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USE OF NATURALLY ACIDIFIED ENVIRONMENTS TO DETERMINE TOLERANCE AND ACCLIMATION POTENTIAL OF MARINE ORGANISMS TO OCEAN ACIDIFICATION

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Abstract

Helen Leigh O'Brien Cooper

Use of naturally acidified environments to determine tolerance and acclimation potential of marine organisms to ocean acidification

Ocean acidification (OA) causes negative responses in numerous marine organisms including declines in calcification, growth and development. However, responses to OA vary, and organisms differ in their tolerance of OA. These variable responses make it difficult to predict how OA will shape marine communities in the future without a greater framework of what traits or mechanisms confer tolerance to OA. This Ph.D. aims to explore some of the patterns in traits associated with tolerance to OA to better predict the acclimation potential of organisms in the future.

Chapters 1 and 2 explore how an organism that currently experiences regular variation in pH responds to chronic ocean acidification by exposing *Euphausia pacifica* to experimental chronic acidification. *E. pacifica* is relatively tolerant of chronic acidification, showing no changes to survival or molting frequency, but slower growth at low pH. Slower growth was likely caused by reduced metabolism at low pH, as oxygen consumption, ingestion, and nutrient excretion rates all declined at low pH.

Chapter 3 explored the potential for OA-sensitive species to acclimate to OA over long time periods by surveying communities of calcifying algae along a pH/saturation gradient at naturally low-pH, low saturation submarine springs off the coast of Puerto Morelos, Mexico. Total percent cover, species richness and diversity

of calcifying algae were all lower at low saturation levels, however tolerance of acidification varied across genera. *Hydrolithon* and *Peyssonnelia* were more tolerant of low saturation, while *Neogoniolithon*, *Amphiroa* and *Lithophyllum* were more sensitive. Results provide mixed support for the hypothesis that early successional species, characterized by fast growing and thin thalli, are more sensitive to OA than later successional species with thicker thalli.

While more work is needed to elucidate relationships between certain traits and OA tolerance, this work is an important step in developing and further testing hypotheses about which traits are important in determining acclimation potential to OA.

1. Introduction

The burning of fossil fuels and deforestation have caused atmospheric carbon dioxide (CO_2) to increase from 290 ppm in pre-industrial times to approximately 400 ppm today, and it is expected to continue to rise to between 800 and 1200 ppm within this century (Caldeira & Wickett, 2005; Solomon, 2007; Doney *et al*, 2009). The rate of atmospheric CO_2 accumulation is slowed by the uptake of CO_2 by the world's oceans, limiting the rate at which we experience effects of global warming (Doney *et al*, 2009). However, the uptake of CO_2 by the oceans has profound effects on ocean chemistry, characterized by declines in ocean pH and carbonate ion (CO_3^{2-}) concentration that are commonly referred to as ocean acidification (OA). On average open ocean pH has dropped by approximately 0.1 since preindustrial times (from 8.2 to 8.1) and is expected to drop another 0.3 to 0.4 pH units by the end of the century (Caldeira & Wickett, 2005; Orr *et al*, 2005; Doney *et al*, 2009; Feely *et al*, 2009; Stocker *et al*, 2013; Edenhofer *et al*, 2014), a rate unprecedented in recent history (Doney & Schimel, 2007). Simultaneously, carbonate ion concentration will decline by 50%, directly reducing the seawater calcium carbonate ($CaCO_3$) saturation state and altering $CaCO_3$ precipitation and dissolution dynamics. At low saturation levels, carbonate minerals dissolve more readily in seawater, and organisms relying on calcified skeletons or shells (e.g., corals, mollusks, coralline algae) have great difficulty maintaining normal calcification rates, and their growth, survival, and reproduction often decline (Kleypas *et al*, 2006; Doney *et al*, 2009; Kroeker *et al*, 2010; Andersson *et al*, 2011; Ross *et al*, 2011).

Research over past decades has established that ocean acidification has overwhelmingly negative effects on marine organisms that include reduced survival, calcification, growth, abundance, fertilization success and abnormal larval development (reviewed in Kroeker *et al*, 2010; Hofmann *et al*, 2010; Doney *et al*, 2012; Kroeker *et al*, 2013). However, the direction and magnitude of these responses vary, with some organisms having either positive or no response to OA (Ries *et al*, 2009; Kroeker *et al*, 2010; Wittmann & Pörtner, 2013). While strong trends of differential responses have been observed among different taxonomic groups (e.g., heavily calcified organisms, such as corals, are more sensitive), much variation between species and even populations has yet to be explained (Kroeker *et al*, 2010; Dupont & Pörtner, 2013). Without a more comprehensive framework of the mechanisms and traits associated with tolerance of OA, this variation makes it difficult to predict long-term ecosystem effects of OA. Several hypotheses advanced to explain variation in organismal tolerances include natural exposure to variable pH in their habitat, acid-base buffering ability, and food availability matching increasing energy demands (Pörtner & Farrell, 2008; Melzner *et al*, 2009; Holcomb *et al*, 2010; Thomsen *et al*, 2010; Maas *et al*, 2012; Pespeni *et al*, 2013; Dupont & Pörtner, 2013).

My research is aimed at understanding some of these patterns with the goal of better predicting tolerance to OA. Specifically, I ask: 1) how do organisms that experience regular variation in pH respond to chronic ocean acidification, and 2) what is the potential for OA-sensitive species to acclimate or adapt to OA? I addressed these questions through a series of laboratory experiments at the Long Marine

Laboratory of the University of California Santa Cruz, and with in-situ field studies in Puerto Morelos, Mexico.

The pH of surface open ocean waters is approximately 8.1, but it declines with increasing depth due to lower temperatures (cold water can absorb more CO_2) and higher CO_2 concentrations produced by decomposition of sinking organic material. Organisms living permanently at depth experience low but stable pH (~7.60 at 500 meters depth (Feely *et al*, 2008), while organisms that make daily vertical migrations between surface waters and depth may experience large daily fluctuations in pH as they move between two depth zones. Similarly, organisms living in areas that undergo seasonal upwelling (in which low pH waters come from depth to the surface) will experience periods of low pH during parts of the year. Limited evidence suggests that natural exposure to variable pH may confer some degree of tolerance to chronic ocean acidification in some organisms (Thomsen *et al*, 2010; Maas *et al*, 2012; Pespeni *et al*, 2013; Pansch *et al*, 2014), possibly due to physiological adaptations that buffer changes in external pCO_2 (Seibel & Walsh, 2003). Understanding how organisms from naturally acidified environments deal with low pH should lead to better predictions of their potential for physiological acclimation or evolutionary adaptation to future OA conditions.

In Chapters 1 and 2, I aimed to answer this question by exposing *Euphausia pacifica*, a krill species in Monterey Bay that performs diel migrations between the deep ocean and surface waters, to chronic acidification. In chapter 1, I explore some basic physical responses to elevated CO_2 including survival, growth and molting

behavior over a 2-month period. I also explore survival at extremely high CO_2 levels to identify OA thresholds causing mortality in krill. I conclude that krill are relatively tolerant to ocean acidification, as their survival was affected only at extremely low pH (high pCO_2), although they did have lower growth rates. Slower growth may be caused by metabolic depression associated with low pH, and this could have large ecological ramifications. In Chapter 2, I explore metabolic responses of krill to short- and long-term acidification to explain their reduced growth rates at low pH. I demonstrate that *E. pacifica* does have lower metabolic rates at low pH, and low rates persist after 3 weeks.

Most previous studies of OA have been short-term experiments exposing present-day organisms to future OA levels. Such studies have limited ability to assess the potential for physiological acclimation or adaptation to OA over the course of several generations (Pansch *et al*, 2014). While possible, evolutionary experiments necessary to answer this question have been limited to short-lived organisms that reproduce quickly and are easily maintained in the laboratory (Collins & Bell, 2004; Lohbeck *et al*, 2012). The fundamental question that remains to be answered is: which organisms (species, populations or individuals) have the potential to acclimate and/or adapt to OA over the next century?

In chapter 3, I address this question by studying communities of calcifying algae occurring along natural pH gradients in a coral reef system on the Caribbean coast of Mexico, and describe differences in the acclimation potential of different algal species. I document overall declines in species richness, diversity and total

abundance of calcifying algae under chronic low saturation/low pH, even though these organisms have spent their entire lives within the low pH zone. Responses vary by genus and at least two genera seem to be either acclimated or adapted to low pH. Which taxa can and cannot adapt may have large repercussions for benthic communities, especially on coral reefs where the dynamics linking coral larval settlement and coralline algae are often species-specific.

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Chapter 1.

Effects of elevated pCO_2 on the survival, growth and molting of the Pacific krill species, *Euphausia pacifica*

1.1 Abstract

While ocean acidification (OA) is expected to have wide-ranging negative effects on marine species, organisms currently living in variable pH environments that expose them intermittently to pH values approaching those predicted for the future, may be well positioned to tolerate prolonged exposure to high pCO_2 levels caused by OA. Seasonal upwelling brings low pH water to the surface along the Pacific Coast of North America. In Monterey Bay, California *Euphausia pacifica*, a key species supporting a diverse multi-trophic level ecosystem, currently experiences broad pCO_2 and pH ranges due to both diel vertical migrations and seasonal upwelling. We determined tolerances of *E. pacifica* to prolonged exposure to pH levels predicted for 2100 by maintaining adults at two pCO_2 levels (380 and 1200 µatm) for 2 months. Rates of survival and molting were the same at both pCO_2 levels. High pCO_2 slowed growth in all size classes. In additional experiments to determine pCO_2 threshold levels above which *E. pacifica* is adversely affected, survival was not affected down to pH 6.96 (6050 µatm), but declined rapidly at pH 6.92 (7228 µatm) and lower, with 100% mortality within 10 days (at pH 6.89).

1.2 Introduction

Ocean acidification (OA) is the decline in ocean pH and carbonate ion concentration caused by the uptake of atmospheric CO_2 by ocean surface waters (Orr *et al*, 2005; Fabry *et al*, 2008; Doney *et al*, 2009). Many biological investigations of OA show adverse effects on marine organisms, including reduced growth, lower calcification rates, reduced fertilization success, and abnormal larval development (Kurihara *et al*, 2004; Kleypas *et al*, 2006; Andersson *et al*, 2011; Ross *et al*, 2011), although these effects vary across taxa and habitats (Kroeker *et al*, 2010), and many studies show positive or no effects of OA (Ries *et al*, 2009; Kroeker *et al*, 2010; Wittmann & Pörtner, 2013).

A quickly expanding area of research is the role of natural environmental variation in determining the tolerances of organisms to future OA levels. Near shore habitats around Monterey Bay, California experience highly variable pH and pCO_2 levels caused by seasonal wind-driven upwelling that brings deep, CO_2-rich waters to the surface (Feely *et al*, 2008). These habitats can fluctuate by approximately 0.35 pH units over several days (Hofmann *et al*, 2011). Organisms living in such upwelling regions may already be well adapted to future OA, due to histories of repeated exposure to lower pH water for much of the year (Thomsen *et al*, 2010; Lewis *et al*, 2013). Conversely, these organisms may already be living near their limits of tolerance (Yu *et al*, 2011), and may face increasing pH stress in the future, since OA is likely to increase the frequency, duration and intensity of low pH conditions (Feely *et al*, 2008; Hauri *et al*, 2009; Gaylord *et al*, 2011; Barton *et al*, 2012).

As a group, crustaceans have mixed responses to OA, but overall, they seem more tolerant of OA than many other taxa (Kroeker *et al*, 2010; Wittmann & Pörtner, 2013; Kroeker *et al*, 2013). At ecologically relevant levels of elevated pCO_2 crustaceans may experience declines in egg production, hatching success, growth and survival and/or changes in mineral content of structures (Kurihara *et al*, 2008; Arnold *et al*, 2009; Findlay *et al*, 2009; Long *et al*, 2013; Lewis *et al*, 2013; Sperfeld *et al*, 2014; Cripps *et al*, 2014; Zheng *et al*, 2015), although many species show no negative effects at these levels (Arnold *et al*, 2009; McConville *et al*, 2013) and within-taxon differences in responses to increased pCO_2 are often attributed to differences in metabolic rates, habitat variation and/or life stages (Pane & Barry, 2007; Kroeker *et al*, 2013; Cripps *et al*, 2014).

Euphausiids, commonly known as krill, are pelagic marine crustaceans with global distributions (Mauchline & Fisher, 1969). Krill form ecologically and economically important links between primary producers (phytoplankton) and numerous forage and commercial fish species (e.g. salmon, herring) that feed directly on krill, as well as to the seabirds and great whales at the top of these food chains. Despite their importance in marine food webs, responses of krill to OA remain largely unknown. Previous studies have focused primarily on the Antarctic krill*, Euphausia superba*, in which embryonic development and larval behavior were unaffected at pCO_2 of 1000 μatm, but eggs completely failed to hatch at 2000 μatm (Kawaguchi *et al*, 2011; Kawaguchi *et al*, 2013). Adult *E. superba* respond to elevated pCO_2 by increasing feeding rates and excretion of nutrients, both of which suggest OA

increases metabolic rates (Saba *et al*, 2012). The only Northern Hemisphere species studied to date is *Nyctiphanes couchii*, which lives in the northern Atlantic and is abundant in the North Sea and Celtic Sea. Elevated pCO_2 reduced survival and increased the proportion of deaths associated with molting in sub-adult *N. couchii,* but did not affect intermolt period or growth rate (Sperfeld *et al*, 2014).

Euphausia pacifica is a dominant krill species in the north-east Pacific, with a North American range extending from Southern California to Alaska (Brinton, 1962). *E. pacifica* is abundant year-round in Monterey Bay, California, where it is associated with the deep waters of the submarine Monterey Canyon. Major population increases of *E. pacifica* occur following upwelling in late spring and early summer (Brinton, 1976). These dense aggregations of *E. pacifica* support large populations of resident and migratory seabirds (Ainley *et al*, 1996), pinnipeds and cetaceans, including the largest ocean predator (blue whale), that are attracted to Monterey Bay in summer months to feed (Croll *et al*, 2005).

Adult *E. pacifica* typically undergo diel vertical migrations, often from hundreds of meters depth during the day to the surface at night where they feed (Brinton, 1976; Bollens *et al*, 1992; Marinovic & Mangel, 1999). Off the Pacific coast of North America, where strong summer winds induce upwelling of cold, high-pCO_2 and low-pH water, vertical migration of *E. pacifica* exposes them daily to pH ranges from approximately 8.1 at the surface to 7.6 at depth (Feely *et al*, 2008, supplementary data).While little is known about how *E. pacifica* will be affected by increased pCO_2, juveniles in one study tolerated pH 7.54 for 7 days (pH was lowered

by addition of acid, not increased pCO_2) (Yamada & Ikeda, 1999).

We assessed the effects of continuous exposure of adult *E. pacifica* to high pCO_2/low pH by measuring survival, growth and molting frequency during a 2-month incubation experiment. The results led to additional survival experiments, across broad pH ranges, that were designed to constrain pCO_2 values for the critical thresholds of pH tolerance.

1.3 Materials and methods

Collections

We collected krill after sunset (when krill had migrated to the surface), ~13 kilometers offshore in Monterey Bay by taking oblique tows from ~30 m depth to the surface using a 1 meter diameter plankton net (500 μm mesh) with a 1 litre, non-filtering codend. Krill were sorted on board ship into groups of 8–10 individuals that were placed in 750 ml jars and kept on ice in a cooler for transport back to the Long Marine Laboratory of the University of California, Santa Cruz. Once at the laboratory krill were maintained individually in 750 ml jars of filtered seawater, in darkness in a water table of 9°C for 1 week before the experiments began. Each krill was fed to excess daily by adding 10 ml of a diatom culture (*Thalassiosira*; 3-5 x 10^5 cells ml^{-1}) to each 750 ml jar; this was supplemented every 4–5 days with newly hatched brine shrimp (*Artemia*) nauplii. This feeding regime was continued throughout the experiment. Krill were maintained at ambient pCO_2 prior to the start of the experiment.

Seawater preparation

Mixtures of air and CO_2 with the desired pCO_2 were prepared by adding a small amount of certified pure CO_2 gas (Prax-Air) to a 30 litre steel cylinder, and then adding compressed air until the desired concentration was reached. pCO_2 content was monitored with a CO_2 analyzer (Quibit Systems S151). New gas mixtures were blended twice a week throughout the study. The seawater supply at the Long Marine Laboratory is taken from Monterey Bay, passed through sand filters to header tanks, and then gravity-fed to individual laboratories. We then filtered the water to 0.2 μm, and stored it in 20 litre carboys. The appropriate pCO_2 gas mixture was bubbled into the carboys for 4 days through gel membrane bubblers, until the water equilibrated with the gas, and pH was at the desired level. Experimental seawater was prepared weekly.

Growth Experiment:

Experimental design

We measured effects of pCO_2 on krill survival, intermolt period and growth in a single factor, two treatment experiment. The two pCO_2 levels were selected to represent recent atmospheric levels (pCO_2 = 380 ppm, pH_T = 8.01) and a level above the IPCC scenario RCP8.5 or "high emissions scenario" for 2100 (pCO_2 = 1200 ppm, pH_T = 7.60; Stocker *et al*, 2013; Edenhofer *et al*, 2014). Both treatments were housed in a single water table maintained at 9 $\pm$ 1 ^{o}C, representative of conditions in Monterey Bay at 200 meters depth (Pennington & Chavez, 2000), by recirculating the

water through an inline chiller (Aqua Logic Delta Star). The table held ten 20 litre glass aquaria (five per treatment). Each aquarium was sealed with a gas-tight plexiglass lid, and contained four 1 litre polycarbonate jars standing in seawater. Each jar held one adult krill in approximately 750 ml of seawater (20 individuals per treatment).

The seawater in each aquarium, and in each jar within the aquarium, was pre-equilibrated to the desired pCO_2 level, and the treatment pCO_2/air mixture was pumped slowly through the lid of each aquarium to maintain the desired pCO_2 level in the headspace of each aquarium. The top of each jar was open to the aquarium headspace to maintain the pCO_2. Water in every jar was changed every second day. The krill were maintained in darkness, except for a few minutes each day while they were checked for molts under low light. pH and temperature were measured daily in each jar with an Oakton WD-35613 handheld pH meter. Water samples (100 ml) were taken from each aquarium every seven days, poisoned with mercuric chloride, and stored for subsequent analyses. The experiment ran for 57 days, from 21 August through 16 October 2012. One krill died from trauma and was excluded from all analyses.

Survival, Intermolt Period and Growth

Krill were checked daily for molting and mortality by transferring each animal and its treatment water into a white plastic tub, searching for molted exoskeletons, and then returning the krill and water to the jar. All molts were collected and measured

immediately using a Wild M3 dissecting microscope outfitted with a Canon Rebel 2Ti DSLR camera, and the images were analyzed using ImageJ software (NIH). Growth was determined from the difference in telson length of consecutive molts, measured from the middle of the rounded rise at the anterior end to the posterior tip of the telson (spines excluded). The telson is well preserved in molts, and its length is proportional to total body length (Shaw *et al*, 2010). Between molts, euphausiids can grow larger, maintain size, or shrink (often in response to stressful conditions) (Marinovic & Mangel, 1999), so size is not a continuous monotonic positive progression. Growth rate during the experiment was calculated for each individual by fitting a regression line to repeated telson measurements against number of days since start of the experiment. Rates are reported as change in telson length per day (mm day^{-1}).

