THE EFFECTS OF OCEAN ACIDIFICATION ON WOUND REPAIR IN THE CORAL *PORITES* SPP.

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Running head: Ocean acidification and damage in corals

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Abstract

Scleractinian corals on tropical reefs are exposed to many natural and anthropogenic disturbances, and while much is known about their responses to such conditions, it is unclear whether the responses will remain the same in a future affected by climate change and ocean acidification. To evaluate how one aspect of these effects -- wound repair -- might be influenced by ocean acidification, small colonies of massive *Porites* spp. from the back reef of Moorea, French Polynesia, were damaged to simulate the effects of single bites by corallivorous fishes, and healing was measured under contrasting P_{CO_2} regimes. Using experiments lasting 19–20 d and employing superficial (2013) or deep (2014) lesions, the effects of damage were evaluated at $\sim 400 \mu\text{atm}$ (ambient) and $\sim 1,000 \mu\text{atm}$ P_{CO_2} (both at $\sim 28.5^\circ\text{C}$) using calcification and healing as dependent variables. Damage reduced calcification of massive *Porites* spp. in 2013, but not in 2014, and P_{CO_2} had no effect on area-normalized calcification or healing in both years, although biomass-normalized calcification was reduced by high P_{CO_2} in 2014. Overall these results reveal the physiological resilience of this functional group of corals to the extent of ocean acidification expected by the end of this century, and suggest that over this period small colonies will remain capable of recovery from minor damage arising from fish corallivory.

1. Introduction

In the marine environment, the ability of colonial modular organisms to recover from partial mortality mitigates the negative effects of damage (Jackson and Coates, 1986). This is important for sessile taxa that cannot flee from sources of damage, and for those that are iteroparous, successful repair can increase the likelihood of future reproduction (Henry and Hart, 2005). The ability to survive damage is made possible by the capacity to regenerate damaged tissue, or to survive the loss of tissue if repair is impossible (Henry and Hart, 2005). There is a long history of interest in the regenerative capacity of colonial modular organisms (Jackson, 1979; Palumbi and Jackson, 1982; Hughes, 1983), and tropical scleractinians have served as a model system in advancing this interest (e.g., Nagelkerken and Bak, 1998; Oren et al., 2001).

Scleractinians frequently experience partial mortality through corallivory and breakage (Reese, 1977; Frydl, 1979; Rotjan and Lewis, 2008; Bruckner and Bruckner, 2016), and these events typically create lesions in which tissue and skeleton are removed (Henry and Hart, 2005). The combined effects of these lesions can sum among members of a population to represent greater tissue loss than occurs through the death of whole colonies (Hughes and Jackson, 1985). Lesions attributed to corallivory are a chronic disturbance for those scleractinians targeted by fish corallivores (Rotjan and Lewis, 2005), but the extent of damage inflicted varies among species of fish (Bellwood and Choat, 1990; Rotjan and Lewis, 2008). Some fish remove individual polyps without damaging the skeleton, while others remove large amounts of skeleton and tissue (Cole et al., 2008). For many years there was scant evidence that fish corallivory had strong effects on scleractinian communities (Hixon, 1997; Coles et al., 2008; Rotjan and Lewis, 2008), but this

disturbance is now viewed as an important factor affecting the abundance, distribution, and fitness of scleractinians (Cole et al., 2008; Rotjan and Lewis, 2008).

When a scleractinian is damaged, repair requires the allocation of resources to replace damaged tissue, and subsequently biological functions, including growth and reproduction, may be impaired as resources are prioritized for this purpose (Rinkevich, 1996; Henry and Hart, 2005).

The rate at which tissue is regenerated is important, because the likelihood that fouling organisms will colonize lesions increases with the duration of exposure of skeleton within the lesion (Meesters et al., 1997; Henry and Hart, 2005). Once fouling organisms colonize a lesion, it is unlikely that coral tissue will grow over the site of damage (Meesters et al., 1997). Whether coral tissues regenerate is determined by intrinsic factors, such as corallum morphology, the supply of interstitial cells, and the availability of metabolic energy (Meesters et al., 1996; Henry and Hart, 2005), as well as extrinsic factors including seawater depth and the magnitude of damage (Meesters et al., 1997; Nagelkerken et al., 1999). As global climate change leads to increases in seawater temperature (Bindoff et al. 2007), and reduced seawater pH (i.e., ocean acidification [Doney et al., 2009]), determining how extrinsic factors influence tissue repair in scleractinians will help to understand how they will respond to damage in the future.

