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Sponge erosion under acidification and warming scenarios: differential impacts on living and dead coral

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Abstract

Ocean acidification will disproportionately impact the growth of calcifying organisms in coral reef ecosystems. Simultaneously, sponge bioerosion rates have been shown to increase as seawater pH decreases. We conducted a 20-week experiment that included a 4-week acclimation period with a high number of replicate tanks and a fully orthogonal design with two levels of temperature (ambient and +1 °C), three levels of pH (8.1, 7.8, and 7.6), and two levels of boring sponge (*Cliona varians*, present and absent) to account for differences in sponge attachment and carbonate change for both living and dead coral substrate (*Porites furcata)*. Net coral calcification, net dissolution/bioerosion, coral and sponge survival, sponge attachment, and sponge symbiont health were evaluated. Additionally, we used the empirical data from the experiment to develop a stochastic simulation of carbonate change for small coral clusters (i.e., simulated reefs). Our findings suggest differential impacts of temperature, pH and sponge presence for living and dead corals. Net coral calcification (mg $CaCO_3$ cm^{-2} day^{-1}) was significantly reduced in treatments with increased temperature (+1 °C) and when sponges were present; acidification had no significant effect on coral calcification. Net dissolution of dead coral was primarily driven by pH, regardless of sponge presence or seawater temperature. A reevaluation of the current paradigm of coral carbonate change under future acidification and warming scenarios should include ecologically relevant timescales, species interactions, and community organization to more accurately predict ecosystem-level response to future conditions.

Keywords: boring sponge, climate change, *Cliona*, coral reef ecosystems, ocean acidification, *Porites*, simulation model, sponge bioerosion

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Introduction

Global atmospheric CO_2 ($CO_{2\ atm}$) has risen at an unprecedented rate, bypassing the forewarned tipping point of 350 ppm (Veron *et al*., 2009) and exceeding 400 ppm (NOAA/ESRL). Largely driven by anthropogenic emissions, the increase in $CO_{2\ atm}$ has already begun to disturb climate patterns, raise mean global temperatures, and alter ocean chemistry (IPCC, 2013; Bates *et al*., 2014). The ocean has warmed by approximately 0.5 °C since the 1970s and is projected to increase an additional 1.8–4 °C by the end of the century (IPCC, 2013). The partial pressure of CO_2 in seawater (pCO_2) at multiple ocean observatories has increased by 1.28–2.95 μatm since 1984, with corresponding decreases of 0.0013–0.0026 pH units (Bates *et al*., 2014). Projected decreases in surface ocean pH range from 0.06 to 0.32 by the end of the century (15–109% increase in acidity; IPCC 2013).

Decreases in seawater pH and subsequent reductions in the aragonite saturation state ($\Omega_{aragonite}$), known as ocean acidification (OA), will disproportionately impact calcifying organisms as available carbonate ion (CO_2^{3-}) concentrations decrease (Zeebe & Wolf-Gladrow, 2001; Orr *et al*., 2005). Ocean acidification will have substantial consequences for ecosystems engineered by calcifying organisms, such as coral reefs. Coral reefs are one of the most vulnerable ecosystems to predict seawater chemistry changes; reduced calcification rates have been reported across a range of coral taxa exposed to experimentally altered pH or pCO_2 levels (e.g. Hoegh-Guldberg *et al*., 2007; Anthony *et al*., 2008, 2011). While species vary in degree of vulnerability, the effect of acidification on coral health, calcification, survival, and reproduction has been shown to be overwhelmingly negative (Marubini *et al*., 2008; Anthony *et al*., 2011; Nakamura *et al*., 2011; Dufault *et al*., 2012; McCulloch *et al*., 2012).

Concurrent inflation of sea surface temperatures as a result of elevated $CO_{2\ atm}$ will further impact coral calcification and survival on reefs. Many coral species are residing close to, or at, the limits of their physiological thermal tolerance (Goreau & Hayes, 1994) and may experience reductions in calcification rates as temperatures rise (Jokiel & Coles, 1977). Additionally, corals harboring symbiotic zooxanthellae are particularly sensitive to temperature changes, as many clades of

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zooxanthellae are unable to tolerate short-term anomalous temperature increases (or decreases) and may disassociate themselves from the corals. The expulsion or loss of zooxanthellae, termed coral bleaching, often results in coral mortality (Hoegh-Guldberg, 1999). As shallow coastal waters experience record-breaking summer temperature maxima at a higher frequency and for longer durations (Hoegh-Guldberg, 1999; Erez *et al*., 2011), corals will undoubtedly incur reductions in calcification and survival (Hoegh-Guldberg *et al*., 2007).

As coral calcification rates are reduced by acidification and temperature increases, reef accretion rates are expected to decline, slowing the overall growth rate of reefs and reducing the resilience of these ecosystems. Simultaneously, the physical, chemical, and biological breakdown of carbonate may be accelerated as reefs are subjected to stressors (e.g., rising temperatures) that result in elevated coral mortality and reduced coral cover (DeCarlo *et al*., 2014). Widespread declines in coral coverage facilitate increased bioeroder abundance (Rose & Risk, 1985; Holmes, 2000; Lopez-Victoria & Zea, 2004; Carballo *et al*., 2013) and may result in reefs shifting from net accretion to net erosion (Enochs *et al*., 2015).

Increased bioerosion rates under acidification scenarios have been reported for several bioeroding taxa, including algae (Tribollet *et al*., 2009), polychaetes, lithophagid bivalves (DeCarlo *et al*., 2014), and sponges (Wisshak *et al*., 2012; Fang *et al*., 2013a; Stubler *et al*., 2014). Sponges are often regarded as the most effective taxonomic group of bioeroders in terms of the amount of $CaCO_3$ removed from coral reefs (Neumann, 1966; Macgeachy, 1977; Glynn, 1997; Rützler, 2002; Schönberg, 2002), which can range from 0.84 to 23.00 kg $CaCO_3$ m^{-2} $year^{-1}$ (Hill, 1996; Zundelevich *et al*., 2007; Nava & Carballo, 2008). Recent studies have shown that boring rates of *Cliona orientalis*, an Indo-Pacific boring sponge, increased in pre-infested dead coral skeleton when exposed to predicted future pCO_2 conditions, with no direct negative impacts to the sponge reported (Fang *et al*., 2013; Wisshak *et al*., 2012). In the Caribbean, Stubler *et al.* (2014) induced *Cliona varians* colonization of living *Porites furcata* and found that elevated pCO_2 reduced coral calcification and led to a significant increase in sponge-mediated erosion.

Bioerosion studies incorporating increased temperature have found a variety of sponge responses. Fang *et al.* (2013a,b) observed higher *C. orientalis* bioerosion rates under acidification and warming compared to ambient conditions. However, the design used by Fang *et al.* (2013a,b) was not fully orthogonal (temperature increased in tandem with increasing pCO_2), and thus, the individual effects of temperature and acidification were not assessed. A fully crossed design was used by Wisshak *et al.* (2013) to evaluate sponge erosion at four levels of pCO_2 and temperature, with limited impacts of temperature on bioerosion reported over an experimental period of 72 h. Unfortunately, the main and interactive effects of increased temperature and acidification on sponge bioerosion rates have not yet been thoroughly evaluated at a temporal scale that allows sponges to physiologically acclimate to environmental changes.

Many studies investigating tropical sponge bioerosion have used pre-infested dead substrate and do not account for changes in competitive interactions between sponges and living corals (i.e., attachment; but see Stubler *et al*., 2014). Excavating sponges that harbor zooxanthellae, such as members of the '*Cliona viridis* species complex' (Schönberg, 2000), must engage in a direct spatial interaction with corals as they compete for light and space on the reef (Hill, 1996). Stubler *et al.* (2014) found no difference in *C. varians* attachment rates to a common Caribbean coral as pH decreased and concluded that the competitive interactions between these two species will remain unchanged with projected future pH conditions. However, the effects of temperature increases were not evaluated. In fact, evidence suggests that the competitive vigor of clionaid sponges increases with temperature (Siegrist *et al*., 1992; Rützler, 2002).