For comparison with published growth data, absolute growth rates (mm day^{-1}) were calculated by converting telson length into total body length using the equation from Shaw (2010) for *Euphausia pacifica:*

Total Length (mm) = (4.937 × *telson length* (mm)) – 0.4142

pCO_2 Threshold Experiments

The upper thresholds of tolerance of *E. pacifica* to high pCO_2/low pH were determined in experiments using methods similar to those in the growth experiment. The main differences were that treatment levels were determined by pH instead of pCO_2 because the CO_2 monitor was not rated to test gases above 2000 ppm.

Experimental water was prepared by bubbling very high pCO_2 gas into seawater in plastic carboys and then diluting with ambient pCO_2 seawater until the desired pH was achieved. Each experimental jar was filled with pre-equilibrated water and sealed with a gas-tight lid to prevent CO_2 off-gassing because no additional gas was pumped into the headspace. Jars were placed directly into the water table, which was maintained at 13°C. Each jar was checked every morning for mortality, and the water in the jars was replaced every second day.

Two thresholds were defined: (a) days until 50% of animals were dead; and (b) days until all animals were dead. Eight pH treatments between pH 7.87 and 6.84 were tested, with eight krill per treatment, and the experiment ran for 16 days. pH and temperature were measured daily in each jar (with the krill remaining alive) using an Oakton WD-35613 handheld pH meter, and 100 ml discrete water samples were taken from representative jars approximately every 4 days for complete carbonate analyses and to calibrate the handheld pH meter readings.

Chemical analyses

Water samples were collected and poisoned with mercuric chloride following standard practices (Dickson *et al*, 2007) before being stored. They were analyzed for total inorganic carbon (C_T) using a CM5011 carbon coulometer (UIC, Inc) and total alkalinity (A_T) using an automated open cell titration procedure. Instruments were calibrated using certified seawater standards (Batch 118) from Andrew Dickson's laboratory at the Scripps Institution of Oceanography. pH, pCO_2, and aragonite

saturation state ($\mathbf{\Omega}_{arag}$) were calculated with CO2SYS software (Pierrot *et al*, 2006) using the C_T and A_T data and CO_2 disassociation constants from (Mehrbach *et al*, 1973), refitted by (Dickson & Millero, 1987). pH is expressed in total scale (pH_T). Daily pH meter measurements were calibrated against the CO2SYS calculated carbonate data.

Statistical Analyses

All statistical analyses used the software JMP PRO 12. All chemical data are reported as mean $\pm$ 1 standard deviation (s.d.). In the growth experiment, mean values for all chemical parameters were calculated per aquaria and used to determine the mean for each treatment group.

Total survival over the course of the experiment was first analyzed in a nested proportional hazards model (aquaria nested within pCO_2 treatment). Since there was no significant effect of aquaria on survival (Wald test, p=0.9973), the aquarium term was dropped from the analyses and individual krill were used as replicates to increase the power of subsequent analyses.

Individuals with fewer than 3 molts were excluded from intermolt period and growth analyses. Consecutive intermolt periods were recorded for each individual krill, and the effect of duration of the experiment (days) on intermolt period was tested in a 1-way ANOVA. Because there was no effect of duration ($F_{3,222} = 0.9589$, p=0.4124), the mean intermolt period was calculated for each individual and a nested

1-way ANOVA was run (aquaria nested within treatment) to compare mean intermolt periods in the two pCO_2 treatments.

Growth rates were estimated by regressing telson size on day of the experiment, with aquaria nested within pCO_2 treatment to account for any aquaria level effects. A quantile regression was used to determine whether growth rate, or the effect of pCO_2 on growth, differed along a distribution of sizes in the krill population. Quantile regression estimates the relationship between dependent and predictor variables in different quantiles of the response variable, and not just the mean value as in least squares regression. Because linear growth rates may change with body size, mean values may mask some of the variation in responses; quantile regression techniques allow more detailed examination of the variation in responses across sizes and assessment of different responses (e.g. maximum, average, or minimum). Growth rates of krill in the 90^{th}, 50^{th} and 10^{th} size quantiles were compared.

For the pCO_2 threshold experiments, survival was expressed as number of days until (a) 50% mortality and (b) 100% mortality. Kaplan-Meier survival curves were plotted as the daily proportion alive against number of days from start of the experiment in a stepped format. A Log-Rank test (also known as Mantel-Cox) was used to test for differences in the survival curves of the pH treatments. The Log-rank test compares the observed number of deaths and the expected number of deaths, with the null hypothesis that the expected number of deaths is the same between groups.

1.4 Results

Growth Experiment

Chemical data

Table 1 summarizes water conditions in the growth experiment. In both treatments temperature and salinity remained constant within the limits of instrumental precision throughout the experiment. Mean pCO_2 in the high pCO_2 treatment was 3.3 times greater than in the low pCO_2 treatment (1289 µatm vs 395 µatm; t =10.64, df = 8 p <0.0001), and this maintained a difference between treatments of 0.46 pH_T units (7.60 vs 8.06; t = .-13.90 df = 8, p <0.0001). Total inorganic carbon was higher in the high pCO_2 treatment, (C_T = 2238 vs 2068 µmol $kg^{-1;}$ t=18.95, df=8, p<0.001) while aragonite saturation state was lower (Ω_{arag} = 0.77 vs 1.94, t=-13.32, df=8, p<0.001). Total alkalinity was similar in both treatments (A_T = 2255 vs 2244 µmol kg^{-1}; t=2.19, df=8, p = 0.06).

Survival

Ten of the 39 individual krill died during the experiment (6 in low pCO_2 and 4 in high pCO_2), giving survival of 70% and 79% respectively, but the difference was not significant (Table 2; Wald test, p = 0.3688).

Molting

The intermolt period (time between molts) of individual krill did not change during the experiment (ANOVA, $F_{3,322}$ = 0.9589, p = 0.4124). Krill molted on average every

6 days in both treatments, and the rate was not affected by pCO_2 level (Table 2; $F_{8,9}$ = 1.451,p = 0.2232).

Growth

Mean initial telson length of krill was 2.46 $\pm$ 0.47 mm (range = 1.81 to 3.42 mm), which is equivalent to 11.74 $\pm$ 2.34 mm (range 8.51 to 16.48 mm) total body length calculated using Shaw's equation (Shaw *et al*, 2010). These sizes are associated with both juvenile and adult stages (Brinton *et al*, 1999). There were no differences in initial sizes between the pCO_2 treatments (ANOVA, $F_{1,9}$ = 0.197, p = 0.6646). Growth between molts varied among individuals and many individuals both grew and shrank at different times during the 57-day experiment. Overall growth (across both pCO_2 treatments) was fastest in smaller (10^{th} percentile) krill (0.008 mm day^{-1}) compared with 0.006 mm day^{-1} in the 50^{th} percentile krill, while the largest (90^{th} percentile) krill declined in length over the course of the experiment (-0.002 mm day^{-1}).

Growth in the two pCO_2 treatments was then compared within each quantile. Smaller krill (10^{th} percentile) grew faster in the low pCO_2 treatment (0.009 mm day^{-1}) than in the high pCO_2 treatment (0.006 mm day^{-1}; p<0.001, Fig1, Table 2). The trend was similar in the 50^{th} size quantile, but growth rates in this size class were more variable (Low pCO_2: 0.009 mm day^{-1} vs High pCO_2: 0.005 mm day^{-1}; p=0.06, Fig 1, Table 2). For the largest krill (90^{th} percentile), those at high pCO_2 shrank (-0.005 mm day^{-1}), while krill in the low pCO_2 treatment had slow but positive growth (0.001 mm day^{-1}; p<0.001, Fig 1, Table 2).

pCO_2 Threshold Experiments

Water Chemistry

Water conditions during the threshold experiment are summarized in Table 3. Mean pH for the 8 treatments ranged from 7.87 to 6.84. Total alkalinity (A_T) was independent of pH (ANOVA; $F_{7,34} = 2.02$; $p = 0.0895$), but aragonite saturation (Ω_{arag}) ($F_{7,34} = 8.19$; $p < 0.0001$) declined significantly with declining pH. Total inorganic carbon (C_T) ($F_{7,34} = 21.44$; $p < 0.0001$) and pCO_2 ($F_{7,34} = 9.30$; $p < 0.0001$) both increased with declining pH.

Survival

The median survival thresholds (days until 50% died) for the four pH treatments $\geq$7.02 were greater than 16 days (Table 4). This contrasts with the lowest three pH treatments (pH 6.92, 6.89 and 6.84) in which median thresholds were either 6 or 3 days, and all animals were dead by day 10 (Table 4). The intermediate treatment (pH 6.96) had a median threshold of 8 days, but the 4 remaining krill were still alive at the end of the experiment (16 days; Table 4).

There were significant differences in the survival curves of the 8 pH groups (Log rank test $\chi^2 = 48.5$, df = 7, $p<0.001$; Fig 2). The survival curves of the 4 highest pH groups did not differ significantly from one another ($\chi^2 = 2.09$, df = 3, $p = 0.55$), and they did not differ from the intermediate (pH 6.96) group ($\chi^2 = 4.66$, df = 4, $p = 0.32$). Survival curves in the lowest 3 pH levels also did not differ significantly from one another ($\chi^2 = 4.86$, df = 2, $p = 0.09$), but they were all

significantly lower than at the intermediate level (pH 6.96) ($\chi^2 = 10.57$, df = 3, p = 0.01). Taken together, these results suggest a significant decline in survival occurs between pH 6.96 and 6.92, with mortality increasing rapidly below pH 6.96.

1.5 Discussion

In the growth experiment, a pCO_2 level of 1200 μatm had no effect on either survival or molting frequency. This pCO_2 level corresponds to the IPCC "high emissions" scenario for 2100 and is also the upper pCO_2 level currently experienced by *Euphausia pacifica* during their diel migrations. These findings differ from those reported for the Atlantic krill, *Nyctiphanes couchii,* which had higher mortality at less elevated pCO_2 levels (800 and 1100 μatm), although its highest mortality was at a pCO_2 of 1700 μatm (Sperfeld *et al*, 2014), a level considerably higher than we used in our growth study.

Sperfeld (2014) also found increased pCO_2 had no effect on molt rates in *N. couchii* in a 34 day experiment; this is consistent with our results over 57 days, and supports a more general suggestion that the molting process in krill may be unaffected by pCO_2, at least for short-term exposures of up to 60 days. The average intermolt period in our study (six days) was within the range reported elsewhere for *E. pacifica* (4-10 days) (Pinchuk & Hopcroft, 2007). Although intermolt period in our growth experiment was not affected by increased pCO_2, intermolt period is known to vary in response to other environmental conditions, such as temperature and food availability (Fowler *et al*, 1971; Buchholz, 1991; Chang & Mykles, 2011). Because

intermolt period is physiologically constrained to some degree (Fowler *et al*, 1971; Marinovic & Mangel, 1999), hormonal control of the timing of molting may outweigh environmental cues related to changing pH. It is also possible that hormones respond only to internal osmotic conditions within the organism, and if these are highly regulated, hormones may not be sensitive to environmental pH or pCO_2.

Many individuals both grew and shrank at different times during the growth experiment, and since similarly variable growth has been reported previously for *E. pacifica* (Marinovic & Mangel, 1999; Shaw *et al*, 2010), this variation probably was not an artifact of our experimental system or a response to the experimental manipulations. Another general trend in the growth experiment was the faster growth rates in smaller krill (10^{th} and 50^{th} quantiles) that declined with increasing body size. This is not uncommon, as linear growth often slows with increased body size (Labat & Cuzin-Roudy, 1996; Atkinson *et al*, 2006). The larger krill (90^{th} quantile) had negative net growth rates, possibly due to diversion of energy from growth to lipid accumulation, or to sexual maturation and reproduction (Pinchuk & Coyle, 2008). After converting telson lengths into absolute total body lengths, the whole body growth rates across both pH treatments in the growth experiment ranged from -0.02 mm day^{-1} to 0.04 mm day^{-1}. This is within the range of published rates (0.01 – 0.03 mm day^{-1}) for other laboratory and instantaneous growth rate studies (Shaw *et al*, 2010).

Krill in all size classes had lower growth rates in the elevated pCO_2 treatment than in the low pCO_2 treatment, although the difference had stronger statistical significance in the 10^{th} and 90^{th} quantiles. Reduced growth under elevated pCO_2 has been linked in some organisms to metabolic changes associated with higher energetic costs of acid-base regulation that divert energy away from other physiological processes including growth (Wood *et al*, 2008; Deigweiher *et al*, 2010; Whiteley, 2011; Stumpp *et al*, 2012). At higher pCO_2 levels the metabolic rate of the Antarctic krill (*E. superba*) rises (as measured by increased food consumption and nutrient excretion), which suggests that maintaining normal functioning at high pCO_2 requires additional food to meet increased metabolic demands (Saba *et al*, 2012). If food is limited, or if krill cannot maintain feeding rates high enough to compensate for increased energetic costs of acid-base regulation, growth may be the first process to be affected.

Alternatively, survival time in many vertically migrating species exposed to low pO_2 /high pCO_2 at depth is extended by metabolic suppression achieved by shutting down such energy-demanding processes as protein synthesis (Guppy & Withers, 1999; Seibel & Walsh, 2003). While temporary metabolic suppression may be an advantageous short-term strategy, long-term suppression by OA can lead to reduced growth or reproduction, and may become detrimental not only to individuals but also for population-level processes (Guppy & Withers, 1999; Seibel & Walsh, 2003). The effects of pCO_2 on reproduction in *E. pacifica* are unknown, but metabolic changes (e.g., reduced metabolism or diversion of energy to acid-base regulation) that

lower growth in *E. pacifica* could also affect their reproduction. Because larger females often have larger broods, possibly due to increased carapace volume available for ovaries (Gómez-Gutiérrez *et al*, 2006), lower growth rates may also affect krill fecundity via smaller brood sizes.

The pCO_2 threshold experiments indicate that there is a critical threshold between pH 6.96 and pH 6.92 (pCO_2 equivalent to 6050 μatm and 7200 μatm) below which *E. pacifica* experiences rapid mortality. At pH 6.96 and above, survival was similar to that in the highest pCO_2 levels that krill experience naturally (approximately 1200 μatm). This suggests that *E. pacifica* survival is unlikely to decline as a direct response to future OA levels, since even at their deepest diel migrations (~500 meters), models predict pCO_2 will not exceed 3500 μatm by the year 2100 (Brewer & Peltzer, 2009). In our experiments, *E. pacifica* survived short term exposure to pCO_2 of 6000 μatm, nearly two times the maximum predicted level.

While mortality rates did not differ at intermediate pCO_2 levels, we did see some behavioral changes, including periods of inactivity, abnormal swimming and periods lying on their backs. This suggests that, although the critical threshold for mortality is near pH 6.92, non-lethal physiological effects are present at higher pH levels. It is known that some organisms are less able to buffer changes in pCO_2 when exposed simultaneously to hypoxic conditions (Pane & Barry, 2007), and since pO_2 also declines with depth, increasing hypoxia with depth may increase the vulnerability of *E. pacifica* to additional OA stresses. In our experiments, pO_2 was always equivalent to that of surface water in equilibrium with the atmosphere;

therefore we cannot predict how relatively hypoxic conditions at their migration depths may affect *E. pacfica*'s tolerance of elevated pCO_2.

While responses of larval *E. pacifica* to pCO_2 remain unknown, they do remain near the surface until late larval or juvenile stages, and are not exposed to the daily variations in pH and temperature that adults experience during vertical migrations. It is possible that early developmental stages may be more susceptible than adults to harm from increasing OA. In the Antarctic *E. superba*, embryonic development and larval behavior were unaffected at pCO_2 of 1000 μatm, but eggs completely failed to hatch at 2000 μatm (Kawaguchi *et al*, 2011; Kawaguchi *et al*, 2013).

Because organisms living where natural variation in pCO_2/pH is common tend to have broader tolerances of fluctuations in pCO_2/pH, it is possible that the natural (e.g., pre-industrial) regimes of pCO_2 variation experienced by organisms may determine their sensitivity to future changes in ocean chemistry, and hence be a good predictor of likely responses to OA (Hofmann *et al*, 2011; Lewis *et al*, 2013). This may be because organisms living in more variable environments are more likely to have evolved well-developed acid-base regulation systems for active maintenance of internal osmotic and ionic balances, and these systems may enable them to buffer changes in pH due to OA (Seibel & Walsh, 2003; Pane & Barry, 2007).

Since *E. pacifica* regularly experiences a range of pCO_2 values during its diel migration, it probably has well developed acid-base regulation systems, and these may enhance its survival when exposed to elevated pCO_2 for long periods, and also during extreme though short-term pH declines. Whether *E. pacifica* uses increased

acid base regulation or metabolic suppression to tolerate exposures to low pH, the reduced growth rates in our experiments suggest that even organisms likely to be well-adapted for acid-base regulation under high pCO_2 may be adversely affected by OA. Consequently, the central role of *E. pacifica* in coastal ecosystems may be affected indirectly by elevated pCO_2 acting on growth or other processes (e.g., reproduction) or on early life stages (eggs, larvae). Because *E. pacifica* inhabits an ecosystem that will see increased frequency and intensity of acidic events (Feely *et al*, 2008), understanding its responses to high pCO_2 is critical for understanding the ecosystem-wide effects of OA.

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1.8 Tables and Figures

Table 1.1. Carbonate chemistry parameters (mean ± 1 s.d.) of seawater in krill growth experiment. Data were compared with 2-sample t-tests. Mean values ± 1 s.d. of pCO_2 and aragonite saturation state (Ω_{Arag}) calculated using CO2sys with measured input variables total alkalinity (A_T), total dissolved inorganic carbon (C_T), salinity and temperature. Temperature was measured directly daily using handheld meter.

Parameter	Low pCO_2			High pCO_2			p value
pH_T	8.06	±	0.05	7.60	±	0.06	< 0.0001
pCO_2 µatm	395	±	58	1289	±	179	< 0.0001
A_T µmol kg^{-1}	2244	±	9	2255	±	8	0.06
C_T µmol kg^{-1}	2068	±	16	2238	±	12	< 0.001
Ω_{Arag}	1.94	±	0.17	0.77	±	0.09	< 0.001
Temp °C	8.9	±	0.2	8.9	±	0.2	1
Salinity ppt	33	±	0.5	33	±	0.5	1
N	5			5			

Table 1.2. Survival, intermolt period (mean ± s.d.) and estimated growth rates (quantile ± s.e.) of *E. pacifica* in the growth experiment.