Most research on the effects of ocean acidification on scleractinians has focused on calcification (Erez et al., 2011), but this phenomenon has the potential to affect other biological functions (Doney et al., 2009). As studies of the response of scleractinians to ocean acidification have broadened their consideration of treatment conditions, there has been growing attention to interactive effects, such as might occur in synergy with elevated temperature, feeding, light

intensities, and flow speeds (Erez et al., 2011; Chan and Connolly, 2013; Edmunds et al., 2012; Comeau et al., 2014a). To date, the possibility that ocean acidification could modulate recovery from partial mortality has rarely been considered (but see Renegar et al., 2008; Horwitz and Fine, 2014; Hall et al., 2015). Since changes in environmental conditions associated with ocean acidification could influence the availability of cellular and energetic resources for tissue regeneration, it is reasonable to hypothesize that tissue regeneration will be affected (Henry and Hart, 2005).

This study investigated the effects of ocean acidification on the recovery of a scleractinian from partial mortality. Two experiments were performed to address the effects of modest (in 2013) and severe (in 2014) damage on the common Indo-Pacific coral, massive *Porites* spp.

Experiment 1 tested the effects of ocean acidification on calcification and tissue regeneration in corals recovering from damage created with a scar extending 2–3 mm into the skeleton and tissue. Experiment 2 was similar, except that damage was more severe and consisted of a scar removing tissue and skeleton extending 5–6 mm into the skeleton and tissue. Both experiments were conducted with small colonies of massive *Porites* spp. from the back reef in Moorea, French Polynesia, and colonies were incubated in mesocosms supplied with flowing seawater, and controlled for P_{CO_2} , temperature, and irradiance. Corals were damaged to simulate corallivory resulting from fishes feeding in scraping (Experiment 1) and excavation (Experiment 2) feeding guilds (Bellwood and Choat, 1990).

2. Materials and Methods

Collection and acclimation

Experiments took place at the Richard B. Gump South Pacific Research Station on Moorea, and used small colonies (~ 4 cm diameter) of massive *Porites* spp. from 2–3 m depth in the back reef. Massive *Porites* spp. was used because small colonies of this taxon are common throughout the back reef, where they are subject to corallivory (Lenihan and Edmunds, 2010). Moreover, throughout the Indo-Pacific, members of this group play important roles in coral reef community structure. In Moorea, the back reef is populated by at least three species of massive *Porites* spp., with *P. lutea* and *P. lobata* particularly common (Bosserele et al., 2014). These species are distinguished by corallite structure (Veron and Pichon, 1982), but the differences are difficult to detect underwater, and may not conform to species boundaries discerned from genetic tools (Forsman et al., 2009). Therefore, small colonies of similar shape, color, and size were combined as massive *Porites* spp., which previously we have distinguished using morphological features to 85% *P. lutea* and 15% *P. lobata* (Edmunds, 2009).

An experiment was designed in which corals were damaged in a standardized way (i.e., scars of similar area and depth), and grown in mesocosms under two P_{CO_2} levels. Each P_{CO_2} treatment was created in replicate tanks containing multiple corals to alleviate the limitations of pseudoreplication (sensu Hurlbert, 1984) (described below). In Experiment 1, six corals were allocated to each treatment combination (damage versus undamaged $\times P_{CO_2}$) in each of three tanks at both P_{CO_2} treatments (72 corals), and in Experiment 2, 10 corals were allocated to each treatment combination in each of two tanks at both P_{CO_2} treatments (80 corals).