This study investigated the bioerosion rates and competitive interactions (attachment) occurring between the excavating sponge *C. varians* (Duchassaing & Michelotti, 1864), a prominent Caribbean member of the '*Cliona viridis* species complex,' and a common, hermatypic coral, *Porites furcata* (Lamarck, 1816). *Cliona varians* is frequently found overgrowing and/or eroding *P. furcata* and other hermatypic corals (Hill, 1996; Rützler, 2002) throughout the Caribbean region. Gamma-stage (massive growth form) *C. varians* individuals are abundant space occupiers in shallow backreef environments (Wiedenmayer, 1977; Diaz, 2005) and are considered competitively superior to most coral species (Vicente, 1978). Therefore, *C. varians* and *P. furcata* are ideal study organisms for investigating the effects of acidification and warming on bioerosion and spatial competition.

In this study, we employed a fully orthogonal design with two levels of temperature (ambient and +1 °C), three pH levels (ambient: 8.1, moderate: 7.8, and high: 7.6), and two levels of sponge (present and absent) to evaluate the effects of acidification and warming on the net calcification/dissolution of living and dead corals. Net calcification of living corals, sponge and coral survival, sponge attachment, and sponge symbiont (zooxanthellae) health were evaluated within each of the crossed treatment levels. Net dissolution/bioerosion

and the relevant sponge metrics were similarly evaluated for dead corals. We predicted that the interactive effects of increased acidification and temperature would amplify the erosional activity of *C. varians* and lead to rapid dissolution of dead coral substrate. For the living corals, we anticipated that any loss in carbonate due to sponge bioerosion would be exceeded by coral growth in the ambient temperature and pH treatments and that sponge bioerosion would only shift net calcification below zero at the extreme pH and temperature levels.

To integrate the findings of our controlled experiments into the context of the entire reef and further interpret the results of the study, we used empirical observations of individual coral–sponge interactions to develop a stochastic simulation of carbonate change for small coral clusters (i.e., simulated reefs). This approach used empirical data obtained from our full suite of treatment conditions to simulate future scenarios of reef development and provided a holistic assessment of calcification, survival, and competitive dynamics between *P. furcata* and *C. varians*.

Materials and methods

Flow-through system and treatments

The experiment began in July 2013 and ran for a total of 20 weeks. This included a 4-week acclimation period, followed by 16 weeks of full-treatment conditions. Using the outdoor, unfiltered seawater system at the Smithsonian Tropical Research Institute's Bocas del Toro Station in Panama, a flow-through pH-stat system was constructed using 12 reservoirs (200 l each) that each fed 12 aquaria (1.8 l). Aquaria (n = 144) were gravity-fed treated seawater at a rate of roughly 0.2 l min^{-1}; seawater residence time in each aquarium was ~10 min. Reservoirs were randomly assigned pH and temperature treatments, although some modification of temperature treatment assignment was necessary to accommodate position of electrical outlets. Aquariums connected to reservoirs were randomly positioned and assigned sponge treatments (present or absent; Figure S1a).

Three acidification treatments were employed; target pH values (NBS scale) were 8.1, 7.8, and 7.6 (hereafter: ambient, moderate, and high acidification, respectively). These values roughly corresponded with ambient pCO_2 and the projected levels of pCO_2 for the years 2100 and 2300 guided by the IPCC A1FI/RCP8.5 emissions scenario (IPCC, 2007; Meinshausen *et al.*, 2011) and models by Caldeira & Wickett (2003). In each reservoir, pH was regulated continuously using a pH controller (Reef Fanatic) connected to a CO_2 regulator (Milwaukee MA957; see Figure S1b). When reservoir pH levels exceeded the target values for the moderate and high acidification treatments, the controller opened a valve that delivered CO_2 gas until target values were restored (Anthony *et al.*, 2008; Stubler *et al.*, 2014). Calibration of the pH controllers occurred every 2–3 days using NIST standards to ensure minimal drift. No direct CO_2 manipulations occurred for the ambient pH treatments.

Two dynamic levels of temperature were applied, ambient (no temperature alteration) and increased (+1 °C over ambient). While tropical water temperatures are expected to rise 1–3 °C by the end of the century (multimodel ensemble; IPCC 2007), a conservative temperature increase of only 1 °C above ambient was used. Temperature increases were achieved by placing two 800W titanium heaters connected to a temperature controller within the appropriate reservoirs. Due to limitations in electrical supply to the facility, the heat treatments could only be applied to three reservoirs (n = 12 aquaria at each acidification level), and the remaining reservoirs (n = 9) were held at ambient temperature (n = 36 aquaria at each acidification level) (Figure S1a). The flow-through system allowed experimental temperature treatments to mirror the diurnal and seasonal (rainy vs. dry) temperature variations occurring in the bay where the seawater was drawn; therefore, temperature ranged from 28.0–30.71 °C to 29.38–31.64 °C for the ambient and increased temperature treatments, respectively. Sponge treatments (present or absent) were applied within the aquariums directly.

Daily measurements of pH (NBS scale), temperature, salinity, and dissolved oxygen were recorded within each aquarium. HOBO® temperature loggers were placed in one representative aquarium from each reservoir to continuously record temperatures. To characterize full water chemistry parameters of treatments, discrete water samples (300 ml) were taken monthly from each reservoir (n = 12 each month; n = 36 total) following NOAA's dissolved inorganic carbon (DIC) sampling protocols. Water samples were immediately preserved by adding 200 μl of a saturated mercuric chloride ($HgCl_2$) solution and stored at 4 °C. DIC measurements were made using an EGM-4 Environmental Gas Analyzer ® (PP Systems, Amesbury, MA, USA) after acidification and separation of the gas phase from seawater using a LiquiCel ® Membrane (Membrana, Charlotte, NC, USA). The instrument was calibrated using standards made from sodium bicarbonate and yielded a full recovery of Dr. Andrew Dickson's (UCSD, Scripps Institute of Oceanography) certified reference material for total inorganic seawater (Batch 135: 2036 μmol DIC kg seawater^{-1}). Levels of pCO_2, total alkalinity, and $\Omega_{aragonite}$ were calculated by the CO2SYS program based on measured levels of dissolved inorganic carbon, pH (NBS scale), temperature, and salinity using the GEOSECS constants (designed for pH measured on the NBS scale).

Acclimation period

To minimize stress attributable to sudden environmental changes and reduce physiological shock, a 4-week acclimation period was used to slowly bring subject organisms to full-treatment conditions. Once organisms were placed in their respective treatment tanks, pH was reduced by 0.1 units per week for all acidification treatments; pH reductions were staggered by treatment intensity to simultaneously reach target values at the same rate. Therefore, reductions for the high acidification treatment began immediately to reduce the pH

from ambient to 7.6 over 4 weeks, followed by moderate which was reduced over the last 2 weeks of the acclimation period from ambient to 7.8. Temperature treatments were elevated biweekly by 0.5 °C, regardless of acidification treatment.

Study species

Approximately 30 small colonies of *P. furcata* were collected from a continuous reef system on Isla Pastores, Panama (9° 13.551′ N, 82° 19.538′ W); colonies were separated by a minimum distance of 5 m to increase the likelihood of obtaining multiple genets. After collection, corals were placed in flow-through seawater tables and the growing tips from healthy branches were excised to create smaller fragments (3–6 cm in length). Any fragments exhibiting necrotic tissue, bleaching, disease, or infestations of bioeroders were discarded. It should be noted that the complete exclusion of endolithic microbioeroders living symbiotically within corals (Gutner-Hoch & Fine, 2011) was not possible due to the difficulty in visually detecting their presence deep within the carbonate material. After 1 week of recovery, each *P. furcata* fragment was tagged and attached to a glass microscope slide using CorAffix® ethyl cyanoacrylate glue. Corals were randomly distributed across treatment tanks (n = 7 per aquarium) during the 4-week acclimation period.