Treatment group	Low pCO_2	High pCO_2	p value
Survival (%)	70.0	78.9	0.3688
Intermolt period (days)	6.2 ± 0.4	6.1 ± 0.2	0.2232
Growth rate (mm telson day^{-1})			
10^{th} Quantile	0.009 ± 0.0013	0.006 ± 0.0009	<0.001
50^{th} Quantile	0.009 ± 0.0023	0.005 ± 0.0016	0.06
90^{th} Quantile	0.001 ± 0.0020	-0.005 ± 0.0013	<0.001

Table 1.3. Carbonate chemistry of experimental seawater in threshold experiments. Mean values $\pm$ 1 s.d (n= 3 – 6) of pCO_2 and aragonite saturation state (Ω_{Arag}) calculated using CO2sys with measured input variables total alkalinity (A_T), total dissolved inorganic carbon (C_T), salinity (33.5 + 0.5 ppt), and temperature (14.0 $\pm$ 0.8°C). Mean values of pH_T and temperature (n=26 – 118) were measured directly daily using handheld meter, and then corrected against calculated pH using full carbonate parameters in CO2sys. Correction factor applied was 2.367 + (0.6856 x pH meter value), R^2= 0.80, p<0.0001, n=35. The experiment was performed as two consecutive runs (4 pH levels at a time) as indicated by run number.

Mean pH_T	pCO_2 µatm	A_T µmol kg^{-1}	C_T µmol kg^{-1}	Ω_{Arag}	run
7.87 ± 0.04	712 ± 200	2257 ± 48	2138 ± 43	1.53 ± 0.46	1
7.82 ± 0.09	938 ± 800	2289 ± 58	2154 ± 98	1.80 ± 1.31	2
7.05 ± 0.05	5364 ± 2343	2295 ± 44	2479 ± 89	0.3 ± 0.23	1
7.02 ± 0.06	4349 ± 677	2289 ± 42	2429 ± 30	0.29 ± 0.04	2
6.96 ± 0.06	6052 ± 1256	2254 ± 39	2467 ± 33	0.21 ± 0.05	2
6.92 ± 0.04	7228 ± 2022	2326 ± 9	2588 ± 85	0.19 ± 0.06	1
6.89 ± 0.02	9906 ± 4720	2249 ± 37	2606 ± 152	0.17 ± 0.13	2
6.84 ± 0.02	8889 ± 340	2300 ± 13	2631 ± 3	0.15 ± 0.01	1

Table 1.4. Survival of *E. pacifica* in the pCO_2 threshold experiments.

pH_T	Days until 50% dead	Days until 100% dead	Overall Survival (%)
7.87	>16	>16	66.7
7.82	>16	>16	62.5
7.04	>16	>16	75
7.02	>16	>16	75
6.96	8	>16	50
6.92	6	10	0
6.89	6	6	0
6.84	3	6	0

Figure 1.1. Fitted growth rates of 10th, 50th and 90th size quantiles of krill in high and low pCO_2 treatments. Solid lines represent estimated growth rates; shaded areas are 95% confidence intervals. Intercept and slopes are fitted using estimated parameters from quantile regression.

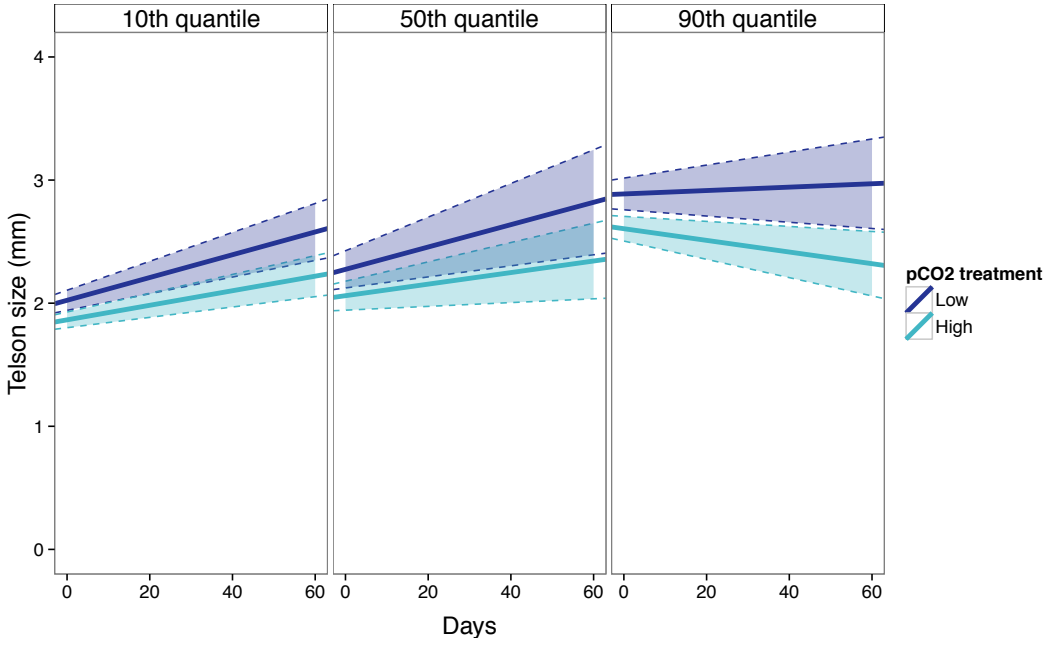
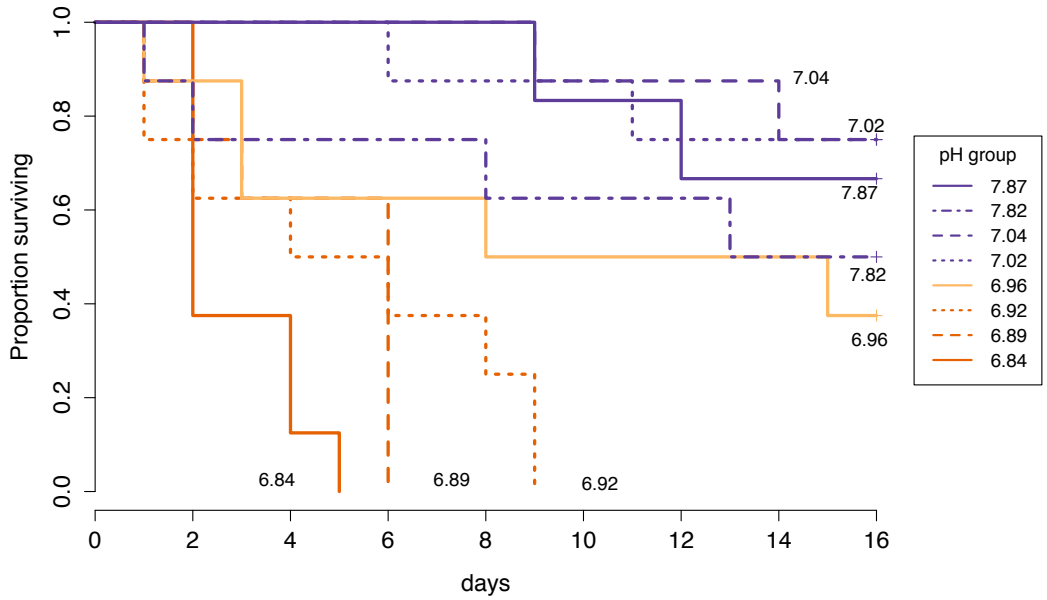


Figure 1.2. Kaplan-Meier survival curves for *E. pacifica* in the pCO_2 threshold experiments.



Chapter 2:

Metabolic responses of a vertically migrating planktonic species to short and long term pCO_2 exposure

2.1 Abstract

While ocean acidification is likely to have major effects on many marine organisms, those species that regularly experience variable pCO_2 environments may be more tolerant of future changes in ocean chemistry. *Euphausia pacifica* is an abundant krill species along the Pacific coast of North America where it is exposed to elevated pCO_2 seasonally during upwelling periods, and daily during vertical migrations to depths where pCO_2 is higher. We measured three metabolic responses (oxygen consumption, ingestion rate, and nutrient excretion rates) of *E. pacifica* to two pCO_2 levels (400 and 1200 μatm). Oxygen consumption declined by 31% in the first 24 hours exposure to high pCO_2, and remained low after 21 days. Oxygen consumption at low pCO_2 was low for the first 12 hours, increased by 34% at 24 hours, but returned to initial values by 21 days. After 3 weeks continuous exposure, oxygen consumption rates were 32% lower in the high pCO_2 group. Ingestion and ammonium excretion were both significantly lower in the high pCO_2 group after 24 hours exposure, but not after 7 or 21 days. There was no effect of pCO_2 on phosphate excretion. Taken together, these results indicate *E. pacifica* has a lower metabolic rate when exposed to high pCO_2, and this is maintained even after 3 weeks exposure. This decrease in metabolism may explain previously reported declines in growth

when exposed to high pCO_2, and suggest *E. pacifica* could face ecological challenges under future ocean acidification.

2.2 Introduction

Absorption of atmospheric CO_2 into the world's oceans is causing declines in ocean pH and carbonate ion concentration, a phenomenon known as ocean acidification (OA) (Orr *et al*, 2005). After declining by 0.1 unit since the pre-industrial era, ocean pH is projected to decline another 0.2 to 0.3 units over the next century (Feely *et al*, 2009; Stocker *et al*, 2013; Edenhofer *et al*, 2014), a rate unprecedented in recent history (Doney & Schimel, 2007). These projected changes to ocean chemistry are likely to alter the survival, distribution, growth, reproduction, gene expression, and behavior of many marine organisms (Kurihara *et al*, 2004; Kleypas *et al*, 2006; Dupont *et al*, 2008; Fabry *et al*, 2008; Kurihara, 2008; Andersson *et al*, 2011; Ross *et al*, 2011). While known effects of OA on marine organisms are overwhelmingly negative, this generalization may be biased by the focus of OA research on calcifying organisms. Responses to OA differ among taxa, populations, habitats and life stages (Kroeker *et al*, 2010). Recent evidence suggests that organisms living in variable pCO_2 habitats are also more tolerant of pCO_2 levels under future OA scenarios (Pespeni *et al*, 2013), possibly due to physiological adaptations that buffer changes in external pCO_2 (Seibel & Walsh, 2003).

Most marine organisms maintain narrow internal pH and ion concentrations that are optimal for their basic biological and physiological functions, and most eliminate excess CO_2 via diffusion gradients across their external cell membranes from higher internal pCO_2 to lower external pCO_2. As environmental pCO_2 increases, the slope of this gradient declines, leading to lower extra- and intracellular pH (i.e.,

acidosis) that may be countered either by passive buffering (e.g., bicarbonate buffering system or respiratory proteins) or by active transport of ions across membranes (Heisler, 1986). Complete compensation (maintaining pH at pre-disturbance levels) may be limited by the necessity of also maintaining ionic homeostasis for biological functions (Cameron & Wood, 1985).

Disturbances in the acid-base balance have varied metabolic and physiological effects, and inability to compensate for pH disturbances may lead to metabolic depression, disrupted enzyme functioning, or altered gene expression (Seibel & Walsh, 2003). Low pH of extracellular fluids reduces the affinity of respiratory proteins for oxygen, may limit oxygen supply to body tissues, and can lower metabolic activity (Seibel & Walsh, 2003). Conversely, organisms with efficient compensation for acidosis may incur high metabolic costs linked to the continual, ATP-intensive active pumping of ions, which diverts energy away from other important processes such as growth or reproduction (Wood *et al*, 2008). Increased ventilation rates in response to reduced oxygen transport can also increase metabolic costs (Pörtner *et al*, 2004). While increasing feeding rates is one way to compensate for elevated metabolic expenditures that may enable organisms to better tolerate changes in pCO_2 (Holcomb *et al*, 2010), they may simultaneously become more susceptible to fluctuations in food availability. Long-term consequences of these metabolic changes may include altered growth, development rates, feeding or swimming behavior, or reproduction (Pörtner *et al*, 2004).

Different metabolic responses in similar OA experiments have been attributed

to prior exposure to different natural pCO_2 regimes, iono-regulatory abilities, and respiratory pigment (Maas *et al*, 2012). Organisms that experience natural fluctuations in pCO_2 during seasonal upwelling, or during diel vertical migrations to deeper water, may also be well adapted to OA due to their frequent and prolonged exposure to lower pH waters (Thomsen *et al*, 2010). For example, pteropods that descend vertically each day to the oxygen minimum zone, where pCO_2 is high, were not affected by elevated pCO_2 in the laboratory, but oxygen consumption and ammonium excretion both declined in a non-migrating species (Maas *et al*, 2012). Metabolic responses vary even among diel migrators. Metabolic rates in the Antarctic krill, *Euphasia superba*, increased under high pCO_2, and this was attributed to acceleration of iono-regulatory processes that compensate for altered pH (Saba *et al*, 2012), but metabolism declined in the jumbo squid, *Dosidicus gigas*, probably due to pH-induced declines in the affinity of respiratory pigments (Rosa & Seibel, 2008). These and other studies of metabolic responses of diel migrators to elevated pCO_2 only examined the first 24 hours of exposure, and it is unknown whether these initial responses to high pCO_2 can be maintained or whether there are other negative effects of long-term exposure to OA conditions.

Along the California coast, seasonal upwelling brings cold, low pH/high pCO_2 waters to the surface, exposing many planktonic and pelagic organisms to substantial variation in pCO_2. One of these is the Pacific krill, *Euphausia pacifica*, a small planktonic marine crustacean widely distributed throughout the northeastern Pacific Ocean (Brinton, 1962). As the most abundant krill species in Monterey Bay,

California, it is an ecologically and economically important link between primary producers (phytoplankton) and higher trophic levels. *E. pacifica* also makes daily vertical migrations to depths of up to 250 meters during the day (and sometimes to 500 m) in the Monterey submarine canyon, returning to the surface at night to feed (CIMT, 2008). At these depths it is exposed daily to lower oxygen (low as 20% saturation; Tremblay, 2014), lower pH (approximately 7.60) and higher pCO_2 (1200 μatm) than at the surface (Feely *et al*, 2008).

Many crustaceans are more tolerant of OA conditions than other phyla (Pane & Barry, 2007) and many characteristics of *E. pacifica* (metabolic activity, variable environment) suggest that this species should have strong acid-base regulation. We demonstrated previously that molting and survival of *E. pacifica* from Monterey Bay did not change when exposed to elevated pCO_2 (1200 μatm), but that growth rates declined (Chapter 1; Cooper, in review). While changes in growth rates may well be linked to changing metabolic rates, it remains unknown whether *E. pacifica*'s metabolism is stimulated in response to pH compensation demands (possibly requiring increased feeding rates), is suppressed by uncompensated pH disturbances (possibly reducing feeding rates), or remains unaffected by pH changes. In this paper, we describe two experiments that examine both the initial metabolic responses of *E. pacifica* to short-term (first 24 hours) exposure to elevated pCO_2 and compare them with long-term responses after acclimation during 21 days exposure. The first experiment measured oxygen consumption, and the second measured rates of ingestion and nutrient excretion.

2.3 Materials and methods

Collections

Euphausia pacifica (Hansen, 1911) were collected after sunset, when they had migrated to the surface, ~13 km offshore in Monterey Bay. We used a 1meter diameter plankton net (500 μm mesh) with a solid non-filtering codend (1 liter volume) to take oblique tows from ~30 meter depth to the surface. Krill were divided on board ship into groups of 8–10 individuals that were placed in 750 ml jars of seawater and kept on ice in a cooler for transport back to the Long Marine Laboratory of the University of California, Santa Cruz. At the laboratory, healthy krill were sorted into 4 liter glass jars, with 6 – 8 krill per jar and placed in an 11±1°C recirculating water table maintained by an inline chiller (Aqua Logic Delta Star). They were kept in darkness, except for a few minutes each day when the jars were checked for mortality under low light. Krill were fed daily with 25 ml of a phytoplankton mixture of *Thalassiosira, Isochrysis* and *Rhodomonas*, and water in the jars was changed every second day. All krill were acclimated to the laboratory for a minimum of 1 week before experiments began.

Basic experimental design

Both experiments used two pCO_2 treatments, one representing recent "ambient" atmospheric CO_2 levels (pCO_2 = 400 μatm, pH_t = 8.01) and the other exceeding the IPCC RCP8.5 or "high emissions" scenario for 2100 (pCO_2 = 1200 ppm, pH_T = 7.60) (Stocker *et al*, 2013). In Experiment 1, the "Respiration

Experiment," oxygen consumption of individual krill was measured after short-term (0, 12 and 24 hours) and long-term (21 days) exposure to the experimental pCO_2 treatments. In Experiment 2, the "Nutrition Experiment," ingestion and nutrient excretion rates of individual krill were measured after exposure to the same two pCO_2 conditions for 1, 7 or 21 days.

In both experiments, krill were exposed to the experimental treatments by maintaining groups of 6-10 individuals in 4 l glass jars containing pre-treated water at the desired pCO_2 and then continuously supplying small amounts of air + CO_2 gas mixtures to maintain a slight positive pressure and a constant pCO_2. Before taking measurements, krill were starved for over 12 hours and then transferred to individual chambers containing water with the same pCO_2 as in their treatment jars. Different individuals were used for each treatment, and each individual was measured on only one day.

Carbonate chemistry

Water preparation: Gases with the desired pCO_2 were prepared by adding a small amount of certified pure CO_2 gas (Prax-Air®) to a 30-liter steel cylinder, and then diluting it with bursts of compressed air while measuring the CO_2 content with a CO_2 analyzer (Quibit Systems S151) until the desired concentration was reached. New gas mixtures were blended twice a week for the duration of the study. Seawater taken from Monterey Bay via the flow-through seawater system at the Long Marine Laboratory was filtered to 0.2 μm and stored in 20 l carboys. The appropriate CO_2 mixtures were then bubbled into the carboys for 4 days using gel membrane bubblers,

until the seawater equilibrated with the gas mixture and seawater pH reached the desired level. Experimental seawater was prepared weekly.

Water analyses: In both experiments, pH and temperature were measured daily with an Oakton WD-35613 handheld pH meter. Water samples were taken from the experimental chambers of all animals during each of the trials, and every 5 – 8 days from the holding jars of the long-term groups (7 or 21 days). Water was analyzed for total inorganic carbon (C_T) using a CM5011 carbon coulometer (UIC, Inc) and total alkalinity (A_T) using an automated open cell titration procedure. Instruments were calibrated using certified seawater standards (Batch 135) from Andrew Dickson's laboratory at the Scripps Institution of Oceanography. pH, pCO_2, and aragonite saturation state (Ω_{arag}) were calculated with CO2SYS software (Pierrot *et al*, 2006) using the C_T and A_T data and the CO_2 disassociation constants from Mehrbach (Mehrbach *et al*, 1973) refitted by Dickson and Millero (Dickson & Millero, 1987). pH is expressed as total scale (pH_T). Daily pH meter readings were calibrated against the full carbonate calculated values and reported as pH_T lab and pH_T calculated, respectively. The calculated pH_T is used in all statistical tests.

Experiment 1: Respiration

Oxygen consumption was measured by closed-chamber respirometry after placing individual krill in 25 ml scintillation vials fitted with optical oxygen sensors (Sensor Spots O2, Presens, Regensburg, Germany) and a magnetic stir bar (separated from the krill by fine mesh). Vials (9 at a time) were placed in a circulating water

bath above a magnetic stir plate. After acclimating for 30 minutes, the vials were flushed with seawater of the appropriate pCO_2 and then sealed. Oxygen concentration (μmol O_2 L^{-1} and % saturation) in each vial was measured every 10 minutes by placing the fiber-optic reader to the sensor (Fitbox4 transmitter, Presens, Regensburg, Germany). 1-2 vials without krill were used as controls in each run. Each trial lasted 2-3 hours, and oxygen saturation never dropped below 75%. Oxygen consumption rates were calculated as the slope of the declining oxygen concentration inside each vial over the course of the experiment.

