



Impacts of food availability and $p\text{CO}_2$ on planulation, juvenile survival, and calcification of the azooxanthellate scleractinian coral *Balanophyllia elegans*

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Abstract. Ocean acidification, the assimilation of atmospheric CO_2 by the oceans that decreases the pH and CaCO_3 saturation state (Ω) of seawater, is projected to have severe adverse consequences for calcifying organisms. While strong evidence suggests calcification by tropical reef-building corals containing algal symbionts (zooxanthellae) will decline over the next century, likely responses of azooxanthellate corals to ocean acidification are less well understood. Because azooxanthellate corals do not obtain photosynthetic energy from symbionts, they provide a system for studying the direct effects of acidification on energy available for calcification. The solitary azooxanthellate orange cup coral *Balanophyllia elegans* often lives in low-pH, up-welled waters along the California coast. In an 8-month factorial experiment, we measured the effects of three $p\text{CO}_2$ treatments (410, 770, and 1220 μatm) and two feeding frequencies (3-day and 21-day intervals) on “planulation” (larval release) by adult *B. elegans*, and on the survival, skeletal growth, and calcification of newly settled juveniles. Planulation rates were affected by food level but not $p\text{CO}_2$. Juvenile mortality was highest under high $p\text{CO}_2$ (1220 μatm) and low food (21-day intervals). Feeding rate had a greater impact on calcification of *B. elegans* than $p\text{CO}_2$. While net calcification was positive even at 1220 μatm (~ 3 times current atmospheric $p\text{CO}_2$), overall calcification declined by ~ 25 – 45 %, and skeletal density declined by ~ 35 – 45 % as $p\text{CO}_2$ increased from 410 to 1220 μatm . Aragonite crystal morphology changed at high $p\text{CO}_2$, becoming significantly shorter

but not wider at 1220 μatm . We conclude that food abundance is critical for azooxanthellate coral calcification, and that *B. elegans* may be partially protected from adverse consequences of ocean acidification in habitats with abundant heterotrophic food.

1 Introduction

As aqueous CO_2 concentrations continue to rise over the next century, pH of oceanic surface waters will decline in the process known as ocean acidification (Caldeira and Wicket, 2003, 2005; Sabine et al., 2004). Growing evidence suggests that calcifying organisms, including reef-building scleractinian corals, will be heavily impacted by a decrease in pH from 8.1 to 7.8 (Orr et al., 2005; Hoegh-Guldberg et al., 2007; Fabry et al., 2008; Doney et al., 2009). In many laboratory and field investigations, calcification rates of tropical corals declined as pH and aragonite saturation state (Ω_{arag} , a measure of ease of CaCO_3 formation) decreased (Fine and Tchernov, 2007; Anthony et al., 2008; Jokiel et al., 2008; Krief et al., 2010). Almost all tropical corals have algal symbionts (zooxanthellae) whose photosynthesis contributes to the host’s nutrition and increases calcification rates. Most deep and cold-water corals lack zooxanthellae, but their potential responses to ocean acidification are largely unknown; as the saturation state of seawater decreases, their calcification rates are also likely to decline and their geographical

distributions may change (Turley et al., 2007; Andersson et al., 2008; Fabry et al., 2009; Maier et al., 2009). Experiments indicate that cold-water species vary in sensitivity to CO_2 manipulation, and some deep-water species can maintain positive net calcification at or below the carbonate saturation horizon, the depth below which $\Omega < 1$ and CaCO_3 can dissolve readily (Form and Riebesell, 2012; McCulloch et al., 2012; Maier et al., 2011). Because azooxanthellate corals lack symbionts and rely solely on heterotrophy for energy, they provide a simplified system for exploring the roles of nutrition (and energy) in coral calcification.

Ocean acidification is predicted to have especially severe impacts in upwelling regions where low-saturation waters occur naturally (Feely et al., 2008; Fabry et al., 2009; Hauri et al., 2009). Strong seasonal upwelling along the western North American coast during summer months brings CO_2 -rich, low-pH water from intermediate depths to the surface, and coastal organisms may be exposed to low-saturation and even undersaturated waters for several months of the year (Feely et al., 2008; Hauri et al., 2009). With anticipated increases in surface ocean $p\text{CO}_2$ and shoaling of the carbonate saturation horizon, calcifying organisms living in these coastal waters are likely to experience seasonal increases in the magnitude, duration, and extent of low-pH waters (Feely et al., 2008; Hauri et al., 2009). Understanding the impacts of ocean acidification on calcification and on the interplay between calcification and nutritional status is critically important in upwelling regions where many organisms may already be living near their lower thresholds for pH tolerance (Barton et al., 2012) and therefore may be particularly vulnerable to ocean acidification. Several recent studies have described likely negative consequences of future ocean acidification events for several key species in the California coastal upwelling zone (Gaylord et al., 2011; Barton et al., 2012; Hettlinger et al., 2012; Timmins-Schiffman et al., 2012), which often experiences seawater pH as low as 7.8 during the summer.

