potentially at the cost of other critical life processes (Wood et al., 2008). A flexible energy budget would enable corals to vary the energy expended to raise the pH and saturation states of the calcifying fluids, and perhaps enable them to maintain calcification despite acidification. Some research indicates that zooxanthellate coral calcification is energetically costly and that a coral's energy budget is not flexible enough to raise the pH of the calcifying fluid under acidic conditions (i.e., the energy budget is fixed) (Cohen and Holcomb, 2009), but some species can maintain up to 100% of their calcification rates in near under-saturated conditions when provided with excess nutrients (Langdon and Atkinson, 2005; Holcomb et al., 2010, Ries et al., 2009; Cohen et al., 2009). Nutrients may stimulate increased photosynthesis by zooxanthellae, giving the coral more energy for calcification. Similarly, increased

heterotrophic feeding by certain zooxanthellate corals can reduce acidification impacts on calcification (Drenkard et al., 2013, Edmunds, 2011). All corals in these studies contained energy-producing zooxanthellae, whereas azooxanthellate corals cannot obtain extra photosynthetic energy from symbionts. Therefore azooxanthellate corals provide a system in which it is possible to study the direct effects of ocean acidification and energy availability on calcification.

Balanophyllia elegans is a solitary, azooxanthellate scleractinian coral common in shallow coastal waters around Monterey Bay, California, where it is exposed seasonally to low pH, high pCO_2 upwelling waters. We assessed the effects of pCO_2 and food availability on planulation rates of adult *B. elegans*, and then explored the effects of the same treatments on survival, growth, and calcification of juvenile *B. elegans* during an 8-month incubation experiment. The duration was based on recommendations of Doney et al. (2009) and Widdicombe et al. (2010) for long-term manipulation experiments. Recent evidence suggests that long-term exposure more accurately predicts responses to acidification than short-term experiments (Form and Riebesell, 2012). We address how azooxanthellate corals may respond to lower ocean pH, and the roles of nutrition on their calcification and survival in low saturation, upwelling regimes.

V.2. Materials and Methods

V.2.a. Organisms

The orange cup coral *Balanophyllia elegans* Verrill, 1964 is a solitary (single polyp) species living on rocky substrates from the low intertidal to ~300 m depth along the west coast of North America, from southern Alaska to Baja California. It is gonochoric (has separate sexes) with a sex ratio of approximately 1:1, and has an annual gametogenic cycle with gametes maturing in mid-summer. Fertilization is internal and zygotes are brooded for about 15 months in the mother's coelenteron where they develop into mature planula larvae that are released in autumn to early winter (Fadlallah and Pearse, 1982). On release (planulation), the larvae crawl down the mother's column and settle and metamorphose into juvenile corals (Fig. V.1), usually within a few centimeters of the mother (Gerrodette, 1981). *B. elegans* is an azooxanthellate species (lacking photosynthetic symbiotic algae) depending on heterotrophic feeding on zooplankton or dissolved organic molecules for all of its energy and nutrients. It grows slowly and can survive for months without feeding.

V.2.b. Experimental design

The experiment used a full factorial design with two factors: pCO_2 (3 levels) and feeding frequency (2 levels). The seawater pCO_2 levels were based on recent atmospheric concentrations (380 ppm, $pH_T = 8.0$) and two IPCC emissions scenarios projected for the year 2100, the A1B "business as usual" (750 ppm, $pH_T = 7.8$), and a high emissions scenario (A1F1) approximately 3 times current atmospheric pCO_2

(1200 ppm, pH_T = 7.6) (Solomon et al., 2007). Experimental pCO_2 levels were slightly higher than these targets at: 410 µatm (pH_T = 8.0), 770 µatm (pH_T = 7.8), and 1220 µatm (pH_T = 7.6). High Food corals were fed newly hatched nauplii larvae of brineshrimp (*Artemia*) every 3 d to represent a plentiful food supply, while Low Food corals were fed once every 21 d, corresponding to a minimal maintenance food supply (Beauchamp, 1989).

Each experimental unit was a 4 L glass jar (approximately 240 mm high and 180 mm in diameter) with an airtight screw cap (lined with a double-layered rubber membrane) containing an inlet for CO_2-enriched air, an outlet for excess air and a sampling port (stoppered rubber valve) for taking pH readings and water samples. Without breaking the airtight seal of the lid, a plastic paddle (90 x 72 mm) on a 110 mm long plastic rod was inserted through the double-layered rubber membrane to provide continuous water movement by mechanical stirring; it oscillated about 30 times per min. There were 2 replicate jars per treatment for a total of 12 jars.

Temperature was maintained close to ocean ambient by placing the jars in a water table with running seawater. A daily light regime of 12 h light (overhead fluorescent bulbs) and 12 h dark was maintained throughout the experiment.

Corals were fed through the sampling port using a syringe to inject 50 mL of concentrated *Artemia* nauplii in filtered seawater (approximately 10,000-15,000

nauplii per jar). The brineshrimp remained in the jars for several hours to ensure the corals had eaten their fill. Every jar, lid, and paddle was cleaned once every 3 d after feeding ended. During cleaning, corals were removed for approximately 30 min and placed in small glass dishes with filtered seawater equilibrated to their experimental pCO_2. The jars and lids were then scrubbed and rinsed, and new filtered, equilibrated seawater was siphoned into the jars to prevent air exchange.

V.2.c. Experimental corals

Two groups of corals were exposed simultaneously to the experimental treatments. The first group consisted of adults to determine whether pCO_2 and feeding treatment affected planulation rates. Ten adult corals of equal size (sex unknown) were assigned to each treatment (i.e., 5 adults per jar) for the first 3 months of the experiment (6 November 2011 through 31 January 31 2012). The 60 adults had been held in the same tank for more than two years with flowing ambient seawater (pH_T ~7.9-8.0) at the UCSC Long Marine Laboratory (LML). Because they brood larvae for ~15 months (Fadlallah and Pearse, 1982), all females should have been equally likely to produce larvae. Every 3 d all larvae produced were counted and removed from the experimental jars.

The second group consisted of newly settled juveniles to determine whether CO_2 and feeding levels affect juvenile survival, growth, and calcification. The juveniles came from a stock of adult *B. elegans* maintained for several generations in the laboratory.

Approximately 85 adults were sequestered in a tank with flowing ambient seawater during the peak planulation season (November to December 2011). Emerging larvae were collected weekly for a month (6 November to 15 December 2011) and placed in glass dishes in a separate tank with flowing seawater, where they were allowed to settle on polypropylene plastic sheets pre-conditioned with a living biofilm and crustose coralline algae. After settlement, small pieces of plastic, each holding one or two larvae, were cut and glued to 5 x 5 cm ceramic tiles (approximately 5 per tile), and immediately transferred to their randomly assigned experimental treatments and jars (Fig. V.1). Since skeletal formation does not begin for at least two weeks after settlement, there was no calcification before exposure to the experimental conditions. All juvenile corals (a total of 202 individuals) started with approximately equal weights and volumes, and initial skeletal weights of 0 mg (i.e., no calcium carbonate). The newly settled juveniles were introduced from mid-November to mid-December 2011 and the experiment ran for approximately 8 months until July 2012. Juvenile mortality was monitored by recording deaths every 3 d.

V.2.d. Seawater carbonate chemistry

The LML is on the open coast north of Monterey Bay where it is exposed to oceanic water driven by prevailing onshore winds. Seawater supplied to LML is sand-filtered down to 30 μm before being pumped into elevated water towers from which it flows under gravity to individual laboratories: it is an open flow-through water system with no seawater recirculation. Experimental seawater was filtered to 0.2 μm in ~800 L

batches to ensure the experiment was not subject to ambient fluctuations in seawater chemistry (4 water batches were prepared at approximate 8 week intervals during the experiment). Cylinders of certified CO_2-air mixtures were obtained from PraxAir (CO_2 at 380, 750, and 1200 ppmV). Filtered water was sampled for salinity and nutrient analyses, transferred to 20 L carboys, and then bubbled with the appropriate gas mixture for at least 4 d to equilibrate pCO_2 and stabilize pH before capping and storing until needed. Water was siphoned from the carboys into the experimental jars which were sealed except for the appropriate pCO_2 gas mixture flowing continuously into the headspace.

pH and temperature were measured daily in each jar with an Oakton WD-35613 hand-held meter calibrated using NIST standards. 40 mL water samples were taken from each jar every 3 d for dissolved inorganic carbon (C_T) and total alkalinity (A_T) analyses (samples were obtained on cleaning days, before feeding and before the water was changed). C_T was measured using a CM5011 Carbon Coulometer (UIC, Inc.) and A_T was measured with an automated, open-cell potentiometric titration procedure. Certified Reference Materials (batch 118) from the Dr. Andrew Dickson laboratory at Scripps Institution of Oceanography were used to calibrate each instrument. C_T and A_T were used to calculate aragonite saturation state (Ω_{arag}) and pH via CO_2sys software (Pierrot et al., 2006), using CO_2 dissociation constants from Merhbach et al. (1973) refit by Dickson and Millero (1987). pH is reported in total scale (pH_T). Salinity was measured with a salinometer (Guildline 8410 PortaSal), and

nutrients were analyzed on a flow injection autoanalyzer (FIA, Lachat Instruments Model QuickChem 8000) for each of the four batches of water using standard operating procedures.

V.2.e. Skeletal growth

At the end of the experiment, each living coral was imaged under a microscope at 40X, then dried in a 50˚C oven for 48 h. All tissue was removed from skeletons in a 1:1 solution of 30% H_2O_2 buffered with 0.1 M NaOH before measuring dimensions and weight. Juvenile *B. elegans* are elliptical cylinders (Fig. V.1c, d). After measuring skeletal height (h) and major (X) and minor (Y) diameters with vernier calipers (± 0.1 mm), major (x) and minor (y) radii were calculated, and the volume (V) of each coral skeleton was estimated as:

$$V = \pi \, x \, y \, h \qquad \text{(Eq. V.1)}$$

Skeletal weight (± 0.01 mg) was determined on an analytical balance and the bulk density of each skeleton calculated by dividing weight by volume.

Septa (vertical elements partially dividing the cavity of the skeleton) from five randomly selected skeletons from each of the four most extreme treatments (High Food and Low Food with pCO_2 of 410 and 1220 μatm) were imaged with 10kx magnification on a Hitachi TM1000 Tabletop Scanning Electron Microscope (SEM)

at the UCSC MACS facility at NASA/Ames. For the SEM analysis, only the extreme pCO_2 groups were used to ensure maximum differences between treatments were captured. The lengths and widths of individual aragonite crystals were measured from the SEM images using Imaging Processing and Analysis in Java (Image J, U.S. National Institute of Health, Fig. V.2).

V.2.f. Statistics

The software R was used for all statistical analyses (R Core Team 2013). Planulation, volume, weight, density and crystal dimensions were analyzed using 2-factor ANOVAs with pCO_2 and feeding frequency as fixed factors. For planulation, data from replicate jars were combined, so an additive model was applied since interaction terms could not be assessed without replication. All other ANOVAs used a full model including both the main effects and the interactions between pCO_2 and feeding frequency. Coral volumes and weights were log-transformed to satisfy normality assumptions. For crystal dimension analyses, the individual corals from which crystals were sampled were treated as a random factor nested within the two main effects. Where statistical significance was indicated, Tukey's HSD tests were used to compare treatments. Juvenile survival was assessed by a logistic regression against the two categorical predictor variables, pCO_2 and feeding frequency. Since the interaction term was not significant (Tukey's test for additivity; $p = 0.41$), it was excluded from the logistic regression model. All water chemistry is reported as mean ± standard deviation (s.d.).

V.3. Results

V.3.a. Water Chemistry

Average water conditions in the six treatments during the 8 month experiment are summarized in Table 5.1. The average pH ± s.d. (expressed as total scale, pH_T) of the three pCO_2 treatments, calculated from measurements of C_T and A_T from discrete water samples, were 8.02 ± 0.02 (410 ± 21 µatm), 7.78 ± 0.03 (770 ± 75 µatm), and 7.59 ± 0.02 (1220 ± 80 µatm). The corresponding aragonite saturation states (Ω_{arag}) of each pCO_2 treatment were 2.1 ± 0.05 (pH_T 8.0), 1.3 ± 0.1 (pH_T 7.8), and 0.9 ± 0.04 (pH_T 7.6). C_T and A_T measurements varied slightly among treatments, but these differences were not significant (Student's t-tests) and they did not affect average values of the other carbonate parameters. Temperature in the jars varied seasonally with the ambient temperature of the water table containing the jars, but averaged 13.6 ± 1.5 ˚C over the duration of the experiment.

V.3.b. Planulation

B. elegans planula larvae were collected as they emerged from adults every 3 d for 3 months (6 November 2011 to 15 January 2012). Total planula numbers from each treatment were counted (Fig. 5.3) and compared in a 2-way ANOVA (Table V.2). Adult corals in the High Food treatments released more than twice as many larvae (120% more overall) than those in the Low Food treatments ($p = 0.064$), but pCO_2 had no effect on numbers of larvae released ($p = 0.628$, Table V.2).

V.3.c. Juvenile Mortality

During the 8 month experiment, 14% of the total 202 experimental juveniles died (Fig. V.4). Approximately 5-15% more juvenile corals died in Low Food than in High Food treatments ($p = 0.043$, Table V.3). In both the Low Food and High Food groups, 10-20% more juveniles died in the lowest pCO_2 treatment (pH_T 7.6) than in the control (pH_T 8.0) treatment ($p = 0.011$); mortality in the pH_T 7.8 treatment was intermediate, but did not differ significantly from the control ($p = 0.38$, Table V.3).

V.3.d. Skeletal characteristics

Juvenile corals from High Food treatments had significantly larger skeletons (6-7 times by volume; $p < 0.001$, Table V.4, Fig. 5.5a) and were also heavier (4-5 times; $p < 0.001$, Table 5.5, Fig. V.5b) than those from Low Food treatments. Although pH_T had no significant effects on skeletal volume ($p = 0.303$), it did affect skeletal weight under the Low Food regime: skeletons of corals grown at pH_T 8.0 weighed significantly more (by ~45%) than those at pH_T 7.8 ($p < 0.001$) and pH_T 7.6 ($p = 0.001$) (Tukey's HSD tests). Under the High Food regime, corals grown at pH_T 8.0 also weighed more than those in lower pH_T treatments, but the differences were smaller (~25%) and not statistically significant ($p = 0.350$, $p = 0.060$).

Bulk densities of Low Food coral skeletons were approximately 35-40% greater than High Food skeletons in both the pH_T 7.8 ($p = 0.008$) and pH_T 7.6 treatments ($p = 0.04$), but density did not differ significantly between feeding treatments at pH_T 8.0 (p

= 0.11, Table 5.6, Fig. V.5c). At pH_T less than 8.0, skeletal density was approximately 35-45% lower in both the High Food ($p = 0.032$, $p = 0.001$ at pH_T 7.8 and 7.6 respectively) and Low Food ($p = 0.010$, $p = 0.043$) treatments (Tukey's HSD tests).

V.3.e. Crystal structure

Aragonite crystals were significantly longer (~18%) at pH_T 8.0 than at pH_T 7.6 ($p < 0.001$; Table V.7, Fig V.6a). There was also a significant effect of Food due mainly to a strong interaction between pH_T and Food level ($p < .001$): at pH_T 7.6, crystals were ~15% longer in High Food than Low Food treatments ($p < 0.001$), but lengths were almost identical at pH_T 8.0. By contrast, there were no significant effects of either pH_T ($p = 0.93$) or food level ($p = 0.41$) on crystal width (Table V.8, Fig. V.6b).

V.4. Discussion

Responses of calcifying organisms to ocean acidification are likely to vary at different stages of their life cycles, and several studies provide evidence that early stages (larvae and juveniles) of many marine taxa are particularly sensitive to acidification (Kroeker et al., 2010, 2013; Hettinger et al., 2012). Therefore, early-stage organisms in upwelling regions may be particularly susceptible to negative stressors associated with decreasing pH. Hettinger et al. (2012) also showed that adverse effects of stress during larval stages can "carry-over" to successive developmental stages and be compounded by the time adulthood is reached. In the present study, pCO_2 had no

effect on the numbers of brooded planulae larvae released, but higher food levels increased the numbers of larvae released by 50-200% (Fig. V.3). Because females brood for approximately 15 months, and because the adults were exposed to the experimental conditions for only a few days to weeks immediately preceding the peak planulation season, this suggests that females may delay planulation until conditions are optimal. It may be that that high food levels are needed by the female to complete maturation of larvae conceived many months earlier, or to sustain non-feeding larvae after release during dispersal, settlement and metamorphosis into a feeding polyp. It is also possible that food level acts indirectly as a proxy for correlated environmental conditions (biotic or abiotic) that may enhance juvenile survival after metamorphosis.

Due to the 15 months of brooding before release, this experiment provides no information about possible impacts of prolonged high pCO_2 or low food on adult nutrition or reproduction, nor about whether prolonged exposure to high pCO_2 negatively impacts such processes as gametogenesis, fertilization, cleavage or early larval development that have been seen in other organisms (Kurihara, 2008; Kroeker et al., 2010, 2013; Nakamura et al., 2011). It also does not address whether prolonged exposure to low pH or high pCO_2 act directly on reproductive processes, or indirectly via diverting energetic resources away from reproduction.

While pCO_2 did not affect numbers of planulae released, mortality of newly-settled juvenile corals was substantially greater at the highest pCO_2 level (1220 μatm, pH_T =

7.6), with average mortality about 10% higher (across both food levels) than in the other pCO_2 treatments (410 and 770 µatm) (Fig. V.4). However, High Food did seem to alleviate some of the stress associated with high pCO_2: at 1220 µatm, High Food increased juvenile survival by 15% over Low Food conditions, but not by enough to counter the decline due to low pCO_2. This pattern suggests that, if atmospheric pCO_2 increases beyond the projected 750 ppm over the next century, the numbers of corals surviving to become adults may decline; even if food is always plentiful, it is unlikely that juvenile mortality will be unaffected by pCO_2.

Food availability was the major factor controlling the growth (and final size) of newly settled *Balanophyllia elegans* juveniles in this experiment (Fig. V.5a); pCO_2 had no significant effects on final volume. After 8 months of growth, High Food skeletons were up to 7 times larger (by volume) than Low Food skeletons at every pCO_2 level. Although corals within a food level had similar volumes across all three pCO_2 treatments (Fig. V.5a), their skeletal weights (and hence bulk densities) significantly decreased from 410 µatm to 1220 µatm pCO_2 (Fig. V.5b,c). This suggests that with increasing pCO_2, either the shapes or spacing of skeletal elements changed, or there was less secondary thickening of the initial skeleton. One possible mechanism is that energy available for calcification is allocated first to ensuring full skeletal extension, at the cost of a less heavily calcified skeleton.