For the short-term exposure group, O_2 consumption rate was measured 3 times for each of 13 individuals: first before any exposure to pCO_2 (hour 0), and then 12 and 24 hours following initiation of pCO_2 exposure. For the long-term exposure group, 27 krill were maintained at the experimental conditions for 21 days and then O_2 consumption was measured once for each krill. After measurement, each krill was rinsed, frozen and later dried and weighed. The effects of pCO_2 and exposure time on oxygen consumption in the short-term group were analyzed in a repeated measures multivariate ANOVA (MANOVA) which is robust to violations of sphericity assumptions. Mean oxygen consumption rates of the long-term groups were compared with a 2-sample t-test.

Experiment 2: Nutrition

Feeding and nutrient excretion by individual krill were measured over a 24 hour period for each exposure duration (1, 7 or 21 days). For each duration, 31

polycarbonate chambers (750 ml) were prepared containing seawater pre-equilibrated to the desired pCO_2 levels. Phytoplankton (50 ml of a *Thalassiosira* culture) were added to each chamber and mixed well. Five chambers were sampled immediately to count initial phytoplankton cell concentrations and 26 chambers were used in the trials. Individual krill were placed in 10 of the 13 chambers at each pCO_2 level and the other 3 chambers were phytoplankton-only controls. All chambers had gas-tight caps, were placed in the water table at 11°C and kept in darkness. Chambers were inverted manually every 2 hours to maintain even suspension of phytoplankton. After 24 hours, the pH was measured and discrete water samples were taken for phytoplankton cell counts (200 ml), carbonate chemistry (C_T and A_T; 40 ml each), and nutrient (NH_4^+ and PO_4^{3-}; 20 ml each) analyses. The krill were then rinsed, frozen, and later dried and weighed on a microbalance.

Ingestion rates of phytoplankton: Phytoplankton cell concentrations in each chamber were counted in 200 ml water samples preserved with 5% Lugol's solution. Three 1 ml subsamples were placed on a Sedgewick rafter and 3 replicate transects of >100 cells were counted for each 1 ml sample. Ingestion rates (I) were calculated from the change in phytoplankton cell concentrations over 24 hours using an equation from Conover (1978):

$$I = (C_0 - C_t + [C]e^{(k \times t)} - 1)/t$$

where C_0 is initial cell concentration (cells ml^{-1}); C_t is cell concentration (cells ml^{-1}) at time t; k is the algal population growth coefficient calculated as $\frac{\ln (C_t - C_0)}{t}$; g′ is the

grazing coefficient for krill calculated as $\frac{-\ln \frac{C_t}{C_0}}{t}$; t is the duration of incubation in hours; and [C] is the mean cell concentration (cells ml^{-1}) calculated as $\frac{C_0 \times \left(1-e^{(-g' \times t)}\right)}{t \times g'}$.

Conover's equation contains a term (k) for phytoplankton growth by cell division. Because k is calculated from control jars containing only phytoplankton, negative ingestion rates are possible if phytoplankton growth accelerates in experimental jars and outpaces krill ingestion, due to the phytoplankton taking up nutrients excreted by krill (Lehman, 1980).

Nutrient excretion rates: Seawater samples (20 ml) for nutrient analysis were frozen at the end of each trial. NH_4^+ and PO_4^{3-} were analyzed on a Lachat Quickchem flow injection analyzer (FIA+ 800 series), using the single end point method which assumes beginning nutrient levels are equivalent, since all water was taken from the same source and filtered at the same time. Apparent excretion rates (E) of ammonium and phosphate were calculated from Ikeda's equation (Harris *et al*, 2000)

$$E = [(C_{t'} - C_0) - (C_t - C_0)] \times \frac{(V_c - V_z)}{t \times N}$$

which simplifies to

$$E = [(C_{t'} - C_t) \times \frac{(V_c - V_z)}{t \times N}$$

where C_0 is the concentration of nutrients at start of incubation; C_t and C_t' are the concentrations of nutrients in control and experimental containers respectively at the

end of the incubation; V_c is the volume of experimental bottles; and V_z is the volume of krill (V_z is calculated from wet weight of krill assuming 1 ml = 1 g wet weight); t is duration of incubation in hours; and N is the number of krill per chamber (N=1).

This rate represents the apparent excretion rate, and the gross excretion rate is equal to the apparent excretion rate plus the nutrient uptake rate by phytoplankton. Because phytoplankton uptake rate is not measured directly, differences in phytoplankton uptake rates between the control and experimental containers due to the presence of krill (Takahashi & Ikeda, 1975) can cause an underestimation of gross excretion rates (e.g. negative apparent excretion rates).

2.4 Results

Experiment 1: Respiration

Water chemistry: Chemical data for water in the Respiration experiment are summarized in Table 1. Partial pressures of CO_2 in the high pCO_2 treatment waters were 1079 μatm (short-term) and 1027 (long-term), both slightly lower than the target of 1200 μatm but still approximately 2.5 times pCO_2 in the low pCO_2 treatments (435 and 431 μatm). This maintained differences of 0.36 and 0.35 pH units respectively within the short-term and long-term treatments (2-sample t-test, $p < 0.0001$ for both pCO_2 and pH). Within each pCO_2 level, there were no significant differences in either pCO_2 or pH of the water used for short-term and long-term exposures (2 sample t-tests, $p > 0.62$ for all comparisons).

Oxygen consumption: Oxygen consumption rates ranged from 0.162 to 0.322 μmol O_2 per mg dry weight (mgdw) per hour (Figure 1). In the initial exposure group,

mean oxygen consumption rates for individuals exposed to high pCO_2 declined by 42% over the first 12 hours (from 0.289 $\pm$ 0.13 to 0.168 $\pm$ 0.07 μmol mgdw^{-1}) and remained low (31% lower than initial) after 24 hours (0.198 $\pm$ 0.11 μmol mgdw^{-1}). Consumption by individuals in the low pCO_2 treatment did not change in the first 12 hours (from 0.239 $\pm$ 0.05 to 0.221 $\pm$ 0.05 μmol mgdw^{-1}), but then increased by 46% after 24 hours (to 0.322 $\pm$ 0.05 μmol mgdw^{-1}). The two groups differed in their responses to pCO_2 treatment over time (significant MANOVA interaction term, $F_{2,9}$ = 7.3317, p = 0.0129; Figure 1a) and mean consumption rates in the two treatments were significantly different by hour 24 (2 sample t-test: t = 2.68, df = 9.899, p = 0.03). After 21 days, oxygen consumption by individuals in the high pCO_2 treatment (0.162 $\pm$ 0.07 μmol mgdw^{-1}) was essentially the same as after 12 -24 hours, and was 32% lower than consumption by low pCO_2 individuals after 21 days (0.240 $\pm$ 0.109 μmol mgdw^{-1}; 2 sample t-test: t = -2.19, df = 22.3, p-value = 0.04; Figure 1b). Oxygen consumption by low pCO_2 krill after 21 days was essentially the same as during the first 12 hours of day 1.

Experiment 2: Nutrition

Water chemistry

In the low pCO_2 treatment, both pCO_2 (485 - 503 μatm) and pH of the water were almost constant over the 21 days of the experiment. However, in the high pCO_2 treatment, pCO_2 was significantly higher on Day 1 (1732 μatm) than on Day 7 (1042 μatm) or Day 21 (1063 μatm; Tukey HSD $p<0.03$), resulting in a lower pH on Day 1

(7.52 on Day 1 vs 7.66 and 7.67 on Days 7 and 21). Day 7 and Day 21 water conditions were very similar to one another (2-sample t-tests. pCO_2: $p = 0.830$; pH: $p = 0.786$). Including the elevated value on Day 1, mean pCO_2 in the high CO_2 treatment (1042 - 1732 μatm) was always 2 - 3.5 times greater than in the low CO_2 treatment (485 - 503 μatm) throughout the Nutrition experiment (Table 2; 2-sample t-test $p < 0.002$ for every exposure duration), and pH differed by 0.3 – 0.5 pH units throughout the experiment (2 sample t-test, $p < 0.001$ for all exposure durations).

Ingestion rates

Mean ingestion rates of phytoplankton ranged from a high of 112 to a low of -81.6 cells $mgdw^{-1}$ $hour^{-1}$ across the three durations (Figure 2). Negative ingestion rates imply that the phytoplankton culture divided at a faster rate than the krill ingestion rate. While neither pCO_2 (2-way ANOVA, $F_{1,61} = 0.2475$, $p = 0.6208$) nor duration ($F_{2,61}=1.0912$, $p = 0.3429$; Table 3a) affected ingestion rates, there was a strong, though not significant, interaction ($p = 0.08$).

Ingestion rates were much more variable on Day 21 than on Days 1 and 7 (Fig. 2), and declines in phytoplankton cell counts in control jars without krill on Day 21 suggest the phytoplankton culture may have become less healthy. Therefore, we repeated the analyses after excluding Day 21 from the model (Table 3b). With Day 21 excluded, ingestion rates on both Day 1 and Day 7 were higher in the low pCO_2 treatment ($F_{1,41}=12.05$ $p = 0.0013$, Table 3b), and ingestion rates were marginally

lower on Day 7 than on Day 1 ($F_{1,41} = 3.9983$, $p = 0.053$; Table 3b). In post-hoc comparisons, the ingestion rate on Day 1 was significantly lower at high pCO_2 than at low pCO_2 (Post hoc Tukey HSD, $p = 0.008$), but there was no significant difference between pCO_2 treatments on Day 7 (Figure 2, Post hoc Tukey HSD, $p = 0.481$).

Nutrient excretion rates

Mean apparent ammonium excretion rates ranged from -0.16 to 0.20 μg NH_4 $mgdw^{-1}$ hr^{-1} (Figure 3a) and mean apparent phosphate excretion rates ranged from 0.07 to 0.39 μg PO_4 $mgdw^{-1}$ hr^{-1} (Figure 3b). Negative excretion rates imply that phytoplankton uptake of nutrients was greater in the experimental containers (with krill) than the control containers (no krill), causing an underestimation of the true excretion rate. The effects of pCO_2 and duration of exposure were evaluated in separate 2-way ANOVAs for ammonium and phosphate excretion (Table 4), after deleting four extreme NH_4^+ values (each >3 standard deviations from the mean) to improve normality and homogeneity of variances. The interaction between exposure and pCO_2 for NH_4^+ excretion was significant ($F_{2,47} = 4.7786$, $p = 0.0135$, Table 4a; Figure 3a), but neither factor alone was significant. NH_4^+ excretion was lower at high pCO_2 during the first 24 hours of exposure (Tukey HSD, $p = 0.007$), but not when exposed for longer periods of time (Tukey HSD, Day 7: $p = 0.994$ and Day 21: $p = 0.9353$, Table 4a, Figure 3a). Although NH_4^+ excretion rates were more variable after 21 days exposure, results of analyses were similar when Day 21 was excluded from the analyses (Table 4b). Neither pCO_2 ($F_{1,51} = 0.0605$, $p = 0.8067$) nor exposure ($F_{2,51} =$

2.0203, p = 0.1442) significantly affected phosphate (PO_4^{3-}) excretion (Table 5a, Figure 3b). Phosphate excretion was also much more variable after 21 days than on days 1 or 7, but excluding Day 21 from the model did not alter the results (Table 5b).

2.5 Discussion

The diel vertically migrating krill species *E. pacifica* responded physiologically to both short- and long-term exposure to enriched pCO_2. In the Respiration experiment, oxygen consumption rates were approximately 30 to 40% lower at high pCO_2 after both the initial and long term exposures. Rates were within the range previously reported for *E. pacifica* (Harris *et al*, 2000), but varied over the 24 hour diel cycle in the initial exposure group. Specifically, oxygen consumption declined during the first 12 hours in the high pCO_2 treatment and then remained lower through hour 24. In contrast, the rates remained relatively unchanged between hours 0 and 12 in the low pCO_2 group, and then increased between hours 12 and 24. Declines in oxygen consumption rates in *E. pacifica* reflect a reduction in aerobic metabolic rate in response to high pCO_2.

In the Nutrition experiment, food ingestion rates were elevated on Day 1 at low pCO_2 were significantly higher than rates in the high pCO_2 group. On Day 7, the trend between pCO_2 treatments persisted, but was not significant. Ammonium excretion rates followed a similar pattern to ingestion rates: excretion was lower at elevated pCO_2 on Day 1, but the trend was not significant at Day 7. There was no difference in phosphate excretion rates across pCO_2 or exposure treatments.

Reported ammonium excretion rates for *E. pacifica* are limited, but range from 0.001 to 0.06 ug N $mgdw^{-1}$ hr^{-1}(Harris *et al*, 2000). Our values spanned the entire range, and our highest values (0.389 ug N $mgdw^{-1}$ hr^{-1}) were substantially higher than any previously reported. Similarly, phosphate excretion levels were 1 to 2 orders of magnitude higher than the only rate reported previously for *E. pacifica* (Takahashi & Ikeda, 1975).

The high pCO_2 treatment was higher (1733 μatm) during Day 1 of the Nutrition experiment compared to both Day 7 (1042 μatm) and Day 21 (1063 μatm), and at all times during the Respiration experiment (1027 -1078 μatm). While this high pCO_2 on Day 1 could have driven the differences in ingestion and excretion rates on Day 1, a similar decline was seen in oxygen consumption at a pCO_2 of 1078 μatm in the Respiration experiment, indicating that a similar effect was seen at the lower level.

Interactions between ingestion and nutrient excretion rates, and subsequently with phytoplankton growth rates, may mask some of the actual processes of ingestion and excretion. Phytoplankton can divide and take up nutrients even in the dark (Vaulot & Chisholm, 1987), therefore the equations used to calculate ingestion rates used controls without krill to account for phytoplankton growth rates. However, the presence of krill in the experimental containers may contribute additional nutrients through excretion, causing the phytoplankton to divide faster in containers with krill, and leading to underestimates of true ingestion rates (Roman & Rublee, 1980) and even negative ingestion rates. Additionally, cell counts are likely to be less accurate

than other methods (e.g., Chl a measurements), and may have introduced more variability into our algal concentrations and the estimation of ingestion rates.

Ammonium and phosphate excretion rates also were variable, and negative excretion rates were recorded in several instances. These suggest that increased uptake of nutrients by phytoplankton also led to underestimation of the true krill excretion rates in containers with krill, and likely contributed to the apparently negative rates (Takahashi & Ikeda, 1975). Methods may have been improved by conducting the excretion experiment separately from the feeding experiment, with no phytoplankton present.

The phytoplankton culture was not consistent between Day 1, 7 and 21 and the algal quality was likely declining by Day 21, as indicated by negative growth rates in algal controls, and this contributed to the highly variable ingestion and nutrient excretion rates on Day 21. Therefore, ingestion rates at Day 21 must be interpreted with caution and comparisons between Days 1, 7 and 21 may be hindered by the independent effect of culture density on ingestion rates. However, comparisons between the low and high pCO_2 groups on Days 1 and 7 are still valid, and indicate overall trends of declining ingestion and NH_4^+ release at elevated pCO_2 that are indicative of the lowered energy demands and protein synthesis associated with metabolic suppression (Seibel & Walsh, 2003).

Both experiments are consistent with E. *pacifica* having lower metabolic rates at pCO_2 elevated above that typical of surface waters, and this difference is maintained after 3 weeks of continuous exposure. This suggests that *E. pacifica* is

not tolerant of declines in pCO_2, even to levels that it currently experiences at depth.

Interpreting these results is complicated by *E. pacifica*'s regular migrations between the low pCO_2 waters at the surface and high pCO_2 waters at depth, during which it experiences conditions likely to increase and decrease metabolic activity on a daily cycle. While our study shows high pCO_2 is associated with a lower metabolic rate compared to low pCO_2, we cannot say which is *E. pacifica*'s "natural" pH habitat or metabolic rate. If we assume surface waters represent *E. pacifica*'s normal habitat, our results suggest that *E. pacifica* undergoes metabolic suppression in response to elevated pCO_2. This may indicate that *E. pacifica* is unable to completely compensate for the disturbance of pH in intra- and/or extra-cellular fluids. Although *E. pacifica* has many traits that indicate it should be a strong acid-base regulator (e.g., crustacean, variable environment, metabolically active) (Melzner *et al*, 2009), its ability to compensate for acidosis may be limited by the simultaneous need to maintain ion homeostasis.

Diel migrations are thought to be driven by the need to feed on photosynthetic organisms in surface waters and to seek protection from visual predators at depth (Zaret & Suffern, 1976). Many diel migrators use metabolic suppression of energetically costly processes (such as protein synthesis or ion transport) to survive the short-term extreme conditions they experience at depth (Seibel & Walsh, 2003). Other hypotheses suggest that the metabolic suppression at depth provides a metabolic advantage to migrating organisms by allowing them to maximize their energy efficiency by feeding at night when food is most abundant and then returning

to depth to reduce energy expenditure via metabolic suppression (Enright, 1977). In this case, short exposures to high pCO_2 waters may be beneficial to *E. pacifica*. However, experimental support for the metabolic advantage hypothesis is lacking, and evidence suggests migrating organisms have slower development and lower fecundity than non-migrating organisms (review in Lampert, 1989).

Under future OA, moderate metabolic suppression may be an adaptive strategy, as it permits species to survive in low pH habitats (Calosi *et al*, 2013). However, this strategy comes with trade-offs. Specifically, the direct link between protein synthesis and growth and reproduction means metabolic suppression cannot be a long-term adaptive strategy without negative effects on the fitness of the individual or species (Seibel & Walsh, 2003; Langenbuch & Pörtner, 2004). In one study, polychaetes living in low pH areas near volcanic vents were 80% smaller than individuals living outside the vent area (Calosi *et al*, 2013).

Conversely, if we assume *E. pacifica*'s normal habitat is the deep ocean and characterized by high pCO_2/low pH, then our results would suggest an increase in metabolic rate when exposed to surface waters. A similar increase in metabolism was seen in polychaetes adapted to the perimeter of low pH volcanic vents (Ischia, Italy) when moved to a high pH environment (Calosi *et al*, 2013).

With OA, *E. pacifica* will experience higher pCO_2 values throughout their entire diel migration as pCO_2 increases in both surface and deeper ocean waters. This may mean *E. pacifica* will have lower metabolic rates across its entire habitat range; not just at depth as it does currently. Greater duration of exposure to conditions

causing lower metabolism could result in reduced growth or reproduction; and continuous exposure to high pCO_2 was associated with reduced growth in *E. pacifica* (Chapter 1). At *E. pacifica*'s current depth range of 250 to 500 meters, ocean pCO_2 is expected to increase to as high as 3500 μatm by the year 2100, and that will be combined with simultaneous declines in oxygen concentration (Brewer & Peltzer, 2009). These pCO_2 levels are much higher than levels tested here, and such increases may have additional physiological effects on *E. pacifica*, and possibly narrow their effective habitat ranges by making the deep ocean uninhabitable to them with ecological ramifications.