Responses of calcifying corals to ocean acidification depend on species-specific energy allocations for calcification. Corals expend energy to remove protons from their calcifying compartments, the extracellular medium between the coral's basal cell membrane and the skeleton below (Al Horani et al., 2003; Allemand et al., 2004; Cohen and McConnaughey, 2003). Removing protons facilitates calcification by increasing pH and CaCO_3 saturation state in the calcifying fluid. In tropical zooxanthellate corals, proton pumping raises the saturation state in the calcifying fluid up to 5–10 times ambient (Al Horani et al., 2003; Cohen and Holcomb, 2009), and deep-water azooxanthellate corals can create even steeper gradients (McCulloch et al., 2012). Lower saturation in the external seawater requires corals to expend more energy to remove excess protons (Ries, 2011; McCulloch et al., 2012), potentially at the cost of other critical life processes (Wood et al., 2008). A flexible energy budget would enable corals to vary the energy expended to raise the pH and satu-

ration states of the calcifying fluids, and perhaps enable them to maintain calcification despite acidification. Some research indicates that zooxanthellate coral calcification is energetically costly and that a coral's energy budget is not flexible enough to raise the pH of the calcifying fluid under acidic conditions (i.e., the energy budget is fixed) (Cohen and Holcomb, 2009), but some species can maintain up to 100% of their calcification rates in near-undersaturated conditions when provided with excess nutrients (Langdon and Atkinson, 2005; Holcomb et al., 2010; Ries et al., 2009; Cohen et al., 2009). Nutrients may stimulate increased photosynthesis by zooxanthellae, giving the coral more energy for calcification. Similarly, increased heterotrophic feeding by certain zooxanthellate corals can reduce acidification impacts on calcification (Drenkard et al., 2013; Edmunds, 2011). All corals in these studies contained energy-producing zooxanthellae, whereas azooxanthellate corals cannot obtain extra photosynthetic energy from symbionts. Therefore azooxanthellate corals provide a system in which it is possible to study the direct effects of ocean acidification and energy availability on calcification.

Balanophyllia elegans is a solitary, azooxanthellate scleractinian coral common in shallow coastal waters around Monterey Bay, California, where it is exposed seasonally to low-pH, high- $p\text{CO}_2$ upwelling waters. We assessed the effects of $p\text{CO}_2$ and food availability on planulation rates of adult *B. elegans*, and then explored the effects of the same treatments on survival, growth, and calcification of juvenile *B. elegans* during an 8-month incubation experiment. The duration was based on recommendations of Doney et al. (2009) and Widdicombe et al. (2010) for long-term manipulation experiments. Recent evidence suggests that long-term exposure more accurately predicts responses to acidification than short-term experiments (Form and Riebesell, 2012). We address how azooxanthellate corals may respond to lower ocean pH, and the roles of nutrition on their calcification and survival in low-saturation, upwelling regimes.

2 Materials and methods

2.1 Organisms

The orange cup coral *Balanophyllia elegans* Verrill, 1864, is a solitary (single polyp) species living on rocky substrates from the low intertidal to ~ 300 m depth along the west coast of North America, from southern Alaska to Baja California. It is gonochoric (has separate sexes) with a sex ratio of approximately 1 : 1, and has an annual gametogenic cycle with gametes maturing in mid-summer. Fertilization is internal, and zygotes are brooded for about 15 months in the mother's coelenteron where they develop into mature planula larvae that are released in autumn to early winter (Fadlallah and Pearse, 1982). On release (planulation), the larvae crawl down the mother's column and settle and metamorphose into

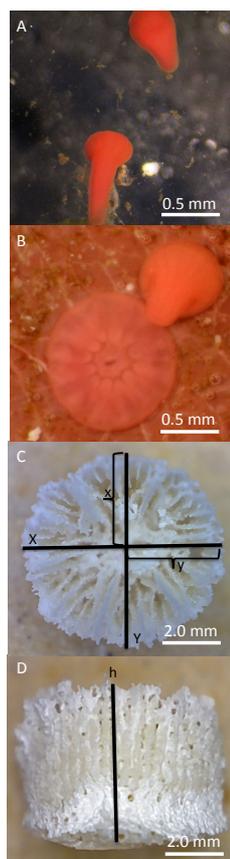


Fig. 1. *Balanophyllia elegans* planula larvae (a), newly settled juveniles (b), and a juvenile skeleton after 8 months of growth from above (c), and in longitudinal section (d). Skeletal measurements are indicated in (c) and (d). The major and minor axes of the elliptical cylinder (X, Y) were measured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

juvenile corals (Fig. 1a, b), usually within a few centimeters of the mother (Gerrodette, 1981). *B. elegans* is an azooxanthellate species (lacking photosynthetic symbiotic algae) depending on heterotrophic feeding on zooplankton or dissolved organic molecules for all of its energy and nutrients. It grows slowly and can survive for months without feeding.