Corals were brought to the laboratory and attached to plastic tiles using epoxy (Z-Spar A-788, Splash Zone Compound Los Angeles, CA, USA) without damaging marginal tissues to induce a stress response. To allow recovery following collection, corals were retained in a 1000 L tank for 7 d in Experiment 1 (at 28.8°C and 481 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ irradiance) and 6 d in Experiment 2 (at 28.9°C and 489 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ irradiance). Temperature was measured with a certified digital thermometer ($\pm 0.05^\circ\text{C}$ model 15-077, Thermo Fisher Scientific, Waltham, MA, USA), and light intensity with a 4- π quantum light sensor (LI-193SA sensor and LI-1400 meter, LI-COR, Inc., Lincoln, NE, USA), and the levels of these factors were similar to the conditions at the site of collection in April when the experiments were conducted. Light was provided using four LED lamps (LED System Model: Sol Blue, AquaIllumination®, Ames, IA, USA).

Experimental treatments

In both experiments, half of the corals were damaged using snub-nose pliers to scrape a rectangular lesion on their upper surface, and care was taken to ensure the lesions were similar in size. Pliers were rinsed in clean seawater between damaging colonies to avoid cross-contaminating replicate corals with mucus and microbes. In Experiment 1, which involved superficial damage, lesions had a mean area of 0.99 cm^2 and penetrated ~ 1–2 mm into the colony surface. In massive *Porites* spp., the coral tissue forms a layer 5–6 mm thick, and therefore this damage did not extend across the full width of the tissue. In Experiment 2, which involved deeper scars and more severe damage, lesions again were created with snub-nose pliers, but their area was increased to 2.5 cm^2 and they were scraped to a depth of ~ 5–6 mm into the skeleton. The dimensions of lesions were similar to those created by parrotfish feeding on

massive corals (Bellwood and Choat, 1990; Rotjan and Lewis, 2005), with those created in Experiment 1 resembling bites of fishes in the scraping guild, and those in Experiment 2 resembling bites of fishes in the excavating guild (Bellwood and Choat, 1990). The other half of the corals was left undamaged and served as control for the damaged treatment.

Following damage, corals were retained in the acclimation tank for 1 day until mucus release attributed to damage ceased, and then control and damaged corals were randomly assigned to 150 L tanks (AquaLogic, San Diego, CA, USA) that were pre-set to treatment conditions (ambient and elevated P_{CO_2}). Six tanks were used in Experiment 1 and four in Experiment 2, and all were supplied with fresh seawater at 100-200 mL min⁻¹. Half of the tanks were bubbled with ambient air, and the other half with CO₂-enriched air to maintain P_{CO_2} at target values of ~ 400 μatm (ambient) and ~ 1,000 μatm (elevated). The elevated P_{CO_2} treatment corresponds to the pessimistic representative concentration pathway (RCP 8.5) that projects atmospheric P_{CO_2} to increase to ~ 1,370 ppm by 2100 (van Vuuren et al., 2011). Tanks were maintained at 28.0°C in Experiment 1, and 28.8°C in Experiment 2, with the slight difference reflecting logistical constraints. Corals were incubated for 19 d in Experiment 1 and 20 d in Experiment 2, and were randomly repositioned within the tanks daily to account for position effects.

Tanks were monitored for temperature, irradiance, salinity, pH, and carbonate chemistry.

Temperature was measured twice daily using a digital thermometer and irradiance was measured using a 4- π quantum PAR sensor and meter (both described above). In Experiment 1, irradiance in the tanks was measured weekly, and in Experiment 2 it was measured daily.

In Experiment 1, salinity was recorded every 2 d using a conductivity meter (YSI 3100), and pH was measured 3 times d⁻¹ (using a DG115-SC probe (Mettler-Toledo, Columbus OH, USA], fitted to an Orion 3 star meter [Thermo Fisher Scientific, Waltham, MA, USA], calibrated on the total scale using TRIS/HCl buffers [Dickson et al., 2007]). Total alkalinity (TA) was measured by potentiometric titration (T50, Mettler-Toledo) following SOP 3b (Dickson et al., 2007), and initially was calculated daily and, once stable, every 2 d. Carbonate chemistry parameters and P_{CO_2} were calculated using the SeaCarb package (Lavigne and Gattuso, 2012) in R software (R foundation for statistical computing), using TA, pH, temperature, and salinity. In Experiment 2, salinity and pH again were measured daily, using a refractometer and certified thermometer (as above), respectively. TA was calculated every 2–4 d by potentiometric titration, and carbonate chemistry parameters and P_{CO_2} were calculated daily using the same methods as Experiment 1.