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2.7 Tables and Figures

Table 2.1. Water chemistry during respiration experiment

Mean chemical properties (± 1 SD) of water in low and high pCO_2 treatments during short-term (initial 24 hours) and long-term (21 days) exposures. Temperature and $pH_{T\ lab}$ were measured daily; A_T and C_T were measured in discrete water samples collected from experimental chambers for all respiration trials, and every 5 – 8 days from maintenance jars during the long-term exposures. All other parameters were calculated from A_T and C_T using CO2Sys Software. $pH_{calculated}$ is used in all statistical analyses.

Exposure		Low CO_2		High CO_2	
		N		N	
Initial	Salinity	7	33.4 ± 0.2	9	33.4 ± 0.2
	Temp °C	11	11.1 ± 0.7	13	11.1 ± 0.6
	$pH_{T\ lab}$	11	7.96 ± 0.13	13	7.60 ± 0.02
	$pH_{T\ calculated}$	7	8.02 ± 0.10	9	7.66 ± 0.10
	pCO_2 µatm	7	434.6 ± 133	9	1078.9 ± 288
	A_T µmol kg_{-1}	7	2231.1 ± 29	9	2239.9 ± 14
	C_T µmol kg^{-1}	7	2060.3 ± 19	9	2195.4 ± 38
	Ω_{arag}	7	1.94 ± 0.38	9	0.92 ± 0.18
Long term	Salinity	13	33.4 ± 0.2	13	33.4 ± 0.2
	Temp °C	143	10.2 ± 0.5	119	10.1 ± 0.6
	$pH_{T\ lab}$	143	8.02 ± 0.10	119	7.60 ± 0.13
	$pH_{T\ calculated}$	13	8.02 ± 0.08	13	7.67 ± 0.10
	pCO_2 µatm	13	430.8 ± 97	13	1027.2 ± 253
	A_T µmol kg^{-1}	13	2259.5 ± 15	13	257.4 ± 12
	C_T µmol kg^{-1}	13	2092.6 ± 37	13	2209.0 ± 28
	Ω_{arag}	13	1.90 ± 0.26	13	0.95 ± 0.22

Figure 2.1. Oxygen consumption by *Euphausia pacifica* in respiration experiment

Mean oxygen consumption rates (±1 SE) of individual krill a) during the initial 24 hours, and b) after 21 days of exposure to low or high pCO_2. Units are µmol oxygen normalized for dry body weight of krill ($mgdw^{-1}$) per hour. Letters indicate significant differences in two sample t-tests (Tukey HSD, $p < 0.05$).

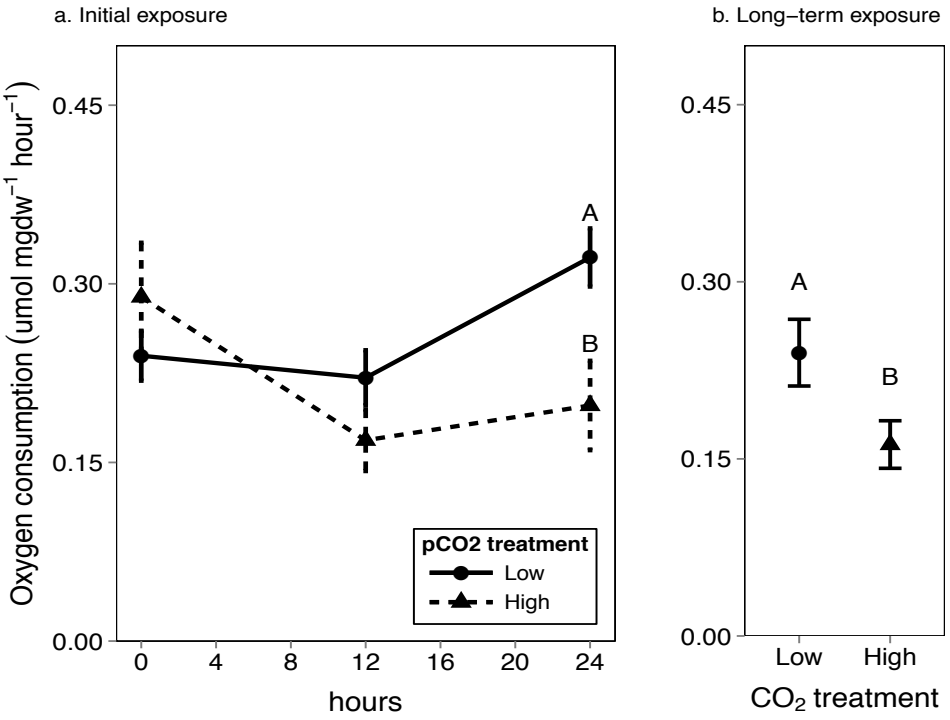

Table 2.2. Water chemistry during nutrition experiment
Mean chemical properties (±1 SD) of water low and high pCO_2 treatments. Temperature and $pH_{T\ lab}$ were measured daily. A_T and C_T were measured in discrete water samples collected from experimental chambers on days of ingestion/excretion trials and every 5 – 8 days from maintenance jars. All other parameters were calculated from A_T and C_T using CO2Sys Software.

Exposure		Low CO_2		High CO_2	
		N		N	
1 Day	Salinity	11	33.4 ± 0.2	9	33.4 ± 0.2
	Temp °C	32	11.6 ± 0.5	27	11.7 ± 0.4
	$pH_{T\ lab}$	32	8.14 ± 0.07	27	7.53 ± 0.10
	$pH_{T\ calculated}$	11	7.97 ± 0.07	9	7.52 ± 0.27
	pCO_2 µatm	11	487.4 ± 92	9	1732.8 ± 835
	A_T µmol kg_{-1}	11	2277.0 ± 18	9	2282.0 ± 13
	C_T µmol kg^{-1}	11	2118.8 ± 22	9	2275.8 ± 80
	Ω_{arag}	11	1.84 ± 0.3	9	0.83 ± 0.6
7 Days	Salinity	6	33.4 ± 0.2	13	33.4 ± 0.2
	Temp °C	59	10.8 ± 0.6	42	10.9 ± 0.5
	$pH_{T\ lab}$	59	8.11 ± 0.06	42	7.62 ± 0.07
	$pH_{T\ calculated}$	6	7.98 ± 0.08	13	7.67 ± 0.07
	pCO_2 µatm	6	485 ± 112	13	1042.4 ± 173
	A_T µmol kg_{-1}	6	2272.6 ± 10	13	2265.7 ± 13
	C_T µmol kg^{-1}	6	2119.3 ± 35	13	2219.0 ± 18
	Ω_{arag}	6	1.79 ± 0.30	13	0.93 ± 0.16
21 Days	Salinity	16	33.4 ± 0.2	16	33.4 0.2
	Temp °C	155	10.2 ± 0.5	131	10.3 ± 0.6
	$pH_{T\ lab}$	155	8.00 ± 0.11	131	7.61 ± 0.09
	$pH_{T\ calculated}$	16	7.97 ± 0.12	16	7.66 ± 0.10
	pCO_2 µatm	16	503.0 ± 179	16	1063.4 ± 260
	A_T µmol kg_{-1}	16	2265.7 ± 20	16	2258.9 ± 21
	C_T µmol kg^{-1}	16	2114.7 ± 58	16	2214.2 ± 31
	Ω_{arag}	16	1.77 ± 0.37	16	0.93 ± 0.21

Figure 2.2. Ingestion rates of *Euphausia pacifica* in nutrition experiment
Mean ingestion rate (±1 SE) of phytoplankton cells per hour, normalized for dry body weight of each animal ($mgdw^{-1}$ hr^{-1}) after 1, 7 and 21 days exposure to high and low pCO_2. N = 10 for each group. Letters indicate significant differences in an ANOVA of days 1 and 7, after excluding day 21 data ($p < 0.05$).

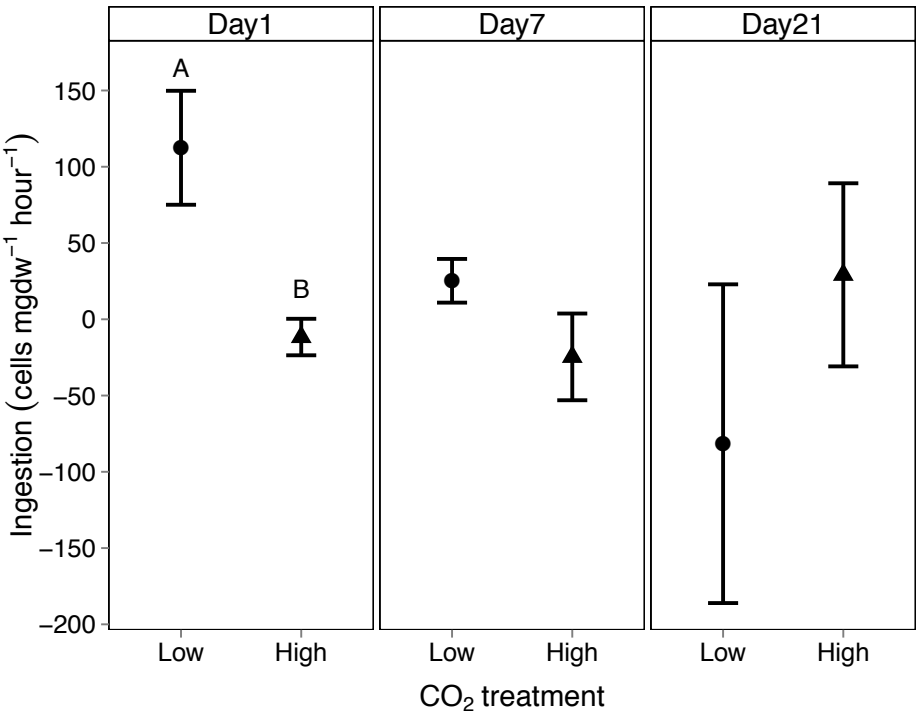

Table 2.3. ANOVA of ingestion rates of phytoplankton by *Euphausia pacifica*. Summary tables for 2-way ANOVAs comparing different durations of exposure at two pCO_2 levels. A. Three exposures (1, 7, 21 days). B. Two exposures (1, 7 days).

A. Three exposure durations

Source	**DF**	**SS**	**F**	**P**
Exposure	2	60668.93	1.0912	0.3429
pCO_2	1	6881.44	0.2475	0.6208
pCO_2*Exposure	2	144461.05	2.5982	0.0834
Error	56	1556808.8		
Total	61	1769455.1	1.5298	0.1954

B. Two exposure durations (excluding Day 21 data)

Source	**DF**	**SS**	**F**	**P**
Exposure	1	26299.584	3.9983	0.0527
pCO_2	1	79286.410	12.0539	0.0013*
pCO_2 x Exposure	1	14414.707	2.1915	0.1470
Error	38	249951.49		
Total	41	366938.15	5.9285	0.0020*

* $p < 0.05$

Figure 2.3. Apparent nutrient excretion by *Euphausia pacifica* in nutrition experiment

Mean apparent excretion rates (± 1 SE) of individual krill, normalized for dry bodyweight, of a) ammonium and b) phosphate after 1, 7 and 21 days exposure to high and low pCO_2. N = 10 for each group on Day 1 and 7, and N = 5 for each group on Day 21. Letters indicate significant differences (Tukey HSD, $p<0.05$).

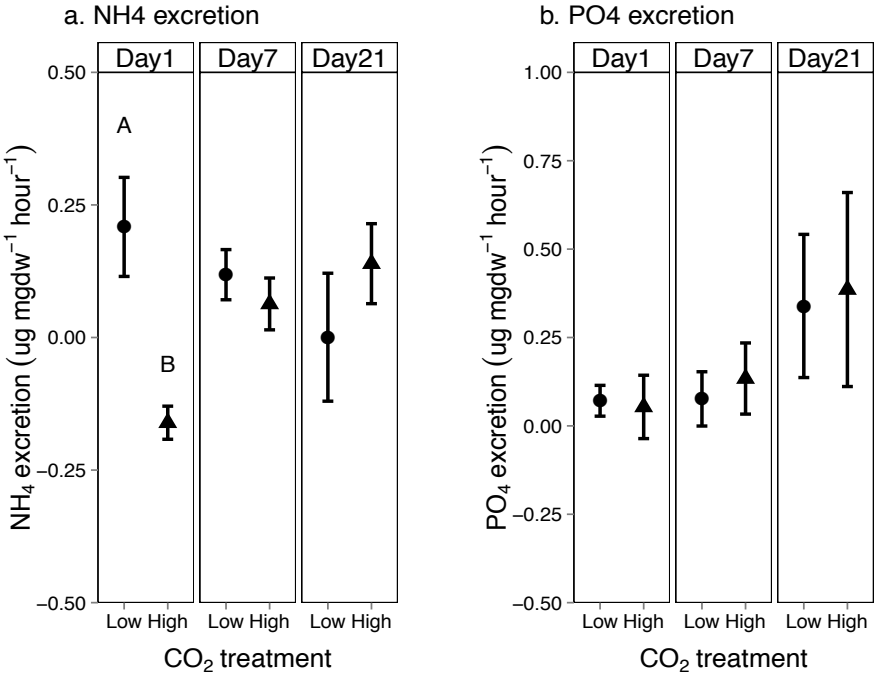

Table 2.4. ANOVA of NH_4 excretion rates in nutrition experiment. Summary tables for 2-way ANOVAs comparing different durations of exposure at two pCO_2 levels. A. Three exposures (1, 7, 21 days). B. Two exposures (1, 7 days)

A. Three exposure durations

Source	**DF**	**SS**	**F**	**P**
Exposure	2	0.04493881	0.4588	0.6352
pCO_2	1	0.09479403	1.9355	0.1715
pCO_2*Exposure	2	0.46807027	4.7786	0.0135*
Error	42	2.0569624		
Total	47	2.8415162	3.2039	0.0154*

* *$p < 0.05$*

B. Two exposure durations (excluding Day 21 data)

Source	**DF**	**SS**	**F**	**P**
Exposure	1	0.04364074	1.1713	0.2865
pCO_2	1	0.43807150	11.7579	0.0016*
pCO_2*Exposure	1	0.23992143	6.4395	0.0158*
Error	35	1.3040185		
Total	38	2.0456617	6.6353	0.0011*

* *$p < 0.05$*

Table 2.5. ANOVA of PO_4 excretion rates in nutrition experiment. Summary tables for 2-way ANOVAs comparing different durations of exposure at two pCO_2 levels. A. Three exposures (1, 7, 21 days). B. Two exposures (1, 7 days).

A. Three exposure durations

Source	**DF**	**SS**	**F**	**P**
Exposure	2	0.64188299	2.0203	0.1442
pCO_2	1	0.00961612	0.0605	0.8067
pCO_2*Exposure	2	0.01594535	0.0502	0.9511
Error	46	7.3074353		
Total	51	7.9744804	0.8398	0.5285

B. Two exposure durations (excluding Day 21 data)

Source	**DF**	**SS**	**F**	**P**
Exposure	1	0.01919054	0.2746	0.6033
pCO_2	1	0.00423889	0.0606	0.8068
pCO_2*Exposure	1	0.01472580	0.2107	0.6488
Error	38	2.6559225		
Total	41	2.6948750	0.1858	0.9054

Chapter 3:

Changes in algal cover and algal community structure along a natural acidification gradient

3.1 Abstract

Ocean acidification is altering species distributions and community dynamics in coral reef ecosystems. We surveyed calcareous algae and water chemistry along an aragonite saturation (Ω_{arag}) gradient at naturally occurring, low pH submarine springs near Puerto Morelos, Mexico. Total percent cover, species richness and species diversity of calcareous algae were lower at $\Omega_{arag} < 2$. The relative abundance of two coralline genera, *Neogoniolithon* and *Lithophyllum* declined at low saturation levels, while the relative abundance of *Hydrolithon* and *Peyssonnelia* were largely unaffected, or even higher, suggesting these genera are more tolerant of low saturation levels. In a 10 month recruitment study, the percent cover of *Lithophyllum* and abundance of serpulid worms were lower at $\Omega_{arag} < 2$. On surfaces exposed for two years the total percent cover of calcareous algae was 91% lower at low Ω_{arag}. Because many coral larvae settle preferentially on certain coralline algae species, the varied responses of algal species to ocean acidification have ecological implications for coral reefs if species that are preferred substrates for coral settlement are less tolerant of OA.

3.2 Introduction

Anthropogenic carbon dioxide dissolving into the world's oceans is causing profound and rapid shifts in ocean chemistry, characterized by a drop in the pH of ocean waters, commonly referred to as "ocean acidification" (OA). Ocean pH is predicted to decline about 0.4 pH units (to ~7.7) by 2100, causing carbonate ion concentration to drop by 50% (Caldeira & Wickett, 2005; Orr *et al*, 2005; Doney *et al*, 2009; Feely *et al*, 2009). A decline in surface carbonate ion concentration directly affects the saturation states (Ω) of two $CaCO_3$ minerals, aragonite and calcite, with major impacts on $CaCO_3$ precipitation and dissolution dynamics. Current Ω_{arag} levels in tropical surface waters (e.g., near coral reefs) are approximately 3.5 to 4, but expected to drop to approximately 2.3 Ω_{arag} by the end of the century (Feely *et al*, 2009). At low saturation levels, carbonate minerals dissolve more readily in seawater and organisms with calcified skeletons or shells (e.g., corals, mollusks, coralline algae) have greater difficulty maintaining normal calcification rates, and their growth, survival, and reproductive rates often decline (Kleypas *et al*, 2006; Doney *et al*, 2009; Kroeker *et al*, 2010; Andersson *et al*, 2011; Ross *et al*, 2011). Organisms differ in their tolerances to OA, and some species may be able to acclimate or even adapt to future CO_2 levels (Kroeker *et al*, 2010; Kelly & Hofmann, 2012). Determining how organisms respond to OA, and identifying the species (or populations within species) that are more tolerant of low saturation, has major implications for marine conservation. If habitats containing OA tolerant populations and species can be protected, they may form refuges that will act as sources for replacing those unable to

survive OA conditions elsewhere.

Calcareous algae (*e.g.,* coralline algae, *Halimeda*) are essential on coral reefs: they often provide much of the carbonate mass, bind the framework together, increase the strength of the reef, and help protect coastlines from heavy wave action. Coralline algae also have important ecological roles, facilitating the recruitment of larval corals and mollusks. In almost all laboratory experiments, coralline algae are more adversely affected by low pH/low Ω_{arag} water than most other organisms, with severe reductions in recruitment, survival, growth, productivity, ability to induce larval settlement, and with 100% mortality at pH $\leq$ 7.2 (Kuffner *et al*, 2007). The high magnesium calcite of coralline algae is more soluble than the aragonite composition of corals and hence it is expected that they will be more sensitive to dissolution in low saturation waters (Ries, 2011). Responses of *Halimeda* and other calcifying green algae (Chlorophyta) are more variable, with sensitivity varying by species and location (Comeau *et al*, 2014; Campbell *et al*, 2014; Vogel *et al*, 2015).

While laboratory studies are valuable for determining individual organisms' tolerance of OA, field studies are needed to understand long-term effects of OA on both organisms and communities (Andersson *et al*, 2015). Recent field studies at naturally occurring low pH/high CO_2 vents in Papua New Guinea and Ischia, Italy have revealed patterns in recruitment, competition and community dynamics of large suites of benthic organisms in response to low Ω_{arag} (Hall-Spencer *et al*, 2008; Kroeker *et al*, 2012Kroeker *et al*, 2013; Fabricius *et al*, 2015).