Thirty large gamma-stage *Cliona varians* forma *varians* individuals (each >300 cm^2 surface area) were collected at Isla Pastores; standard spicule preparations confirmed species identification. Sponge tissues (no carbonate material) were cut into smaller explants (~8 cm^3) which included approximately 4 cm^2 of the ectosome, where the majority of zooxanthellae reside, as well as a portion of the choanosome. Explants were allowed a 7-day recovery period in a large raceway to ensure that all sponges were healed before being placed in treatment tanks. Aquaria designated as sponge treatments (n = 72) were supplied 10 sponge explants. Sponges were placed in the same tanks as corals but were separated within treatment tanks to prevent premature interactions from occurring during acclimation. All coral and sponge fragments were elevated off the bottom using plastic grating, and aquaria were siphoned every 3 days to remove any accumulated sediment or debris; any algal growth was also dislodged and removed by siphoning. Aquaria and tubing were cleaned as necessary to maintain consistent water flow and light levels throughout the experiment.

To detect differences in the effects of temperature, pH, and sponge presence for living and dead corals, skeletal corals were also included within each tank. Dead coral specimens of *P. furcata* were collected from the reef system along Isla Pastores, Panama. Dead corals were soaked in a 10% sodium hypochlorite (bleach) solution for 3 days to remove any coral tissue and epibiota. Skeletal fragments were then soaked in deionized water (DIW) for 24 h, rinsed, and dried to a constant mass at 60 °C. Fragments were tagged with unique IDs and dry weights recorded. Dead corals were not assigned to or placed in tanks until after the acclimation period was over to prevent premature carbonate dissolution.

After the acclimation period, initial buoyant weights of the living corals were determined following methods of Jokiel *et al.* (1978) and Davies (1989). Sponges were then loosely secured to coral fragments with cable ties (Schönberg & Wilkinson, 2001) and returned to designated treatment tanks (Figure S3b). *Porites furcata* fragments serving as controls (no sponge attached) were also supplied a small marker cable tie that mimicked the contact area of sponges (3.75 cm^2) to partially account for any abrasion or shading artifacts (Figure S3a). Once acclimation was completed, the skeletal fragments were placed in treatment tanks (n = 3 per aquarium). Dead coral fragments that were assigned to sponge treatments had sponges attached, while fragments designated as controls received a cable tie as a procedural control.

Net change in calcium carbonate: living and dead corals

Living corals were buoyant-weighed – without any sponge material attached – before and after the experiment to determine the net change in calcium carbonate, hereafter referred to as net calcification. After obtaining final buoyant weights, living corals were soaked in a 10% sodium hypochlorite solution for a minimum of 24 h, or until all tissue was removed, and skeletal buoyant weights were obtained for tissue correction (Davies, 1989). Corals were then soaked overnight in DIW and triple-rinsed before being placed in the drying oven for 72 h at 60 °C. Dry weights were obtained for each coral. To quantify surface area of corals, a standard paraffin wax-dipping procedure was used (Stimson & Kinzie, 1991; Holmes, 2008), which is an accurate and efficient method for processing large quantities of corals (Veal *et al.*, 2010). Net calcification (mg $CaCO_3$) was then standardized to surface area and number of days the coral spent in the experiment.

After the conclusion of the experiment, dead corals were bleached and dried as previously described for the living corals. The variability in the size and surface area of control dead corals (no sponges present) prevented the application of a constant correction for abiotic dissolution of carbonate material as described by Fang *et al.* (2013a,b). It is important to note that this study does not provide sponge bioerosion rates (defined as the amount of carbonate material removed per area of sponge contact over time; mg $CaCO_3$ cm^{-2} day^{-1}) because the sponge contact area on living and dead corals could not accurately be measured (see Figure S3d). Previous studies reporting sponge bioerosion rates (Fang *et al.*, 2013a,b) have used standardized, pre-infested carbonate material that (i) enabled the accurate measurement of the sponge contact area, (ii) allowed for the application of a correction factor for passive dissolution, and (iii) distinguished between passive abiotic dissolution and sponge bioerosion. Due to our inability to measure sponge contact area and distinguish between abiotic and biotic dissolution in the present experiment, the net change in carbonate material for the dead corals will hereafter be referred to as 'net dissolution + bioerosion'. For each coral, net dissolution + bioerosion (mg $CaCO_3$) was standardized to the surface area of the coral and number of days spent in the experiment.

Sponge attachment and symbiont health

Prior to experimental breakdown, an underwater pulse amplitude-modulated (PAM) fluorometer (Walz, Germany) was used to assess photosynthetic yield of sponges across all pH and temperature treatments. Measurements of photosynthetic yield (*Fv/Fm*) were taken at midnight to allow sponge symbionts to dark-adapt naturally for ~6 h. After PAM measurements were taken, sponges were removed from corals and classified as either attached or not attached (Figure S3c). Two small portions from the surface of each sponge (approximately 0.25 g) were excised for zooxanthellae and chlorophyll *a* analysis. Each portion was gently blotted and weighed. The chlorophyll *a* portion was placed in a small, labeled foil packet and immediately frozen at −20 °C. Samples were then lyophilized for 24 h and dry weights recorded. Dried sponge pieces were added to aluminum-wrapped vials containing 10 ml of 90% acetone and stored at 4 °C overnight. After 18 h, each sample was centrifuged and the absorbance of the supernatant was measured at wavelengths 630, 647, 664, and 750 nm on a scanning spectrophotometer (SpectraMax® Plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA). Concentrations of chlorophyll *a* were calculated based on equations provided by Parsons *et al*. (1984) and standardized to dry and blotted wet weights.

The second sponge portion was used to quantify the density of zooxanthellae cells. Blotted wet weight was recorded, and sponge piece was immediately homogenized using a mortar and pestle and centrifuged at 2000 rpm for 5 min. Supernatant was discarded, and 5 ml of filtered seawater was added to resuspend the pellet; 1 ml of homogenized, resuspended fluid was removed, and 200 μl of Lugol's solution was then added for cell staining and preservation. Zooxanthellae densities were counted under the microscope using a hemocytometer, and counts (5 replicates) were normalized to wet tissue weights (cells g^{-1} sponge tissue). The amount of chlorophyll per zooxanthellae cell was calculated by normalizing the chlorophyll *a* concentration to wet mass and dividing by the number of cells found in one gram of wet sponge tissue (μg $cell^{-1}$).

Stochastic simulation study

To fully integrate the data from the living and dead corals and to understand how our results might translate to the reef level, we used empirical values obtained from individual corals within the experiment to simulate a series of reef replicates exposed to the future conditions. The simulation incorporated the following values for all treatment combinations obtained from the actual experiment: coral survival (number of days), net calcification rates for living coral (with and without sponges present), net dissolution + bioerosion rates for dead coral (with and without sponges present), and proportion of successful attachment in sponge treatments. The simulation incorporated the data that were not included in the analysis of the mesocosm data, such as the net calcification of corals in the sponge treatments that did not experience any erosion because the sponge never attached. Input values for the model were from individual corals (living/dead), rather than tank means. Miniature reefs (hereafter, 'trays') consisted of 10 corals initialized at a standardized weight (600 mg) and surface area (25 cm^2). Change in calcium carbonate of trays (n = 10 replicate trays per treatment) was simulated under a fully orthogonal combination of temperature, pH, and sponge treatment for 113 days (the duration of the mesocosm experiment from which empirical values were drawn). All simulated corals within the trays began as living corals and were assigned a daily net calcification rate (mg cm^{-2} day^{-1}) randomly sampled with replacement from all calcification rates recorded during the experiment for that treatment combination; thus, calcification rates varied daily for each of the modeled coral individuals. Each coral within the trays was randomly appointed a number of days to survive (based on actual survival data). If corals were slated to expire before the end of the model period, corals were assigned a randomly sampled daily net dissolution rate appropriate for the specific treatment combination once they 'died'. A final, traywide change in carbonate was then calculated and compared between each treatment combination. Note that because the simulation was based on empirical values obtained from the mesocosm experiments, this model did not capture new sponge recruitment to corals that were not interacting with a sponge (which may occur in a real-world scenario, but logistically could not occur during the mesocosm experiments). The goal of the simulation was to integrate the survival and sponge attachment data with different calcification/dissolution rates observed between living and dead corals under experimental conditions of temperature, pH, and sponge presence, thereby providing insight into how these effects might propagate to higher levels of organization (i.e., the community).