2.2 Experimental design

The experiment used a full factorial design with two factors: $p\text{CO}_2$ (3 levels) and feeding frequency (2 levels). The seawater $p\text{CO}_2$ levels were based on recent atmospheric concentrations (380 ppm, $\text{pH}_T = 8.0$) and two IPCC emissions scenarios projected for the year 2100, the A1B “business as usual” (750 ppm, $\text{pH}_T = 7.8$), and a high emissions scenario (A1F1) approximately 3 times current atmospheric $p\text{CO}_2$ (1200 ppm, $\text{pH}_T = 7.6$) (Solomon et al., 2007). Experimental $p\text{CO}_2$ levels were slightly higher than these targets at 410 μatm ($\text{pH}_T = 8.0$), 770 μatm ($\text{pH}_T = 7.8$), and

1220 μatm ($\text{pH}_T = 7.6$). High-food corals were fed newly hatched nauplii larvae of brine shrimp (*Artemia*) every 3 days to represent a plentiful food supply, while low-food corals were fed once every 21 days, corresponding to a minimal maintenance food supply (Beauchamp, 1989).

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

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

Corals were fed through the sampling port using a syringe to inject 50 mL of concentrated *Artemia* nauplii in filtered seawater (approximately 10 000–15 000 nauplii per jar). The brine shrimp remained in the jars for several hours to ensure the corals had eaten their fill. Every jar, lid, and paddle was cleaned once every 3 days after feeding ended. During cleaning, corals were removed for approximately 30 min and placed in small glass dishes with filtered seawater equilibrated to their experimental $p\text{CO}_2$. The jars and lids were then scrubbed and rinsed, and newly filtered, equilibrated seawater was siphoned into the jars to prevent air exchange.

2.3 Experimental corals

Two groups of corals were exposed simultaneously to the experimental treatments. The first group consisted of adults to determine whether $p\text{CO}_2$ and feeding treatment affected planulation rates. Ten adult corals of equal size (sex unknown) were assigned to each treatment (i.e., five adults per jar) for the first 3 months of the experiment (6 November 2011 through 31 January 2012). The 60 adults had been held in the same tank for more than 2 yr with flowing ambient seawater ($\text{pH}_T \sim 7.9$ –8.0) at the UCSC Long Marine Laboratory (LML). Because they brood larvae for ~15 months (Fadlallah and Pearse, 1982), all females should have been equally likely to produce larvae. Every 3 days all larvae produced were counted and removed from the experimental jars.

The second group consisted of newly settled juveniles to determine whether CO_2 and feeding levels affect juvenile survival, growth, and calcification. The juveniles came from a stock of adult *B. elegans* maintained for several generations in the laboratory. Approximately 85 adults were sequestered in a tank with flowing ambient seawater during the peak planulation season (November to December 2011). Emerging

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

2.4 Seawater carbonate chemistry

The LML is on the open coast north of Monterey Bay where it is exposed to oceanic water driven by prevailing onshore winds. Seawater supplied to LML is sand-filtered down to 30 μm before being pumped into elevated water towers from which it flows under gravity to individual laboratories: it is an open flow-through water system with no seawater recirculation. Experimental seawater was filtered to 0.2 μm in \sim 800 L batches to ensure the experiment was not subject to ambient fluctuations in seawater chemistry (4 water batches were prepared at approximate 8-week intervals during the experiment). Cylinders of certified CO_2 -air mixtures were obtained from PraxAir (CO_2 at 380, 750, and 1200 ppmV). Filtered water was sampled for salinity and nutrient analyses, transferred to 20 L carboys, and then bubbled with the appropriate gas mixture for at least 4 days to equilibrate $p\text{CO}_2$ and stabilize pH before capping and storing until needed. Water was siphoned from the carboys into the experimental jars, which were sealed except for the appropriate $p\text{CO}_2$ gas mixture flowing continuously into the headspace.

pH and temperature were measured daily in each jar with an Oakton WD-35613 handheld meter calibrated using NIST standards. In addition, 40 mL water samples were taken from each jar every 3 days for dissolved inorganic carbon (C_T) and total alkalinity (A_T) analyses (samples were obtained on cleaning days, before feeding and before the water was changed). C_T was measured using a CM5011 carbon coulometer (UIC, Inc.), and A_T was measured with an automated, open-cell potentiometric titration procedure. Certified reference materials (batch 118) from the Andrew Dickson laboratory at Scripps Institution of Oceanography were used to calibrate each instrument. C_T and A_T were used to calculate aragonite saturation state (Ω_{arag}) and pH via CO_2 Sys software (Pierrot et al., 2006), using CO_2 dissociation constants from Merzbach et al. (1973) refit by Dickson and

Millero (1987). pH is reported in total scale (pH_T). Salinity was measured with a salinometer (Guildline 8410 PortaSal), and nutrients were analyzed on a flow injection autoanalyzer (FIA, Lachat Instruments Model QuickChem 8000) for each of the four batches of water using standard operating procedures.