Off the coast of the Yucatan Peninsula, Mexico a system of natural submarine

springs, locally called “ojos”, has existed within the Mesoamerican reef for millennia (Beddows *et al*, 2002). These ojos continuously discharge groundwater that is close to seawater salinity but more acidic (pH 6.70-7.30) and under-saturated (0.3 Ω_{arag} to 0.97 Ω_{arag}) than the ambient seawater (pH 8.03, Ω_{arag} 3.60) (Crook *et al*, 2012). The chemical composition of waters discharging from the ojos differs from that of seawater affected by typical mechanisms of ocean acidification (i.e., absorption of atmospheric CO_2). The ojo water has high CO_2 concentrations derived from the groundwater interaction with limestone and soil and mixes with seawater before discharge (Crook *et al*, 2012). The ojo discharge also has high total inorganic carbon, high alkalinity, and lower pH and carbonate saturation states than the ambient lagoon waters. Both pH and Ω_{arag} increase with distance from the ojo center as the ojo discharge mixes with the ambient seawater (Crook *et al*, 2012). These conditions provide an opportunity to study responses of marine communities along gradients of pH and Ω_{arag} that are similar to conditions predicted by future OA scenarios.

Few coral species live in the low saturation water in or near the ojos, although species diversity and coral colony size both decline at lower Ω_{arag} levels (Crook *et al*, 2012). Calcifying algae also live in the discharge area, although dominance shifts from calcareous algae towards fleshy algae in lower saturation zones, which Crook (2015) hypothesizes is due to altered competitive dynamics. Crook (2015) treated all calcareous algae as a single functional group. The objective of the present study was to determine how saturation state affects the distributions, abundance and richness of different taxa in communities of calcifying algae. This study consists of two parts, 1)

an in-situ field survey of calcifying algae and 2) a recruitment experiment.

3.3 Materials and methods

Ojos:

There are approximately 13 ojos within the shallow lagoon (usually in 5 to 7 meters depth) between the shore and the Mesoamerican Reef in the National Maritime Park at Puerto Morelos, Mexico. These ojos typically discharge either from fractures several meters long or from circular seeps that vary in size from a few centimeters to a few meters diameter. Five ojos were sampled in this study. Ojo Agua and Ojo Pargos are large circular ojos with cave-like openings 1-2 meters across and strong water flow from the center of the ojo. Ojo Gorgos consists of several small round ojos, each about 0.5 meters across. Ojo Laja and Ojo Norte are fracture-type ojos with water discharging from beneath linear, horizontal limestone outcrops overhanging a sandy seafloor (Figure 1A, 1B).

Field survey:

Species distribution

Surveys were conducted at the 5 ojos in December 2013 to determine the type and abundances of calcifying algae across environmental gradients (e.g., pH, Ω_{arag}). For fracture ojos, a 30-meter measuring tape was laid along the ledge above the fracture and a 25 cm x 25 cm quadrat was placed at 25 cm intervals. At circular ojos the transects began at the center of the ojo and moved away from the center for 10 to

15 meters. At all ojos, additional quadrats were placed within the same habitat, but approximately 10 meters away from the area of discharge influence for additional control samples. At each quadrat, divers recorded all algae morphologies, measured the length and width of surface covered by each algae, and collected samples of each morpho-type. In the lab the algal specimens were grouped based on morphological characteristics and identified to the lowest possible taxonomic level. Because some samples could not be identified to species, all analyses were performed on genera.

Environmental variables

Discrete water samples were collected near the substrate in each quadrat using 200 ml syringes and immediately transferred to a boat where water was filtered (0.2μm) into acid-washed vials for analyses of nutrients, Ca^{2+} and carbonate chemistry. Samples for total inorganic carbon and total alkalinity were poisoned with Hg and stored for later analysis following standard procedures (Dickson *et al*, 2007). In-situ measurements of pH, salinity and temperature were also made aboard the boat immediately after collection.

Each water sample was analyzed in triplicate for total inorganic carbon (C_T) using a CM5011 carbon coulometer (UIC, Inc) and total alkalinity (A_T) using an automated open cell titration procedure. Instruments were calibrated using certified seawater standards (Batch 135) from the Andrew Dickson laboratory at the Scripps Institution of Oceanography. Nutrient analyses (PO_4^{-3}, Si, NO_2^-, NO_3^-, NH_4^+) were performed on a flow injection autoanalyzer (FIA, Lachat Instruments Model

QuickChem 8000). Ca^{2+} concentration was measured by spectroscopy on an ICP-OES (Perkin Elmer Optia 43000). Salinity was measured on a salinometer (Guideline 8410). Saturation states (Ω_{cal} and Ω_{arag}) were calculated using the measured parameters (C_T, A_T, temperature, salinity and nutrients) in the CO_2Sys software program (Pierrot *et al*, 2006) and CO_2 disassociation constants from (Mehrbach *et al*, 1973), refitted by (Dickson & Millero, 1987). Some samples had higher Ca^{2+} values than expected based on their salinity, and their Ω_{cal} and Ω_{arag} values were corrected using (observed Ca^{2+} /expected Ca^{2+}) as a correction factor. In addition to these discrete samples pH, temperature and salinity were monitored hourly at some of the Ojos Laja and Norte and control sites using SeapHOx sensors.

Total percent cover, species richness and diversity

Percent cover of each algal genus was calculated for each 25 x 25 cm quadrat. Several quadrats that were predominantly sand were excluded from the analyses as unsuitable habitat for calcifying algae. Total percent cover was calculated by summing the areas of all algae within each quadrat. The Shannon diversity index was calculated using the diversity function (vegan package in R), and richness was calculated as the number of unique genera in each quadrat. Algae that could not be identified to genus level were included in the total percent cover calculation only, and excluded from diversity analyses.

Data analysis

Due to the different area influenced by the discharge at each ojo, sampling quadrats were assigned to 3 groups based on calculated Ω_{arag}: low saturation ($\Omega_{arag} < 2$; n = 14), medium saturation ($2 \leq \Omega_{arag} < 4$; n = 34) and high saturation ($\Omega_{arag} \geq 4$; n = 9), and all statistical analyses were conducted on these groups. Ω_{arag} was used instead of Ω_{cal} because although our samples were predominantly coralline algae which deposit high-Mg calcite in their skeletons, the solubility of high-Mg calcite is more similar to aragonite than calcite. The equivalent Ω_{cal} values are provided in Table 1 and Table 3 for comparison. Nonparametric Kruskal-Wallis tests were used to test for differences in total algal cover, richness and diversity among the three saturation levels using Jmp Pro 12.

Community structure and composition

To compare patterns in community composition (presence or absence of genera) and community structure (relative abundances of genera) among saturation levels and among ojos we conducted Permutational Multivariate Analysis of Variance (PERMANOVA) on zero-adjusted Bray-Curtis (BC) dissimilarity matrices of community data (adonis in vegan package in R). A Bray-Curtis dissimilarity matrix calculates dissimilarities between all possible pairs of quadrats within the community (genus) matrix using the equation:

$$BC_{ij} = 1 - \left[\frac{2C_{ij}}{(S_i + S_j)}\right]$$

where C_{ij} is the abundance of species that quadrats i and j have in common, and S_i and S_j are the abundance of quadrats i and j, respectively (McCune *et al*, 2002). To account for quadrat pairs that were completely denuded of algae a "bare" category (dummy variable) was added to the community matrix and all cells were given the same value (0.04%; smallest non-zero value in original matrix (Clarke *et al*, 2006).

The community composition analysis used a BC dissimilarity matrix of the presence/absence of each genus, while the community structure analysis used a BC dissimilarity matrix of square root transformed relative percent cover of genera. Ojo and Ω_{arag} level were categorical fixed factors in the PERMANOVA, which calculated 99,999 permutations of the transformed data. SIMPER analyses were used to determine which genera contributed the most to overall dissimilarities in community composition and structure.

A nonmetric dimensional scaling (nMDS) ordination plot was used to visualize differences in community structure among saturation states and ojos. nMDS graphically represents relationships between different genera and sampling quadrats based on the ranked dissimilarities between pairwise comparisons of quadrats (Quinn & Keough, 2002). All nMDS ordinations were constrained to 2 dimensions and created using the metaMDS function in vegan package for R (version 2.3.0) (Oksanen *et al*, 2015).

Recruitment experiments:

A recruitment experiment was deployed in December 2013 at Ojo Laja to determine the recruitment rates of calcifying algae along the environmental gradients. Small circular, numbered, aluminum discs (area 8.55 cm^2) were attached with cable ties through a small hole to a 30-meter long chain (Figure 1C) at 50 centimeter intervals. The chain was anchored along the edge of the fracture. The discs were located approximately 5 cm from the edge of the ledge: most were on top of the ledge, but several hung down directly into the ojo discharge. Algae recruited to both sides of the discs. The discs were collected 10 months later (October 2014), rinsed with fresh water and dried. All calcifying algae were identified and the area of each was measured using ImageJ software. Other calcifying organisms (e.g., spirobids, serpulids and vermetid worms) present were enumerated.

Discrete water samples were collected from each of the disc locations at the end of the 10 month deployment, using the same methods as used in the field survey and analyzed in the same manner. SeapHOx sensors collecting hourly samples were deployed at the ojo center and at a control site throughout the experiment. The discs (N = 65) were grouped into the same three Ω_{arag} levels as the field survey analysis (low N = 9; medium N = 36; high N= 20). The percent cover or count for each genus or organism in the three Ω_{arag} levels was analyzed using non-parametric Kruskal-Wallis tests.

Stainless steel grids that had been placed previously at the ojo were also sampled for calcifying algae to assess the effects of long-term exposure to low Ω_{arag}

on recruitment of calcifying algae. Each grid (approximately 75 cm x 40 cm; with 2.5 cm mesh) was attached to the limestone substrate at Ojo Laja either directly in the ojo discharge or in a control region 10 meters away. The grids were deployed for 2 years (November 2012 to January 2015). After collection, the grids were rinsed and air dried, and total area cover of algae was calculated. Percent cover of calcareous algae in the ojo and control areas was compared in a nonparametric Kruskal-Wallis test. Discrete water samples were taken from the ojo and control areas at deployment and approximately monthly over the 2-year period, and saturation state was calculated for each treatment group.

3.4 Results

Field survey

Water analyses of the three saturation groups are summarized in Table 1. C_T and A_T were significantly higher in the low saturation group (ANOVA, $p < 0.001$ for both). Dissolved nutrients (silicate, phosphate and ammonium) were 2 to 5 times higher in the low saturation group than in the other two saturation levels (ANOVA, $p < 0.001$ for all comparisons). Mean salinities did not differ among Ω_{arag} group ($p > 0.05$).

Distribution and abundance

We identified 4 genera of coralline algae (*Neogoniolithon, Hydrolithon, Amphiroa* and *Lithophyllum)*, 1 other genus of Rhodophyta (*Peyssonnelia),* and 2

genera of Chlorophyta (*Halimeda* and *Rhipidosiphon*). *Neogoniolithon, Hydrolithon* and *Peyssonnelia* were abundant, while *Amphiroa* and *Lithophyllum* were both rare.

Algal abundance

Total percent cover of all algae combined was significantly lower in low saturation waters ($\Omega_{arag} < 2$) than in medium saturation waters ($2 \leq \Omega_{arag} < 4$; Kruskal-Wallis test: H (5.01, p = 0.08, post-hoc comparison p = 0.03; Figure 2A). Total cover was also lower at the high saturation levels than medium levels, but was not significant (p = 0.30). A quadratic equation was fit to the raw data (quadratic regression, $F_{2,53} = 4.366$, p = 0.02, Adj $R^2 = 0.11$; Figure 2B); percent cover increased from $\Omega_{arag} = 0$ to a peak at $\Omega_{arag} = 3$, then declined again at $\Omega_{arag} > 3$. The quadratic relationship suggests that saturation state may be the primary driver of percent cover at $\Omega_{arag} < 2$, but at $2 < \Omega_{arag} < 4$ other environmental or ecological factors may dictate percent cover. There were no differences in mean total percent cover of calcifying algae among the 5 ojos (Kruskal-Wallis, p>0.05).

Community composition

Both main effects, Ω_{arag} level (PERMANOVA, $F_{2,50} = 2.24$, p = 0.05) and ojo ($F_{4,50} = 3.95$, p = 0.0002) were strong predictors of community composition of genera (presence/absence data). Specifically, community composition in low Ω_{arag} differed from that in medium Ω_{arag} waters (p = 0.02), but community composition in low Ω_{arag} and medium Ω_{arag} waters did not differ from those in high Ω_{arag} waters (p >0.05 for

both comparisons). SIMPER analyses identified that *Neogoniolithon*, *Hydrolithon* and *Peyssonnelia* were the three most influential genera in determining community composition differences by saturation level.

To understand these effects of Ω_{arag} on community composition we examined the relative frequencies (percentage of quadrats containing each genus) at each Ω_{arag} level; Table 2A). *Hydrolithon* was the most abundant genus, occurring in almost half of all quadrats, regardless of saturation level. *Neogoniolithon*, and *Peyssonnelia* were also abundant at all saturation levels, but occurred less frequently in low saturation waters. *Halimeda* occurred less often (approximately 20% of quadrats across all saturation levels), but occurred slightly less often in low and high saturation areas. *Lithophyllum* and *Rhipidosiphon* were extremely rare outside the medium saturation zone (7% of quadrats), but occurred in 21-26% of quadrats in medium waters. *Amphiroa* was absent from both low and high saturation waters, and was the rarest genus (15%) in medium saturation quadrats.

The community composition at Ojos Agua and Gorgos differed from that at Ojos Laja, Norte and Pargos (PERMANOVA $F_{1,53} = 8.7737$, $p = 0.0002$). This difference was primarily driven by the same three genera (SIMPER analysis); specifically *Hydrolithon* was absent from Ojo Agua (0% occurrence compared to 50 to 80% at other ojos; Table 2B), *Neogoniolithon* occurred more frequently at Ojos Agua and Gorgos (53 to 75%) compared to Ojos Laja, Norte and Pargos (10 to 17%), and *Peyssonnelia* occurred less frequently at Ojo Agua (25%) compared to the other ojos (44 to 83%). Other differences that existed, but contributed less to the overall

community differences, were the absence of both *Amphiroa* and *Halimeda* from Ojos Agua and Gorgos.

Genus richness and diversity

There were fewer genera in low saturation ($\Omega_{arag} < 2$) than in medium saturation waters ($2 \leq \Omega_{arag} < 4$; Kruskal-Wallis test, H (5.75) p= 0.05, post hoc comparison, p=0.015; Figure 3A). Richness in the high saturation area did not differ from that in the low or medium waters ($p > 0.05$ for both comparisons).

Diversity also differed by saturation state (Kruskal-Wallis test, H(7.02), p = 0.03), and was lower at low saturation levels ($\Omega_{arag} < 2$) than medium levels ($2 \leq \Omega_{arag} < 4$; post hoc comparison, p = 0.007; Figure 3B). Diversity at the highest saturation level ($\Omega_{arag} > 4$) did not differ from the medium or low saturation groups ($p > 0.05$ for both comparisons). There were no differences in genus richness or diversity among ojos (Kruskal-Wallis p value > 0.05 for both).

Community structure

Both main effects, Ω_{arag} level (PERMANOVA, $F_{2, 50} = 2.35$, p = 0.020) and ojo (PERMANOVA, $F_{4,50} = 3.30$, p = 0.0005) were strong predictors of community structure of genera (sqrt relative abundance data). Specifically, community structure differed in low Ω_{arag} waters compared to medium Ω_{arag} waters (PERMANOVA, $F_{1,46} = 3.2609$, p = 0.0138, Figure 4). SIMPER analysis indicated that the same 3 genera (*Neogoniolithon*, *Hydrolithon*and *Peyssonnelia*) contributed the most to

dissimilarities in overall community structure between the saturation levels (Figure 5A).

Of the three genera that contributed most to overall community dissimilarity, relative percent cover of *Hydrolithon* was highest in low saturation waters, and decreased with increasing saturation level (Figure 5B). *Peyssonnelia* had higher relative percent cover at both low and high saturation as compared to the medium saturation level. These results suggest that *Hydrolithon* and *Peyssonnelia* may have some tolerance to low saturation levels. Conversely, *Neogoniolithon* had much lower relative percent cover at the low saturation level compared to both high and medium levels, indicating that it is highly sensitive to low saturation level. *Lithophyllum* and *Amphiroa* were rare in all saturation levels, and thus did not contribute much to the overall community dissimilarity. However, both genera had lower relative cover, or were completely absent, in both low and high saturation levels than in the medium saturation level. This finding suggests that these genera were also sensitive to low saturation level. Both *Halimeda* and *Rhipidosiphon* were also minor contributors to the overall community dissimilarities, and their relative percent cover did not vary much between saturation levels.

Community structure also differed among ojos (PERMANOVA $F_{4,50}$ = 3.0811, p = 0.0005; Figure 6) Specifically, community structure at Ojo Agua and Ojo Gorgos differed from Ojos Laja, Pargos and Norte (which were all similar to one another; $F_{1,53}$ =8.046, p = 0.0001; Figure 6). This difference in community structure was primarily driven by the dominance of the genus *Neogoniolithon* at both Ojo Agua

and Ojo Gorgos, while it was rare at the other 3 ojos, and the absence of *Hydrolithon* from Ojo Agua.

Recruitment experiment

Water chemistry data are summarized in Table 3. C_T and A_T were higher in the low saturation waters (ANOVA, $p<0.0001$ for both). Dissolved nutrients (silicate, ammonium and phosphate) were 2 to 8 times higher in the low saturation waters (ANOVA, $p < 0.0001$ for all). Salinities ranged from 26.7 to 36.3, and were slightly lower in the low saturation waters (ANOVA, $p<0.0001$).

After 10 months, calcifying algae occurred on 92% of recruitment tiles (total N = 65) and consisted of only *Peyssonnelia* and 2 coralline genera *Amphiroa* and *Lithophyllum* (Figure 7A). *Peyssonnelia* was the most abundant genus, occurring on almost 90% of tiles with a mean percent cover of 12.07 $\pm$ 12.17%). *Lithophyllum* was also very abundant, occurring on nearly 60% of tiles (mean percent cover = 9.52 $\pm$ 17.68 %). *Amphiroa* was relatively rare, occurring only on 20% of tiles and occupying much less space (mean percent cover 0.37 $\pm$ 1.29%).

Percent cover of each algal species was compared among aragonite saturation levels. Both *Amphiroa* and *Lithophyllum* had higher cover in medium saturation waters, but neither was significantly different from the other saturation levels ($p>0.05$, Figure 7A). Similarly, *Peyssonnelia* cover and total cover (all calcareous algae) did not differ by saturation level, likely due to the high variability in percent cover between samples.