Data analysis

All data analysis was performed using R statistical software (R Development Core Team, 2008). In summary, 1008 living corals and 432 dead corals were distributed across 144 tanks (seven live corals per tank and three dead corals per tank); 72 tanks were designated as sponge treatments, and 504 sponges were attached to live corals and 216 sponges attached to dead corals. The experimental design required the analysis of tank means (n = 144), rather than individual coral/sponge units (which were planned pseudoreplicates within tanks; Hurlbert, 1984). Only corals with sponges that had successfully attached (as opposed to merely cable-tied on, but not attached) were considered in the analysis of sponge treatments for survival and calcification response; this reduced the number of tanks included in the analysis (see Table 3 for final N analyzed per treatment). Due to the resultant imbalance of the design, survival of corals (number of days in experiment) and net calcification/dissolution + bioerosion (mg $CaCO_3$ cm^{-2} day^{-1}) of dead and living corals were analyzed by permutation of the residuals (n = 5000, without replacement) in a three-way analysis of variance with three fixed factors (temperature, pH, and sponge) following the guidelines of Anderson & ter Braak (2003). Homogeneity of variances was tested for each term using the nonparametric Fligner–Killeen test prior to permuta-

tion tests, as this is the only assumption of permutation tests. Changes in photosynthetic yield, zooxanthellae density, and chlorophyll *a* (μg cell^{-1}) were analyzed by permutation of the residuals (n = 5000, without replacement) in a two-way analysis of variance with temperature and pH as fixed factors (Anderson & ter Braak, 2003). To determine whether attachment was a response to the treatments, the proportion of sponges that were attached to corals in each tank was calculated, and tank means were used to assess attachment for each treatment. The mean proportion of sponges attached to corals was analyzed using a binomial generalized linear model permutation test (package: glmperm) with temperature and pH as factors. Water chemistry parameters that were not directly measured were calculated using the CO2SYS package, and a two-way ANOVA was used to confirm that unique treatments were established.

Simulated data from the stochastic model were analyzed using regression with empirical variable selection (REVS), which uses a branch-and-bound all-subsets regression to quantify the empirical support for predictor variables (Goodenough *et al*., 2012). The REVS model is more effective than stepwise, full, or all-subsets regression models (Goodenough *et al*., 2012) because it selects models by the amount of empirical support, rather than systematically adding or removing variables based on AIC or *P*-values. The REVS model was run using the *leaps* function in R following the code provided by Goodenough *et al*. (2012). Mean tray carbonate change from the simulated data was the dependent variable, and parameters included as predictor variables were temperature, pH, sponge presence, and coral survival. Because carbonate loss was directly related to survival (due to very different empirical living coral calcification and dead coral dissolution + bioerosion rates), the mean of the lower quartile of days that corals survived in each tray was used to represent the traywide survival in the regression model. Once the best fit model was determined, tests were run to check the assumptions of (i) normality for the dependent variable and residuals, (ii) multicollinearity, (iii) homogeneity of variance, and (iv) independence of the residuals. After failing to meet the assumptions of homogeneity of variance and normality of the residuals, the *avas* function in the R package acepack was used to fit the additive, variance-stabilized models (AVAS) to the optimally transformed dependent variable so that the additive models fit well and had constant variance. Transformed predictor variables were then once again tested for all aforementioned assumptions.

Results

Water chemistry and treatment parameters

Mean temperature (± SD hereafter, unless otherwise specified) of the ambient temperature treatment aquaria was 29.7 ± 0.4 °C (range was 28.0–30.71 °C) and 30.7 ± 0.5 °C in the elevated temperature treatment (range was 29.38–31.64 °C) over the 16-week experimental period. The increased temperature treatment tracked natural fluctuations and was consistently ~ 1 °C higher than the ambient values. Mean pH (NBS scale) was 8.13 ± 0.01, 7.77 ± 0.02, and 7.57 ± 0.03 for the ambient, moderate, and high acidification treatments, which corresponded to mean pCO_2 of 539 ± 48 μatm, 1294 ± 107 μatm, and 2245 ± 263 μatm. Seawater carbonate chemistry parameters for each treatment are found in Table 1. It is worth noting that ambient pCO_2, total alkalinity, and DIC values are slightly higher than expected for coral reef ecosystems; this is likely due to the influence of mangroves surrounding the bay where the seawater intake lies for the flow-through system (Zablocki *et al*., 2011).

Survival

All sponges survived for the entire duration of the experiment. In general, moderate mortality was observed for the corals, with 64% of all corals surviving until the end of the experiment. Permutation of the residuals from a three-way ANOVA analyzing coral survival as a function of sponge presence, temperature, and pH revealed that the main effects of temperature and sponge both significantly affected the number of days that coral survived; no interactions were found (Table 2). Surprisingly, higher temperatures significantly increased coral survival ($F_{(1,98)}$ = 10.422, $P < 0.001$) from 85.3 days (±29.4) to 103.1 (±15.9). Sponge presence also positively affected coral survival ($F_{(1,98)}$ = 4.62, P = 0.03); mean coral survival was 85.4 (±28.7) days in no sponge treatments and 98.0 (±23.8) days in sponge treatments.

Attachment

Within sponge treatments, we attempted to induce *C. varians* colonization of *P. furcata*. Some sponges readily attached to corals (24% experiment-wide attachment success), while the remainder did not physically attach to corals. All donor sponges were gamma-stage individuals, which may have predisposed the explants to exist independent of substrate. The percentage of sponges that attached to either living or dead corals was not significantly affected by pH or temperature (see Table 3 for summary of attachment percentages by treatment).

Net calcification and dissolution + bioerosion

Living coral calcification (mg $CaCO_3$ cm^{-2} day^{-1}) was significantly affected by temperature and sponge presence ($F_{(1,100)}$ = 7.68, P = 0.007 and $F_{(1,100)}$ = 11.26, P = 0.001, respectively; Table 2A). Interestingly, pH did not affect the net calcification of living corals (P = 0.38). In general, increased temperatures sup-

pressed coral calcification rates compared to ambient temperatures (Fig. 1). Positive net calcification was observed in all corals when no sponge was present, regardless of pH or temperature treatment (Fig. 1, Table 3). However, when sponges were present, corals experienced decreased or negative net calcification (Fig. 1, Table 3). The corals without attached sponges exhibited the highest mean calcification rates in the ambient temperature*high acidification treatment (0.22 ± 0.16 mg $CaCO_3$ cm^{-2} day^{-1}) and the lowest mean calcification rates in the increased temperature*ambient acidification treatment (0.08 ± 0.12 mg $CaCO_3$ cm^{-2} day^{-1}; Table 3). For corals with attached sponges, the greatest loss of $CaCO_3$ was found in the increased temperature*moderate acidification treatment (-0.13 ± 0.22 mg $CaCO_3$ cm^{-2} day^{-1}).

Net dissolution + bioerosion of dead coral was significantly affected by pH ($F_{(2,92)} = 4.80$, $P = 0.007$, Table 2B); neither sponge presence nor temperature was significant effects (Fig. 2). Net dissolution + bioerosion (mg $CaCO_3$ cm^{-2} day^{-1}) in the dead corals was approximately 60% greater in the moderate and high acidification treatments (-0.041 ± 0.06 and -0.039 ± 0.03, respectively) than the ambient (-0.015 ± 0.02; Table 3).