Calcification rates in reef-building corals are often measured as the annual linear

extension multiplied by the bulk density, and expressed in g cm^{-2} yr^{-1}. Because *B. elegans* is a solitary species, its radial expansion must be considered in the calculation of calcification rates. Therefore, we express calcification as the change in the total skeletal weight measured over known intervals and normalized to g yr^{-1} per coral. In every pCO_2 treatment, higher food led to both greater linear extension and greater calcification (skeletal weight) over the 8 month experiment, a trend that is consistent with more energy being allocated to skeletal formation. While linear dimensions were unaffected by pCO_2, well-fed corals had heavier skeletons; in particular, calcification by high pCO_2, High Food corals was 4 times greater than in Low Food corals at ambient pCO_2. However, higher pCO_2 negatively impacted bulk densities (Fig. V.5c) and the reduction of skeletal density by ~35% with increasing pCO_2 suggests that structural integrity of the skeletons may be weakened, leaving these corals more vulnerable to predation, bioerosion, and dislodgement (Hoegh-Guldberg et al., 2007).

While high pCO_2 caused overall reductions in density (~35%), Low Food skeletons were actually denser than High Food skeletons in all pCO_2 treatments (Fig. V.5c), even though the former grew less and weighed less after 8 months. This response may be analogous to that of many colonial, zooxanthellate reef-building corals in which rapid linear extension and low density skeleton dominates under favorable conditions, while slower growing, denser skeleton forms during less favorable conditions (Highsmith, 1979).

The aspect ratio (length divided by width) of aragonite crystals in coral skeletons has been used as an indirect proxy for aragonite saturation state of seawater in a coral's calcifying compartment (Cohen and Holcomb, 2009; Holcomb et al., 2009). Longer, thinner crystals are associated with high saturation states while shorter, broader crystals are indicative of low saturation states (Cohen and McConnaughey, 2003). Cohen and Holcomb (2009) used abiogenic aragonites precipitated in seawater with known saturation states to derive a formula in which crystal aspect ratio linearly approximates the saturation state of the calcifying fluid (Ω_{cf}) in a coral's calcifying compartment (Cohen and Holcomb, 2009):

$$\Omega_{cf} = 0.93\ (\pm\ 0.06) \times \text{crystal aspect ratio} + 0.20\ (\pm\ 0.89) \qquad \text{(Eq. V.2)}$$

In our experiment, high pCO_2 significantly reduced crystal length, with crystals being approximately 18% longer in 410 µatm than in 1220 µatm pCO_2 corals (Fig. V.6a). Crystal width did not vary significantly between feeding frequency or pCO_2 treatments (Fig. V.6b), but the crystal aspect ratio was higher at ambient pCO_2 ($p=0.028$, Fig. V.7). Using Eq. V.2, we calculated that Ω_{cf} was ~20 and ~19 for High and Low Food corals respectively at 410 µatm, and ~18 and ~17 for High and Low Food corals at 1220 µatm. Even in well-fed corals at 1220 µatm, calculated Ω_{cf} was slightly lower than in corals grown at ambient pCO_2. One explanation is that these corals were unable to expel enough protons under high pCO_2 conditions to calcify at rates similar to those at ambient pCO_2, even when provided with plentiful high energy

food. However, the fact that crystals from High Food corals at 1220 μatm were significantly longer (by about 15%) than crystals from Low Food corals caused slight increases in both measured crystal aspect ratio and estimated Ω_{cf}. This is consistent with our calcification data and may indicate that excess food enables corals to partially counteract some of the negative impacts of lower saturation states under higher pCO_2 conditions.

Combining these lines of evidence suggests that *Balanophyllia elegans* is able to maintain moderate calcification rates even during extreme acidification conditions, provided they also have a plentiful nutritional supply. *B. elegans* and other efficient filter-feeders that do not have zooxanthellae may be able to maintain their energy reserves under physiologically stressful conditions by increasing their feeding rates (provided sufficient prey are available). Our experiment suggests that even feeding on planktonic crustacean only once every 21 d was still sufficient to maintain positive growth at high pCO_2, albeit very slowly. Removing protons to increase pH and saturation state in the calcifying compartment may be energetically costly, and energetic demands for maintaining the saturation state of calcifying fluids are likely to rise with increasing pCO_2, so the total amount of $CaCO_3$ deposited is likely to decline even though extension rates are maintained. The decreased calcification at moderate to high pCO_2 that we observed regardless of feeding amount, suggests that even well-fed corals cannot entirely overcome the stresses of ocean acidification.

Our observation that heterotrophic feeding rate has a greater impact on calcification than pH may explain the ability of *B. elegans* and other calcifying organisms to survive in upwelling waters and tolerate low saturation. When pH is lowest during upwelling events, nutrient and plankton concentrations are often at their highest in Monterey Bay. Indeed, nutrient concentrations during the upwelling months can be up to 20 times greater than during non-upwelling periods (Pennington and Chavez, 2000). This nutrient surplus accelerates phytoplankton (and subsequent zooplankton) production, and should increase the amounts of heterotrophic food available to corals and other benthic filter-feeders. Zooplankton concentrations in Monterey Bay can be up to 10 times higher during upwelling than during non-upwelling months, and often peak about the time of maximum planulation by *B. elegans* (Marinovic et al., 2002). This may suggest that, should acidification be decoupled from upwelling, *B. elegans* calcification may be more negatively impacted by lower food concentrations than low pH, but as long as food availability remains high, *B. elegans* may be able to largely compensate for the extra energy required for calcification at low saturations, even if calcification occurs at slightly lower rates than at modern pCO_2.

V.5. Acknowledgments

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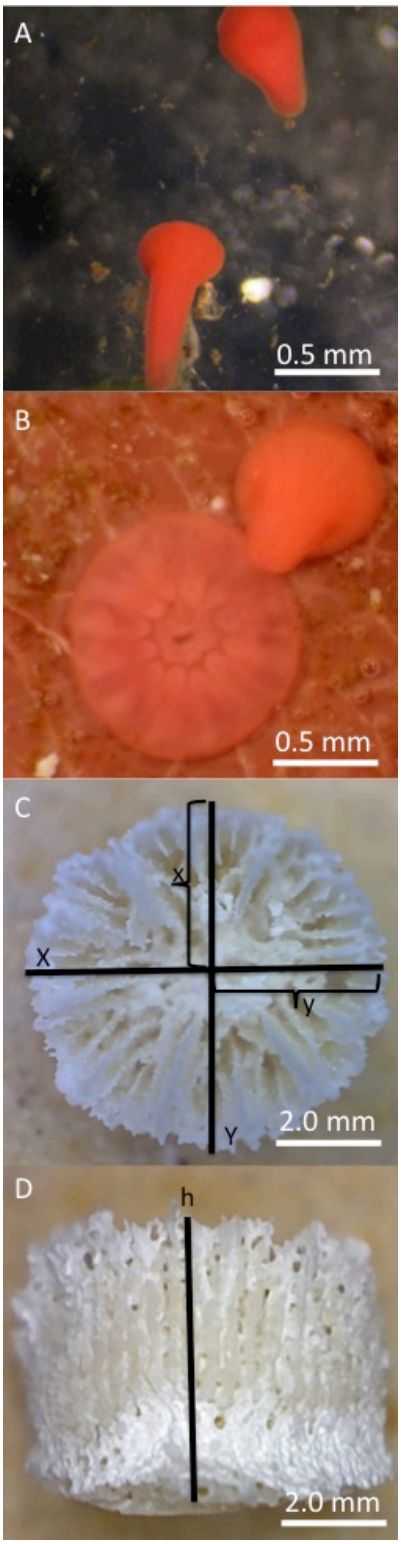
Figure V.1. *Balanophyllia elegans* planula larvae (**a**), newly settled juveniles (**b**), and a juvenile skeleton after 8 months of growth from above (**c**), and in longitudinal section (**d**). Skeletal measurements are indicated in (**c**) and (**d**). The major and minor axes of the elliptical cylinder (**X, Y**) were measured with vernier calipers, and the major and minor radii (**x, y**) were calculated (**c**). The height (**h**) was measured (**d**) and volume was calculated using Eq. 1.


A
0.5 mm
B
0.5 mm
C
x
X
y
Y
2.0 mm
D
h
2.0 mm

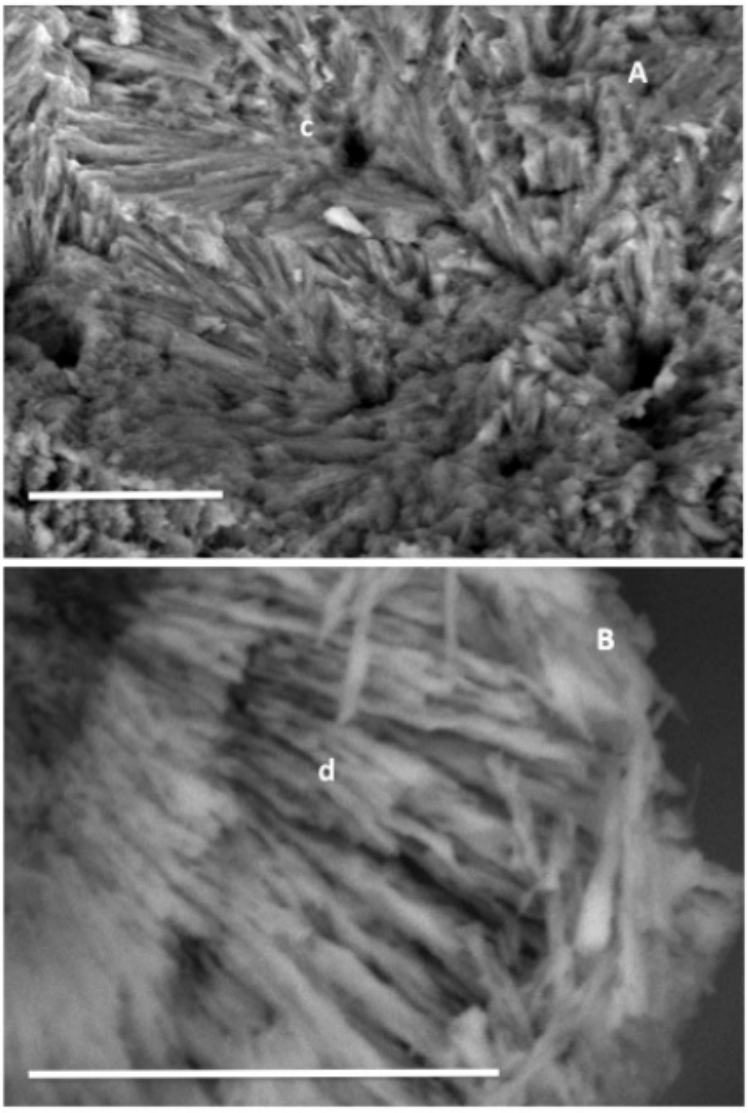


Figure V.2. Scanning Electron Microscope (SEM) images of coral septa under 5 kx (**A**) and 10 kx (**B**) magnification. Skeletal crystals were imaged on a Hitachi TM1000 Tabletop SEM at the UCSC MACS facility at NASA/Ames. Under 5 kx magnification (**A**), it is clear that *B. elegans* skeletons are an aggregate of very densely packed, fine needle-like crystals that fan outward from a central point (**c**) (e.g. Cohen and McConnaughey, 2003; Cohen and Holcomb, 2009). The lengths of individual aragonite crystals were obtained from the 10 kx images using Imaging Processing and Analysis in Java (Image J, U.S. National Institute of Health) by measuring from the base to the tip of each needle-like crystal (**d**). Scale bars are 10 μm.

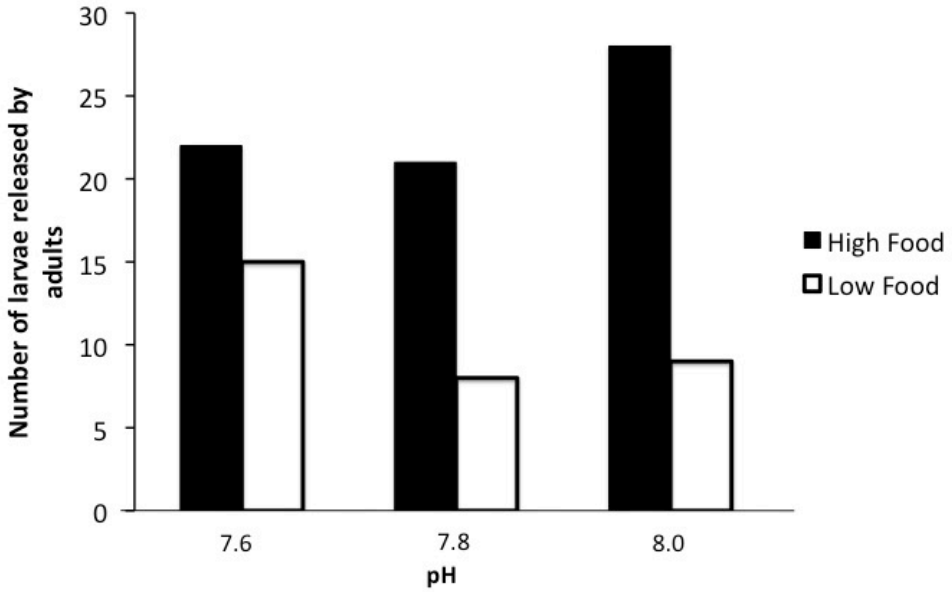


Figure V.3. Numbers of planula larvae released by 10 *Balanophyllia elegans* adults over 3 months in each pH_T X food treatment.

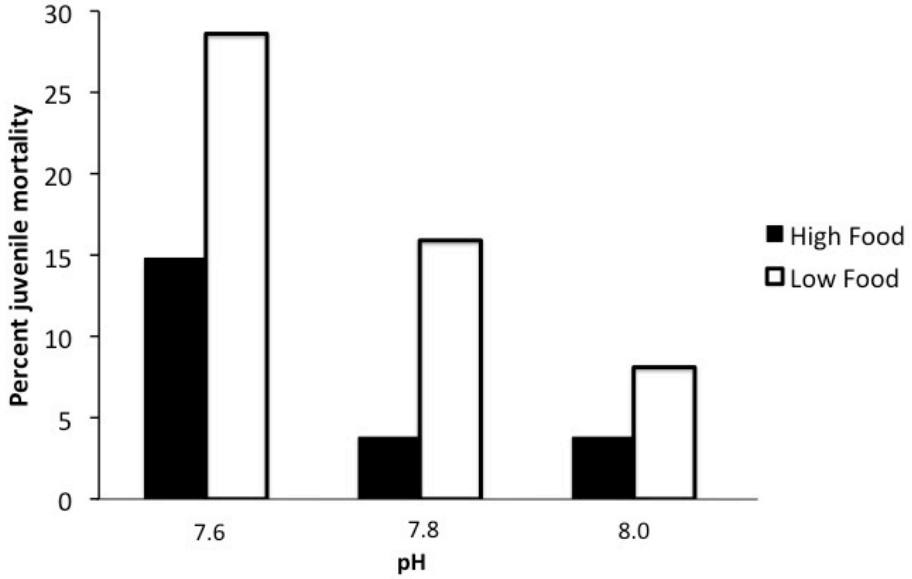


Figure V.4. Percent mortality of juvenile *Balanophyllia elegans* over 84 days in each pH_T X food treatment. Initial numbers varied from 26 to 44 for a total of 202 juveniles.

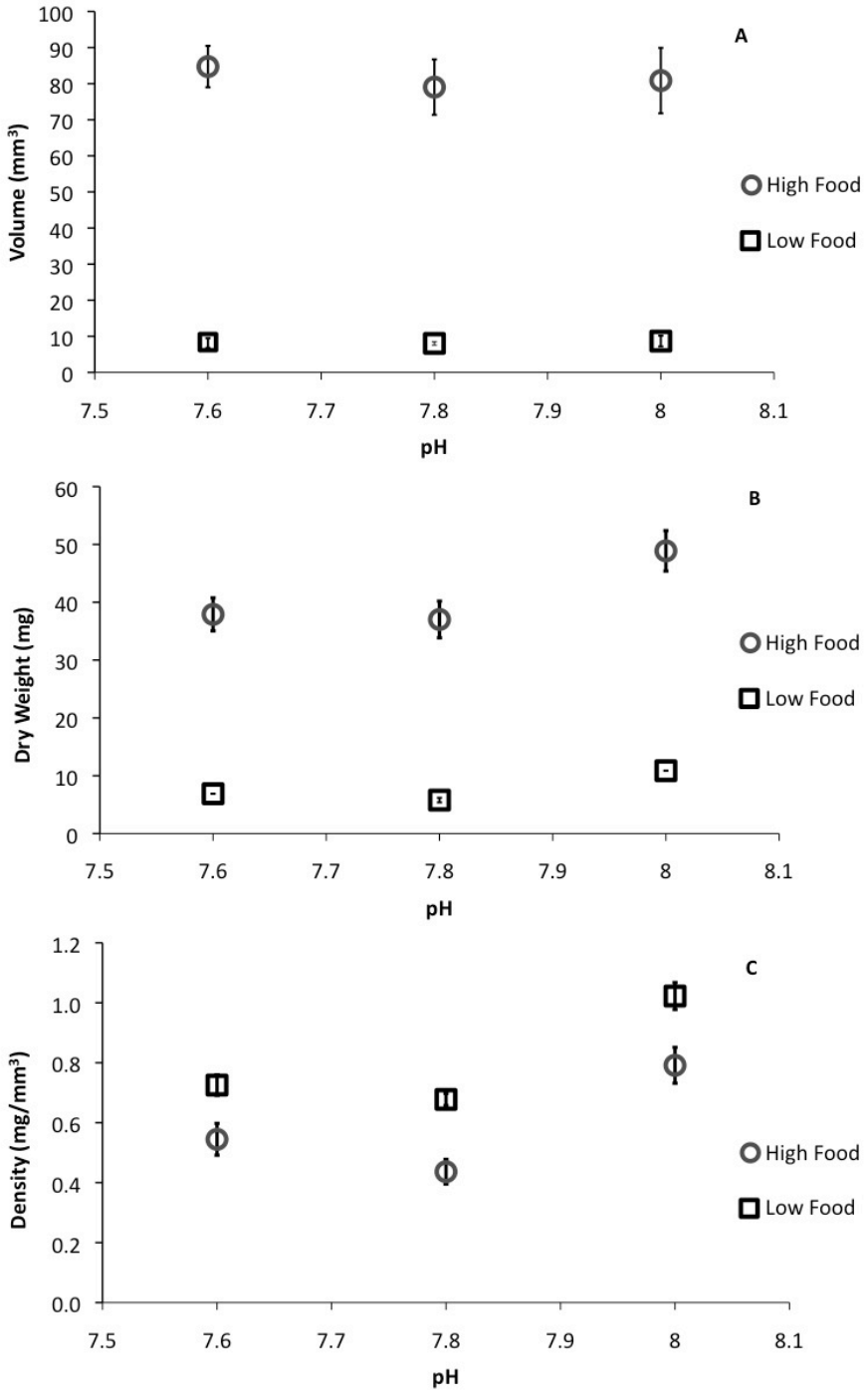


Figure V.5. Means ± s.e.m. for volumes (**a**), dry weights (**b**), and bulk densities (**c**) of skeletons of juvenile *Balanophyllia elegans* after 8 months growth.