Calcifying worms and snails also occurred on the tiles. Spirobids and serpulids were each found on approximately 78% of tiles, but spirobids were more abundant (spirobids 19.68 + 25.05 per tile, serpulids 4.89 $\pm$ 8.28 per tile; Figure 7B). Vermetid snails were less common, occurring only on 23% of tiles (mean abundance 0.446 $\pm$ 1.14 per tile). Serpulid abundance was significantly lower in the low saturation waters compared to medium saturation waters (Kruskal-Wallis H(6.88), p=0.03, post hoc comparison p = 0.01; Figure 7B). Spirobid and vermetid abundance did not differ by saturation state.

On the stainless steel mesh that was exposed for two years, total cover was significantly lower in the low saturation waters of the ojo discharge area than in adjacent, high saturation control waters (Kruskal-Wallis H(5.44), p = 0.02). Mean percent cover in the control area was 81.13 $\pm$ 4.45% (N = 6), compared to 6.68 $\pm$ 12.84% inside the ojo area (N = 3).

3.5 Discussion

Surveys at low-pH ojos indicate that while some genera of calcareous algae can survive at low saturation levels, total percent cover of all calcifying algae in the surveys was greatly reduced at low Ω_{arag}, with percent cover 59% to 91% that of high saturation waters ($\Omega_{arag} > 2$) in the field survey and the long-term recruitment experiment, respectively. These rates are similar to studies of other in-situ systems, in which the percent cover of calcifying organisms declined by 25 to 100% (Hall-Spencer *et al*, 2008; Kroeker *et al*, 2012; Porzio *et al*, 2013; Kroeker *et al*, 2013;

Crook, 2015; Fabricius *et al*, 2015). The relationship between the total percent cover and Ω_{arag} is best described using a quadratic regression. Total percent cover increased from $\Omega_{arag} = 0$ to $\Omega_{arag} = 2$, indicating that Ω_{arag} may be the primary predictor of calcifying algal cover at low saturation levels. At $2 < \Omega_{arag} < 4$, there was no relationship between saturation level and percent cover, which suggests that other environmental and ecological factors likely play a greater role in medium saturation levels. While not significant, there was a tendency for percent cover, species richness and diversity all to be lower in the highest saturation level ($\Omega_{arag} > 4$). The mechanism is unknown, but could be due to a physiological limitation at higher saturation levels or competition with other organisms.

Loss of calcifying algal cover has significant implications for numerous marine ecosystems. Coralline algae are primary settlement substrates for corals and other invertebrates, and contribute to reef structure by binding coral and other skeletons together. Calcifying green algae, such as *Halimeda,* contribute to the overall production of carbonate sediments in tropical systems, and provide habitat for other small organisms (Campbell *et al*, 2014). Declines in the percent cover of these calcifying algae affect coral reef ecosystems via reduced substrate available to corals and less calcareous material contributed to the reef framework and sediments (McCoy & Kamenos, 2015).

Our studies also demonstrated that both community composition and structure were different at low saturation sites, where the genus *Amphiroa* was absent and most other species occurred less frequently. Little is known about what drives responses of

different species, but there is some evidence that early successional coralline species (characterized by thin walls and rapid growth; e.g., *Hydrolithon boreale*, *H. farinosum*, *Titanoderma spp.)* are more sensitive to OA than later successional species (characterized by thicker crusts; e.g., *Sporolithon*, *Neogoniolithon*, *Porolithon)* (Steneck, 1986; Doropoulos *et al*, 2012). The *Lithophyllum* in our study all had thin crusts and it was one of only two coralline genera present on recruitment tiles after 10 months, both of which are consistent with being early successional species. *Lithophyllum* appeared relatively sensitive to OA. In our surveys, relative percent cover was lower in low saturation than medium saturation waters. In the recruitment experiment there also was a (non significant) trend for lower total percent cover in the low saturation water.

The two species within the genus *Hydrolithon (H. boergesenii* and *H. munitum*) both had thick crusts (González *et al*, 2009) and appeared to be tolerant of low saturation, since it was the only genus of coralline in our study that had a similar frequency of occurrence across all saturation levels and its relative percent cover was highest in low saturation waters. Studies of other thick-encrusting species within the genus have mixed results. *H.* (= *Porolithon*) *okodes* may tolerate OA by altering its mineralogy under elevated pCO_2 (Diaz-Pulido *et al*, 2012; Nash *et al*, 2012), but in other studies its percent cover decreased at elevated pCO_2 (Fabricius *et al*, 2015). Increased mortality of *H. okodes* (Diaz-Pulido *et al*, 2014), and increased vulnerability to grazing pressure of urchins (due to decreased structural integrity), have also been reported (Johnson & Carpenter, 2012). Some of these negative effects

were seen in populations where *H. okodes* were also very thin, possibly due to young age (Fabricius *et al*, 2015). Percent cover of other thin-crust *Hydrolithon* species (*H. reinboldii, H. boreale*, and *H. farinosum)* declined at low pH (Doropoulos *et al*, 2012; Porzio *et al*, 2013; Fabricius *et al*, 2015).

In contrast to the previous pattern, all the *Neogoniolithon* surveyed appeared to be late successional and thick-crusted species. They all had lower relative frequencies and relative percent cover in the low saturation zones, indicating that they also were sensitive to saturation level. All the *Peyssonnelia* was thin-crusted and present in the Recruitment experiment, indicating it is an early successional species. However, *Peyssonnelia* appeared tolerant to OA, as its relative percent cover has highest at low saturation levels. These findings suggest the reported relationship of crust thickness and sensitivity to OA is not universal, and more studies on different coralline species are needed to identify valid relationships between functional traits and sensitivity to OA, and to identify possible mechanisms for their response to OA.

Halimeda and *Rhipidosiphon* were both rare in our study, and while they occurred with lower frequency at low saturation state, there was no significant change in relative percent cover. Reported effects of OA on *Halimeda* are varied, and may be species- and location-dependent (Hall-Spencer *et al*, 2008; Ries *et al*, 2009; Comeau *et al*, 2014; Campbell *et al*, 2014; Vogel *et al*, 2015).

The varied responses of different species to ocean acidification may alter communities by reducing the percent cover of certain species. Because many coral larvae have preferences for particular coralline species as settlement sites (Morse *et*

al, 1988; Raimondi & Morse, 2000; Harrington *et al*, 2004; Ritson-Williams *et al*, 2010), OA may disproportionately affect coral recruitment and reef resilience, if preferred coralline species are sensitive to low saturation levels. *H. boergesenii and Peyssonnelia*, both important recruitment substrates for some corals (Heyward & Negri, 1999; Raimondi & Morse, 2000), were relatively tolerant of acidified conditions in our study. However, *Titanoderma prototypum* (= *Lithophyllum prototypum*), a species closely associated with corals on the Great Barrier Reef (Harrington *et al*, 2004), was very sensitive to OA in our study and others (Doropoulos *et al*, 2012). In addition, the affinity of coral larvae for *T. prototypum,* their preferred substrate, declined under acidified conditions (Doropoulos *et al*, 2012), possibly due to altered chemical cues or microbial communities associated with the corallines (Webster *et al*, 2013). Thus OA may limit coral recruitment both directly through reduced availability of preferred settlement substrate, and indirectly by altering the interaction between larvae and corallines.

Our study demonstrated a reduction in both richness (number of unique genera) and diversity of calcifying algae with lower saturation state and other studies report similar declines in diversity and richness of calcareous species between 4 and 30% (Hall-Spencer *et al*, 2008; Crook *et al*, 2012; Porzio *et al*, 2013). Diversity and species richness can affect ecosystem function, reduce variability in such community properties as biomass, and increase resistance to, or recovery from, environmental disturbances and species invasions (Stachowicz *et al*, 2007).

In our 10-month recruitment study cover of calcifying algae was 28% lower at low saturation levels than medium levels, although the trend was not statistically significant (Figure 7A). This agrees with previous laboratory and field studies in which recruitment of calcifying algae declined at low saturation states (Kuffner *et al*, 2007; Jokiel *et al*, 2008; Porzio *et al*, 2013; Fabricius *et al*, 2015). In some in-situ studies calcareous algae did recruit and grow in low saturated waters for a short time, but development ceased after 3 months, coinciding with increases in fleshy algae (Crook, 2015). These studies suggest that while capable of recruitment under acidified conditions, calcifying algae are outcompeted by fleshy algae, resulting in lower percent cover of calcareous algae. We did not measure fleshy algal cover, so we cannot determine whether the reduced calcareous cover seen at low saturation was due to reduced recruitment, slower growth rates, or altered competition with fleshy algae. However, altered competitive dynamics and reduced coralline substrate driven by ocean acidification will only exacerbate other existing stressors and may push coral reefs further towards algal dominance. Systems dominated by fleshy algae have less substrate available for coralline algae, which in turn reduces coral settlement and establishment, resulting in a reef system with decreased diversity, altered ecosystem functions and less able to recover from environmental disturbances (McManus, 2000; Hughes *et al*, 2007).

Fewer serpulid worms settled on discs at low saturation levels in our experiment. The mineralogy of calcareous tubes formed by serpulids varies from entirely aragonitic to entirely high Mg-calcite, making them vulnerable to ocean

acidification (Ries, 2011; Smith *et al*, 2013). Low saturation states change the mineralogy and structure of serpulid tubes resulting in less elasticity and strength of the tubes (Chan *et al*, 2012). Weakened tubes may lead to more predation and lower survival of the worms, and may cause wider ecosystem effects in places where abundant calcareous tubes form complex habitats contributing to the diversity of the community (Smith *et al*, 2005). Vermetid molluscs were rare while spirobid worms were abundant at all saturation levels in our study. Responses varied in the few previous studies of the effects of ocean acidification on these organisms (Jokiel *et al*, 2008; Kroeker *et al*, 2013; Milazzo *et al*, 2014; Peck *et al*, 2015; Crook, 2015). As with Jokiel *et al (*2008), large variances in vermetid abundances may have obscured any trend linking vermetid abundance to saturation state.

In-situ systems like the ojos at Puerto Morelos permit studies of the responses of organisms to elevated CO_2 over time scales long enough to allow for acclimation or adaptation to low saturation waters. Further, they permit the study of community processes such as competition, predation and reproduction under OA scenarios. However, in-situ studies may be confounded by other environmental variables that may co-vary with saturation state. In our study, nutrient concentrations (phosphate, silicate and ammonium) were higher in low saturation waters than in higher saturation levels, although differences in salinity were either small or absent. These nutrients originate from the groundwater (Null *et al*, 2014; Hernández-Terrones *et al*, 2015). While we cannot tease out effects of these extra nutrients from that of saturation state

on responses of calcifying algae, our results are consistent with other controlled field studies that show reductions in percent cover and diversity at low saturation states.

The ojo system also differs from future ocean acidification scenarios in that low pH waters are localized rather than regional or global. Therefore, ambient saturation sites in close proximity may be the source of spores and larvae settling in the low saturation zones. This could affect our projections of OA responses in two ways. If non-acclimated individuals from source populations can survive under low saturation, but their reproduction is affected, then the declines we saw in algal species are likely to underestimate total population effects. Conversely, if most of the algae in low saturation zones originated from ambient waters, our findings may overestimate species responses to OA if species currently in ambient waters are able to adapt to gradual declines in saturation state over the coming years.

In our study total calcareous algal cover and species diversity declined because some species were highly sensitive to low saturation. However, at least two calcifying algae had a degree of tolerance of low saturation levels (*i.e., Hydrolithon* and *Peyssonnelia*), suggesting that some species are likely to persist in marine communities under future OA scenarios. Differences in acclimation potentials among species are likely to lead to shifts in coral reef and other communities, as species better acclimated to low saturation increase in relative abundance. The resulting effects on coral reef ecosystems will depend partly on which algal species tolerate low saturation, and partly due to species-specific relationships between coral larvae and coralline.

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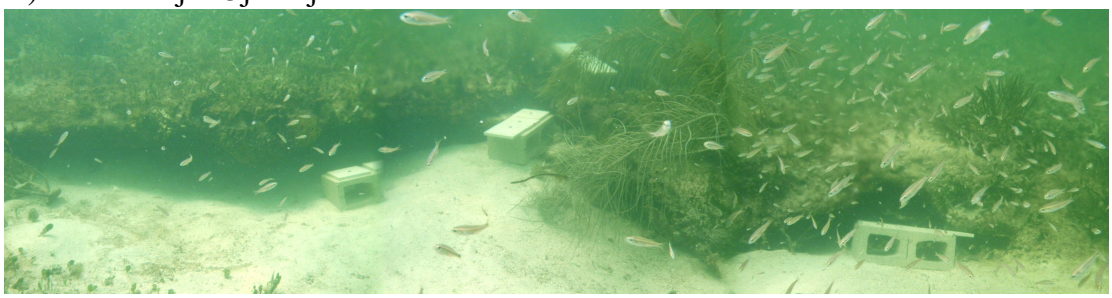
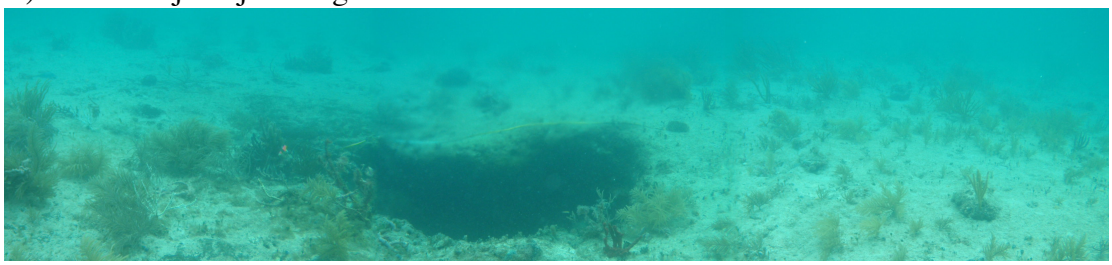
3.7. Tables and Figures

Figure 3.1. Examples of different types of ojos. A) A fracture-type ojo approximately 12 meters long. Fracture is below a limestone shelf that overhangs the outflow. B) A circular ojo approximately 4 meters diameter in the sandy seafloor. C) Chain and settlement disc from recruitment experiment at Ojo Laja.

A) Fracture ojo: Ojo Laja



B) Circular ojo: Ojo de Agua



C) Short-term recruitment tiles

Table 3.1. Water chemistry parameters for field survey. Mean ± 1 s.d. of water chemistry parameters by saturation level.

	Aragonite saturation state		
	Low	Medium	High
Temperature	27.28 ± 0.32	27.25 ± 0.32	27.28 ± 0.51
Salinity	35.01 ± 2.07	35.29 ± 0.84	35.40 ± 0.67
C_T (µmol/kgsw)	2595 ± 240	2098 ± 104	2014 ± 127
A_T (µmol/kgsw)	2643 ± 198	2367 ± 68	2389 ± 89
Phosphate (µmol/kgsw)	0.31 ± 0.20	0.12 ± 0.14	0.08 ± 0.06
Silicate (µmol/kgsw)	16.75 ± 10.33	5.41 ± 7.08	3.20 ± 3.94
pH	7.44 ± 0.19	7.93 ± 0.10	8.12 ± 0.05
pCO_2 (µatm)	2564 ± 1384	571 ± 187	348 ± 68.4
Ω_{cal}	2.07 ± 0.74	4.73 ± 0.81	6.50 ± 0.43
Ω_{arag}	1.37 ± 0.48	3.14 ± 0.54	4.32 ± 0.29
N	14	34	9

Figure 3.2. Total percent cover of calcareous algae in field survey by aragonite saturation level. A) Means (± 1 s.e.) of total percent cover. Letters indicate groups that are statistically different. B) Quadratic regression of total percent cover by saturation level. Quadratic regression fitted to raw data.

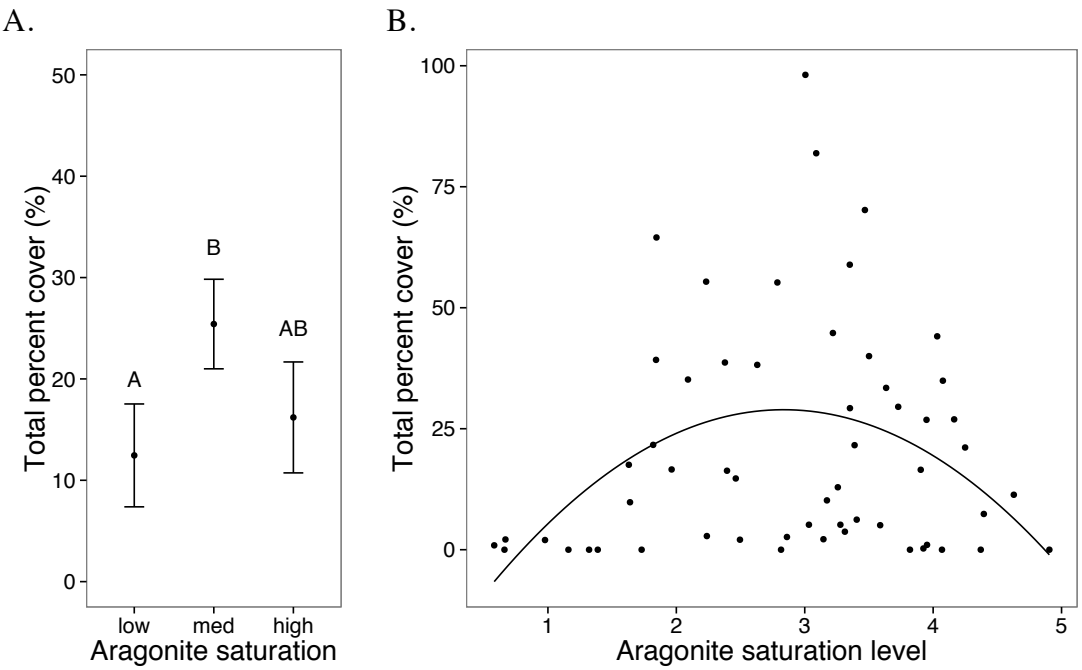


Table 3.2. Community composition. Percent of quadrats containing each genus (relative frequency of occurrence) by A) saturation level and b) Ojo.

A.

	Low	Medium	High
Hydrolithon	43 %	50 %	56 %
Peyssonnelia	36 %	53 %	56 %
Neogoniolithon	7 %	44 %	44 %
Halimeda	14 %	21 %	11%
Rhipidosiphon	7 %	21 %	11%
Lithophyllum	7 %	26 %	0 %
Amphiroa	0 %	15 %	0 %

B.

	Agua	Gorgos	Laja	Norte	Pargos
Hydrolithon	0 %	54%	83 %	50 %	80 %
Peyssonnelia	25 %	61 %	83 %	44 %	50 %
Neogoniolithon	75 %	53 %	17 %	13 %	10 %
Halimeda	0 %	0 %	33 %	19 %	50 %
Rhipidosiphon	17 %	8 %	17 %	6 %	40 %
Lithophyllum	17 %	8 %	33 %	13 %	30 %
Amphiroa	0 %	0 %	17 %	25 %	0 %

Figure 3.3. Genus richness and Shannon diversity index by aragonite saturation state.