2.5 Skeletal growth

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

$$V = \pi xyh. \quad (1)$$

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

Septa (vertical elements partially dividing the cavity of the skeleton) from five randomly selected skeletons from each of the four most extreme treatments (high food and low food with $p\text{CO}_2$ of 410 and 1220 μatm) were imaged with 10kx magnification on a Hitachi TM1000 tabletop scanning electron microscope (SEM) at the UCSC MACS facility at NASA/Ames. For the SEM analysis, only the extreme $p\text{CO}_2$ groups were used to ensure maximum differences between treatments were captured. The lengths and widths of individual aragonite crystals were measured from the SEM images using imaging processing and analysis in Java (Image J, US National Institute of Health, Fig. S1).

2.6 Statistics

The software R was used for all statistical analyses (R Core Team 2013). Planulation, volume, weight, density and crystal dimensions were analyzed using 2-factor ANOVAs (analyses of variance) with $p\text{CO}_2$ and feeding frequency as fixed factors. For planulation, data from replicate jars were combined, so an additive model was applied since interaction terms could not be assessed without replication. All other ANOVAs used a full model including both the main effects and the interactions between $p\text{CO}_2$ and feeding frequency. Coral volumes and weights were log-transformed to satisfy normality assumptions. For crystal dimension analyses, the individual corals from which crystals were sampled were treated as a random factor nested within the two main effects. Where statistical significance was indicated, Tukey's HSD tests were used to compare treatments. Juvenile survival was assessed by a logistic regression against the two categorical predictor variables, $p\text{CO}_2$ and feeding frequency. Since the

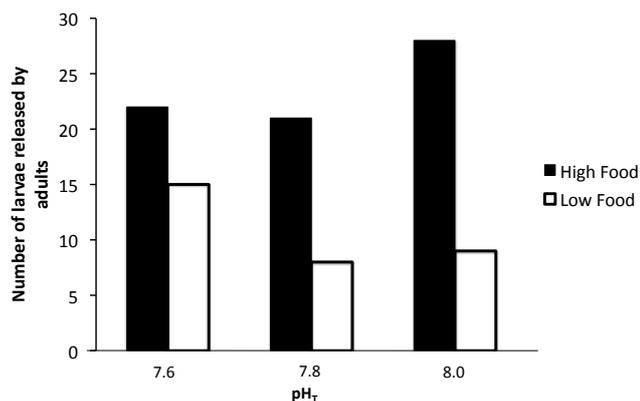


Fig. 2. Numbers of planula larvae released by 10 *Balanophyllia elegans* adults over 3 months in each pH_T X food treatment.

interaction term was not significant (Tukey's test for additivity; $p = 0.41$), it was excluded from the logistic regression model. All water chemistry is reported as mean \pm standard deviation (s.d.).

3 Results

3.1 Water chemistry

Average water conditions in the six treatments during the 8-month experiment are summarized in Table 1. The average $\text{pH} \pm \text{s.d.}$ values (expressed as total scale, pH_T) of the three $p\text{CO}_2$ treatments, calculated from measurements of C_T and A_T from discrete water samples, were 8.02 ± 0.02 ($410 \pm 21 \mu\text{atm}$), 7.78 ± 0.03 ($770 \pm 75 \mu\text{atm}$), and 7.59 ± 0.02 ($1220 \pm 80 \mu\text{atm}$). The corresponding aragonite saturation states (Ω_{arag}) of each $p\text{CO}_2$ treatment were 2.1 ± 0.05 (pH_T 8.0), 1.3 ± 0.1 (pH_T 7.8), and 0.9 ± 0.04 (pH_T 7.6). C_T and A_T measurements varied slightly among treatments, but these differences were not significant (Student's t tests), and they did not affect average values of the other carbonate parameters. Temperature in the jars varied seasonally with the ambient temperature of the water table containing the jars, but averaged $13.6 \pm 1.5^\circ\text{C}$ over the duration of the experiment.

3.2 Planulation

B. elegans planula larvae were collected as they emerged from adults every 3 days for 3 months (6 November 2011 to 15 January 2012). Total planula numbers from each treatment were counted (Fig. 2) and compared in a 2-way ANOVA (Supplement Table 1S). Adult corals in the high-food treatments released more than twice as many larvae (120% more overall) compared to those in the low-food treatments ($p = 0.064$), but $p\text{CO}_2$ had no effect on numbers of larvae released ($p = 0.628$, Table 1S).

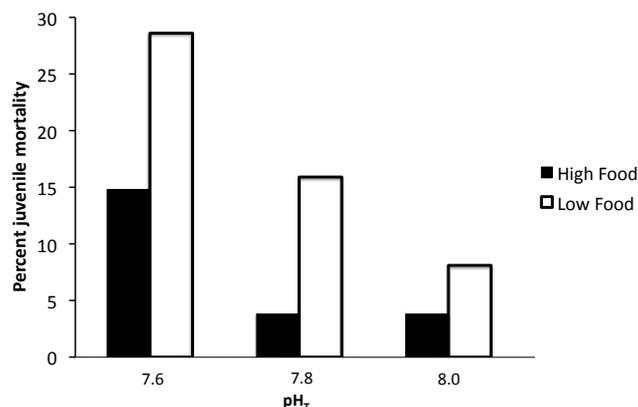


Fig. 3. Percent mortality of juvenile *Balanophyllia elegans* over 84 days in each pH_T X food treatment. Initial numbers varied from 26 to 44 for a total of 202 juveniles.