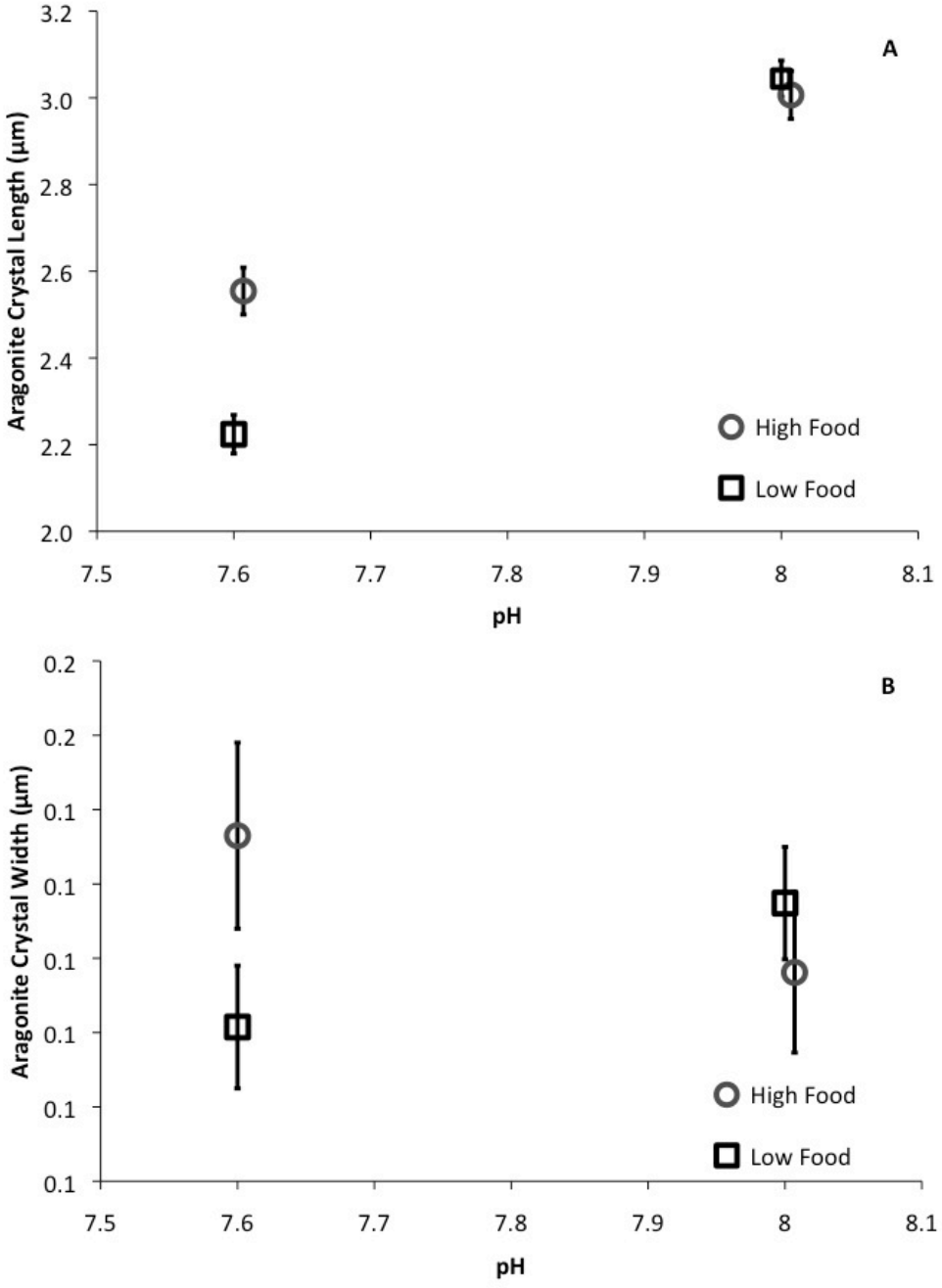


Figure V.6. Means ± s.e.m. of aragonite crystal lengths (**a**), and crystal widths (**b**) obtained from scanning electron microscope (SEM) images of septa from 20 juvenile *Balanophyllia elegans*.

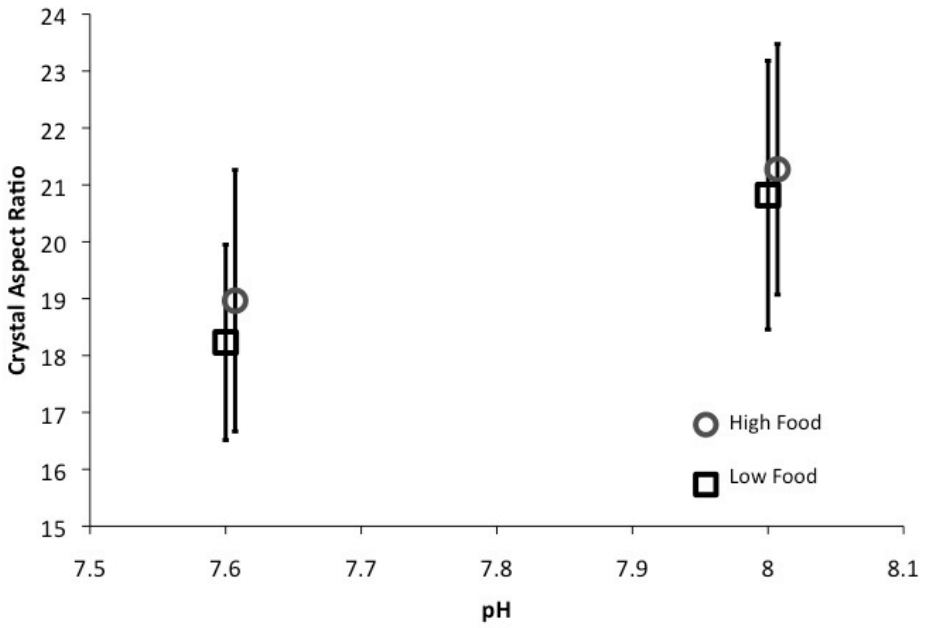


Figure V.7. Mean crystal aspect ratios ± s.d. of aragonite crystals in septa of juvenile *Balanophyllia elegans*, calculated by dividing the crystal length by the crystal width for individual crystals.

	1200 ppm High food	1200 ppm Low food	750 ppm High food	750 ppm Low food	380 ppm High food	380 ppm Low food
A_T mol/kg	2217 ± 9	2219 ± 9	2221 ± 12	2222 ± 9	2220 ± 8	2221 ± 11
C_T mol/kg	2183 ± 12	2184 ± 15	2127 ± 10	2129 ± 12	2034 ± 10	2037 ± 12
pCO_2 µatm	1220 ± 80	1220 ± 80	770 ± 75	770 ± 75	410 ± 21	410 ± 21
pH_T	7.59 ± 0.02	7.59 ± 0.02	7.78 ± 0.03	7.78 ± 0.03	8.02 ± 0.02	8.02 ± 0.02
Ω_{arag}	0.9 ± 0.04	0.9 ± 0.04	1.3 ± 0.10	1.3 ± 0.10	2.1 ± 0.08	2.1 ± 0.08
Temp (°C)	13.6 ± 1.5	13.6 ± 1.5	13.6 ± 1.5	13.6 ± 1.5	13.6 ± 1.5	13.6 ± 1.5
Salinity	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1
SiO_2 µmol kg^{-1}	7.6 ± 0.9	7.6 ± 0.9	7.6 ± 0.9	7.6 ± 0.9	7.6 ± 0.9	7.6 ± 0.9
PO_4^{3-} µmol kg^{-1}	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.05
NO_3^- µmol kg^{-1}	3.4 ± 0.6	3.4 ± 0.6	3.4 ± 0.6	3.4 ± 0.6	3.4 ± 0.6	3.4 ± 0.6

Table V.1. Mean ± standard deviation (s.d.) of experimental chemical conditions from November 2011 to July 2012. High Food corals were fed once every 3 d, and Low Food corals were fed once every 21 d

Feeding	pCO_2 (µatm)			
Interval	410	770	1220	Total
3 d	22	21	28	71
21 d	15	8	9	32
Total	37	29	37	103

2-way Additive ANOVA, no nesting

Source	Df	SS	MS	F	P	
pCO_2	2	21.33	10.67	0.593	0.628	ns
Food	1	253.50	253.50	14.083	0.064	ns
Residuals	2	36.00	18.00			

Table V.2. Total numbers of planula larvae released over 3 months by 10 adults in each treatment (combining jars within treatments).

Feeding	pCO_2 (µatm)		
Interval	410	770	1220
3 d	0.038 (26)	0.038 (26)	0.148 (27)
21 d	0.081 (37)	0.159 (44)	0.285 (42)

Additive logistic regression model

	Estimate	S.E	Z	P	
(Intercept)	-3.3790	0.6459	-5.231	< < 0.001	***
CO_2 750	0.6164	0.6431	0.958	0.338	ns
CO_2 1200	1.5069	0.5955	2.531	0.011	*
Food Low	1.0021	0.4944	2.027	0.043	*

Table V.3. Proportions of juvenile corals dying over 8 months in each treatment (combining jars within treatments). Initial numbers of juveniles are shown in parentheses.

Feeding	pCO_2 (µatm)		
Interval	410	770	1220
3 d	65.7 ± 9.8 (23)	101.2 ±11.1 (23)	82.0 ± 8.8 (22)
21 d	10.8 ± 1.5 (33)	8.7 ±0.9 (36)	9.6 ± 1.1 (30)

Nested ANOVA (log transformed data)

Source	Df	SS	MS	F	P	
pCO_2	2	1.06	0.53	1.202	0.303	ns
Food	1	184.40	184.40	419.966	< < 0.001	***
pCO_2 X Food	2	2.98	1.49	3.390	0.036	*
pCO_2 X Food X Jar	6	0.91	0.15	0.347	0.911	ns
Residuals (Coral)	156	68.50	0.44			

Table V.4. Mean volumes (mm^3) ± s.e.m. of juvenile skeletons after 8 months growth. Numbers of individuals are shown in parentheses.

Feeding Interval	pCO_2 (µatm) 410	770	1220
3 d	53.7 ±5.9 (18)	37.0 ±3.2 (23)	41.8 ±3.8 (22)
21 d	11.2 ±1.1 (22)	5.8 ±0.4 (28)	6. 9 ±0.6 (24)

Nested ANOVA (log transformed data)

Source	Df	SS	MS	F	P	
pCO_2	2	5.45	2.73	16.031	< < 0.001	***
Food	1	104.59	104.59	614.761	< < 0.001	***
pCO_2 X Food	2	0.35	0.17	1.023	0.363	ns
pCO_2 X Food X Jar	6	0.30	0.05	0.293	0.939	ns
Residuals (Coral)	125	21.27	0.17			

Table V.5. Mean weights (mg) ± s.e.m. of juvenile skeletons after 8 months growth. Numbers of individuals are shown in parentheses.

Feeding Interval	pCO_2 (µatm) 410	770	1220
3	0.79 ±0.06 (18)	0.44 ±0.04 (23)	0.54 ±0.05 (22)
21	1.02 ±0.04 (22)	0.68 ±0.02 (28)	0.73 ±0.03 (24)

Nested ANOVA (log transformed data)

Source	Df	SS	MS	F	P	
pCO2	1	1.357e-06	1.357e-06	36.637	< < 0.001	***
Food	1	1.534e-06	1.535e-06	41.441	< < 0.001	***
pCO2 X Food	1	1.800e-08	1.810e-08	0.488	0.486	ns
pCO2 X Food X Jar	8	1.697e-06	2.122e-07	5.730	< < 0.001	***
Residuals (Coral)	123	4.554e-06	3.700e-08			

Table V.6. Mean densities (mg mm^{-3}) ± s.e.m. of juvenile skeletons after 8 months growth. Numbers of individuals are shown in parentheses.

Feeding Interval	pCO_2 (μatm) 410	1220
3	3.0 ±0.07 (33)	2.6 ±0.05 (44)
21	3.0 ±0.05 (29)	2.2 ±0.04 (42)

Nested ANOVA

Source	Df	SS	MS	F	P	
pCO2	1	15.037	15.037	153.328	< < 0.001	***
Food	1	1.266	1.266	12.913	< 0.001	***
pCO2 X Food	1	1.073	1.073	10.939	0.001	**
pCO2 X Food X Jar	14	2.901	0.207	2.112	0.015	
Residuals (Coral)	130	12.750	0.098			

Table V.7. Mean lengths (μm) ± s.e.m. of aragonite crystals in septa of juvenile skeletons after 8 months growth. Numbers of measurements are shown in parentheses.

Feeding Interval	pCO_2 (µatm) 410	1220
3	0.13 ±0.006 (24)	0.14 ±0.006 (27)
21	0.14 ±0.005 (32)	0.13 ±0.004 (32)

Nested ANOVA

Source	Df	SS	MS	F	P	
pCO_2	1	0.00001	0.0000063	0.009	0.924	ns
Food	1	0.00050	0.0005029	0.729	0.395	ns
pCO_2 X Food	1	0.00228	0.0022789	3.303	0.072	ns
pCO_2 X Food X Jar	16	0.01757	0.0010980	1.591	0.086	ns
Residuals (Coral)	95	0.06556	0.0006901			

Table V.8. Mean widths (µm) ± s.e.m. of aragonite crystals in septa of juvenile skeletons after 8 months growth. Numbers of measurements are shown in parentheses.

VI. Conclusions

VI.1. The Coral Calcification Response to Ocean Acidification

Results from the *in-situ* field investigations at Puerto Morelos, Mexico lend considerable insight into the impact of ocean acidification on coral calcification. The coral cores (Chapter III) enabled the direct measurement the extension, density, and calcification of corals growing in a gradient of saturation conditions, from under-saturated to super-saturated waters. Using a linear regression model, I predict that *Porites* calcification may decrease by 22% from preindustrial values by the year 2065, when saturation of the surface oceans reach $\Omega_{arag} \approx 3.1$. If atmospheric CO_2 concentrations triple ($\Omega_{arag} \sim 2$) a loss of approximately 46% could result.

It is important to note that *Porites* are not the dominant species of the Meso-American Barrier Reef framework. Evidence from Chapter II suggests that as seawater saturation nears 2.5, today's larger, dominant, framework-building corals of the Meso-American Reef (e.g., *Acropora, Montastraea*) may be replaced by smaller, patchily distributed colonies of only a few species (i.e. *Porites* and *Siderastrea*). Therefore, this projected drop in calcification of *Porites* by 22% in the next 50 years is likely a conservative estimate, as they appear to be particularly tolerant of low saturation conditions. Because species richness increased with increasing saturation state at the ojos, it is likely that the less tolerant species will experience more extreme drops in calcification.

This work illustrates that while the effects of ocean acidification on coral reefs may be severe, the impacts will differ considerably across various species and ecosystems. It is possible, therefore, that ocean acidification will result in ecosystem shifts, in which today's frame-building colonies are replaced by more tolerant species (i.e. *Porites* and *Siderastrea*). Combined with significant drops in calcification (22-46%) of these more tolerant species in the coming decades, the altered composition of reefs may have severe consequences for the extent of coral reefs in the future and the important ecosystem services they provide.

VI.2. Insights on the Acclimation Potential of Corals to Acidification

The coral cores from Chapter III and the laboratory experiment of Chapter V provide evidence for the acclimation potential of corals to ocean acidification. Specifically, the *Porites* corals found at the ojos, which settle and spend their entire lives in low saturation waters, appear unable to calcify at the same rates as their ambient conspecifics. While it is heartening that certain coral species are able to withstand extreme acidification conditions, closer examination of calcification rates (i.e. up to a 65% drop in calcification) suggests that corals are not, in fact, able to acclimate to ocean acidification. The coral cores allow us to look closely at the calcification process to determine where corals are allocating their energy. It is possible, for instance, that in response to the harsher environment encountered at the ojos, the corals in low or under-saturated waters utilize more energy to maintain their linear

extension rates, but at the cost of skeletal density. These "stretch modulation" observations fit well with my data, in which density decreases while linear extension rates are maintained.

While our data suggests that certain corals may be able to maintain their linear extension under the ocean acidification conditions expected by the year 2100, when considering the impact of density on bioerosion the situation is disheartening. The extent of erosion and predation by boring organisms was found to be significantly greater in corals where $\Omega_{arag} < 2.0$. The observed increase in total volume eroded at low saturation ($\Omega_{arag} < 2$), which is likely caused by the lower carbonate density, indicates that future acidification events may not only decrease calcification rates, but reduce coral coverage via boring organisms and mechanical erosion. A decrease in skeletal density combined with an increase in susceptibility to bioerrosion may indicate a weakening of the reef framework in the future and subsequent degradation of the complex coral reef ecosystem. Low structural integrity caused by a reduction in density could leave reefs more vulnerable to wave action leading to a weaker framework and the further degradation of coral reefs.

Chapter V further addresses the calcification mechanism of corals, as *Balanophyllia elegans* serves as a model organism for calcification due to its lack of symbiotic xooxanthellae. As with the *Porites* corals of Chapter III, high pCO_2 caused overall reductions in density in the 8-month experiment at Long Marine Lab. However, *B.*

elegans appear able to maintain moderate calcification rates even during extreme acidification conditions, provided they also have a plentiful nutritional supply. Therefore, when considering the energy budget for calcification, *B. elegans* appears to have an energy budget that is somewhat flexible: they may be able to maintain their energy reserves under physiologically stressful conditions by increasing their feeding rates (provided sufficient prey are available). However, removing protons to increase pH and saturation state in the calcifying compartment may be energetically costly, and energetic demands for maintaining the saturation state of calcifying fluids are likely to rise with increasing pCO_2. Thus, the total amount of $CaCO_3$ deposited is likely to decline even though extension rates are maintained (similar to the *Porites* corals). The decreased calcification at moderate to high pCO_2 that we observed regardless of feeding amount, suggests that even well-fed corals cannot entirely overcome the stresses of ocean acidification.

VI.3. Benthic Reef Community Response to Ocean Acidification

Results from the year-long *in-situ* recruitment investigation at Puerto Morelos illustrate that benthic reef community responses to ocean acidification may be complex. While low saturation tiles were able to recruit coralline algae at the beginning of the study, there was a decrease in crustose coralline algal (CCA) coverage of up to 80% in the low saturation by the end of the 14-month experiment. This decrease in coralline coverage was coincident with an increase in fleshy algal coverage, indicating that fleshy algae have a competitive edge over CCA, and are

able to outcompete CCA for space in acidification conditions. Thus, while acidification will have significant direct impacts on calcification, the altered competitive interactions between organisms will also impact community assemblages in the future. That is, we expect to see a shift in communities from coralline algal coverage to fleshy algae over time as pCO_2 increases over the 21st century.