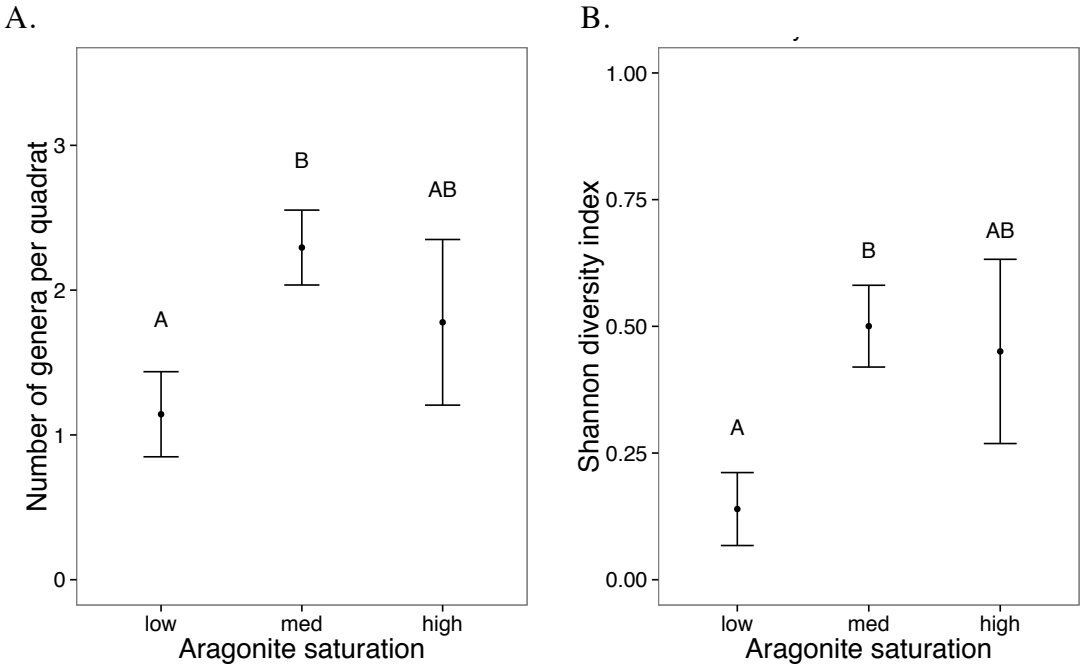


Figure 3.4. nMDS ordination: community structure by aragonite saturation level. nMDS ordination of community structure based on relative percent cover of genera. Each point is an individual quadrat. Ellipses represent 95% confidence intervals of mean location of points from each saturation level. nMDS ordination displays points relative to how similar they are to one another, thus points that are closer together represent quadrats with similar community structure.

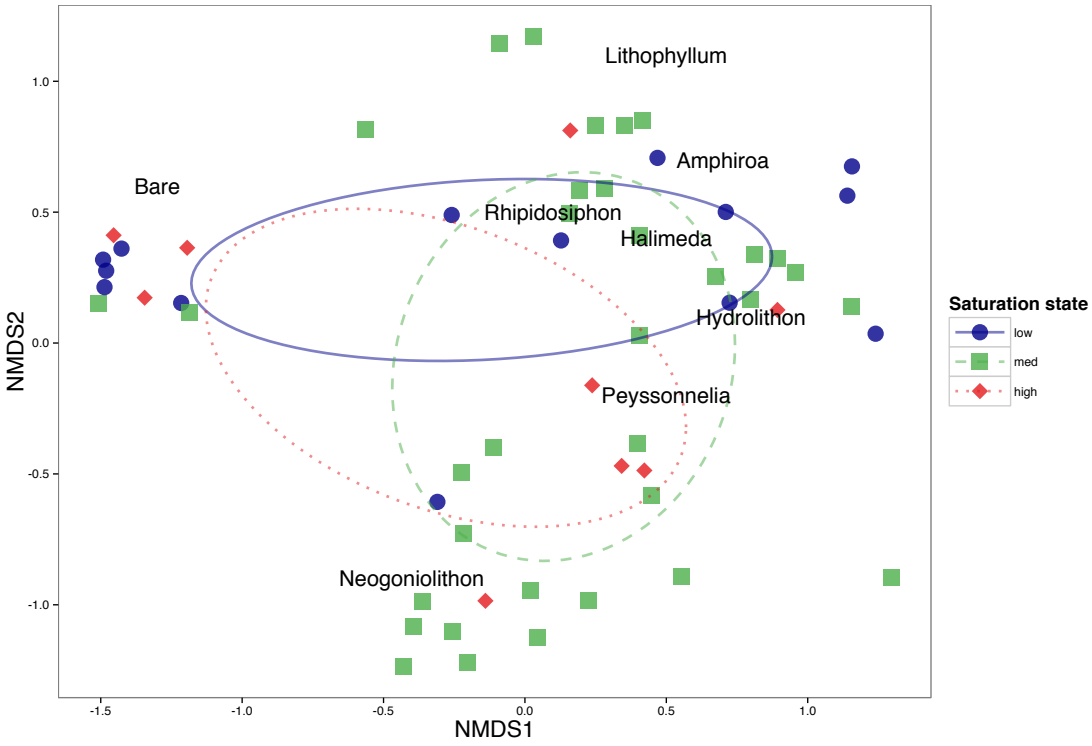
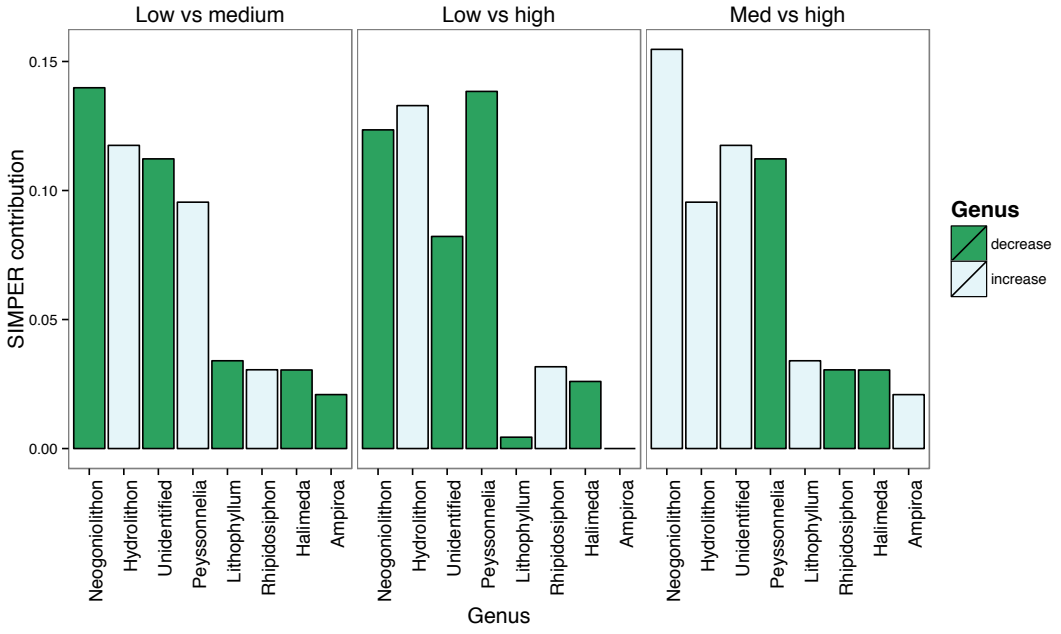


Figure 3.5. Community structure. A) Simper contribution plot. Bars represent the average contribution of each genus to overall community dissimilarity. Colors indicate whether the relative mean cover for each genus is lower or higher at the aragonite level being compared. B) Mean relative percent cover of each genus grouped by saturation level.

A) SIMPER contribution



B) Means of relative cover

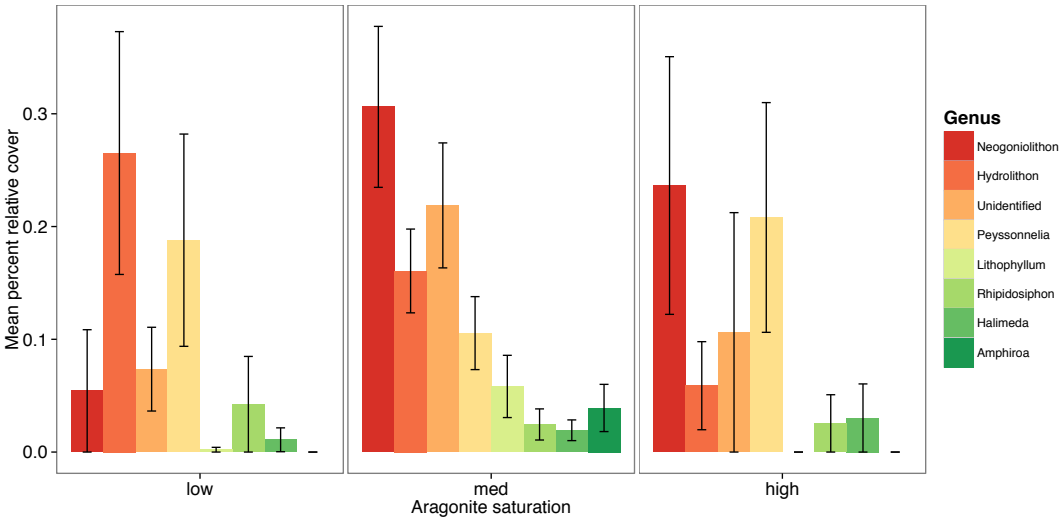


Figure 3.6. nMDS ordination: community structure by ojo. nMDS ordination of community structure based on relative percent cover of genera. Each point is an individual quadrat. Ellipses represent 95% confidence intervals of mean location of points from each ojo. nMDS ordination displays points relative to how similar they are to one another, thus points that are closer together represent quadrats with similar community structure.

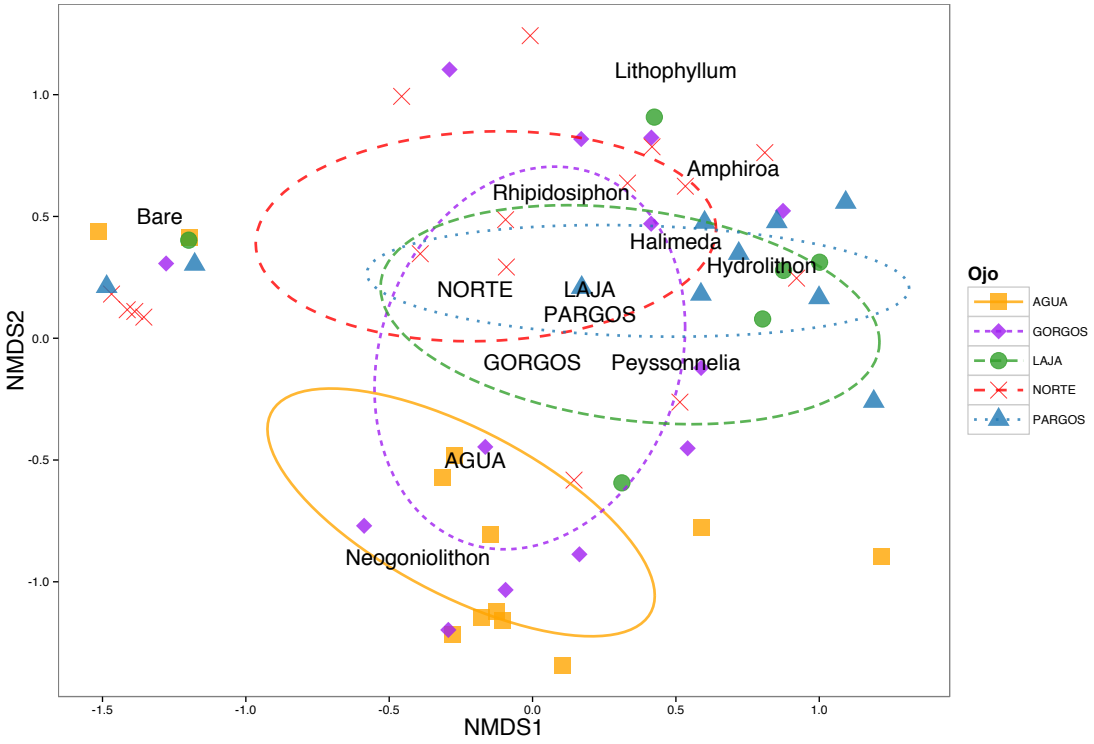
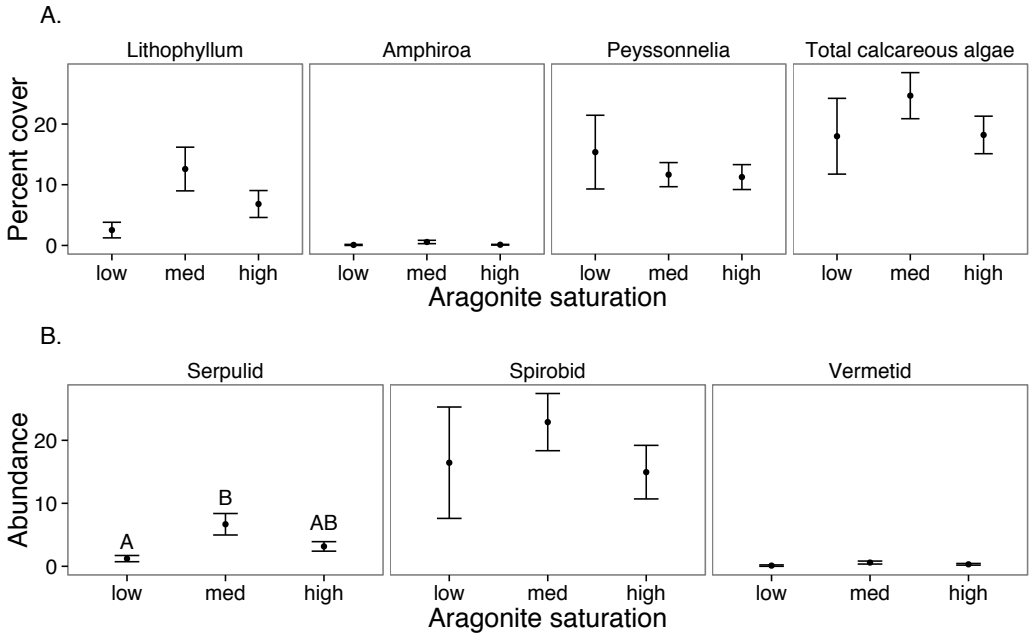


Table 3.3. Water chemistry parameters for Recruitment experiment.

	Low			Medium			High		
Salinity	32.01	±	2.55	35.39	±	1.59	34.48	±	1.42
C_T (µmol/kgsw)	2729	±	139	2146	±	223	2064	±	142
A_T (µmol/kgsw)	2690	±	210	2373	±	209	2501	±	207
Phosphate (µmol/kgsw)	0.34	±	0.16	0.08	±	0.06	0.15	±	0.09
Silicate (µmol/kgsw)	22.85	±	10.50	4.09	±	5.12	6.64	±	5.22
Ammonium (µmol/kgsw)	31.49	±	14.07	3.83	±	7.23	7.24	±	7.30
pH	7.23	±	0.26	7.92	±	0.07	8.18	±	0.13
pCO_2 (µatm)	4253	±	1694	592	±	162	300	±	84
Ω_{cal}	1.25	±	0.85	4.64	±	0.61	7.45	±	1.89
Ω_{arag}	0.81	±	0.55	3.09	±	0.41	4.72	±	1.66
N	9			36			20		

Figure 3.7. Recruitment on discs in 10-month experiment. A) Mean (± 1 s.e.) percent cover of calcifying algae by genus and B) abundance of other calcifying organisms by saturation state**.** Letters indicate groups that are significantly different from one another in Kruskal-Wallis non-parametric tests.



Conclusions

While ocean acidification is expected to have wide-ranging negative effects on marine species, differences in responses among species are likely to result in altered marine communities, although the traits explaining different responses to OA are largely unknown. The main objective of this thesis was to increase understanding of some of the patterns that may predict tolerance of future CO_2 levels. Specifically, I explored the responses to chronic acidification of one species that experiences regular variation in pH, *Euphausia pacifica*, and assessed the acclimation potential of different genera of calcifying algae. Understanding which traits confer tolerance of OA is a first step towards predicting how OA will shape future communities.

In Chapter 1, I focused on organisms currently living in environments that expose them intermittently to low pH values approaching those predicted for the future. These organisms may have physiological adaptations that permit them to tolerate prolonged exposure to high pCO_2 levels. Rates of molting and survival in *E. pacifica* were unaffected when exposed to chronic acidification levels predicted for 2100, suggesting at least a moderate tolerance of OA, compared to some other taxa (*e.g.,* heavily calcified corals). These results corroborate other studies on crustaceans and organisms that move regularly between high and low pH areas. We showed that growth of *E. pacifica* slowed at high pCO_2. Slower growth can result in increased predation, lower reproduction, and longer time to maturity, all factors that can negatively affect krill populations and the food webs they support.

In chapter 2 we demonstrated that the slower growth in *E. pacifica* is likely caused by lower metabolic rates (lower oxygen consumption, ingestion and nutrient excretion) under high pCO_2. Previous studies show varied metabolic responses of organisms to OA, with metabolism increasing, decreasing or showing no response to OA. Metabolic rate of organisms with superior acid-base regulating capabilities may increase, but then they increase their energy intake to accommodate the high ATP demands (*e.g.,* Antarctic krill). Conversely, reductions in metabolism may be due to insufficient acid-base buffering capacities, or passive changes in oxygen-binding proteins. Both of these strategies are successful during short-term exposures to elevated pCO_2, as with diel migration to depth, but with long-term OA exposure these strategies will either be limited by food availability (high metabolism) or by reduced growth and/or reproduction (lower metabolism). In general, *E. pacifica* tolerate OA and will likely fare better than many organisms, but as OA increases in surface and deep waters, fitness of *E. pacifica* may decline due to metabolic responses and/or the need to shift their vertical range that will require tradeoffs between predator protection and physiological needs.

In chapter 3, I explored the potential for OA-sensitive species of calcifying algae to acclimate or adapt to OA, by studying communities of calcifying algae occurring along a saturation (Ω_{arag}) gradient at naturally occurring, low pH submarine springs in Puerto Morelos, Mexico. Total percent cover, species richness and species diversity of calcareous algae all decreased with low saturation state. These results suggest that, as a functional group, calcifying algae may have little acclimation

potential, resulting in less substrate available for coral recruitment, less stable reef framework and less resilient reef ecosystems. However, not all species responded similarly to low saturation. Three genera, *Ampihroa, Neogoniolithon* and *Lithophyllum,* were absent or less abundant at low saturation levels suggesting that they are less tolerant of OA or are outcompeted by other species under low saturation conditions. Conversely, relative abundance was higher for *Hydrolithon* and *Peyssonnelia*, suggesting these genera may be able to persist in a low saturation environment. Our results provide mixed support for the hypothesis that early successional species, characterized by fast growth, but thin thalli, are more sensitive to OA than later successional species with thicker thalli. While much work is still needed to elucidate relationships among growth, thallus thickness and tolerance of high pCO_2, this research contributed important groundwork for describing species responses to OA. This work is an important first step in developing and testing further hypotheses about which traits are important in determining acclimation potential. Varied responses to ocean acidification among different algal species could have ramifications for coral reefs if vulnerable species are preferred substrate for coral settlement. Understanding which species perform well at low saturation and which do not will enhance our predictions of how coral reefs may perform in the future, and lead to make better management decisions for protecting coral reefs.