Response variables

Net calcification was measured using change in buoyant weight for each coral between the start and end of the incubation. Change in buoyant weight was converted to dry weight using the density of aragonite (2.93 g cm⁻³ [Davies, 1989]) and seawater density, and values were standardized to area of the coral tissue (cm²) and time (d) in units of mg CaCO₃ cm⁻² d⁻¹. As lesions were deeper and larger in Experiment 2 versus Experiment 1, lesion area in Experiment 2 was subtracted from the colony area to obtain the tissue area used for normalizing calcification. In Experiment 2, net calcification (mg) also was standardized to biomass (mg) and time (d) in units of d⁻¹. Tissue area was determined at the conclusion of the experiment using aluminum foil (Marsh, 1970), and in Experiment 2, biomass was determined by fixing corals in 5% formalin in seawater for 72 h, decalcifying in 5% HCl in seawater, and drying the tissue tunic to a constant

weight at 60°C. Prior to drying, endolithic taxa were removed with forceps, and the tissue was rinsed in deionized water. Biomass was standardized to area of the coral tissue (cm^2) to obtain units of mg cm^{-2} , and we assumed the tissue area-biomass relationship was consistent among treatment groups.

Regeneration of tissue within the lesions was evaluated photographically as the rate at which new coral tissue filled in the damage (see Fig. 1 in Cameron and Edmunds, 2014). In Experiment 1, pictures of the lesions were recorded with a camera (Olympus 850 SW, 8 megapixels) prior to, and following, the incubation. These pictures revealed damaged tissue on the floor of the lesion (scars did not penetrate the full width of the coral tissue layer), and the final photographs were used to evaluate healing based on a two-category assessment of tissue color. Lesions were categorized as “partially healed” when their margins were visible, the lesion area and shape largely were unchanged relative to the first day of the incubation, and the return of normal tissue color was mostly restricted to individual polyps (i.e., not the coenosarc). Lesions were categorized as “mostly healed” when their margins were not clearly visible, and normal tissue color had returned to individual polyps and the coenosarc. There were no corals that could be categorized as fully healed based on the lesion being indistinguishable from the rest of the colony surface.

In Experiment 2, pictures of damaged corals were recorded (Nikon D70 camera, 6 megapixels) prior to incubation, and every 4 d thereafter until the experiment finished. Pictures were recorded from a fixed distance above the coral, and included a scale bar, and revealed exposed skeleton within the lesion at the start of the experiment. The area of the lesions (cm^2) was

calculated at each sampling using ImageJ software (Abramoff et al., 2004), and was used to calculate the rate of healing.

Statistical analysis

In both experiments, conditions in the tanks (temperature, pH, P_{CO_2} , TA, and irradiance) were analyzed using two-way, partly nested ANOVAs, with treatment (P_{CO_2}) a fixed factor, and tank a random factor nested within treatment. In Experiment 1, area-normalized net calcification was analyzed with a three-way, partly nested ANOVA with P_{CO_2} and damage (scarred versus undamaged) as fixed factors, and tank a random factor nested within P_{CO_2} . The frequency of corals categorized as partially healed versus mostly healed as a function of treatment (i.e., P_{CO_2}) was analyzed using a 2×2 contingency table with χ^2 -test. In Experiment 2, area- and biomass-normalized net calcification, as well as biomass, were analyzed with three-way, partly nested ANOVAs with P_{CO_2} and damage (scarred versus undamaged) as fixed factors, and tank as a random factor nested within P_{CO_2} . Lesion areas were expressed as a percentage of initial area, and repeated-measures ANOVA was used to test the effects of time, P_{CO_2} , and their interaction on the change in relative lesion area.