It is important to note that the tropical benthic calcifying organisms were able to recruit and grow in low Ω_{arag} conditions. However, competition for space as the community developed rather than physiological limitations were leading drivers in the community shifts observed. Thus, our study illustrates the importance of observing the response of entire communities to OA, as interactions between organisms will compound the direct effects of acidification and likely increase reef degradation beyond the estimates derived from species-specific observational studies.

Coralline algae are important ecological components of a coral reef, as they cement the reef framework and provide chemical settlement cues and settlement substrate for coral larvae. Understanding the response of coralline algae to ocean acidification is therefore of critical importance. A dramatic decline in coverage under conditions of ocean acidification indicates that both the basic framework of reefs and the recruitment of corals could decrease with decreasing Ω_{arag}.

This dissertation illustrates the need to consider both the direct and indirect effects of

acidification on corals and coral reef communities. Not only will the impacts of acidification on corals be immediate and direct, but competition between organisms may intensify the acidification problem. As the oceans become more acidic over time, it will become essential to alleviate human impacts that have the potential to cause further direct harm to coral reef communities, or to compound competitive interactions between organisms. The collective negative impacts of other stressors, including rising sea surface temperatures, pollution, and overfishing may deal a significant blow to the health of coral reefs in the coming decades. Combined, these lines of evidence indicate a rather grim outlook for the future of coral calcification and coral reef accretion as surface ocean pH decreases over the 21st century.

Appendix A. Chapter II Supporting Data

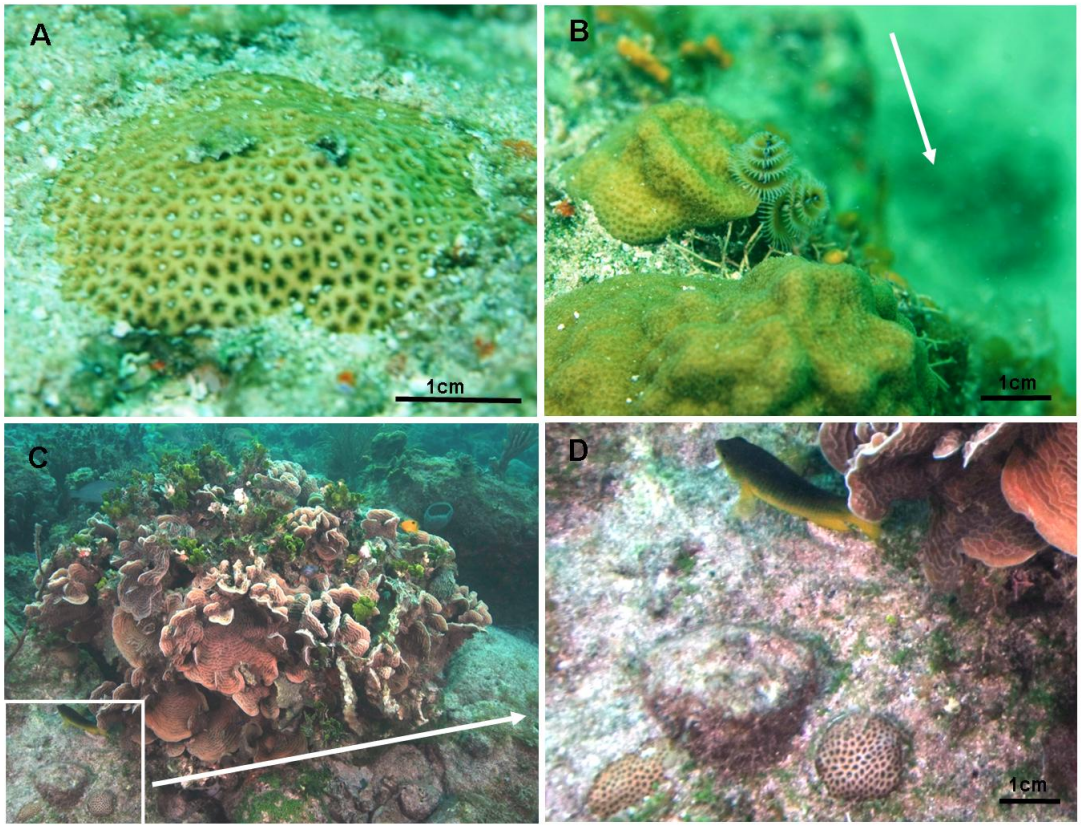


Figure A.1. Examples of corals from the ojos (A,B) and ambient transects (C,D). The arrow in (B) alongside *Porites astreoides* notes the presence of low aragonite saturation discharge. *Siderastrea radians* (A,D) was one of three species present both at the ojos and control sites.

Table A.1.Water Chemistry Data, all sampling periods. The ojo, date, and distance to the ojo center are identified. pH values were in-situ measurements from a hand-held pH meter accurate to 0.01 pH units. Salinity was measured on a salinometer from collected samples. Temperature was taken as an in-situ measurement from a YSI 85.

Ojo	DATE	Distance from Ojo	pH (in-situ)	Salinity	Temp-C	DIC (umol/kg)	TA
Pargos	11/10/09	inside hole	7.25	27.9	27.5	2889	3008
Pargos	11/10/09	0	7.23	27.3	26.5	3122	3230
Pargos	11/10/09	0.25	7.21	24.3	24.9	3156	3095
Pargos	11/10/09	0.5	7.26	24.7	27	2981	3007
Pargos	11/10/09	0.75	7.34	24.7	25.2	2989	2962
Pargos	11/10/09	1	7.24	27	26.6	2773	2767
Pargos	11/10/09	1.25	7.29	27.7	26.1	2956	2968
Pargos	11/10/09	1.5	7.31	27.5	25.9	2939	2982
Pargos	11/10/09	1.75	7.36	27.7	26.4	2798	2863
Pargos	8/30/10	SeaPhOx	7.22	28.3	27.5	3262	3082
Pargos	8/30/10	SeaPhOx	7.22	28.1	27.5	3333	3079
Pargos	8/30/10	SeaPhOx	7.27	28	27.5	3310	3079
Pargos	8/30/10	SeaPhOx	7.25	28.3	27.5	3290	3096
Pargos	8/30/10	0.75	7.49	29.7	29.7	2910	3006
Pargos	8/30/10	0.5	7.41	29.7	29.6	2848	2989
Pargos	8/30/10	0.25	7.42	28.6	30	3284	2871
Pargos	8/30/10	0	7.42	33.8	28	2798	2543
Pargos	8/30/10	SeaPhOx	7.17	29.1	27.4	2905	3003
Pargos	8/30/10	SeaPhOx	7.17	29	27.6	2999	2955
Pargos	8/30/10	SeaPhOx	7.16	29.2	27.8	2919	2985
Pargos	8/30/10	CORAL HEAD	7.23	30.4	28.6	2880	2810
Pargos	3/1/11	Coral Core_Center 07	7.2	32.7	27.5	3169	3096
Pargos	10/20/11	Pargos 100cm	8.00	34.20	27.50	2084	2354
Pargos	10/20/11	Pargos 25cm	7.23	28.00	27.70	3047	3055
Pargos	10/20/11	Pargos 50cm	7.72	32.00	27.60	2160	2165
Pargos	10/20/11	Pargos Center	7.15	27.90	27.60	3016	3006
Pargos	10/20/11	Pargos Center	7.18	29.00	27.60	2960	2994
Pargos	10/20/11	Pargos Surface	7.70	34.40	27.70	2342	2540
Pargos	10/20/11	Pargos Center Core (12 cm)	7.20	29.40	27.70	2949	2929
Pargos	4/16/12	Pargos Center	7.97	35.99	27.3	1976	2764
Pargos	4/18/12	Pargos Center	7.15	31.89	28.2	2521	3011
Pargos	4/20/12	Pargos Center	7.81	33.76	27.8	2338	2912
Pargos	4/19/12	Pargos Ojo	7.65	31.33	28.3	2638	2743

		(time series)					
Pargos	4/19/12	Pargos Ojo (time series)	7.92	33.43	28.3	2300	2530
Pargos	4/19/12	Pargos Ojo (time series)	7.58	31.41	28.4	2613	3105
Pargos	4/19/12	Pargos Ojo (time series)	7.65	31.99	27.7	2560	3059
Pargos	4/19/12	Pargos Ojo (time series)	7.6	30.81	28.4	2755	2845
Pargos	4/19/12	Pargos Ojo (time series)	8.11	35.91	27.6	2061	2363
Pargos	4/19/12	Pargos Ojo (time series)	8.15	35.85	27.6	2037	2355
Pargos	4/19/12	Pargos Ojo (time series)	8.01	20.28	27.6	2268	2461
Pargos	4/19/12	Pargos Ojo (time series)	8.1	35.42	27.5	1999	2394
Pargos	4/19/12	Pargos Ojo (time series)	7.67	32.18	27.2	2491	2605
Pargos	4/19/12	Pargos Ojo (time series)	8.09	35.12	27.6	2147	2447
Pargos	4/19/12	Pargos Ojo (time series)	7.71	32.21	27.8	2489	2629
Pargos	4/19/12	Pargos Ojo (time series)	7.71	31.55	27.8	2570	2765
Pargos	4/19/12	Pargos Ojo (time series)	7.72	32.07	27.7	2502	2636
Pargos	4/18/12	Pargos Ojo (time series)	8.13	35.12	27.8	2043	2146
Pargos	4/18/12	Pargos Ojo (time series)	8.01	35.89	27.2	2028	2352
Pargos	4/18/12	Pargos Ojo (time series)	8.18	35.59	27.7	2017	2332
Pargos	4/18/12	Pargos Ojo (time series)	8.18	35.95	27.8	1912	2323
Pargos	4/18/12	Pargos Ojo (time series)	8.18	34.46	28.1	2017	2871
Pargos	4/18/12	Pargos Ojo (time series)	8.23	35.93	27.8	1999	2337
Pargos	4/18/12	Pargos Ojo (time series)	7.66	32.31	29.2	2479	2651
Pargos	4/18/12	Pargos Ojo (time series)	7.77	32.29	29.3	2438	2665
Pargos	4/18/12	Pargos Ojo (time series)	7.78	32.28	28.1	2478	2632
Pargos	4/18/12	Pargos Ojo (time series)	7.45	32.09	30	2492	2714
Pargos	4/18/12	Pargos Ojo (time series)	8.23	35.91	27.9	1953	2354
Pargos	11/10/09	-0.75	7.82	29.7	28.3		
Pargos	11/10/09	-0.5	7.58	26.5	28.1		
Pargos	11/10/09	-0.25	7.32	23.5	28.3		

Pargos	11/10/09	0	7.41	21.9	28.3		
Pargos	11/10/09	0.25	7.29	22.9	28.1		
Pargos	11/10/09	0.5	7.29	23.4	28.2		
Pargos	11/10/09	1.5	7.84	26.9	28.2		
Pargos	11/11/09	2-Pargos	8	32.6	26	2373	2491
Pargos	11/11/09	3-Pargos	8.11	33	23.9	2023	2332
Pargos	11/10/09	1.75-Pargos	8.15	31.5	28.2		
Pargos	11/10/09	0.25-Pargos	8.2	28.11	28.6		
Pargos	11/10/09	0.25-Pargos	8.05	29.5	29		
Pargos	11/10/09	0.5-Pargos	8.14	31.8	28.3		
Pargos	3/1/11	Pargos_Cont1	8.09	34.9	28.6	2020	2388
Pargos	10/20/11	Pargos Control	7.99	33.20	27.90	1937	2318
Pargos	10/20/11	Pargos Control	8.02	34.00	27.40	2078	2354
Pargos	10/20/11	Pargos Surface	7.99	32.85	28.80	2124	
Pargos	10/20/11	Pargos Control Core (4-6 cm)	8.14	34.30	27.10	2036	2355
Pargos	4/16/12	Pargos Control	8.28	36.03	27.8	2006	2760
Pargos	4/16/12	Pargos Control	8.28	35.92	28.2	1949	2324
Pargos	4/16/12	Pargos Control	8.16	35.94	28.7	1996	2674
Pargos	4/16/12	Pargos Control	8.12	35.59	27.5	2077	2389
Pargos	4/16/12	Pargos Control	8.18	35.95	28.6	1981	2333
Pargos	4/16/12	Pargos Control	8.17	35.94	28.6	1999	2352
Pargos	4/16/12	Pargos Control	8.15	35.94	27.7	1947	2345
Pargos	4/16/12	Pargos Control	8.15	35.95	27.6	2044	2360
Pargos	4/16/12	Pargos Control	8.15	35.96	27.8	2038	2364
Pargos	4/16/12	Pargos Control	8.15	35.94	27.8	2015	2357
Pargos	4/16/12	Pargos Control	8.15	35.93	27.5	2059	2436
Pargos	4/16/12	Pargos Control	8.13	35.95	27.6	2060	2348
Pargos	4/16/12	Pargos Control	8.18	35.91	27.6	2002	2343
Pargos	4/16/12	Pargos Control	8.15	35.95	27.5	2028	2351
Pargos	4/16/12	Pargos Control	8.15	35.95	27.7	2084	2342
Pargos	4/16/12	Pargos	8.17	35.92	27.8	2053	2332

		Control (time series)					
Pargos	4/16/12	Pargos Control	8.17	35.92	27.7	1946	2369
Pargos	4/16/12	Pargos Control	8.21	35.26	27.7	2053	2333
Pargos	4/16/12	Pargos Control	8.22	35.92	27.8	1959	2339
Pargos	4/16/12	Pargos Control	8.2	35.91	27.7	1960	2355
Pargos	4/16/12	Pargos Control	8.23	35.9	28.1	1900	2321
Pargos	4/16/12	Pargos Control	8.25	35.93	28.1	1974	2335
Pargos	4/16/12	Pargos Control	8.18	36	28.4	1851	2331
Pargos	4/16/12	Pargos Control	8.18	32.98	29.3	1932	2326
Pargos	4/16/12	Pargos Control	8.18	35.95	29.2	1933	2336
Pargos	4/16/12	Pargos Control	8.15	35.98	29.5	1965	2297
Pargos	4/16/12	Pargos Control	8.15	35.53	27.6	2025	2340
Gorgos	6/23/09	>30 m	8.19	34.6	29	2057	2385
Gorgos	6/23/09	>30 m	8.2	34.6	29	2032	2434
Gorgos	6/23/09	>30 m	8.18	34.8	29	2007	2399
Gorgos	6/23/09	>30 m	8.17	34.7	29	2023	2405
Gorgos	6/23/09	>30 m	8.17	34.7	29	1998	
Gorgos	6/23/09	>30 m	8.14	34.7	29	2023	2397
Gorgos	6/23/09	>30 m	8.17	34.6	29	2015	
Gorgos	6/23/09	>30 m	8.18	34.7	29	2016	2496
Gorgos	6/23/09	>30 m	8.25	35.4	29	2007	2346
Gorgos	6/23/09	>30 m	8.18	34.7	29	2015	2394
Gorgos	6/23/09	>30 m	8.17	34.8	29	1996	2392
Gorgos	6/23/09	>30 m	8.17	34.9	29	2032	2383
Gorgos	6/23/09	>30 m	8.16	33.2	29	1998	2387
Gorgos	6/23/09	>30 m	8.12	34.7	29	1998	2387
Gorgos	6/23/09	>30 m	8.16	34.9	29	2023	2396
Gorgos	6/23/09	>30 m	8.17	34.9	29	2007	2401
Gorgos	6/23/09	>30 m	8.14	35.6	29	1973	2382
Gorgos	6/23/09	>30 m	8.14	35.2	29	1940	2387
Gorgos	6/23/09	>30 m	8.28			1973	2369
Gorgos	6/23/09	>30 m	8.1	35	29	1990	2381
Gorgos	6/23/09	>30 m	8.23	35.8	29	1990	2362
Gorgos	6/23/09	>30 m	8.3	35.6	29	1998	2390
Gorgos	6/23/09	>30 m	8.28	35.2	29	1973	2384
Gorgos	6/23/09	>30 m	8.07	35	29	1957	2393
Gorgos	6/23/09	>30 m	8.25	35.5	29	2023	2332

Gorgos	6/23/09	>30 m	8.08	35.4	29	2023	
Gorgos	6/23/09	>30 m	8.08	35.5	29	2032	2374
Gorgos	6/23/09	>30 m	8.09	35.3	29	2023	2408
Gorgos	6/23/09	>30 m	8.08	35	29	2032	2364
Gorgos	6/23/09	>30 m	8.08	33.4	29	2057	2338
Gorgos	6/23/09	>30 m	8.1	35.3	29	2115	2349
Gorgos	6/23/09	>30 m	8.08	35.2	29	2040	
Gorgos	6/23/09	>30 m	8.08	35.4	29	2065	2357
Gorgos	6/20/09	<2 (Gorgos)	8.16	35	29	2015	2346
Gorgos	6/20/09	<2 (Gorgos)	8.16	34	29	2032	2394
Gorgos	6/20/09	<2 (Gorgos)	8.12	35	29	2032	2404
Gorgos	6/20/09	<2 (Gorgos)	8.05	35.5	29	2007	2387
Gorgos	6/20/09	<2 (Gorgos)	8.17	35.6	29	1998	2382
Gorgos	6/20/09	<2 (Gorgos)	8.08	35	29	2057	2447
Gorgos	6/20/09	<2 (Gorgos)	8.16	35	29	2269	2383
Gorgos	6/20/09	<2 (Gorgos)	8.16	35	29	2026	2397
Gorgos	6/20/09	<2 (Gorgos)	8.16	35	29	2023	
Gorgos	11/11/09	2-Gorgos	8.03	28.36	25		
Gorgos	11/11/09	1-Gorgos	8.02	28.46	25		
Gorgos	11/11/09	1-Gorgos	8.05	28.66	25	2087	
Gorgos	11/11/09	2-Gorgos	8.1	30.91	27.2	2154	
Gorgos	11/11/09	2-Gorgos	8.15	31.27	25	2095	2402
Gorgos	11/11/09	1-Gorgos	8.1	32.5	25	2087	2424
Gorgos	11/11/09	1.75-Gorgos	8.13	30.1	27.2	2070	2376
Gorgos	11/11/09	0.25-Gorgos	8.07	31.9	27.8	2070	2365
Gorgos	11/11/09	0.5-Gorgos	8.07	33.8	27.6	2053	
Gorgos	11/11/09	0.25-Gorgos	8.09	32.1	27.6	2120	
Gorgos	11/11/09	0.5-Gorgos	8.09	32	27.5	2112	
Gorgos	8/28/10	>10m-Gorgos	8.11	35.1	29.7	2055	2393
Gorgos	8/28/10	>10m-Gorgos	8.13	35.8	29.9	2075	2399
Gorgos	10/20/11	Gorgos 100cm	8.09	34.40	26.90	2044	
Gorgos	10/20/11	Gorgos 50cm	8.07	34.80	26.80	2004	2364
Gorgos	10/20/11	Gorgos Control	8.00	33.80	29.00	2036	2309
Gorgos	10/20/11	Gorgos Control	8.12	34.80	26.60	2008	2341
Gorgos	10/20/11	Gorgos Surface	8.13	35.00	28.60	2028	2340
Gorgos	10/20/11	Gorgos Surface	8.04	34.60	29.50	2001	2333
Gorgos	10/20/11	Gorgos Surface	7.95	34.00	27.50	2060	2419
Gorgos	11/11/09	0.5	7.39	23.32	25		
Gorgos	11/11/09	0.25	7.29	22.35	25		
Gorgos	11/11/09	0	7.17	27.32	25		
Gorgos	11/11/09	-0.25	7.16	25.12	25	3466	
Gorgos	11/11/09	-0.5	7.37	22.27	25	3399	