Sponge symbiont parameters

Neither zooxanthellae densities (cells g^{-1} sponge tissue) nor the amount of chlorophyll *a* per zooxanthellae cell (μg $cell^{-1}$) differed significantly due to either pH or temperature for sponges attached to living or dead corals. There was a significant main effect of pH on photosynthetic yield (*Fv*/*Fm*) of sponges attached to living corals ($F_{(2,59)} = 4.72$, $P = 0.01$); there was also a significant interaction between pH and temperature ($F_{(2,59)} = 5.37$, $P = 0.008$). In the ambient temperatures, photosynthetic yield plateaued between moderate and high acidification levels; however, when temperatures were increased, the photosynthetic yield was highest in the high acidification treatment. For the photosynthetic yield of sponges attached to dead corals, the main effect of pH was significant ($F_{(2,63)} = 3.771$, $P = 0.03$); photosynthetic yield of the symbionts increased with acidification, regardless of temperature. See Table 4 for mean values (±1 SD) of all symbiont parameters in each treatment combination.

Simulation model

The regression model that met all assumptions and best explained reef-level carbonate change ($\Delta CaCO_3$) included temperature and coral survival as predictor variables (adjusted $R^2 = 0.14$, $P < 0.001$). Temperature played an important role in reducing calcification of living corals in the mesocosm experiment; it is therefore intuitive that within the simulated data, temperature along with the amount of time a coral survived ultimately dictated $\Delta CaCO_3$ at the reef level. Increased temperature also resulted in statistically higher survival days in the mesocosms (Table 2); therefore, the relationship between temperature and survival lends itself to complicated results. The maximum $\Delta CaCO_3$ of any tray with 100% coral survival in an increased temperature treatment was 23% less than the maximum $\Delta CaCO_3$ in a tray with 100% coral survival in ambient temperature. In trays where coral survival was <100%, $\Delta CaCO_3$ was 56% lower in the increased temperature trays, as compared to the ambient temperature (although caution should be used when interpreting

Table 1 Summary of measured (*) and calculated (**) seawater chemistry parameters represented as means ± 1 SD. Reported values of temperature, salinity and pH (NBS scale) were measured daily in aquaria. Total scale pH and carbonate chemistry values were calculated from preserved DIC samples and represent the mean values from treatment reservoirs at three time-points collected midday at the beginning, middle, and end of the experiment

	Ambient temperature			Increased temperature		
	Ambient acidification	Moderate acidification	High acidification	Ambient acidification	Moderate acidification	High acidification
Temperature (°C)*	29.77 ± 0.4	29.76 ± 0.4	29.76 ± 0.4	30.67 ± 0.5	30.79 ± 0.5	30.62 ± 0.4
Salinity*	33.38 ± 0.5	33.35 ± 0.6	33.34 ± 0.6	33.35 ± 0.5	33.29 ± 0.5	33.37 ± 0.5
pH (NBS scale)*	8.14 ± 0.01	7.78 ± 0.02	7.56 ± 0.03	8.13 ± 0.01	7.76 ± 0.04	7.58 ± 0.03
pH (Total scale)**	8.04 ± 0.01	7.68 ± 0.04	7.48 ± 0.04	8.03 ± 0.01	7.66 ± 0.05	7.50 ± 0.04
Total Alkalinity** (μmol kg^{-1} seawater)	2724 ± 209	2535 ± 162	2595 ± 156	2612 ± 131	2538 ± 113	2704 ± 214
DIC* (μmol kg^{-1} seawater)	2438 ± 190	2448 ± 152	2578 ± 154	2338 ± 114	2448 ± 110	2679 ± 215
pCO_2** (μatm)	540 ± 54	1284 ± 90	2253 ± 232	534 ± 14	1337 ± 185	2234 ± 328
$\Omega_{aragonite}$ **	3.4 ± 0.2	1.56 ± 0.18	1.04 ± 0.11	3.26 ± 0.03	1.56 ± 0.16	1.13 ± 0.11

Table 2 Results of permutation tests; the interactive and main effects of temperature, pH, and sponge are evaluated for (a) living coral calcification (mg cm^{-2} day^{-1}), (b) dead coral dissolution + bioerosion (mg cm^{-2} day^{-1}), and (c) coral survival (days)

ANOVA	df	SS	MS	*F*-value	*P* (perm)
(a) Net Calcification					
Temperature	1	0.20	0.20	7.68	0.007***
pH	2	0.05	0.03	0.98	0.38
Sponge	1	0.29	0.29	11.26	0.001***
Temp*pH	2	0.01	0.004	0.15	0.86
Temp*Sponge	1	0.02	0.02	0.81	0.37
pH*Sponge	2	0.03	0.01	0.37	0.59
Temp*pH*Sponge	2	0.04	0.02	0.59	0.45
Residuals	100	2.56	0.03	—	—
(b) Net Dissolution + bioerosion					
Temperature	1	0.0001	0.0001	0.085	0.78
pH	2	0.014	0.007	4.803	0.007***
Sponge	1	0.0001	0.0001	0.084	0.77
Temp*pH	2	0.0003	0.0001	0.093	0.91
Temp*Sponge	1	0.003	0.003	1.995	0.16
pH*Sponge	2	0.002	0.001	0.689	0.50
Temp*pH*Sponge	2	0.009	0.004	2.901	0.07
Residuals	92	0.138	0.0014	—	—
(c) Coral Survival					
Temperature	1	6932.5	6932.5	10.42	<0.001***
pH	2	319.3	159.6	0.24	0.80
Sponge	1	3075.4	3075.4	4.62	0.03***
Temp*pH	2	1783.0	891.5	1.34	0.27
Temp*Sponge	1	839.8	839.8	1.26	0.26
pH*Sponge	2	3961.9	1981.0	2.98	0.054
Temp*pH*Sponge	2	458.1	229.1	0.34	0.72
Residuals	98	65186.9	665.2	—	—

***Significant factors.

this value, as only $n = 2$ trays in the increased temperature treatments experienced less than 100% survival). Averaging over all treatments, when survival was <100%, reef calcification was 17% less than when coral survival was 100%. Mean values of $\Delta CaCO_3$ in each treatment remained positive and experienced net accretion over the modeled 113-day period; however, the $\Delta CaCO_3$ was lowest in the increased temperature*high acidification*sponge treatment, which was an order of magnitude less than any other treatment, even though survival was 100% for all trays. When compared to the ambient temperature*ambient acidification*sponge treatment, coral trays exhibited 90% less calcification, indicating that, although not statistically significant, the combination of high temperatures, acidification, and sponge erosion will reduce calcification even when all corals survive.