All statistics were carried out with Systat software (Version 11, Systat Software, Inc., San Jose, CA, USA) operating in a Windows environment. The normality and homoscedasticity assumptions of ANOVA were tested through graphical inspection of residuals.

3. Results

Experiment 1: Response to superficial damage

Treatment conditions. Tanks were maintained close to the targeted conditions (Table 1).

Salinity was consistent across tanks and averaged 35.2 (SE < 0.1, n = 6). Irradiance did not vary among tanks ($F_{4,12} = 0.191$, $P = 0.938$) or treatments ($F_{1,4} = 0.002$, $P = 0.968$), and had a grand mean of 373 ± 2 mmol photons $\text{m}^{-2} \text{s}^{-1}$ ($\pm$ SE, n = 6). Temperature varied among tanks ($F_{4,240} = 3.262$, $P = 0.013$) but not treatments ($F_{1,4} = 0.056$, $P = 0.825$), yet differences among means were $\leq 0.3^\circ\text{C}$; the grand mean was $28.3 \pm 0.1^\circ\text{C}$ ($\pm$ SE, n = 6). TA varied among tanks ($F_{4,108} = 2.946$, $P = 0.024$) but not treatments ($F_{1,4} = 0.019$, $P = 0.898$), and differences between means were ≤ 10 $\mu\text{mol kg}^{-1}$. Both P_{CO_2} and pH varied among treatments ($F_{1,4} = 6001$, $P < 0.001$ and $F_{1,4} = 10819$, $P < 0.001$, respectively), but not tanks ($F_{4,317} = 0.195$, $P = 0.941$, and $F_{4,498} = 0.301$, $P = 0.877$, respectively); these treatments contrasted 420 ± 3 μatm versus $1,050 \pm 57$ μatm P_{CO_2} , and a pH of $8.02 \pm < 0.01$ versus 7.69 ± 0.02 (all mean $\pm$ SD, n = 2).

Response variables. Mean calcification ranged from 1.07 ± 0.13 $\text{mg cm}^{-2} \text{d}^{-1}$ (damaged corals, elevated P_{CO_2}) to 1.67 ± 0.14 $\text{mg cm}^{-2} \text{d}^{-1}$ (undamaged corals, ambient P_{CO_2} , both $\pm$ SE) (Fig. 1A). Mean calcification was depressed 26% (pooled across P_{CO_2} treatments) by damage ($F_{1,4} = 21.205$, $P = 0.010$), but not by P_{CO_2} ($F_{1,4} = 2.247$, $P = 0.208$), the interaction between the two ($F_{1,4} = 3.093$, $P = 0.153$), or tank ($F_{4,64} = 0.598$, $P = 0.666$). For damaged corals, elevated P_{CO_2} reduced mean calcification 24%, while the effect of P_{CO_2} on undamaged corals was negligible (mean difference < 0.01%). At the end of the experiment, the categorization of the 36 damaged corals

based on the extent of lesion healing (partially versus mostly healed) was independent of P_{CO_2} ($\chi^2 = 1.003$, $P = 0.317$).

Experiment 2: Response to deeper scaring

Treatment conditions. Tanks were maintained close to the targeted treatment conditions (Table 1). Salinity averaged 33.8 across tanks ($\text{SE} < 0.1$, $n = 4$). Irradiance and temperature did not vary among tanks ($F_{2,80} = 0.388$, $P = 0.680$, and $F_{2,156} = 2.732$, $P = 0.068$, respectively) or treatments ($F_{1,2} = 8.173$, $P = 0.104$, and $F_{1,2} = 0.212$, $P = 0.690$, respectively) and had grand mean values of $627 \pm 9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and $28.8 \pm 0.1^\circ\text{C}$, respectively ($\pm \text{SE}$, $n = 4$). P_{CO_2} varied among tanks ($F_{2,84} = 3.370$, $P = 0.039$) and treatments ($F_{1,2} = 228$, $P = 0.004$), however, differences between tanks were $\leq 81 \mu\text{atm}$. pH varied among treatments ($F_{1,2} = 378$, $P = 0.003$) but not tanks ($F_{2,80} = 2.214$, $P = 0.116$), and TA was consistent among tanks ($F_{2,24} = 0.879$, $P = 0.428$) and treatments ($F_{1,2} = 1.223$, $P = 0.384$). These treatments contrasted $388 \pm 6 \mu\text{atm}$ versus $970 \pm 7 \mu\text{atm}$ P_{CO_2} , and a pH of $8.06 \pm < 0.01$ versus $7.74 \pm < 0.01$ (all mean $\pm \text{SE}$, $n = 3$).