Gorgos	11/11/09	-0.5	7.22	23.55	25	3340	3279
Gorgos	11/11/09	-0.25	7.5	24.09	25	3138	3210
Gorgos	11/11/09	0	7.12	23.45	25	3408	3264
Gorgos	11/11/09	0.25	7.18	26.17	25	3332	3291
Gorgos	11/11/09	0.75	7.81	28.65	25.8	3130	2620
Gorgos	8/28/10	SAMI	7.49	29.6	27.2	3194	3097
Gorgos	8/28/10	SAMI	7.81	30.4	27.2	3326	2996
Gorgos	10/20/11	Gorgos 25cm	7.97	34.40	27.30	2065	2350
Gorgos	10/20/11	Gorgos Center	7.32	31.70	29.20	2740	
Gorgos	10/20/11	Gorgos Center	7.18	29.30	29.10	3039	
Gorgos	10/20/11	Gorgos Center	7.59	31.80	27.10	3095	3424
Gorgos	4/20/12		7.74	34.01	28	2475	2577
Gorgos	4/18/12		7.89	32.6	28.8	3047	2990
Norte	6/21/09	<2m-Norte	8.08	34.2	29	2331	2677
Norte	6/21/09	<2m-Norte	8.05	34.6	29	2007	2421
Norte	6/21/09	<2m-Norte	8.09	34.4	29	2057	2404
Norte	6/21/09	<2m-Norte	8.05	34.3	29	2161	2406
Norte	6/21/09	<2m-Norte	8.06	34.5	29	2048	2354
Norte	6/21/09	<2m-Norte	8.1	34.4	29	2015	2382
Norte	8/30/10	8.0-Norte	8.09	35.1	30	2051	2377
Norte	8/30/10	6.1-Norte	8.02	33.8	30	2560	2602
Norte	8/30/10	8.6-Norte	8.05	34	30	2272	2496
Norte	8/30/10	7.2-Norte	8.03	33.8	29.8	2592	2657
Norte	3/1/11	Norte_Cont01	8.04	35.1	29.2	2052	2399
Norte	3/1/11	Norte_Cont02	8.04	35.3	29.4	2056	2404
Norte	3/1/11	Norte_Cont03	8.05	35.3	29.4	2050	2406
Norte	6/21/09	Norte	7.47	26.7	29	2773	3134
Norte	6/21/09	Norte	7.32	23.4	29	3122	3293
Norte	6/21/09	Norte	7.43	26.6	29	3089	3226
Norte	6/21/09	Norte	7.6	28	29	3106	2937
Norte	6/21/09	Norte	7.7	29	29	2806	2951
Norte	6/21/09	Norte	7.8	30.1	29	2906	
Norte	8/31/10	5.0m	7.26	28.6	27	3267	3184
Norte	8/31/10	2.5m	7.51	27.7	27.5	2883	2810
Norte	8/31/10	4.5m	7.59	30.6	28	2953	2858
Norte	8/31/10	5.7m	7.55	31.1	28.5	2624	2475
Norte	8/31/10	1.8m	7.24	29.2	27	3326	2842
Norte	8/31/10	8.5m	7.91	32.8	29	2671	2644
Norte	8/31/10	5.3m	7.57	30.8	28	2932	2810
Norte	8/31/10	0.6m	7.97	33.6	29	2803	2321
Norte	8/31/10	3.7m	7.37	28.7	27	2810	2848

Norte	3/1/11	Norte_Center_01	7.3	32.6	27.3	2492	2518
Norte	3/1/11	Norte_Center_02	7.56	33.1	27.6	2900	2997
Norte	3/1/11	Norte_Center_03	7.57	31.1	27.4	2904	2999
Norte	10/20/11	Norte 100cm	7.66	31.70	27.00	2314	
Norte	10/20/11	Norte 25cm	7.35	29.70	27.20	2734	2753
Norte	10/20/11	Norte 50cm	7.34	29.70	27.20	2694	
Norte	10/20/11	Norte Center	7.17	30.50	27.50	2517	2611
Norte	8/30/10	2.5m	7.6	31.67	27.9	2874	2782
Norte	8/30/10	2.5m	7.99	33.9	28	2259	2470
Norte	8/30/10	2.0m	7.43	29.7	27.2	3145	2946
Norte	8/30/10	1.5m	7.91	34.5	28.7	2974	2412
Norte	8/30/10	1.0m	7.86	34.5	28.2	2257	2402
Norte	8/30/10	2.0m	7.89	33.4	28	2880	2471
Norte	10/20/11	Norte control	8.00	34.40	27.00	2055	2354
Norte	10/20/11	Norte Surface	7.84	34.60	27.90	2094	2337
Laja	6/20/09	<2m (Laja)	8.07	33	29	2040	2379
Laja	6/20/09	<2m (Laja)	8.11	35	29	2073	2417
Laja	6/20/09	<2m (Laja)	8.08	33	29	2040	2390
Laja	6/20/09	<2m (Laja)	8.04	35.7	29	2015	2414
Laja	6/20/09	<2m (Laja)	8.09	29.5	29	2073	2401
Laja	6/20/09	<2m (Laja)	8.11	35	29	2040	2410
Laja	6/20/09	<2m (Laja)	8.09	33	29	2040	2391
Laja	6/20/09	<2m (Laja)	8.08	27	29	2090	2385
Laja	6/20/09	<2m (Laja)	8.08	27	29	2023	2407
Laja	6/20/09	<2m (Laja)	8.07	33	29	2028	2391
Laja	8/30/10	>10m-Laja	8.07	34.3	31.6	2233	2362
Laja	8/30/10	>10m-Laja	8.14	34.3	31.7	2206	2373
Laja	8/30/10	>10m-Laja	8.14	35.8	30.6	2199	2357
Laja	8/30/10	3.9-Laja	8.02	34.2	31.5	2606	2634
Laja	8/30/10	4.5-Laja	8.16	34.8	30.8	2124	2390
Laja	8/30/10	3.4-Laja	8.06	33	30.7	2176	2639
Laja	8/30/10	1.0-Laja	8.15	34.4	30.4	2051	2394
Laja	8/30/10	0-Laja	8.12	34	30.8	2060	2442
Laja	8/30/10	4.7-Laja	8.11	34	30.4	2291	2365
Laja	10/20/11	Laja 100cm	8.16	34.80	26.50	2051	2319
Laja	10/20/11	Laja 25cm	8.04	33.70	26.10	1998	
Laja	10/20/11	Laja 50cm	8.10	35.00	26.40	2013	2354
Laja	10/20/11	Laja Control	8.02	34.00	31,1(on deck)	2148	2364
Laja	10/20/11	Laja Control	8.18	35.10	26.60	2013	2359
Laja	10/20/11	Laja Control II	8.05	33.90	29.70	2095	2350
Laja	10/20/11	Laja Surface	8.05	34.40	27.20	2130	
Laja	6/20/09	Laja	7.4	21.9	29	3314	3528
Laja	6/20/09	Laja	7.49	23.5	29	2798	2948

Laja	30/08/10	ojo center, by block 6	7.6	33	28.6	3101	2687
Laja	30/08/10	ojo center, by block 6	7.42	30.1	28.6	2724	2589
Laja	30/08/10	ojo center, by block 6	7.66	30.4	28.6	2846	2803
Laja	31/08/10	5.2m	7.29	28.7	27.5	3187	3027
Laja	31/08/10	3.2m	7.38	29.9	28	2713	2708
Laja	20-Apr-12	67	7.37	31.07	28.4	2930	3134
Laja	18-Apr-12	9	7.23	32.31	28.1	2624	2852
Laja	20-Oct-11	Laja Center	7.01	30.10	29.10	2083	
Laja	20-Oct-11	Laja Center	7.21	29.30	26.70	3385	3379
H-10 Fractura	6/19/09	<2m	8.01			2561	2504
H-10 Fractura	6/19/09	<2m	8.03			2107	2506
H-10 Fractura	6/19/09	<2m	8.05			2331	2509
H-10 Fractura	6/19/09	<2m	8.16			2007	2382
H-10 Fractura	6/19/09	<2m	8.2			2032	2393
H-10 Fractura	6/19/09	<2m	8.16			2053	2396
H-10 Fractura	6/19/09	<2m	8.18			1948	2365
H-10 Fractura	6/19/09	<2m	8.18			2024	2383
H-10 Fractura	6/19/09	<2m	8.2	35.7	29	2008	2370
H-10 Fractura	8/30/10	3.7-H-10	8.01	34.1	29.6	2263	2406
H-10 Fractura	8/30/10	4.0-H-10	8.09	34.1	29.6	2088	2372
H-10 Fractura	8/30/10	0.5-H-10	8.04	34.6	29.4	2168	2373
H-10 Fractura	8/30/10	0-H-10	8.07	33.9	29	2017	2408
Mini	6/23/09	<2 (Mini)	8.03	34.9	29	2057	2519
Mini	11/13/09	0.25-Mini	8.02	28.9	25	2179	2476
Mini	11/13/09	0.5-Mini	8.06	28.31	25.4	2095	2391
Mini	11/13/09	0.25-Mini	8.1	28.86	25.5	2070	2374
Mini	11/13/09	1-Mini	8.11	33.5	25.3	2095	2372
Mini	10/20/11	Mini 100 cm	8.12	34.60	26.60	2113	2365
Mini	10/20/11	Mini 25 cm	8.08	34.40	26.90	2071	
Mini	10/20/11	Mini Control	8.09	35.00	26.40	2063	2356
Mini	6/23/09	Ojo Mini	7.5	28.8	29	3705	3634

Mini	6/23/09	Ojo Mini	7.85	33.1	29	2248	2553
Mini	6/23/09	Ojo Mini	7.44	23.5	29	4246	3771
Mini	6/23/09	Ojo Mini	7.79	26.5	29	2739	3055
Mini	6/23/09	Ojo Mini	7.86	28.3	29	2573	3002
Mini	6/23/09	Ojo Mini	8.03	34.9	29	2057	2519
Mini	11/13/09	0	7.18	16	26.6	5233	4691
Mini	11/13/09	0.25	7.51	22.9	25.6	3845	3648
Mini	11/13/09	-0.25	8.02	28.9	25	2179	2476
Mini	11/13/09	-0.25	7.91	27.7	25	2793	2849
Mini	11/13/09	0.25	7.99	28.11	26.3	2145	2436
Mini	11/13/09	0.5	8.06	28.31	25.4	2095	2391
Mini	11/13/09	-0.25	8.1	28.86	25.5	2070	2374
Mini	11/13/09	1	8.11	33.5	25.3	2095	2372
Mini	20-Apr-12		7.86	22.43	27.6	3552	3129
Mini	18-Apr-12		6.95	34.59	28	2215	2504
Mini	22-Oct-11	Mini Center	7.85	30.70	27.60	3076	3109
Fractura	6/22/09	<2-Fractura	8.12	34.3	29	1982	2338
Fractura	6/22/09	<2-Fractura	8.17		29	1965	
Fractura	6/22/09	<2-Fractura	8.15	35.9	29	1990	2355
Fractura	6/22/09	<2-Fractura	8.13	33.4	29	2015	2370
Fractura	6/22/09	<2-Fractura	8.08	34.1	29	2015	2340
Fractura	6/22/09	<2-Fractura	8.09	34.4	29	1965	2371
Fractura	6/22/09	<2-Fractura	8.13	35.4	29	1957	
Fractura	6/22/09	<2-Fractura	8.14	33.2	29	1998	2329
Fractura	6/22/09	Ojo Fractura	7.62	24.7	29	3399	3375
Fractura	6/22/09	Ojo Fractura	7.27	16.3	29	3997	3864
Fractura	6/22/09	Ojo Fractura	7.41	26.7	29	3556	3498
Parque	6/21/09	<2-Parque	8.05	35.8	29	2007	2385
Parque	6/21/09	<2-Parque	8.01	34.2	29	2032	2394
Parque	6/21/09	<2-Parque	8.09	35.1	29	2065	2389
Parque	6/21/09	<2-Parque	8.12	35.2	29	1982	2347
Parque	6/21/09	<2-Parque	8.09	25.7	29	2023	2394
Parque	6/21/09	<2-Parque	8.09	35.1	29	2048	2383
Parque	6/21/09	<2-Parque	8.1	33.9	29	2048	2385
Parque	6/21/09	<2-Parque	8.11	35.1	29	2015	2333
Parque	6/21/09	Parque	7.8	30.9	29	2540	2670
Bonita	6/22/09	<2-Bonita	8.01	32.8	29	1974	2402
Bonita	6/22/09	<2-Bonita	8.09	35.3	29	1932	2376
Bonita	6/22/09	<2-Bonita	8.09	35.5	29	1998	2389
Bonita	6/22/09	<2-Bonita	8.11	35	29	1998	2391
Bonita	6/22/09	<2-Bonita	8.11	34.6	29	1973	2306
Bonita	6/22/09	<2-Bonita	8.1	35.5	29	2032	2429
Bonita	6/22/09	<2-Bonita	8.11	35	29	1957	2393
Bonita	6/22/09	<2-Bonita	8.1	35.5	29	2040	2396

Bonita	6/22/09	<2-Bonita	8.19	35.1	29	1998	2403
Bonita	6/22/09	<2-Bonita	8.09	35.7	29	1992	2374
Bonita	6/22/09	<2-Bonita	8.1	35.5	29	1990	2450
Bonita	6/22/09	<2-Bonita	8.09	35	29	1998	2359
Bonita	6/22/09	<2-Bonita	8.03	35.5	29	2023	2411
Bonita	6/22/09	<2-Bonita	8.09	34.8	29	2007	2379
Bonita	6/22/09	Bonita	7.26	22.8	29	3997	3964
de Agua	8/30/10	3.5-Agua	8.06	34.4	29.6	2157	2334
de Agua	8/30/10	3.0-Agua	8.05	34.7	30.4	2182	2477
de Agua	8/30/10	2.0-Agua	8.02	33.7	29.5	2190	2451
de Agua	8/30/10	2.0-Agua	8.07	33.9	29.7	2324	2418
de Agua	8/30/10	3.0-Agua	8.09	34.9	29.6	1968	2411
de Agua	8/30/10	2.0-Agua	8.14	34.8	29.6	2021	2383
de Agua	3/1/11	Agua_Cont01	8.02	34.8	28.8	2069	2398
de Agua	3/1/11	Agua_Cont02	8	35.4	28.8	2083	2392
de Agua	3/1/11	Agua_Cont03	8	35.3	28.2	2076	2387
de Agua	10/20/11	de Agua 100cm	8.10	34.20	28.20	2063	2347
de Agua	10/20/11	de Agua 25cm	8.13	34.30	28.00	2015	2364
de Agua	10/20/11	de Agua 50cm	8.13	34.20	28.40	2088	2314
de Agua	10/20/11	de Agua Surface	8.09	34.90	27.60	2016	
de Agua	10/20/11	deAgua Control	8.15	34.40	27.70	2049	2363
de Agua	3/1/11	Agua_Center _01	7.41	33.8	28.7	2559	2601
de Agua	3/1/11	Agua_Center _02	7.63	33.9	27.8	2409	2533
de Agua	3/1/11	Agua_Center _03	7.63	34.2	28.2	2483	2609
de Agua	10/20/11	de Agua Center	7.89	33.70	27.40	2167	2444
de Agua	4/20/12	de Agua Center	7.95	32.3	27.2	2986	2791
de Agua	4/20/12	de Agua Center	6.81	36	27.4	2072	2409
de Agua	6/20/09	de Agua Center	7.15	23.45	29	3545	3427
de Agua	6/20/09	de Agua Center	7.19	23.45	29	2939	3469
H-10 Fractura	6/19/09	H-10 Fractura	7.91			2373	2638
H-10 Fractura	6/19/09	H-10 Fractura	7.36	35.7	29	2723	2740
H-10 Fractura	6/19/09	H-10 Fractura	7.83			2431	2652
H-10 Fractura	6/19/09	H-10 Fractura	7.7			2590	2774
H-10	6/19/09	H-10 Fractura	7.91			2356	2571

Fractura							
H-10 Fractura	6/19/09	H-10 Fractura	7.95			2240	2548
H-10 Fractura	1/10/14	4.0m	7.37	30.4	28	2999	2735
H-10 Fractura	1/10/14	5.0m	7.62	32.2	28	2777	2639
H-10 Fractura	1/10/14	4.5m	7.84	31.7	29	2418	2617
H-10 Fractura	1/10/14	4.0m	7.72	32.6	29.2	2112	2567
H-10 Fractura	1/10/14	3.25m	7.52	31.45	28.8	2717	2704
H-10 Fractura	1/10/14	3.9m	7.68	31.5	28.9	2528	2557
H-10 Fractura	1/10/14	1.0m	7.92	33.6	28.7	2219	2371
H-10 Fractura	1/10/14	3.5m	7.67	31.8	28.3	2550	2529
H-10 Fractura	1/10/14	2.0m	7.36	29.4	27.9	3407	2736
H-10 Fractura	1/10/14	1.5m	7.38	31	28	2886	2662
H-10 Fractura	1/10/14	3.0m	7.35	30.3	27.8	2713	2708
H-10 Fractura	1/10/14	2.5m	7.42	30.5	28		2613

Appendix B. Chapter III Supporting Data

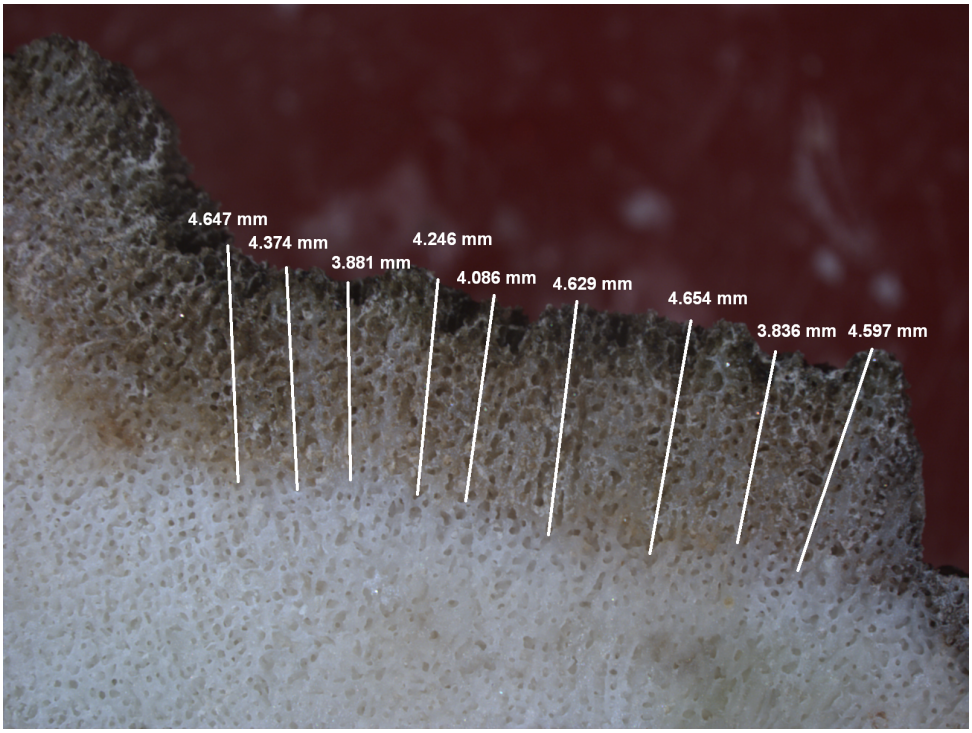


Figure B.1. **Example measurements of tissue thickness from coral cores**. Tissue thickness, a measure of the volume of coral soft tissue occupying the skeleton, was measured on each core half using a Nikon SMZ1500 Stereo microscope and SPOT imaging software. We define tissue thickness as the distance between the last (most recently accreted) dissepiment and the tip of the calical walls.