3.3 Juvenile mortality

During the 8-month experiment, 14% of the total 202 experimental juveniles died (Fig. 3). Approximately 5–15% more juvenile corals died in low-food than in high-food treatments ($p = 0.043$, Table 2S). In both the low-food and high-food groups, 10–20% more juveniles died in the lowest $p\text{CO}_2$ treatment (pH_T 7.6) than in the control (pH_T 8.0) treatment ($p = 0.011$); mortality in the pH_T 7.8 treatment was intermediate, but did not differ significantly from the control ($p = 0.38$, Table 2S).

3.4 Skeletal characteristics

Juvenile corals from high-food treatments had significantly larger skeletons (6–7 times by volume; $p < 0.001$, Table 3S, Fig. 4a) and were also heavier (4–5 times; $p < 0.001$, Table 4S, Fig. 4b) than those from low-food treatments. Although pH_T had no significant effects on skeletal volume ($p = 0.303$), it did affect skeletal weight under the low-food regime: skeletons of corals grown at pH_T 8.0 weighed significantly more (by $\sim 45\%$) than those at pH_T 7.8 ($p < 0.001$) and pH_T 7.6 ($p = 0.001$) (Tukey's HSD tests). Under the high-food regime, corals grown at pH_T 8.0 also weighed more than those in lower pH_T treatments, but the differences were smaller ($\sim 25\%$) and not statistically significant ($p = 0.350$, $p = 0.060$).

Bulk densities of low-food coral skeletons were approximately 35–40% greater than high-food skeletons in both the pH_T 7.8 ($p = 0.008$) and pH_T 7.6 treatments ($p = 0.04$), but density did not differ significantly between feeding treatments at pH_T 8.0 ($p = 0.11$, Table 5S, Fig. 4c). At pH_T less than 8.0, skeletal density was approximately 35–45% lower in both the high-food ($p = 0.032$, $p = 0.001$ at pH_T 7.8 and 7.6 respectively) and low-food ($p = 0.010$, $p = 0.043$) treatments (Tukey's HSD tests).

Table 1. Mean \pm standard deviation (s.d.) of experimental chemical conditions from November 2011 to July 2012. High-food corals were fed once every 3 days, and low-food corals were fed once every 21 days.

	1200 ppm High food	1200 ppm Low food	750 ppm High food	750 ppm Low food	380 ppm High food	380 ppm Low food
A_T mol/kg	2217 \pm 9	2219 \pm 9	2221 \pm 12	2222 \pm 9	2220 \pm 8	2221 \pm 11
C_T mol/kg	2183 \pm 12	2184 \pm 15	2127 \pm 10	2129 \pm 12	2034 \pm 10	2037 \pm 12
$p\text{CO}_2$ μatm	1220 \pm 80	1220 \pm 80	770 \pm 75	770 \pm 75	410 \pm 21	410 \pm 21
pH_T	7.59 \pm 0.02	7.59 \pm 0.02	7.78 \pm 0.03	7.78 \pm 0.03	8.02 \pm 0.02	8.02 \pm 0.02
Ω_{arag}	0.9 \pm 0.04	0.9 \pm 0.04	1.3 \pm 0.10	1.3 \pm 0.10	2.1 \pm 0.08	2.1 \pm 0.08
Temp ($^\circ\text{C}$)	13.6 \pm 1.5	13.6 \pm 1.5	13.6 \pm 1.5	13.6 \pm 1.5	13.6 \pm 1.5	13.6 \pm 1.5
Salinity	33.3 \pm 0.1	33.3 \pm 0.1	33.3 \pm 0.1	33.3 \pm 0.1	33.3 \pm 0.1	33.3 \pm 0.1
SiO_2 $\mu\text{mol kg}^{-1}$	7.6 \pm 0.9	7.6 \pm 0.9	7.6 \pm 0.9	7.6 \pm 0.9	7.6 \pm 0.9	7.6 \pm 0.9
PO_4^{3-} $\mu\text{mol kg}^{-1}$	0.3 \pm 0.05	0.3 \pm 0.05	0.3 \pm 0.05	0.3 \pm 0.05	0.3 \pm 0.05	0.3 \pm 0.05
NO_3^- $\mu\text{mol kg}^{-1}$	3.4 \pm 0.6	3.4 \pm 0.6	3.4 \pm 0.6	3.4 \pm 0.6	3.4 \pm 0.6	3.4 \pm 0.6

3.5 Crystal structure

Aragonite crystals were significantly longer ($\sim 18\%$) at pH_T 8.0 than at pH_T 7.6 ($p < 0.001$; Table 6S, Fig. 5a). There was also a significant effect of food due mainly to a strong interaction between pH_T and food level ($p < 0.001$): at pH_T 7.6, crystals were $\sim 15\%$ longer in high-food than low-food treatments ($p < 0.001$), but lengths were almost identical at pH_T 8.0. By contrast, there were no significant effects of either pH_T ($p = 0.93$) or food level ($p = 0.41$) on crystal width (Table 7S, Fig. 5b).