Discussion

The present study tested the relative contributions of the main and interactive effects of acidification, temperature, and sponge presence on net calcification and dissolution + bioerosion of living and dead *P. furcata* fragments. Previous studies of clionaid bioerosion rates using pre-infested carbonate substrate found a positive relationship with acidification and warming but did not aim to include the direct interactions (attachment and subsequent erosion) occurring between living corals and boring sponges (e.g., Wisshak *et al.*, 2012; Fang *et al.*, 2013a,b). Stubler *et al.* (2014) investigated the interactions between living *P. furcata* and *C. varians* but found no difference in sponge attachment rates to coral under experimentally altered pH conditions expected within this century. While Stubler *et al.* (2014) suggested that the interaction between *P. furcata* and *C. varians* may remain unchanged with minor acidification, our understanding of the interactive effects of acute acidification and temperature rise on coral–sponge interactions remains limited. The data presented here address this gap in our understanding and demonstrate differential impacts of sponge bioerosion and attachment for living and dead corals under fully

Table 3 Coral survival, net calcification, or dissolution + bioerosion rates, and the proportion of sponges attached are reported for living and dead corals (mean ± 1 SD). The final number of tanks analyzed is also presented for each treatment combination

	Ambient temperature			Increased temperature		
	Ambient acidification	Moderate acidification	High acidification	Ambient acidification	Moderate acidification	High acidification
Living corals, Sponge present						
Coral Survival (days)	85.2 ± 30.1	105.8 ± 17.3	106.0 ± 17.8	97.2 ± 27.0	112.8 ± 0.5	98.5 ± 24.0
Net Calcification ($mg\ CaCO_3\ cm^{-2}\ day^{-1}$)	0.10 ± 0.12	0.05 ± 0.35	0.12 ± 0.11	0.05 ± 0.07	−0.13 ± 0.22	−0.005 ± 0.085
Proportion Attached	0.23 ± 0.25	0.16 ± 0.2	0.22 ± 0.26	0.28 ± 0.27	0.17 ± 0.14	0.54 ± 0.25
Final *N* (tanks)	12	8	9	4	4	6
Living corals, Sponge absent						
Coral Survival (days)	83.4 ± 28.3	80.1 ± 33.3	82.8 ± 32.6	108.3 ± 7.6	110.3 ± 12.2	92.1 ± 27.0
Net Calcification ($mg\ CaCO_3\ cm^{-2}\ day^{-1}$)	0.17 ± 0.14	0.15 ± 0.13	0.22 ± 0.16	0.08 ± 0.12	0.15 ± 0.13	0.12 ± 0.15
Final *N* (tanks)	18	18	17	4	6	6
Dead Coral Skeletons, Sponge Present						
Net Dissolution + bioerosion ($mg\ CaCO_3\ cm^{-2}\ day^{-1}$)	−0.01 ± 0.01	−0.02 ± 0.01	−0.05 ± 0.06	−0.02 ± 0.01	−0.11 ± 0.01	−0.04 ± 0.01
Proportion Attached	0.33 ± 0.34	0.17 ± 0.26	0.19 ± 0.3	0.22 ± 0.27	0.05 ± 0.14	0.46 ± 0.36
Final *N* (tanks)	11	6	7	3	2	5
Dead Coral Skeletons, Sponge Absent						
Net Dissolution + bioerosion ($mg\ CaCO_3\ cm^{-2}\ day^{-1}$)	−0.02 ± 0.03	−0.05 ± 0.07	−0.03 ± 0.01	−0.01 ± 0.004	−0.02 ± 0.001	−0.03 ± 0.004
Final *N* (tanks)	18	18	17	6	5	6

crossed acidification and warming scenarios expected for the next century and beyond.

Experimental manipulation of acidification, temperature, and sponge presence

The effects of acidification and warming on the interaction between *P. furcata* and *C. varians* were evaluated over an experimental period of 16 weeks (following a 4-week acclimation period). Temperature and sponge presence had the most dramatic impact on net coral calcification. An increase of 1 °C above ambient temperature reduced net coral calcification in the mesocosm experiment (Table 3). While some studies have reported increased coral calcification when temperatures exceeded 28 °C (Edmunds *et al.*, 2012; *Porites rus*), the optimal temperature range for coral calcification is generally considered to be 25–28 °C (Jokiel & Coles, 1977; Coles & Jokiel, 1978; Marshall & Clode, 2004). Corals residing beyond or at the upper limit of this range – such as those in our study – may experience reductions in calcification as temperatures increase (e.g., De'ath *et al.*, 2009). Therefore, it is likely that the addition of 1 °C over ambient caused temperatures to surpass the optimal temperature range for *P. furcata* calcification and resulted in the reduced calcification rates found in the present study.

Surprisingly, acidification did not have a significant effect on net *P. furcata* calcification even though the $\Omega_{aragonite}$ approached undersaturation in the highest acidification treatments. Previous studies of congeners have shown both resilience (*Porites rus*; Edmunds *et al.*, 2012) and sensitivity (*Porites lobata*, Anthony *et al.*, 2008) to acidification. Reduced calcification rates in *P. furcata* were reported by Stubler *et al.* (2014) after 8 weeks of exposure to elevated pCO_2 (~750 μatm), yet no reduction in calcification was observed in the present study of *P. furcata* at pCO_2 levels of ~1200 and 2200 μatm. One explanation for the difference may be that the present study incorporated a longer experimental duration (16 weeks), an acclimation period (4 weeks), and a more robust experimental design with a greater number of replicates than Stubler *et al.* (2014). The acclimation period and longer experimental period may have allowed the corals to physiologically alter their response to pH changes (full acclimatization). There is currently no consensus as to whether an acclimation period should be standard procedure for acidification studies. However, the disparity in the response of *P. furcata* to acidification between Stubler *et al.* (2014) and the present study suggests that the use of an acclimation period may alter coral calcification response.

The literature is replete with discrepancies in coral calcification response to pH changes. McCulloch *et al.*

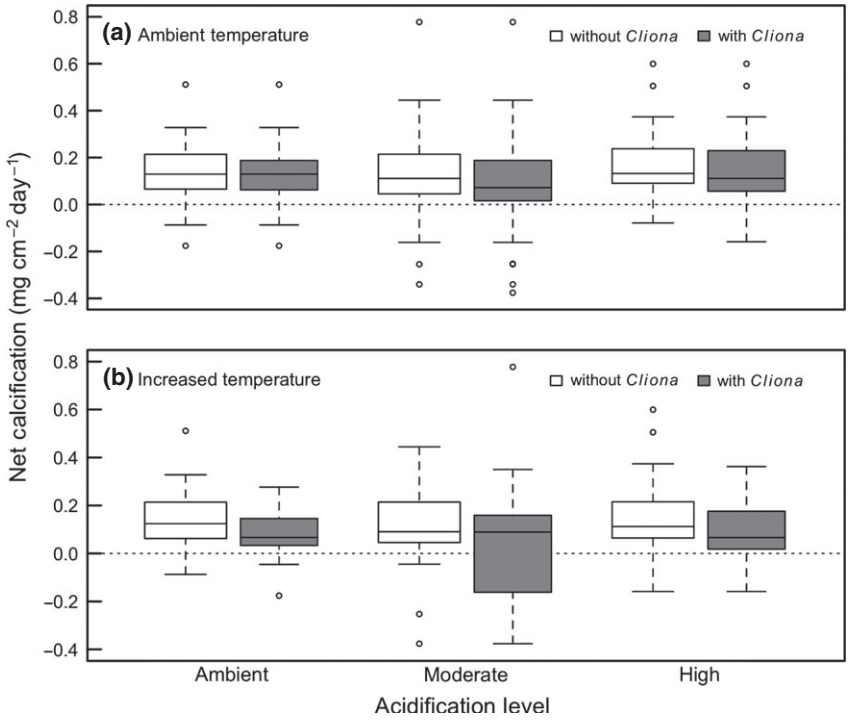

Fig. 1 Net calcification (mg cm^{-2} day^{-1}) of living *Porites furcata* at (a) ambient and (b) increased temperatures in each acidification level over the 113-day experimental period. Corals with and without *Cliona varians* are denoted in gray and white, respectively. Boxplots mark median values with a central bar, the 1st and 3rd quartiles with a box, the ±1.5 interquartile ranges with 'Tukey whiskers', and outliers with open circles.

(2012) related the reported differences in coral response to the species-specific ability of corals to control the pH of their internal calcifying fluid (pH_{int}). Active transport of DIC, Ca^{2+}, and H^+ ions can alter pH_{int} and may facilitate coral calcification even when ambient seawater carbonate chemistry is less than favorable, albeit at an increased metabolic cost (McCulloch *et al.*, 2012). Due to the excess energy supplied by photosynthetic symbionts, zooxanthellate corals may be able to modify their pH_{int} more readily than azooxanthellate corals further enabling calcification in acidified environments (McCulloch *et al.*, 2012). While the pH of the calcifying fluid has not been measured for *P. furcata*, two other *Porites* species exhibit low gradients between pH_{int} and seawater pH, suggesting that the genera may expend less energy calcifying relative to other coral genera (McCulloch *et al.*, 2012). The species-specific physiology may be one reason why the corals in the present experiment did not exhibit large reductions in calcification, even when seawater was nearly undersaturated with respect to aragonite.