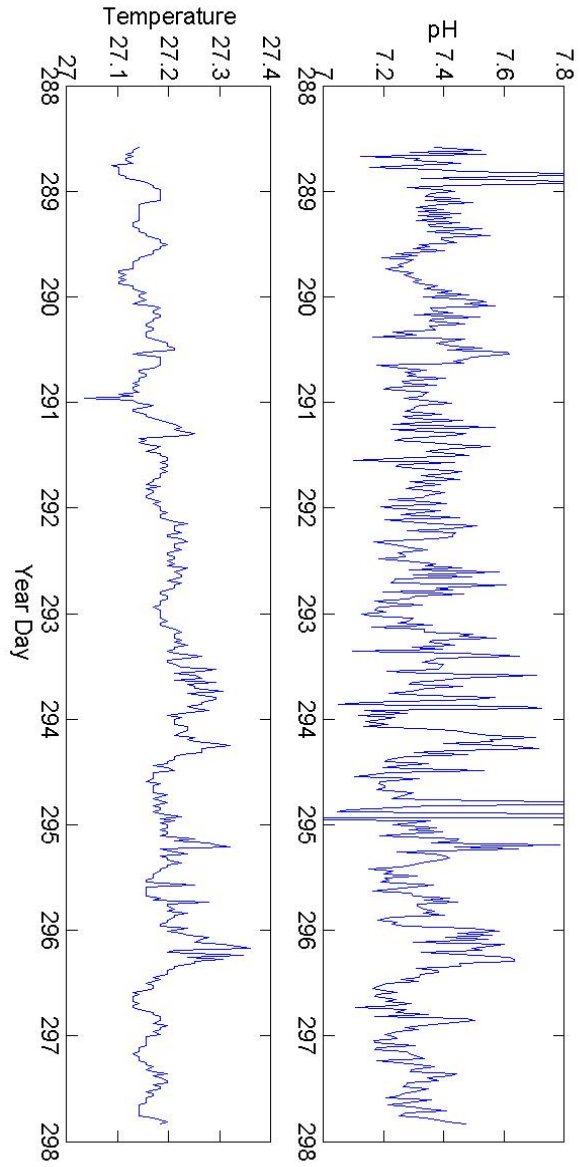


Figure B.2. SAMI Sensor Data. Submersible Automatic Multisampler Instrument data from October 2011 at Ojo B, Ojo Gorgos.

Table B.1. Tissue thickness data, coral cores. Measurements are to the nearest 0.01 mm.

CORAL #	Measurement	Tisse Thickness
Crook_05_2	1	2.546
Crook_05_2	2	2.638
Crook_05_2	3	2.792
Crook_05_2	4	2.417
Crook_05_2	5	2.845
Crook_05_2	6	2.721
Crook_05_2	7	2.203
Crook_05_2	8	2.464
Crook_05_2	9	2.462
CORAL #		
Crook_05_1	1	3.546
Crook_05_1	2	3.073
Crook_05_1	3	3.465
Crook_05_1	4	3.537
Crook_05_1	5	3.552
Crook_05_1	6	3.939
Crook_05_1	7	3.449
Crook_05_1	8	2.926
Crook_05_1	9	2.763
CORAL #		
Crook_13B	1	2.44
Crook_13B	2	2.764
Crook_13B	3	2.505
Crook_13B	4	2.7
Crook_13B	5	2.781
Crook_13B	6	3.153
Crook_13B	7	3.269
Crook_13B	8	3.577
Crook_13B	9	3.583
CORAL #		
Crook_20B	1	4.261
Crook_20B	2	4.249
Crook_20B	3	4.009
Crook_20B	4	4.229
Crook_20B	5	4.35
Crook_20B	6	4.373
Crook_20B	7	4.492
Crook_20B	8	4.459
Crook_20B	9	4.457
CORAL #		
Crook_06	1	2.226
Crook_06	2	2.429
Crook_06	3	2.458
Crook_06	4	3.103
Crook_06	5	3.6

Crook_06	6	4.27
Crook_06	7	3.16
Crook_06	8	3.262
Crook_06	9	2.894
CORAL #		
Crook_01	1	2.802
Crook_01	2	2.809
Crook_01	3	2.941
Crook_01	4	2.602
Crook_01	5	2.72
Crook_01	6	3.588
Crook_01	7	4.158
Crook_01	8	4.096
Crook_01	9	3.279
CORAL #		
Crook_09	1	2.304
Crook_09	2	3.244
Crook_09	3	3.362
Crook_09	4	3.459
Crook_09	5	3.691
Crook_09	6	3.505
Crook_09	7	3.334
Crook_09	8	3.493
Crook_09	9	3.669
CORAL #		
Crook_14	1	3.36
Crook_14	2	3.131
Crook_14	3	3.335
Crook_14	4	3.53
Crook_14	5	4.055
Crook_14	6	4.024
Crook_14	7	4.091
Crook_14	8	5.263
Crook_14	9	3.261
CORAL #		
Crook_16	1	3.012
Crook_16	2	3.422
Crook_16	3	3.163
Crook_16	4	2.954
Crook_16	5	2.989
Crook_16	6	3.435
Crook_16	7	3.417
Crook_16	8	3.492
Crook_16	9	3.722
CORAL #		
Crook_05_2	1	2.793
Crook_05_2	2	2.926
Crook_05_2	3	3.449
Crook_05_2	4	
Crook_05_2	5	3.552
Crook_05_2	6	3.537

Crook_05_2	7	3.465
Crook_05_2	8	3.073
Crook_05_2	9	3.546
CORAL #		
Crook_07	1	4.174
Crook_07	2	4.275
Crook_07	3	4.225
Crook_07	4	4.024
Crook_07	5	3.883
Crook_07	6	3.743
Crook_07	7	3.701
Crook_07	8	3.715
Crook_07	9	3.633
CORAL #		
Crook_02	1	4.647
Crook_02	2	4.374
Crook_02	3	3.881
Crook_02	4	4.246
Crook_02	5	4.086
Crook_02	6	4.629
Crook_02	7	4.654
Crook_02	8	3.836
Crook_02	9	4.597
CORAL #		
Crook_03	1	5.603
Crook_03	2	5.062
Crook_03	3	6.092
Crook_03	4	6.441
Crook_03	5	6.382
Crook_03	6	6.632
Crook_03	7	5.647
Crook_03	8	5.517
Crook_03	9	5.346
CORAL #		
Crook_04	1	4.036
Crook_04	2	3.828
Crook_04	3	4.331
Crook_04	4	3.547
Crook_04	5	3.497
Crook_04	6	3.521
Crook_04	7	3.721
Crook_04	8	4.033
Crook_04	9	4.287
CORAL #		
Crook_08	1	3.515
Crook_08	2	3.787
Crook_08	3	4.291
Crook_08	4	3.938
Crook_08	5	4.002
Crook_08	6	3.948
Crook_08	7	4.036

Crook_08	8	3.899
CORAL #		
Crook_10	1	4.534
Crook_10	2	4.732
Crook_10	3	4.361
Crook_10	4	4.399
Crook_10	5	3.956
Crook_10	6	3.657
Crook_10	7	3.724
Crook_10	8	4.443
CORAL #		
Crook_11	1	2.293
Crook_11	2	2.276
Crook_11	3	2.859
Crook_11	4	3.314
Crook_11	5	3.756
Crook_11	6	3.081
Crook_11	7	2.96
Crook_11	8	2.668
CORAL #		
Crook_15	1	4.218
Crook_15	2	4.263
Crook_15	3	4.173
Crook_15	4	4.824
Crook_15	5	4.585
Crook_15	6	4.442
Crook_15	7	4.224
Crook_15	8	4.331
Crook_15	9	4.572
CORAL #		
Crook_17	1	3.306
Crook_17	2	2.889
Crook_17	3	3.033
Crook_17	4	2.907
Crook_17	5	2.771
Crook_17	6	3.661
Crook_17	7	3.685
Crook_17	8	3.36
Crook_17	9	3.351
CORAL #		
Crook_18	1	4.691
Crook_18	2	4.465
Crook_18	3	3.954
Crook_18	4	4.455
Crook_18	5	5.064
Crook_18	6	4.8
Crook_18	7	4.877
Crook_18	8	4.624
Crook_18	9	4.073
CORAL #		
Crook_19	1	2.952

Crook_19	2	3.15
Crook_19	3	3.037
Crook_19	4	3.14
Crook_19	5	3.253
Crook_19	6	3.284
Crook_19	7	2.805
Crook_19	8	3.322
Crook_19	9	3.167
CORAL #		
Crook_20	1	3.378
Crook_20	2	3.833
Crook_20	3	3.745
Crook_20	4	3.349
Crook_20	5	3.351
Crook_20	6	3.089
Crook_20	7	3.031
Crook_20	8	3.647
Crook_20	9	2.972
CORAL #		
Crook_13A	1	2.154
Crook_13A	2	2.228
Crook_13A	3	2.751
Crook_13A	4	2.851
Crook_13A	5	3.099
Crook_13A	6	3.302
Crook_13A	7	2.892
Crook_13A	8	2.635
Crook_13A	9	2.212

Table B.2. Coral core data summary. Extension and density were obtained from Computed Tomography (CT) scans. Extension was measured as the distance between high density bands in Osirix using the 4 most recent years. Density data were obtained using coral density standards. Saturation of the water from the core was obtained from in-situ measurements taken at the time of sampling. Tissue thickness was measured to 0.1mm accuracy on a digital zoom microscope.

01_Porites_Norte_Center

	Extension (cm yr^{-1})	Density (g $cm^{-3}yr^{-1}$)	Calcification (g $cm^{-2}yr^{-1}$)	Saturation	Tissue Thickness (mm)
mean	0.24	0.29	0.35	1.22	3.22
stdev	0.05	0.10	0.10		0.60
standard error	0.02	0.02	0.02		0.21

02_Porites_Norte_Center

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.26	1.35	0.35	1.85	4.33
stdev	0.08	0.10	0.11		0.33
standard error	0.04	0.04	0.05		0.12

04_Porites_Norte_Center

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.39	1.07	0.42	1.90	3.87
stdev	0.08	0.08	0.07		0.32
standard error	0.04	0.04	0.03		0.11

09_Porites_Agua_Center

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.22	1.08	0.23	0.77	3.34
stdev	0.04	0.05	0.06		0.42
standard error	0.02	0.01	0.01		0.15

10_Porites_Agua_Center

	Extension	Density	Calcification	Saturation	Tissue

					Thickness
mean	0.25	1.09	0.30	1.82	4.31
stdev	0.08	0.17	0.10		0.44
standard error	0.03	0.05	0.03		0.16

11_Porites_Agua_Center

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.30	1.14	0.33	1.82	2.87
stdev	0.01	0.07	0.03		0.48
standard error	0.01	0.03	0.01		0.17

17_Porites_Pargos_Center

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.15	1.06	0.16	0.81	3.22
stdev	0.05	0.06	0.06		0.34
standard error	0.03	0.04	0.04		0.12

05_1_Porites_Norte_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.27	1.61	0.44	4.03	3.36
stdev	0.09	0.17	0.15		0.37
standard error	0.03	0.06	0.05		0.13

05_2_Porites_Norte_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.21	1.52	0.37	4.03	3.29
stdev	0.03	0.09	0.11		0.31
standard error	0.01	0.02	0.02		0.11

06_Porites_Norte_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.42	1.66	0.67	4.12	3.04

stdev	0.06	0.12	0.12		0.64
standard error	0.02	0.03	0.03		0.23

13A_Porites_Agua_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.19	1.74	0.32	3.80	2.68
stdev	0.04	0.06	0.08		0.41
standard error	0.01	0.02	0.02		0.14

13B_Porites_Agua_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.30	1.55	0.46	4.24	2.97
stdev	0.08	0.13	0.13		0.44
standard error	0.03	0.05	0.05		0.15

18_Porites_Pargos_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.39	1.51	0.59	3.60	4.56
stdev	0.08	0.11	0.11		0.36
standard error	0.10	0.03	0.03		0.13

19_Porites_Pargos_Control

	Extension	Density	Calcification	Saturation	Tissue Thickness
mean	0.33	1.47	0.48	3.60	3.12
stdev	0.06	0.06	0.10		0.17
standard error	0.03	0.03	0.05		0.06

Appendix C. Chapter IV Supporting Information

Table C.1. % Cover Data for all taxa, all tiles. Both ojos used for the recruitment study are represented: Ojo Gorgos (Gorg) and Ojo Laja (Laja) for sites A and B. Tiles were removed from one of two saturation (pH) zones: Control (Con) or Ojo Center (Cen) at 3, 6, or 14 months. Tile replicates are shown by tile #.

SITE	pH	TAXA	Month	Tile #	% Cover
Gorg	Con	Erect Red	3	1	0.100
Gorg	Con	Erect Red	3	2	0.000
Gorg	Con	Erect Red	3	3	0.000
Laja	Con	Erect Red	3	1	0.000
Laja	Con	Erect Red	3	2	0.000
Laja	Con	Erect Red	3	3	0.000
Gorg	Cen	Erect Red	3	1	0.000
Gorg	Cen	Erect Red	3	2	0.000
Gorg	Cen	Erect Red	3	3	0.000
Laja	Cen	Erect Red	3	1	0.050
Laja	Cen	Erect Red	3	2	0.000
Laja	Cen	Erect Red	3	3	0.030
Gorg	Con	Erect Red	6	1	0.000
Gorg	Con	Erect Red	6	2	0.000
Gorg	Con	Erect Red	6	3	0.150
Laja	Con	Erect Red	6	1	0.050
Laja	Con	Erect Red	6	2	0.000
Laja	Con	Erect Red	6	3	0.000
Gorg	Cen	Erect Red	6	1	0.450
Gorg	Cen	Erect Red	6	2	0.000
Gorg	Cen	Erect Red	6	3	0.000
Laja	Cen	Erect Red	6	1	0.000
Laja	Cen	Erect Red	6	2	0.138
Laja	Cen	Erect Red	6	3	0.000
Gorg	Con	Erect Red	14	1	0.020
Gorg	Con	Erect Red	14	2	0.350
Gorg	Con	Erect Red	14	3	0.000
Gorg	Con	Erect Red	14	4	0.150
Laja	Con	Erect Red	14	1	0.300
Laja	Con	Erect Red	14	2	0.200
Laja	Con	Erect Red	14	3	0.250
Laja	Con	Erect Red	14	4	0.000
Gorg	Cen	Erect Red	14	1	0.300
Gorg	Cen	Erect Red	14	2	0.600

Gorg	Cen	Erect Red	14	3	0.600
Gorg	Cen	Erect Red	14	4	0.400
Laja	Cen	Erect Red	14	1	0.250
Laja	Cen	Erect Red	14	2	0.500
Laja	Cen	Erect Red	14	3	0.400
Laja	Cen	Erect Red	14	4	0.300
SITE	**pH**	**TAXA**	**Month**	**Tile**	**% Cover**
Gorg	Con	Encrusting Fleshy Algae	3	1	0.200
Gorg	Con	Encrusting Fleshy Algae	3	2	0.200
Gorg	Con	Encrusting Fleshy Algae	3	3	0.150
Laja	Con	Encrusting Fleshy Algae	3	1	0.400
Laja	Con	Encrusting Fleshy Algae	3	2	0.300
Laja	Con	Encrusting Fleshy Algae	3	3	0.300
Gorg	Cen	Encrusting Fleshy Algae	3	1	0.263
Gorg	Cen	Encrusting Fleshy Algae	3	2	0.375
Gorg	Cen	Encrusting Fleshy Algae	3	3	0.200
Laja	Cen	Encrusting Fleshy Algae	3	1	0.300
Laja	Cen	Encrusting Fleshy Algae	3	2	0.300
Laja	Cen	Encrusting Fleshy Algae	3	3	0.250
Gorg	Con	Encrusting Fleshy Algae	6	1	0.300
Gorg	Con	Encrusting Fleshy Algae	6	2	0.400
Gorg	Con	Encrusting Fleshy Algae	6	3	0.375
Laja	Con	Encrusting Fleshy Algae	6	1	0.400
Laja	Con	Encrusting Fleshy Algae	6	2	0.200
Laja	Con	Encrusting Fleshy Algae	6	3	0.300
Gorg	Cen	Encrusting Fleshy Algae	6	1	0.350
Gorg	Cen	Encrusting Fleshy Algae	6	2	0.275
Gorg	Cen	Encrusting Fleshy Algae	6	3	0.500
Laja	Cen	Encrusting Fleshy Algae	6	1	0.150
Laja	Cen	Encrusting Fleshy Algae	6	2	0.625
Laja	Cen	Encrusting Fleshy Algae	6	3	0.700
Gorg	Con	Encrusting Fleshy Algae	14	1	0.300
Gorg	Con	Encrusting Fleshy Algae	14	2	0.300
Gorg	Con	Encrusting Fleshy Algae	14	3	0.300
Gorg	Con	Encrusting Fleshy Algae	14	4	0.200
Laja	Con	Encrusting Fleshy Algae	14	1	0.350
Laja	Con	Encrusting Fleshy Algae	14	2	0.300
Laja	Con	Encrusting Fleshy Algae	14	3	0.400
Laja	Con	Encrusting Fleshy Algae	14	4	0.400
Gorg	Cen	Encrusting Fleshy Algae	14	1	0.500
Gorg	Cen	Encrusting Fleshy Algae	14	2	0.300
Gorg	Cen	Encrusting Fleshy Algae	14	3	0.300
Gorg	Cen	Encrusting Fleshy Algae	14	4	0.300