Response variables. Area-normalized calcification ranged from $0.60 \pm 0.05 \text{ mg cm}^{-2} \text{d}^{-1}$ (damaged corals, elevated P_{CO_2}) to $0.79 \pm 0.08 \text{ mg cm}^{-2} \text{d}^{-1}$ (damaged corals, ambient P_{CO_2} , both mean $\pm \text{SE}$), and was not affected by P_{CO_2} ($F_{1,2} = 3.943$, $P = 0.185$), damage ($F_{1,2} = 2.804$, $P = 0.236$), the interaction between the two ($F_{1,2} = 4.030$, $P = 0.182$), or tank ($F_{2,72} = 0.836$, $P = 0.438$) (Fig. 1B). Mean biomass-normalized calcification ranged from $0.071 \pm 0.008 \text{ d}^{-1}$ (damaged corals, elevated $p\text{CO}_2$) to $0.088 \pm 0.008 \text{ d}^{-1}$ (undamaged corals, ambient P_{CO_2} , both $\pm \text{SE}$) (Fig. 1D), and was reduced by elevated P_{CO_2} ($F_{1,2} = 20.577$, $P = 0.045$), with a 10% decrease across both damage treatments, and was unaffected by damage ($F_{1,2} = 8.583$, $P = 0.099$), the P_{CO_2}

$\times$ damage interaction ($F_{1,2} = 4.716$, $P = 0.162$), and tank ($F_{2,72} = 0.047$, $P = 0.954$). Mean biomass ranged from $8.80 \pm 0.70 \text{ mg cm}^{-2}$ (undamaged corals, elevated P_{CO_2}) to $9.57 \pm 0.49 \text{ mg cm}^{-2}$ (damaged corals, elevated P_{CO_2}) and was not affected by P_{CO_2} ($F_{1,2} = 0.511$, $P = 0.549$), damage ($F_{1,2} = 0.184$, $P = 0.710$), the interaction between the two ($F_{1,2} = 0.004$, $P = 0.954$), or tank ($F_{2,72} = 1.749$, $P = 0.181$) (Fig. 1C).

At the end of the incubation, lesions were reduced to $20.6 \pm 4.1\%$ of their original size at ambient P_{CO_2} , and to $22.7 \pm 4.0\%$ of their original size at elevated P_{CO_2} (both mean $\pm$ SE, $n = 20$) (Fig. 2). The highest rates of decline in lesion area occurred within the first 3 d following damage, averaging $9.0 \pm 0.9\% \text{ d}^{-1}$ in elevated P_{CO_2} , and $7.6 \pm 0.8\% \text{ d}^{-1}$ in ambient P_{CO_2} (both $\pm$ SE, $n = 20$). The reduction in size of lesions differed over time ($F_{4,8} = 9.874$, $P = 0.003$), but was unaffected by P_{CO_2} ($F_{1,2} = 0.014$, $P = 0.917$), the $P_{\text{CO}_2} \times$ time interaction ($F_{4,8} = 1.102$, $P = 0.418$), or tank ($F_{2,180} = 0.994$, $P = 0.372$).