4 Discussion

Responses of calcifying organisms to ocean acidification are likely to vary at different stages of their life cycles, and several studies provide evidence that early stages (larvae and juveniles) of many marine taxa are particularly sensitive to acidification (Kroeker et al., 2010, 2013; Hettlinger et al., 2012). Therefore, early-stage organisms in upwelling regions may be particularly susceptible to negative stressors associated with decreasing pH. Hettlinger et al. (2012) also showed that adverse effects of stress during larval stages can “carry over” to successive developmental stages and be compounded by the time adulthood is reached. In the present study, $p\text{CO}_2$ had no effect on the numbers of brooded planulae larvae released, but higher food levels increased the numbers of larvae released by 50–200% (Fig. 2). Because females brood for approximately 15 months, and because the adults were exposed to the experimental conditions for only a few days to weeks immediately preceding the peak planulation season, this suggests that females may delay planulation

until conditions are optimal. It may be that high food levels are needed by the female to complete maturation of larvae conceived many months earlier, or to sustain non-feeding larvae after release during dispersal, settlement and metamorphosis into a feeding polyp. It is also possible that food level acts indirectly as a proxy for correlated environmental conditions (biotic or abiotic) that may enhance juvenile survival after metamorphosis.

Due to the 15 months of brooding before release, this experiment provides neither information about possible impacts of prolonged high $p\text{CO}_2$ or low food on adult nutrition or reproduction, nor about whether prolonged exposure to high $p\text{CO}_2$ negatively impacts such processes as gametogenesis, fertilization, cleavage or early larval development that have been seen in other organisms (Kurihara, 2008; Kroeker et al., 2010, 2013; Nakamura et al., 2011). It also does not address whether prolonged exposure to low pH or high $p\text{CO}_2$ acts directly on reproductive processes, or indirectly via diverting energetic resources away from reproduction.

While $p\text{CO}_2$ did not affect numbers of planulae released, mortality of newly settled juvenile corals was substantially greater at the highest $p\text{CO}_2$ level (1220 μatm , $\text{pH}_T = 7.6$), with average mortality about 10% higher (across both food levels) than in the other $p\text{CO}_2$ treatments (410 and 770 μatm) (Fig. 3). However, high food did seem to alleviate some of the stress associated with high $p\text{CO}_2$: at 1220 μatm , high-food conditions increased juvenile survival by 15% over low-food conditions, but not by enough to counter the decline due to low $p\text{CO}_2$. This pattern suggests that, if atmospheric $p\text{CO}_2$ increases beyond the projected 750 ppm over the next century, the numbers of corals surviving to become adults may

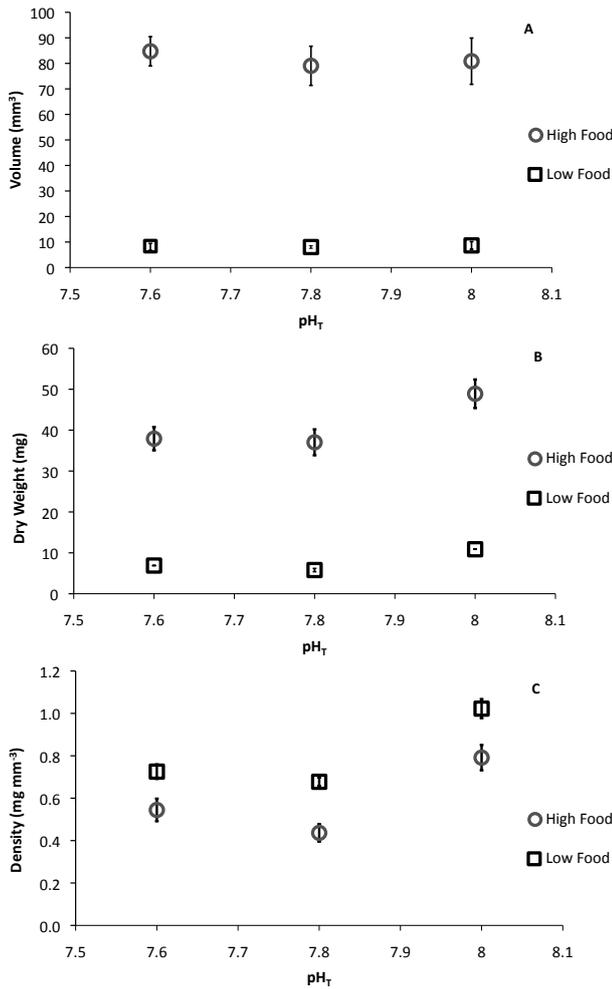


Fig. 4. Means \pm s.e.m. for volumes (a), dry weights (b), and bulk densities (c) of skeletons of juvenile *Balanophyllia elegans* after 8 months growth.

decline; even if food is always plentiful, it is unlikely that juvenile mortality will be unaffected by $p\text{CO}_2$.