Laja	Cen	Encrusting Fleshy Algae	14	1	0.500
Laja	Cen	Encrusting Fleshy Algae	14	2	0.400
Laja	Cen	Encrusting Fleshy Algae	14	3	0.600
Laja	Cen	Encrusting Fleshy Algae	14	4	0.400

SITE	pH	TAXA	Month	Tile	% Cover
Gorg	Con	Biofilm	3	1	0.010
Gorg	Con	Biofilm	3	2	0.050
Gorg	Con	Biofilm	3	3	0.010
Laja	Con	Biofilm	3	1	0.010
Laja	Con	Biofilm	3	2	0.020
Laja	Con	Biofilm	3	3	0.020
Gorg	Cen	Biofilm	3	1	0.075
Gorg	Cen	Biofilm	3	2	0.040
Gorg	Cen	Biofilm	3	3	0.020
Laja	Cen	Biofilm	3	1	0.100
Laja	Cen	Biofilm	3	2	0.050
Laja	Cen	Biofilm	3	3	0.020
Gorg	Con	Biofilm	6	1	0.050
Gorg	Con	Biofilm	6	2	0.200
Gorg	Con	Biofilm	6	3	0.040
Laja	Con	Biofilm	6	1	0.020
Laja	Con	Biofilm	6	2	0.250
Laja	Con	Biofilm	6	3	0.250
Gorg	Cen	Biofilm	6	1	0.100
Gorg	Cen	Biofilm	6	2	0.188
Gorg	Cen	Biofilm	6	3	0.950
Laja	Cen	Biofilm	6	1	0.800
Laja	Cen	Biofilm	6	2	0.110
Laja	Cen	Biofilm	6	3	0.150
Gorg	Con	Biofilm	14	1	0.000
Gorg	Con	Biofilm	14	2	0.000
Gorg	Con	Biofilm	14	3	0.000
Gorg	Con	Biofilm	14	4	0.100
Laja	Con	Biofilm	14	1	0.050
Laja	Con	Biofilm	14	2	0.000
Laja	Con	Biofilm	14	3	0.000
Laja	Con	Biofilm	14	4	0.100
Gorg	Cen	Biofilm	14	1	0.150
Gorg	Cen	Biofilm	14	2	0.100
Gorg	Cen	Biofilm	14	3	0.100
Gorg	Cen	Biofilm	14	4	0.250
Laja	Cen	Biofilm	14	1	0.200

Laja	Cen	Biofilm	14	2	0.100
Laja	Cen	Biofilm	14	3	0.120
Laja	Cen	Biofilm	14	4	0.250

SITE	pH	TAXA	Month	Tile	% Cover
Gorg	Con	Encrusting Calcified Algae	3	1	0.303
Gorg	Con	Encrusting Calcified Algae	3	2	0.155
Gorg	Con	Encrusting Calcified Algae	3	3	0.090
Laja	Con	Encrusting Calcified Algae	3	1	0.320
Laja	Con	Encrusting Calcified Algae	3	2	0.155
Laja	Con	Encrusting Calcified Algae	3	3	0.583
Gorg	Cen	Encrusting Calcified Algae	3	1	0.020
Gorg	Cen	Encrusting Calcified Algae	3	2	0.038
Gorg	Cen	Encrusting Calcified Algae	3	3	0.043
Laja	Cen	Encrusting Calcified Algae	3	1	0.018
Laja	Cen	Encrusting Calcified Algae	3	2	0.183
Laja	Cen	Encrusting Calcified Algae	3	3	0.093
Gorg	Con	Encrusting Calcified Algae	6	1	0.408
Gorg	Con	Encrusting Calcified Algae	6	2	0.492
Gorg	Con	Encrusting Calcified Algae	6	3	0.478
Laja	Con	Encrusting Calcified Algae	6	1	0.512
Laja	Con	Encrusting Calcified Algae	6	2	0.495
Laja	Con	Encrusting Calcified Algae	6	3	0.550
Gorg	Cen	Encrusting Calcified Algae	6	1	0.100
Gorg	Cen	Encrusting Calcified Algae	6	2	0.273
Gorg	Cen	Encrusting Calcified Algae	6	3	0.000
Laja	Cen	Encrusting Calcified Algae	6	1	0.185
Laja	Cen	Encrusting Calcified Algae	6	2	0.220
Laja	Cen	Encrusting Calcified Algae	6	3	0.145
Gorg	Con	Encrusting Calcified Algae	14	1	0.482
Gorg	Con	Encrusting Calcified Algae	14	2	0.523
Gorg	Con	Encrusting Calcified Algae	14	3	0.450
Gorg	Con	Encrusting Calcified Algae	14	4	0.767
Laja	Con	Encrusting Calcified Algae	14	1	0.683
Laja	Con	Encrusting Calcified Algae	14	2	0.930
Laja	Con	Encrusting Calcified Algae	14	3	0.478
Laja	Con	Encrusting Calcified Algae	14	4	0.823
Gorg	Cen	Encrusting Calcified Algae	14	1	0.052
Gorg	Cen	Encrusting Calcified Algae	14	2	0.032
Gorg	Cen	Encrusting Calcified Algae	14	3	0.040
Gorg	Cen	Encrusting Calcified Algae	14	4	0.037
Laja	Cen	Encrusting Calcified Algae	14	1	0.165
Laja	Cen	Encrusting Calcified Algae	14	2	0.198

Laja	Cen	Encrusting Calcified Algae	14	3	0.370
Laja	Cen	Encrusting Calcified Algae	14	4	0.000

SITE	pH	TAXA	Month	Tile	% Cover
Gorg	Con	Erect Brown Algae	3	1	0.000
Gorg	Con	Erect Brown Algae	3	2	0.020
Gorg	Con	Erect Brown Algae	3	3	0.000
Laja	Con	Erect Brown Algae	3	1	0.000
Laja	Con	Erect Brown Algae	3	2	0.020
Laja	Con	Erect Brown Algae	3	3	0.020
Gorg	Cen	Erect Brown Algae	3	1	0.000
Gorg	Cen	Erect Brown Algae	3	2	0.000
Gorg	Cen	Erect Brown Algae	3	3	0.000
Laja	Cen	Erect Brown Algae	3	1	0.000
Laja	Cen	Erect Brown Algae	3	2	0.020
Laja	Cen	Erect Brown Algae	3	3	0.000
Gorg	Con	Erect Brown Algae	6	1	0.000
Gorg	Con	Erect Brown Algae	6	2	0.050
Gorg	Con	Erect Brown Algae	6	3	0.013
Laja	Con	Erect Brown Algae	6	1	0.300
Laja	Con	Erect Brown Algae	6	2	0.050
Laja	Con	Erect Brown Algae	6	3	0.000
Gorg	Cen	Erect Brown Algae	6	1	0.000
Gorg	Cen	Erect Brown Algae	6	2	0.100
Gorg	Cen	Erect Brown Algae	6	3	0.000
Laja	Cen	Erect Brown Algae	6	1	0.000
Laja	Cen	Erect Brown Algae	6	2	0.113
Laja	Cen	Erect Brown Algae	6	3	0.100
Gorg	Con	Erect Brown Algae	14	1	0.050
Gorg	Con	Erect Brown Algae	14	2	0.000
Gorg	Con	Erect Brown Algae	14	3	0.010
Gorg	Con	Erect Brown Algae	14	4	0.000
Laja	Con	Erect Brown Algae	14	1	0.000
Laja	Con	Erect Brown Algae	14	2	0.000
Laja	Con	Erect Brown Algae	14	3	0.000
Laja	Con	Erect Brown Algae	14	4	0.000
Gorg	Cen	Erect Brown Algae	14	1	0.000
Gorg	Cen	Erect Brown Algae	14	2	0.000
Gorg	Cen	Erect Brown Algae	14	3	0.000
Gorg	Cen	Erect Brown Algae	14	4	0.000
Laja	Cen	Erect Brown Algae	14	1	0.000
Laja	Cen	Erect Brown Algae	14	2	0.000
Laja	Cen	Erect Brown Algae	14	3	0.000

Laja	Cen	Erect Brown Algae	14	4	0.150

SITE	pH	TAXA	Month	Tile	% Cover
Gorg	Con	Erect Green	3	1	0.000
Gorg	Con	Erect Green	3	2	0.000
Gorg	Con	Erect Green	3	3	0.050
Laja	Con	Erect Green	3	1	0.000
Laja	Con	Erect Green	3	2	0.000
Laja	Con	Erect Green	3	3	0.000
Gorg	Cen	Erect Green	3	1	0.000
Gorg	Cen	Erect Green	3	2	0.000
Gorg	Cen	Erect Green	3	3	0.000
Laja	Cen	Erect Green	3	1	0.000
Laja	Cen	Erect Green	3	2	0.000
Laja	Cen	Erect Green	3	3	0.000
Gorg	Con	Erect Green	6	1	0.000
Gorg	Con	Erect Green	6	2	0.000
Gorg	Con	Erect Green	6	3	0.013
Laja	Con	Erect Green	6	1	0.010
Laja	Con	Erect Green	6	2	0.050
Laja	Con	Erect Green	6	3	0.200
Gorg	Cen	Erect Green	6	1	0.000
Gorg	Cen	Erect Green	6	2	0.000
Gorg	Cen	Erect Green	6	3	0.000
Laja	Cen	Erect Green	6	1	0.000
Laja	Cen	Erect Green	6	2	0.055
Laja	Cen	Erect Green	6	3	0.200
Gorg	Con	Erect Green	14	1	0.050
Gorg	Con	Erect Green	14	2	0.100
Gorg	Con	Erect Green	14	3	0.010
Gorg	Con	Erect Green	14	4	0.000
Laja	Con	Erect Green	14	1	0.050
Laja	Con	Erect Green	14	2	0.000
Laja	Con	Erect Green	14	3	0.010
Laja	Con	Erect Green	14	4	0.000
Gorg	Cen	Erect Green	14	1	0.000
Gorg	Cen	Erect Green	14	2	0.000
Gorg	Cen	Erect Green	14	3	0.000
Gorg	Cen	Erect Green	14	4	0.000
Laja	Cen	Erect Green	14	1	0.100
Laja	Cen	Erect Green	14	2	0.000
Laja	Cen	Erect Green	14	3	0.500
Laja	Cen	Erect Green	14	4	0.100

SITE	pH	TAXA	Month	Tile	% Cover
Gorg	Con	Turf Algae	3	1	0.450
Gorg	Con	Turf Algae	3	2	0.250
Gorg	Con	Turf Algae	3	3	0.850
Laja	Con	Turf Algae	3	1	0.200
Laja	Con	Turf Algae	3	2	0.300
Laja	Con	Turf Algae	3	3	0.300
Gorg	Cen	Turf Algae	3	1	0.488
Gorg	Cen	Turf Algae	3	2	0.450
Gorg	Cen	Turf Algae	3	3	0.800
Laja	Cen	Turf Algae	3	1	0.300
Laja	Cen	Turf Algae	3	2	0.300
Laja	Cen	Turf Algae	3	3	0.600
Gorg	Con	Turf Algae	6	1	0.350
Gorg	Con	Turf Algae	6	2	0.300
Gorg	Con	Turf Algae	6	3	0.338
Laja	Con	Turf Algae	6	1	0.400
Laja	Con	Turf Algae	6	2	0.250
Laja	Con	Turf Algae	6	3	0.300
Gorg	Cen	Turf Algae	6	1	0.325
Gorg	Cen	Turf Algae	6	2	0.275
Gorg	Cen	Turf Algae	6	3	0.500
Laja	Cen	Turf Algae	6	1	0.150
Laja	Cen	Turf Algae	6	2	0.250
Laja	Cen	Turf Algae	6	3	0.150
Gorg	Con	Turf Algae	14	1	0.250
Gorg	Con	Turf Algae	14	2	0.200
Gorg	Con	Turf Algae	14	3	0.150
Gorg	Con	Turf Algae	14	4	0.100
Laja	Con	Turf Algae	14	1	0.200
Laja	Con	Turf Algae	14	2	0.150
Laja	Con	Turf Algae	14	3	0.150
Laja	Con	Turf Algae	14	4	0.000
Gorg	Cen	Turf Algae	14	1	0.300
Gorg	Cen	Turf Algae	14	2	0.300
Gorg	Cen	Turf Algae	14	3	0.300
Gorg	Cen	Turf Algae	14	4	0.300
Laja	Cen	Turf Algae	14	1	0.200
Laja	Cen	Turf Algae	14	2	0.200
Laja	Cen	Turf Algae	14	3	0.100
Laja	Cen	Turf Algae	14	4	0.050

SITE	pH	TAXA	Month	Tile	% Cover
Gorg	Con	Upright Calcified	3	1	0.000
Gorg	Con	Upright Calcified	3	2	0.000
Gorg	Con	Upright Calcified	3	3	0.000
Laja	Con	Upright Calcified	3	1	0.000
Laja	Con	Upright Calcified	3	2	0.000
Laja	Con	Upright Calcified	3	3	0.000
Gorg	Cen	Upright Calcified	3	1	0.000
Gorg	Cen	Upright Calcified	3	2	0.000
Gorg	Cen	Upright Calcified	3	3	0.000
Laja	Cen	Upright Calcified	3	1	0.000
Laja	Cen	Upright Calcified	3	2	0.000
Laja	Cen	Upright Calcified	3	3	0.000
Gorg	Con	Upright Calcified	6	1	0.080
Gorg	Con	Upright Calcified	6	2	0.080
Gorg	Con	Upright Calcified	6	3	0.050
Laja	Con	Upright Calcified	6	1	0.200
Laja	Con	Upright Calcified	6	2	0.070
Laja	Con	Upright Calcified	6	3	0.050
Gorg	Cen	Upright Calcified	6	1	0.000
Gorg	Cen	Upright Calcified	6	2	0.000
Gorg	Cen	Upright Calcified	6	3	0.000
Laja	Cen	Upright Calcified	6	1	0.100
Laja	Cen	Upright Calcified	6	2	0.000
Laja	Cen	Upright Calcified	6	3	0.000
Gorg	Con	Upright Calcified	14	1	0.150
Gorg	Con	Upright Calcified	14	2	0.090
Gorg	Con	Upright Calcified	14	3	0.080
Gorg	Con	Upright Calcified	14	4	0.070
Laja	Con	Upright Calcified	14	1	0.100
Laja	Con	Upright Calcified	14	2	0.150
Laja	Con	Upright Calcified	14	3	0.070
Laja	Con	Upright Calcified	14	4	0.120
Gorg	Cen	Upright Calcified	14	1	0.020
Gorg	Cen	Upright Calcified	14	2	0.000
Gorg	Cen	Upright Calcified	14	3	0.000
Gorg	Cen	Upright Calcified	14	4	0.020
Laja	Cen	Upright Calcified	14	1	0.000
Laja	Cen	Upright Calcified	14	2	0.000
Laja	Cen	Upright Calcified	14	3	0.000
Laja	Cen	Upright Calcified	14	4	0.050

| SITE | pH | TAXA | Month | Tile | % Cover |

Gorg	Con	All Taxa	3	1	1.063
Gorg	Con	All Taxa	3	2	0.675
Gorg	Con	All Taxa	3	3	1.150
Laja	Con	All Taxa	3	1	0.930
Laja	Con	All Taxa	3	2	0.795
Laja	Con	All Taxa	3	3	1.223
Gorg	Cen	All Taxa	3	1	0.845
Gorg	Cen	All Taxa	3	2	0.903
Gorg	Cen	All Taxa	3	3	1.063
Laja	Cen	All Taxa	3	1	0.768
Laja	Cen	All Taxa	3	2	0.853
Laja	Cen	All Taxa	3	3	0.993
Gorg	Con	All Taxa	6	1	1.188
Gorg	Con	All Taxa	6	2	1.522
Gorg	Con	All Taxa	6	3	1.456
Laja	Con	All Taxa	6	1	1.892
Laja	Con	All Taxa	6	2	1.365
Laja	Con	All Taxa	6	3	1.650
Gorg	Cen	All Taxa	6	1	1.325
Gorg	Cen	All Taxa	6	2	1.111
Gorg	Cen	All Taxa	6	3	1.950
Laja	Cen	All Taxa	6	1	1.385
Laja	Cen	All Taxa	6	2	1.510
Laja	Cen	All Taxa	6	3	1.445
Gorg	Con	All Taxa	14	1	1.732
Gorg	Con	All Taxa	14	2	1.813
Gorg	Con	All Taxa	14	3	1.700
Gorg	Con	All Taxa	14	4	1.737
Laja	Con	All Taxa	14	1	1.733
Laja	Con	All Taxa	14	2	1.730
Laja	Con	All Taxa	14	3	1.358
Laja	Con	All Taxa	14	4	1.443
Gorg	Cen	All Taxa	14	1	1.322
Gorg	Cen	All Taxa	14	2	1.332
Gorg	Cen	All Taxa	14	3	1.340
Gorg	Cen	All Taxa	14	4	1.307
Laja	Cen	All Taxa	14	1	0.985
Laja	Cen	All Taxa	14	2	1.148
Laja	Cen	All Taxa	14	3	1.390
Laja	Cen	All Taxa	14	4	0.950

Table C.2. Average count per tile and average size of vermetid molluscs, foraminifera, and polychaetes. Both ojos used for the recruitment study are represented: Ojo Gorgos (Gorg) and Ojo Laja (Laja) for sites A and B. Tiles were removed from one of two saturation (pH) zones: Control (Con) or Ojo Center (Cen) at 3, 6, or 14 months. Tile replicates are shown by tile #.