Sponge presence resulted in decreased *P. furcata* calcification rates compared to control corals in the same treatment combinations (Fig. 1, Table 3). On the contrary, sponge presence did not affect the net dissolution + bioerosion of dead corals. This finding differs from prior studies examining the impact of sponge presence on carbonate substrate dissolution. Previous studies demonstrated that tropical (*C. orientalis*) and temperate (*C. celata*) sponge bioerosion rates increased with moderate acidification (500–750 μatm) over timescales ranging from 72 h to 8 weeks (Duckworth & Peterson, 2012; Wisshak *et al.*, 2012, 2013, 2014; Fang *et al.*, 2013a,b); however, *C. varians* did not follow the same pattern. The levels of pCO_2 used in the present study were much higher (~1200 and 2200 μatm) and were sustained for more than double the time (16 weeks) used in prior analogous experiments. It is worth consideration that passive dissolution may have superseded any effect of sponge presence over the 16-week exposure to high acidification.

Many microborers, like the ubiquitous *Ostreobium* sp., inconspicuously exist within carbonate material and could have contributed to the net dissolution of coral skeletons. The dead coral skeletons were bleached prior to experimental manipulation, which effectively removed all living organisms; however, 16 weeks is ample time for colonization by endolithic microborers such as algae, cyanobacteria, or fungi (Grange *et al.*, 2014). Measurable rates of biogenic carbonate dissolution due to endolithic organisms have been reported under elevated pCO_2 and temperature scenarios

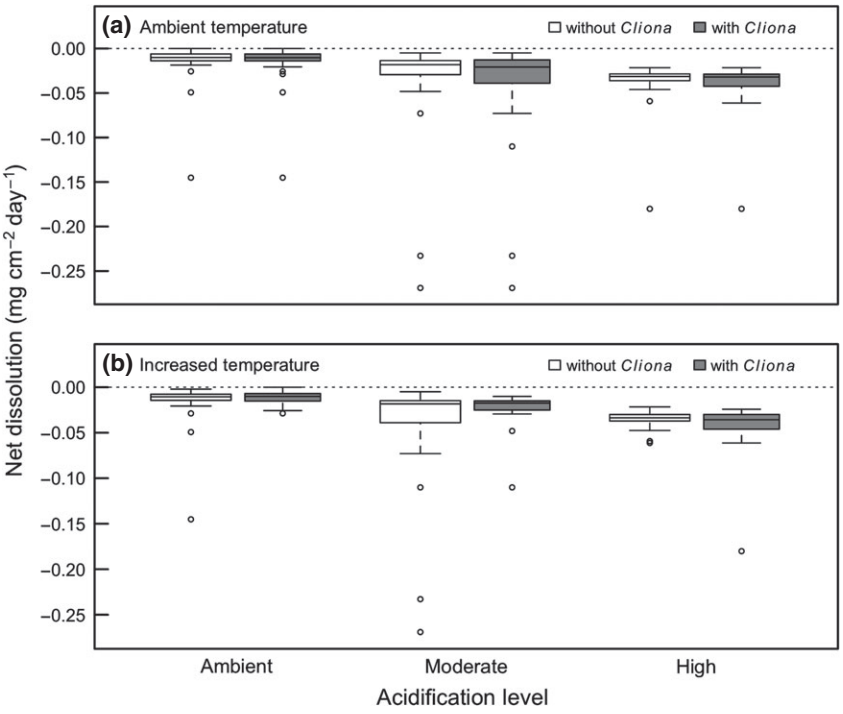

Fig. 2 Net dissolution and bioerosion (mg $CaCO_3$ cm^{-2} day^{-1}) of dead *Porites furcata* skeletons at (a) ambient and (b) increased temperatures in each acidification level over the 113-day experimental period. Skeletons with and without *Cliona varians* present are denoted in gray and white, respectively. Boxplots mark median values with a central bar, the 1st and 3rd quartiles with a box, the ±1.5 interquartile ranges with 'Tukey whiskers', and outliers with open circles.

(Tribollet *et al*., 2009; Reyes-Nivia *et al*., 2013). Thus, endolithic microeroders may have contributed to the changes we attributed to passive dissolution in dead coral skeletons. While no visible signs of microbioerosion or infestations by endoliths were found in the living corals, the possibility that they contributed to the net calcification cannot be discounted. However, net calcification was not significantly different by pH treatment, suggesting that microbioerosion was negligible and did not impart any bias in the interpretation of results.

Photosynthetic symbionts are thought to benefit from acidification due to an increased concentration of HCO_3^-, which is used during photosynthesis (Marubini *et al*., 2008). Yet the photosynthetic response of zooxanthellae to acidification varies widely among invertebrates with negative (Iguchi *et al*., 2012), positive (Langdon & Atkinson, 2005), and null (Kroeker *et al*., 2010) effects reported. Studies of zooxanthellate sponges have shown that bleaching is amplified by increases in temperature and pCO_2 (Fang *et al*., 2013a,b; Wisshak *et al*., 2013); however, no bleaching was observed in the present study. Although sponge symbiotic zooxanthellae did not exhibit significant changes in population density or chlorophyll *a* concentrations in any of the treatment combinations, the photosynthetic yield of symbionts in *C. varians* was higher in acidified treatments (Table 4). This study highlights the need to further elucidate the role that zooxanthellae play in promoting sponge bioerosion (Hill, 1996) under future environmental conditions.

Attachment – one measure of direct competition between *C. varians* and living *P. furcata* – was not affected by either temperature or acidification; the proportion of sponges attached to dead coral skeletons was also shown to be independent of treatments. The experiment-wide attachment percentage (24%) was comparable to other studies attempting to induce *C. orientalis* colonization of coral substrate (25–30%; Schönberg & Wilkinson, 2001). In a similar study to the one presented here, Stubler *et al*. (2014) monitored weekly attachment rates between *C. varians* and *P. furcata* and found no differences due to acidification. Our results support this finding and suggest that the interaction between *C. varians* and *P. furcata* will not be altered as a result of acidification and/or warming.

Interestingly, sponge presence actually increased the mean days of survival for *P. furcata* by 21% across all treatments. While the reason for increased coral survival is unclear, interpretation at a larger reef scale should be cautiously approached as further overgrowth and shading of coral tissue will undoubtedly result in

Table 4 Tank means (±1 SD) of symbiont parameters for sponges attached to living or dead corals in each of the treatment combinations