4. Discussion

This study tested the hypothesis that ocean acidification affects the response of massive *Porites* spp. to damage of differing severities representing the effects of corallivory by fishes in the scraping and gouging functional groups (Bellwood and Choat, 1990). There is a long history of investigating the effects of damage on corals (Cole et al., 2008; Rotjan and Lewis, 2008; Bruckner and Bruckner, 2016), and recent interest in the response of corals to ocean acidification (Chan and Connelly, 2012; Kroeker et al., 2013), but little is known of the interaction between these stressors in terms of their effects on coral performance (but see Renegar et al., 2008;

Horwitz and Fine, 2014; Hall et al., 2015). A prior, it was reasonable to hypothesize that regeneration of coral tissue and skeleton following damage would be affected by ocean acidification, potentially because the physical and chemical consequences of this phenomenon can alter the use of metabolic energy by metazoans (Pan et al. 2015), and in reef corals, can modulate holobiont respiration and photochemical efficiency (Anthony et al., 2008; Crawley et al., 2009; Edmunds, 2012; Kaniewska et al., 2012; but see Schneider and Erez, 2006; Comeau et al., 2016), calcification (Erez et al., 2011; Chan and Connelly, 2012), and protein metabolism (Kaniewska et al., 2012; Edmunds and Wall, 2014). In contrast to this expectation, ocean acidification had no effect on the response to damage, as evaluated by area-normalized calcification, biomass, and rate of closure of the lesion (i.e., tissue regeneration) during short experiments in mesocosms.

The effect of damage on calcification of massive *Porites* spp. varied between experiments, and area-normalized rates were unaffected by $\sim 1,000 P_{CO_2}$, and remained similar to those previously reported for this genus in Moorea (Comeau et al., 2014b), as well as more broadly for other corals (Pratchett et al., 2015). In Experiment 1, damage affecting $\sim 4\%$ of the surface of the corals reduced area-normalized calcification $\sim 26\%$ in ambient and elevated P_{CO_2} , while in Experiment 2, more severe damage affecting $\sim 10\%$ of the surface area of the corals (and removing a deeper layer of skeleton and tissue) did not affect area-normalized or biomass normalized calcification, although P_{CO_2} reduced biomass-normalized calcification $\sim 10\%$ (Fig 1A,B).

The key to reconciling the contrasting results in the two experiments probably lies in differences in the environmental conditions preceding the experiments, and differences between experiments in the physical conditions employed. For instance, while calcification rates in both experiments were within the range of values previously reported for this genus, mean rates in Experiment 1 were double those in Experiment 2 (pooled among P_{CO_2} and damage treatments), which could reflect the effects of seawater temperature both in the mesocosms and on the reef prior to the experiment. In the mesocosms, mean seawater temperature was 0.6°C warmer in Experiment 2 (28.9°C) compared to Experiment 1 (28.3°C), and the warmer conditions in Experiment 2 might have depressed calcification if ~ 28.3°C is close to the thermal threshold for calcification in massive *Porites* spp. (Pratchett et al., 2015). In situ, seawater temperature from April 2012 to March 2013 (1 y prior to Experiment 1) was slightly warmer (mean = 27.7°C, n = 365 d) with a warmer winter minimum (26.1°C) than from April 2013 to March 2014 (~ 1 y prior to Experiment 2), that was cooler (mean = 27.5°C, n = 345 d) with a colder winter minimum (25.3°C) (Washburn 2015). These effects might have established differences in the quality of coral tissue (Fitt et al., 2000; Thornhill et al., 2011) that were associated with reduced calcification in Experiment 2. Light intensity also was higher in Experiment 2 (627 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) than Experiment 1 (372 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) due to logistical constraints, and while this difference is unlikely to have directly depressed calcification in Experiment 2 given the hyperbolic tangent function that relates calcification to light intensity in reef corals (Chalker, 1981), it probably would have accelerated photosynthesis (Hennige et al., 2008). With greater rates of photosynthesis, it is likely that larger quantities of photosynthetically fixed carbon would be translocated to the animal host (Muscatine et al., 1984; Edmunds and Davies, 1986). This enhanced supply of carbon (and the energy it represents) may have eclipsed the metabolic costs

of repair (Henry and Hart, 2005), thereby allowing the calcification rates of damaged corals to remain similar to those of undamaged corals.