SITE	pH	TAXA	Month	Tile	F-Num	F-Size
Gorg	Cen	Forams	3	1	34	0.771
Gorg	Cen	Forams	3	2	7	0.783
Gorg	Cen	Forams	3	3	68	0.775
Laja	Cen	Forams	3	1	9	0.787
Laja	Cen	Forams	3	2	30	0.812
Laja	Cen	Forams	3	3	13	1.002
Laja	Con	Forams	3	1	21	1.111
Laja	Con	Forams	3	2	37	0.615
Laja	Con	Forams	3	3	57	0.848
Gorg	Con	Forams	3	1	84	0.839
Gorg	Con	Forams	3	2	73	0.722
Gorg	Con	Forams	3	3	56	0.880
Gorg	Cen	Forams	6	1	60	1.338
Gorg	Cen	Forams	6	2	119	1.214
Gorg	Cen	Forams	6	3	12	1.780
Laja	Cen	Forams	6	1	76	1.052
Laja	Cen	Forams	6	2	21	1.423
Laja	Cen	Forams	6	3	39	1.383
Laja	Con	Forams	6	1	48	1.730
Laja	Con	Forams	6	2	78	1.545
Laja	Con	Forams	6	3	65	1.545
Gorg	Con	Forams	6	1	88	1.324
Gorg	Con	Forams	6	2	138	1.317
Gorg	Con	Forams	6	3	103	1.664
Gorg	Cen	Forams	14	1	63	1.238
Gorg	Cen	Forams	14	2	106	1.274
Gorg	Cen	Forams	14	3	31	1.448
Gorg	Cen	Forams	14	4	39	1.453
Laja	Cen	Forams	14	1	39	1.460
Laja	Cen	Forams	14	2	86	1.477
Laja	Cen	Forams	14	3	138	1.356
Laja	Cen	Forams	14	4	32	0.866
Gorg	Con	Forams	14	1	22	1.973
Gorg	Con	Forams	14	2	20	1.851

Gorg	Con	Forams	14	3	22	1.593
Gorg	Con	Forams	14	4	44	1.397
Laja	Con	Forams	14	1	9	1.533
Laja	Con	Forams	14	2	5	2.276
Laja	Con	Forams	14	3	20	1.852
Laja	Con	Forams	14	4	69	1.590

SITE	**pH**		**Month**	**Tile**	**F-Num**	**F-Size**
Gorg	Cen	Vermetid Molluscs	3	1	1	5.0
Gorg	Cen	Vermetid Molluscs	3	2	2	2.1
Gorg	Cen	Vermetid Molluscs	3	3	1	2.0
Laja	Cen	Vermetid Molluscs	3	1	3	0.4
Laja	Cen	Vermetid Molluscs	3	2	2	3.4
Laja	Cen	Vermetid Molluscs	3	3	2	1.8
Laja	Con	Vermetid Molluscs	3	1	0	
Laja	Con	Vermetid Molluscs	3	2	0	
Laja	Con	Vermetid Molluscs	3	3	1	0.4
Gorg	Con	Vermetid Molluscs	3	1	1	2.0
Gorg	Con	Vermetid Molluscs	3	2	0	
Gorg	Con	Vermetid Molluscs	3	3	0	
Gorg	Cen	Vermetid Molluscs	6	1	1	4.0
Gorg	Cen	Vermetid Molluscs	6	2	0	
Gorg	Cen	Vermetid Molluscs	6	3	3	7.4
Laja	Cen	Vermetid Molluscs	6	1	7	9.7
Laja	Cen	Vermetid Molluscs	6	2	23	12.7
Laja	Cen	Vermetid Molluscs	6	3	0	
Laja	Con	Vermetid Molluscs	6	1	1	0.5
Laja	Con	Vermetid Molluscs	6	2	0	
Laja	Con	Vermetid Molluscs	6	3	1	9.0
Gorg	Con	Vermetid Molluscs	6	1	1	6.0
Gorg	Con	Vermetid Molluscs	6	2	0	
Gorg	Con	Vermetid Molluscs	6	3	1	9.0
Gorg	Cen	Vermetid Molluscs	14	1	7	6.9
Gorg	Cen	Vermetid Molluscs	14	2	1	21.0
Gorg	Cen	Vermetid Molluscs	14	3	3	19.3
Gorg	Cen	Vermetid Molluscs	14	4	3	9.5
Laja	Cen	Vermetid Molluscs	14	1	10	12.3
Laja	Cen	Vermetid Molluscs	14	2	21	15.4
Laja	Cen	Vermetid Molluscs	14	3	2	5.1
Laja	Cen	Vermetid Molluscs	14	4	4	5.5
Gorg	Con	Vermetid Molluscs	14	1	12	8.0
Gorg	Con	Vermetid Molluscs	14	2	3	5.3

Gorg	Con	Vermetid Molluscs	14	3	5	5.6
Gorg	Con	Vermetid Molluscs	14	4	1	19.0
Laja	Con	Vermetid Molluscs	14	1	7	6.4
Laja	Con	Vermetid Molluscs	14	2	1	11.0
Laja	Con	Vermetid Molluscs	14	3	2	2.5
Laja	Con	Vermetid Molluscs	14	4	1	5.0

SITE	**pH**	**TAXA**	**Month**	**Tile**	**F-Num**	**F-Size**
Gorg	Cen	Polychaetes	3	1	5	3.01
Gorg	Cen	Polychaetes	3	2	6	3.50
Gorg	Cen	Polychaetes	3	3	1	2.04
Laja	Cen	Polychaetes	3	1	4	2.59
Laja	Cen	Polychaetes	3	2	4	2.53
Laja	Cen	Polychaetes	3	3	6	4.09
Laja	Con	Polychaetes	3	1	8	6.03
Laja	Con	Polychaetes	3	2	5	1.56
Laja	Con	Polychaetes	3	3	6	2.59
Gorg	Con	Polychaetes	3	1	21	5.60
Gorg	Con	Polychaetes	3	2	14	2.36
Gorg	Con	Polychaetes	3	3	9	3.78
Gorg	Cen	Polychaetes	6	1	26	2.29
Gorg	Cen	Polychaetes	6	2	4	4.76
Gorg	Cen	Polychaetes	6	3	14	7.07
Laja	Cen	Polychaetes	6	1	15	7.42
Laja	Cen	Polychaetes	6	2	5	5.69
Laja	Cen	Polychaetes	6	3	14	11.29
Laja	Con	Polychaetes	6	1	9	5.37
Laja	Con	Polychaetes	6	2	11	7.12
Laja	Con	Polychaetes	6	3	20	7.91
Gorg	Con	Polychaetes	6	1	22	6.73
Gorg	Con	Polychaetes	6	2	5	10.40
Gorg	Con	Polychaetes	6	3	20	4.72
Gorg	Cen	Polychaetes	14	1	14	8.14
Gorg	Cen	Polychaetes	14	2	4	8.75
Gorg	Cen	Polychaetes	14	3	9	6.05
Gorg	Cen	Polychaetes	14	4	13	7.54
Laja	Cen	Polychaetes	14	1	35	9.34
Laja	Cen	Polychaetes	14	2	25	11.51
Laja	Cen	Polychaetes	14	3	10	12.09
Laja	Cen	Polychaetes	14	4	5	3.02
Gorg	Con	Polychaetes	14	1	19	7.84
Gorg	Con	Polychaetes	14	2	15	10.42
Gorg	Con	Polychaetes	14	3	14	7.47

Gorg	Con	Polychaetes	14	4	18	9.82
Laja	Con	Polychaetes	14	1	14	5.86
Laja	Con	Polychaetes	14	2	18	7.81
Laja	Con	Polychaetes	14	3	35	7.49
Laja	Con	Polychaetes	14	4	19	11.40

Appendix D. Chapter V Supporting Information

Table D.1. Raw data for *Balanophyllia elegans* crystal length as measured by SEM. Lengths (μm) of aragonite crystals in the septa of juvenile *B. elegans* skeletons after 8 months growth under four pCO_2 X food treatments.

1200 ppm High Food		1200 ppm Low Food		380 ppm High Food		380 ppm Low Food	
Coral #	Crystal Length (μm)	Coral #	Crystal Length (μm)	Coral #	Crystal Length (μm)	Coral #	Crystal Length (μm)
1200_HF_1	2.641	1200_LF_1	2.314	380_HF_1	2.529	380_LF_1	2.521
1200_HF_1	2.874	1200_LF_1	2.081	380_HF_1	2.324	380_LF_1	3.252
1200_HF_1	1.836	1200_LF_1	2.366	380_HF_1	2.750	380_LF_1	3.144
1200_HF_1	2.624	1200_LF_1	2.568	380_HF_1	3.172	380_LF_1	3.000
1200_HF_1	2.647	1200_LF_1	2.481	380_HF_1	3.033	380_LF_1	2.729
1200_HF_1	2.170	1200_LF_1	1.777	380_HF_1	2.768	380_LF_1	3.071
1200_HF_1	2.535	1200_LF_1	2.435	380_HF_1	3.840	380_LF_1	2.962
1200_HF_1	2.561	1200_LF_1	1.987	380_HF_1	3.914	380_LF_2	2.545
1200_HF_1	2.833	1200_LF_2	2.437	380_HF_2	3.244	380_LF_2	3.162
1200_HF_2	3.047	1200_LF_2	1.561	380_HF_2	3.302	380_LF_2	2.973
1200_HF_2	2.710	1200_LF_2	2.346	380_HF_2	2.861	380_LF_2	3.034
1200_HF_2	3.335	1200_LF_2	2.891	380_HF_2	3.426	380_LF_2	3.090
1200_HF_2	3.243	1200_LF_2	2.471	380_HF_2	3.091	380_LF_2	2.823
1200_HF_2	2.692	1200_LF_2	2.316	380_HF_2	2.497	380_LF_2	3.119
1200_HF_2	3.035	1200_LF_2	2.417	380_HF_2	3.157	380_LF_2	2.874
1200_HF_2	2.640	1200_LF_2	2.020	380_HF_2	3.426	380_LF_2	2.589
1200_HF_2	2.918	1200_LF_2	2.153	380_HF_2	3.496	380_LF_3	3.375
1200_HF_2	2.750	1200_LF_2	2.257	380_HF_3	2.694	380_LF_3	3.064

1200_HF_3	2.595	1200_LF_2	2.209	380_HF_3	2.390	380_LF_3	3.044
1200_HF_3	2.578	1200_LF_3	2.335	380_HF_3	2.586	380_LF_3	3.468
1200_HF_3	2.574	1200_LF_3	2.171	380_HF_3	2.406	380_LF_3	3.280
1200_HF_3	2.753	1200_LF_3	1.924	380_HF_3	2.874	380_LF_3	3.569
1200_HF_3	2.881	1200_LF_3	2.107	380_HF_3	2.969	380_LF_3	3.472
1200_HF_3	2.008	1200_LF_3	2.014	380_HF_3	2.628	380_LF_4	3.031
1200_HF_3	2.069	1200_LF_3	2.189	380_HF_3	2.897	380_LF_4	2.704
1200_HF_3	1.997	1200_LF_3	2.060	380_HF_3	3.122	380_LF_4	3.516
1200_HF_3	2.468	1200_LF_3	2.425	380_HF_3	3.539	380_LF_4	2.959
1200_HF_3	2.512	1200_LF_3	2.420	380_HF_3	4.011	380_LF_4	3.083
1200_HF_3	2.620	1200_LF_3	2.324	380_HF_4	3.118	380_LF_4	2.855
1200_HF_4	2.430	1200_LF_4	2.161	380_HF_4	3.296		
1200_HF_4	2.554	1200_LF_4	2.364	380_HF_4	3.106		
1200_HF_4	2.735	1200_LF_4	2.217	380_HF_4	2.623		
1200_HF_4	2.081	1200_LF_4	2.381	380_HF_4	2.874		
1200_HF_4	2.652	1200_LF_4	2.277				
1200_HF_4	2.548	1200_LF_4	1.695				
1200_HF_4	2.101	1200_LF_4	2.228				
1200_HF_4	2.371	1200_LF_4	1.757				
1200_HF_4	2.133	1200_LF_4	2.376				
1200_HF_4	2.083	1200_LF_5	2.383				
1200_HF_5	2.279	1200_LF_5	2.269				
1200_HF_5	2.567	1200_LF_5	1.976				
1200_HF_5	2.293	1200_LF_5	2.180				
1200_HF_5	2.672						
1200_HF_5	2.624						

Table D.2. Raw data of *B. elegans* crystal width, as measured by SEM. Widths (µm) of aragonite crystals in the septa of juvenile *Balanophyllia elegans* skeletons after 8 months growth under four pCO_2 X food treatments.

1200 ppm High Food		1200 ppm Low Food		380 ppm High Food		380 ppm Low Food	
Coral #	Crystal Width (µm)	Coral #	Crystal Width (µm)	Coral #	Crystal Width (µm)	Coral #	Crystal Width (µm)
1200_HF_1	0.184	1200_LF_1	0.121	380_HF_1	0.176	380_LF_1	0.211
1200_HF_1	0.165	1200_LF_1	0.110	380_HF_1	0.086	380_LF_1	0.149
1200_HF_1	0.116	1200_LF_1	0.126	380_HF_1	0.087	380_LF_1	0.124
1200_HF_1	0.107	1200_LF_1	0.121	380_HF_1	0.159	380_LF_1	0.185
1200_HF_1	0.107	1200_LF_1	0.133	380_HF_1	0.111	380_LF_1	0.134
1200_HF_2	0.187	1200_LF_2	0.104	380_HF_2	0.124	380_LF_1	0.173
1200_HF_2	0.122	1200_LF_2	0.145	380_HF_2	0.108	380_LF_2	0.133
1200_HF_2	0.089	1200_LF_2	0.136	380_HF_2	0.144	380_LF_2	0.148
1200_HF_2	0.113	1200_LF_2	0.132	380_HF_2	0.148	380_LF_2	0.169
1200_HF_2	0.113	1200_LF_3	0.123	380_HF_2	0.148	380_LF_2	0.132
1200_HF_3	0.119	1200_LF_3	0.140	380_HF_3	0.113	380_LF_2	0.127
1200_HF_3	0.165	1200_LF_3	0.141	380_HF_3	0.101	380_LF_2	0.134
1200_HF_3	0.163	1200_LF_3	0.117	380_HF_3	0.116	380_LF_2	0.136
1200_HF_3	0.193	1200_LF_3	0.134	380_HF_3	0.121	380_LF_3	0.118
1200_HF_3	0.154	1200_LF_3	0.150	380_HF_3	0.130	380_LF_3	0.134
1200_HF_3	0.140	1200_LF_4	0.105	380_HF_4	0.179	380_LF_3	0.132
1200_HF_3	0.188	1200_LF_4	0.190	380_HF_4	0.119	380_LF_3	0.179
1200_HF_3	0.126	1200_LF_4	0.067	380_HF_4	0.165	380_LF_3	0.104
1200_HF_4	0.134	1200_LF_4	0.167	380_HF_4	0.134	380_LF_3	0.119
1200_HF_4	0.140	1200_LF_4	0.197	380_HF_4	0.201	380_LF_3	0.140
1200_HF_4	0.127	1200_LF_4	0.131	380_HF_5	0.091	380_LF_4	0.116
1200_H	0.134	1200_LF_	0.133	380_HF_5	0.146	380_LF_4	0.106

F_4		5					
1200_H		1200_LF_					
F_4	0.137	5	0.126	380_HF_5	0.133	380_LF_4	0.116
1200_H		1200_LF_					
F_5	0.166	5	0.115	380_HF_5	0.168	380_LF_4	0.119
1200_H		1200_LF_					
F_5	0.139	5	0.118			380_LF_4	0.139
1200_H		1200_LF_					
F_5	0.139	5	0.104			380_LF_4	0.127
1200_H		1200_LF_					
F_5	0.196	5	0.139			380_LF_5	0.125
			0.146			380_LF_5	0.117
			0.139			380_LF_5	0.124
			0.111			380_LF_5	0.148
			0.110			380_LF_5	0.132
			0.135			380_LF_5	0.189

Table D.3. Raw data: Weights (g) of juvenile *Balanophyllia elegans* skeletons after 8 months growth under four pCO_2 X food treatments. All measurements are accurate to ±0.01 mg.

1200 ppm High Food	1200 ppm Low Food	750 ppm High Food	750 ppm Low Food	380 ppm High Food	380 ppm Low Food
0.03031	0.00440	0.02115	0.00676	0.04979	0.00713
0.05387	0.01180	0.02732	0.00574	0.05564	0.00453
0.01592	0.00381	0.03009	0.00463	0.03391	0.00456
0.07323	0.00544	0.01622	0.00265	0.03471	0.00658
0.02822	0.01265	0.06370	0.00265	0.03471	0.00615
0.08791	0.00709	0.03382	0.00562	0.05480	0.00672
0.02035	0.01012	0.02967	0.00661	0.09887	0.00678
0.02669	0.00950	0.02969	0.00443	0.03842	0.01210
0.02794	0.00577	0.03371	0.00444	0.09211	0.00668
0.02795	0.00608	0.05200	0.00445	0.02187	0.01386
0.02792	0.00432	0.04583	0.00860	0.04383	0.00464
0.02153	0.00421	0.04782	0.00458	0.03842	0.01272
0.03893	0.00407	0.06121	0.00458	0.05424	0.01234
0.03893	0.00407	0.03159	0.00457	0.05425	0.01244
0.03893	0.00406	0.07466	0.00459	0.05422	0.01242
0.05856	0.00503	0.02842	0.00720	0.11427	0.01243
0.05041	0.00702	0.02794	0.00610	0.04736	0.01453
0.05046	0.00711	0.03554	0.01302	0.04736	0.01723
0.05048	0.00711	0.03554	0.00800		0.01625
0.05042	0.01535	0.05040	0.00602		0.01625
0.05041	0.00758	0.02530	0.00516		0.02073
0.05041	0.00647	0.02530	0.00948		0.02073
	0.00647	0.02530	0.00502		
	0.00646		0.00554		
			0.00504		
			0.00525		
			0.00525		
			0.00662		

Table D.4. Raw data: Volumes (mm^3) of juvenile *Balanophyllia elegans* skeletons after 8 months growth under four pCO_2 X food treatments. Calculated as an elliptical cylinder from measurements made with vernier calipers (0.1 mm accuracy).

1200 ppm High Food	1200 ppm Low Food	750 ppm High Food	750 ppm Low Food	380 ppm High Food	380 ppm Low Food
169.8	8.6	34.2	4.2	114.3	19.2
132.5	6.1	36.1	4.8	35.5	9.9
39.7	6.9	46.4	2.0	27.9	10.3
76.0	4.2	105.3	6.3	138.3	27.2
34.0	6.8	104.1	8.9	89.3	29.8
100.4	17.4	98.0	11.8	41.9	42.6
136.1	29.5	101.4	9.5	97.0	8.6
112.4	3.5	137.9	8.9	137.6	7.4
74.3	6.0	103.7	18.1	28.2	6.6
86.2	9.6	94.3	7.4	74.6	6.1
57.2	9.2	156.5	4.0	35.2	8.3
123.5	9.4	188.2	7.7	19.2	8.0
106.2	12.6	189.3	32.5	10.3	6.6
98.2	11.7	130.2	13.2	63.3	19.2
114.7	6.3	222.1	6.9	71.8	15.3
54.3	12.1	58.1	11.7	153.8	8.6
104.2	21.3	51.6	7.5	49.3	5.7
49.0	17.2	71.3	8.9	44.8	19.2
15.8	15.3	147.8	11.3	169.6	9.1
22.1	6.1	26.7	8.2	14.3	9.0
37.7	4.8	67.2	8.2	26.6	13.9
59.1	7.1	132.4	20.4	33.8	2.5
	5.6	23.8	10.2	35.1	8.5
	7.8		6.8		9.1
	8.5		11.0		13.4
	7.6		11.2		12.8
	4.4		9.2		5.0
	6.6		4.3		6.5
	4.8		6.4		2.2
			4.5		1.2
			1.8		3.4
			6.6		2.3
			2.4		5.7
			6.6		4.7
			4.1		
			5.5		
			8.5		
			7.7		

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