Food availability was the major factor controlling the growth (and final size) of newly settled *Balanophyllia elegans* juveniles in this experiment (Fig. 4a); $p\text{CO}_2$ had no significant effects on final volume. After 8 months of growth, high-food skeletons were up to 7 times larger (by volume) than low-food skeletons at every $p\text{CO}_2$ level. Although corals within a food level had similar volumes across all three $p\text{CO}_2$ treatments (Fig. 4a), their skeletal weights (and hence bulk densities) significantly decreased from 410 μatm to 1220 μatm $p\text{CO}_2$ (Fig. 4b, c). This suggests that with increasing $p\text{CO}_2$, either the shapes or spacing of skeletal elements changed, or there was less secondary thickening of the initial skeleton. One possible mechanism is that energy available for calcification is allocated first to ensuring full skeletal extension, at the cost of a less heavily calcified skeleton.

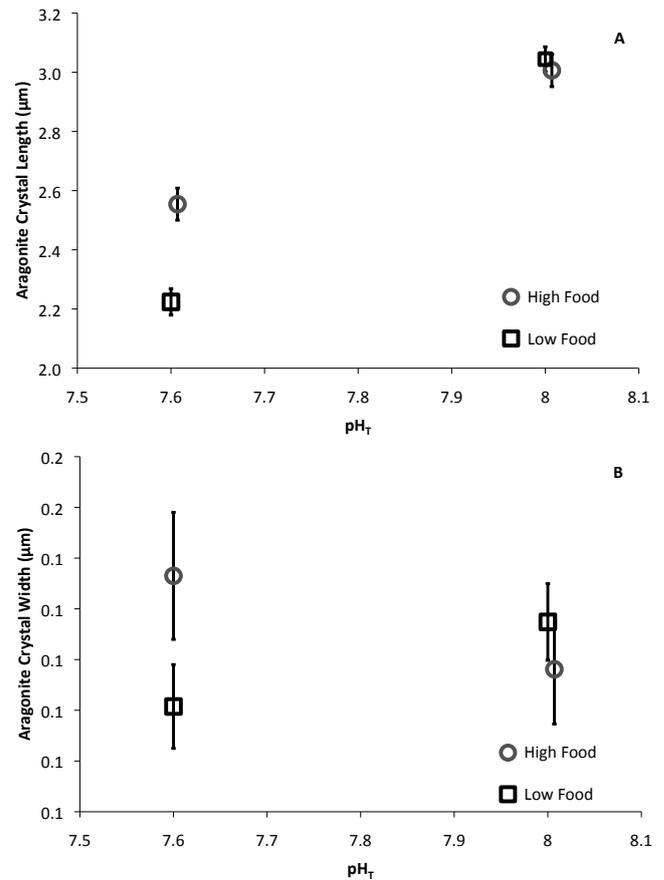


Fig. 5. Means \pm s.e.m. of aragonite crystal lengths (a), and crystal widths (b) obtained from scanning electron microscope (SEM) images of septa from 20 juvenile *Balanophyllia elegans*.

Calcification rates in reef-building corals are often measured as the annual linear extension multiplied by the bulk density, and expressed in $\text{g cm}^{-2} \text{yr}^{-1}$. Because *B. elegans* is a solitary species, its radial expansion must be considered in the calculation of calcification rates. Therefore, we express calcification as the change in the total skeletal weight measured over known intervals and normalized to g yr^{-1} per coral. In every $p\text{CO}_2$ treatment, higher food led to both greater linear extension and greater calcification (skeletal weight) over the 8-month experiment, a trend that is consistent with more energy being allocated to skeletal formation. While linear dimensions were unaffected by $p\text{CO}_2$, well-fed corals had heavier skeletons; in particular, calcification by high- $p\text{CO}_2$, high-food corals was 4 times greater than in low-food corals at ambient $p\text{CO}_2$. However, higher $p\text{CO}_2$ negatively impacted bulk densities (Fig. 4c), and the reduction of skeletal density by $\sim 35\%$ with increasing $p\text{CO}_2$ suggests that structural integrity of the skeletons may be weakened, leaving these corals more vulnerable to predation, bioerosion, and dislodgement (Hoegh-Guldberg et al., 2007).