	Ambient temperature			Increased temperature		
	Ambient acidification	Moderate acidification	High acidification	Ambient acidification	Moderate acidification	High acidification
Sponges attached to living corals						
Zooxanthellae Density (cells g^{-1} wet tissue)	$6.1 \times 10^5 \pm 3.4 \times 10^5$	$5.5 \times 10^5 \pm 3.1 \times 10^5$	$6.1 \times 10^5 \pm 4.6 \times 10^5$	$4.4 \times 10^5 \pm 2.8 \times 10^5$	$6.9 \times 10^5 \pm 2.9 \times 10^5$	$6.5 \times 10^5 \pm 4.4 \times 10^5$
Chlorophyll *a* (μg g^{-1} wet tissue	80.5 ± 56.8	59.2 ± 28.4	57.0 ± 31.7	60.9 ± 27.9	65.2 ± 38.9	66.8 ± 29.9
Chlorophyll *a* (ng $cell^{-1}$)	0.019 ± 0.02	0.017 ± 0.01	0.02 ± 0.03	0.018 ± 0.01	0.01 ± 0.008	0.018 ± 0.02
Fv/Fm (Photosynthetic Yield)	0.67 ± 0.06	0.69 ± 0.05	0.69 ± 0.05	0.67 ± 0.05	0.67 ± 0.04	0.71 ± 0.03
Sponges attached to dead coral skeletons						
Zooxanthellae Density (cells g^{-1} wet tissue)	$6.3 \times 10^5 \pm 3.7 \times 10^5$	$8.6 \times 10^5 \pm 5.7 \times 10^5$	$5.8 \times 10^5 \pm 3.3 \times 10^5$	$4.9 \times 10^5 \pm 4.3 \times 10^5$	$8.0 \times 10^5 \pm 4.7 \times 10^5$	$7.2 \times 10^5 \pm 3.6 \times 10^5$
Chlorophyll *a* (μg g^{-1} wet tissue	79.6 ± 39.7	65.2 ± 44.7	63.9 ± 28.7	51.3 ± 22.6	60.6 ± 23.9	65.3 ± 23.4
Chlorophyll *a* (ng $cell^{-1}$)	0.017 ± 0.01	0.015 ± 0.02	0.014 ± 0.01	0.016 ± 0.01	0.01 ± 0.002	0.012 ± 0.01
Fv/Fm (Photosynthetic Yield)	0.68 ± 0.04	0.69 ± 0.05	0.70 ± 0.03	0.68 ± 0.05	0.69 ± 0.03	0.71 ± 0.02

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coral mortality. Temperature also positively impacted the survival of *P. furcata*. The increased temperature treatment was 30.7 ± 0.5 °C; however, the corals were collected from a very shallow reef that frequently experiences water temperatures in excess of 30 °C in the dry season. Perhaps the corals (and their zooxanthellae) were already adapted to higher temperatures and were therefore unaffected by the heat stress imposed during the experiment, a phenomenon observed in corals inhabiting shallow lagoons in Indonesia (Hoeksema, 1991; Rowan, 2004). Alternatively, the corals may have responded to the temperature stress by shifting their energy toward maintenance and survival, which may also explain the reduced calcification and growth, although further work must be done to support this hypothesis.

Modeling future reef erosion

The experimental portion of the present study looked at sponge erosion of living and dead coral in elevated temperature and acidification scenarios; however, the study did not evaluate impacts at the reef level. Therefore, a stochastic model was developed to empirically simulate the response of a reef system experiencing growth, bioerosion, death, and dissolution. We used the results of the simulation study as a tool to guide the interpretation and discussion of the observed sponge and coral responses to warming and acidification from our controlled experiment, given the relative importance of each factor for living and dead coral substrates.

Our simulation showed that temperature and the demographic survival of corals are the most important factors determining net calcification on future coral reefs. Temperature reduced calcification for the simulated *P. furcata* reef; however, survival was 22% higher in elevated temperatures than in ambient. Caribbean reefs have already begun (and will continue) to experience temperature increases related to climate change (Kuffner *et al.*, 2014); therefore, understanding the relative impact of temperature is paramount. Our study indicates that for *P. furcata* reefs that survive temperature increases, net calcification may not be as detrimentally impacted by acidification or bioerosion as previously postulated. Reefwide changes in calcification will be largely dependent on direct temperature effects and the ability of *P. furcata* to survive these changes, rather than as a function of reduced calcification rates or acidification *per se*.

Due to the relatively short experimental period and the use of a single coral species from which empirical values were drawn, the simulation does not incorporate the long-term or any species-specific responses to elevated physiological stress. Additionally, the impact on reproductive effort and recruitment success was not considered. McCulloch *et al.* (2012) modeled the future response of several coral species to warming (+2 °C) and acidification (~1000 μatm) and found a slight calcification increase, despite acidification, for coral species that are able to upregulate pH_{int}. This suggests that if species capable of altering their pH_{int} are able to maintain upregulation without unforeseen physiological consequences, then acidification may not directly inhibit calcification. However, any species unable to alter the pH_{int} may succumb to reduced calcification and growth as temperature and pCO_2 increase. Further, the intrinsic capacity of corals and zooxanthellae for long-term adaptation is still unknown.

The simulation was parameterized with empirical data from our experimental manipulation and is not meant to be a predictive model; therefore, we caution against extrapolation beyond the scope of the experimental parameters. Additionally, the low adjusted R^2 (0.14) may suggest that additional important parameters were absent from our investigation; we argue against this interpretation as alternative models provided better adjusted R^2 values (from 0.20 to 0.61); however, these models were discounted as they failed to meet one or more assumptions. The model that explained the most variance ($R^2 = 0.61$) included temperature, coral survival, and sponge presence; however, the assumptions of homogeneity of variance and normality of the residuals could not be met.

The sponge presence treatment in the simulation study included all coral calcification data even when a sponge did not successfully attach to a coral; therefore, all coral–sponge interactions – even those that did not lead to bioerosion – were incorporated. This resulted in a wide range of calcification responses to sponge presence, but added an important scenario that was necessarily excluded from the mesocosm experiment analysis. Unfortunately, the addition of the sponge term increased the heterogeneity of variance and led to a subsequent rejection of the model explaining the most variance. Ecologically, sponge contribution may be increasingly important, even if bioerosion is not amplified, as the abundance of boring sponges increases on reefs (Schönberg & Ortiz, 2008) and results in more intense spatial competition with other benthic invertebrates, such as corals.

Summary

Based on our findings, little alteration to the direct interactions between *C. varians* and *P. furcata* will occur as pH and temperatures change. Assuming that the results of this study are applicable to other species of coral and boring sponges, we suggest that boring sponges will

become increasingly important spatial competitors on reefs as projected scenarios of warming and acidification are realized. Temperature-induced reductions in net coral calcification combined with increased sponge bioerosion will synergistically destabilize reef growth, resilience, and ultimately functionality. Meanwhile, passive and biogenic dissolution of nonliving carbonate substrate will occur, further leveling the topographical features that create complexity on reefs. The stochastic simulation model found that reef-level changes in net calcification will be largely dependent on the ability of corals to survive (and perhaps adapt or acclimate to) future environmental changes. Our study demonstrates the need to expand our understanding of the complicated interactions between multiple stressors, bioeroders, and hermatypic organisms.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Schematic illustration of the experimental design and set-up. Round circles represent the reservoirs (n = 12); solid color of the reservoirs indicates acidification level (ambient: 8.1 pH, moderate: 7.8 pH, and high: 7.6 pH). Hatched pattern indicates that the reservoir was designated as a heat treatment. Each reservoir delivered treatment seawater to 12 small randomly distributed aquaria (represented by small rectangles). Sponge treatment was applied within the aquaria and is represented by a bold 'S'. (b) Diagram of direct application of the temperature and pH treatments. If a reservoir (200 l) was designated as an increased temperature treatment, two 800W heaters attached to a temperature controller were placed in the reservoir. These heaters ensured that water within the reservoir remained 1 °C above ambient in the flow-through system. If the reservoir was an acidified treatment (moderate or high), pH was regulated continuously using a pH controller that was connected to a CO_2 regulator with an affixed solenoid valve; whenever reservoir pH levels exceeded the target values for the moderate and high acidification treatments, the controller opened a valve that delivered CO_2 gas until target values were restored. Treatment water was gravity-fed from each reservoir to 12 aquaria (1.8 l).

Figure S2. Photo of the mesocosm experimental set-up at the Smithsonian Tropical Research Institute's Bocas del Toro facility in Panama.

Figure S3. Photos of (a) an individual live *P. furcata* fragment with control marker cable tie, (b) *Cliona varians* attached to an individual *P. furcata* fragment in the mesocosm experiment, (c) *Cliona varians* attached to a dead coral fragment and (d) an extreme example of sponge bioerosion that occurred over the 113 day experiment. Note the difficulty in measuring the sponge contact area in an accurate manner.