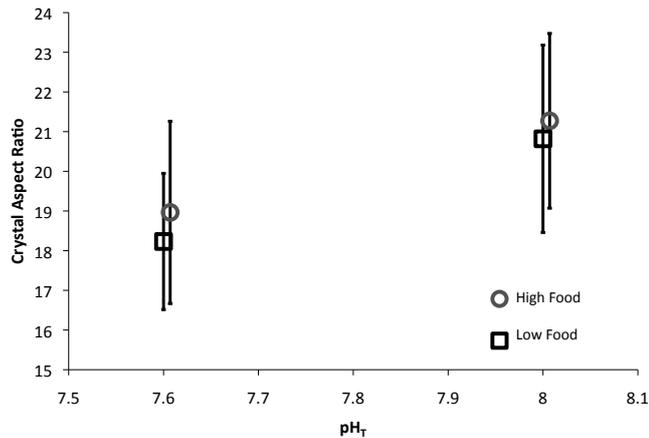


Fig. 6. Mean crystal aspect ratios \pm s.d. of aragonite crystals in septa of juvenile *Balanophyllia elegans*, calculated by dividing the crystal length by the crystal width for individual crystals.

While high $p\text{CO}_2$ caused overall reductions in density ($\sim 35\%$), low-food skeletons were actually denser than high-food skeletons in all $p\text{CO}_2$ treatments (Fig. 4c), even though the former grew less and weighed less after 8 months. This response may be analogous to that of many colonial, zooxanthellate reef-building corals in which rapid linear extension and low-density skeletons dominate under favorable conditions, while slower growing, denser skeletons form during less favorable conditions (Highsmith, 1979).

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

$$\Omega_{\text{cf}} = 0.93 (\pm 0.06) \cdot \text{crystal aspect ratio} + 0.20 (\pm 0.89). \quad (2)$$

In our experiment, high $p\text{CO}_2$ significantly reduced crystal length, with crystals being approximately 18% longer in $410 \mu\text{atm}$ than in $1220 \mu\text{atm}$ $p\text{CO}_2$ corals (Fig. 5a). Crystal width did not vary significantly between feeding frequency or $p\text{CO}_2$ treatments (Fig. 5b), but the crystal aspect ratio was higher at ambient $p\text{CO}_2$ ($p = 0.028$, Fig. 6). Using Eq. (2), we calculated that Ω_{cf} was ~ 20 and ~ 19 for high- and low-food corals respectively at $410 \mu\text{atm}$, and ~ 18 and ~ 17 for high- and low-food corals at $1220 \mu\text{atm}$. Even in well-fed corals at $1220 \mu\text{atm}$, calculated Ω_{cf} was slightly lower than in corals grown at ambient $p\text{CO}_2$. One explanation is that these corals were unable to expel enough protons un-

der high- $p\text{CO}_2$ conditions to calcify at rates similar to those at ambient $p\text{CO}_2$, even when provided with plentiful high-energy food. However, the fact that crystals from high-food corals at $1220 \mu\text{atm}$ were significantly longer (by about 15%) than crystals from low-food corals caused slight increases in both measured crystal aspect ratio and estimated Ω_{cf} . This is consistent with our calcification data and may indicate that excess food enables corals to counteract partially some of the negative impacts of lower saturation states under higher $p\text{CO}_2$ conditions.

Combining these lines of evidence suggests that *Balanophyllia elegans* is able to maintain moderate calcification rates even during extreme acidification conditions, provided they also have a plentiful nutritional supply. *B. elegans* and other efficient filter feeders that do not have zooxanthellae may be able to maintain their energy reserves under physiologically stressful conditions by increasing their feeding rates (provided sufficient prey are available). Our experiment suggests that even feeding on planktonic crustacean only once every 21 days was still sufficient to maintain positive growth at high $p\text{CO}_2$, albeit very slowly. Removing protons to increase pH and saturation state in the calcifying compartment may be energetically costly, and energetic demands for maintaining the saturation state of calcifying fluids are likely to rise with increasing $p\text{CO}_2$, so the total amount of CaCO_3 deposited is likely to decline even though extension rates are maintained. The decreased calcification at moderate to high $p\text{CO}_2$ that we observed regardless of feeding amount suggests that even well-fed corals cannot entirely overcome the stresses of ocean acidification.

Our observation that heterotrophic feeding rate has a greater impact on calcification than pH may explain the ability of *B. elegans* and other calcifying organisms to survive in upwelling waters and tolerate low saturation. When pH is lowest during upwelling events, nutrient and plankton concentrations are often at their highest in Monterey Bay. Indeed, nutrient concentrations during the upwelling months can be up to 20 times greater than during non-upwelling periods (Pennington and Chavez, 2000). This nutrient surplus accelerates phytoplankton (and subsequent zooplankton) production, and should increase the amounts of heterotrophic food available to corals and other benthic filter feeders. Zooplankton concentrations in Monterey Bay can be up to 10 times higher during upwelling than during non-upwelling months, and often peak about the time of maximum planulation by *B. elegans* (Marinovic et al., 2002). This may suggest that, should acidification be decoupled from upwelling, *B. elegans* calcification may be more negatively impacted by lower food concentrations than low pH, but as long as food availability remains high, *B. elegans* may be able to largely compensate for the extra energy required for calcification at low saturations, even if calcification occurs at slightly lower rates than at modern $p\text{CO}_2$.

Supplementary material related to this article is available online at <http://www.biogeosciences.net/10/7599/2013/bg-10-7599-2013-supplement.zip>.

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