In Experiment 2, stable area-normalized calcification and depressed biomass-normalized calcification at high P_{CO_2} also requires explanation, and conceivably could be a result of a P_{CO_2} -induced increase in biomass. This would depress biomass-normalized calcification, but with more tissue calcifying less rapidly, area-normalized calcification could be sustained (as was observed). Massive *Porites* spp. uses this strategy to ameliorate its sensitivity to high P_{CO_2} (Edmunds, 2011), but in the study in which this has been demonstrated, biomass was enhanced at high P_{CO_2} in association with ad libitum feeding on *Artemia* spp. (Edmunds, 2011). In the present study in which corals were not fed plankton and experiments lasted ~ 3 weeks (cf. 1 month in Edmunds, 2011), P_{CO_2} was not associated with a change in biomass (Fig. 1C), yet tissue calcifying 10% less quickly at high P_{CO_2} supported equal area-normalized calcification at elevated and ambient P_{CO_2} . It is not possible to explain this outcome with the present results, but it could reflect a limitation of the assumption that calcification did not occur within lesions that removed the full tissue thickness (described in methods), and therefore, that the area of the scar should be subtracted from colony area before normalizing calcification to area. If the scarring left sufficient tissue deep within the skeleton to support limited calcification within the scar, and this tissue recovered quickly (as observed), then our area-normalized calcification rates would be upwardly biased by the extent to which the scar area functioned like undamaged areas of the coral in terms of calcification. If this is correct, then potentially the high P_{CO_2} regime applied in Experiment 2 could have caused a slight overall reduction in area-normalized calcification.

Previous studies have demonstrated that the effect of damage on the growth of corals varies among species, increasing growth in some (Loya, 1976), while decreasing growth in others (Cox, 1986; Meesters et al., 1994). Loya (1976), for example, found that damaged colonies of the branching coral *Stylophora pistillata* in the Gulf of Eilat, grew twice as fast as undamaged colonies, and in Moorea, Edmunds and Lenihan (2009) found that the effects of damage (~ 67 mm² gouges) on the calcification of massive *Porites* spp. depended on environmental conditions; damage accelerated calcification 25% at 26.7°C, but depressed calcification 25% at 29.6°C. Decreased calcification in damaged corals, as in Experiment 1, is consistent with a trade-off model involving limited resources, for example energy and nitrogen, which are diverted from calcification to tissue regeneration to accelerate recovery (Henry and Hart, 2005). In Experiment 2, calcification and biomass were not affected by damage, which suggests that the energy used for tissue regeneration was not being diverted from calcification or tissue reserves. It is possible that in Experiment 2, carbon resources either were in excess of needs (described above), or were reallocated to repair from other processes, such as reproduction, which can be depressed in damaged corals (Rinkevich 1996).

In the present study, a P_{CO_2} of ~ 1,000 μatm did not affect repair in massive *Porites* spp. over 19–20 d, after which damage appeared almost fully healed. Healing was evaluated every 4 d in Experiment 2, and initially was rapid, but slowed over time as predicted from an exponential decay function that describes healing in corals (Meesters et al., 1994; Denis et al., 2011). Rates of healing during Experiment 2 were 4.0% d⁻¹ in ambient P_{CO_2} and 3.9% d⁻¹ in elevated P_{CO_2} , which are similar to rates previously reported for massive *Porites* spp. For example, Cameron and Edmunds (2014) found that small colonies with single sites of excavation damage (each ~

105 mm² and 2–5 mm deep) healed at $\sim 2.5\% \text{ d}^{-1}$ over 21 d, while Edmunds and Lenihan (2009) reported tissue healing of $4\text{--}5\% \text{ d}^{-1}$ over 10 d for small colonies with lesions $\sim 67 \text{ mm}^2$ and 1–2 mm deep.

The conclusion of this study that healing in massive *Porites* spp. is unaffected by high P_{CO_2} , has several caveats relating to the extent of damage, the duration of the experiment, the magnitude of the P_{CO_2} regime applied, and the potential for calcification to occur in severe (i.e., deep) scars such as those created in Experiment 2. First, the present results can only be applied to larger corals (i.e., > 4 cm diameter), cases of more severe damage (e.g., multiple bites on each colony), and longer durations of exposure to high P_{CO_2} , with caution, because all three cases require extrapolation beyond the scope of this study. Second, while the treatments were designed to be ecologically relevant, with the P_{CO_2} regime simulating a pessimistic projection for the next ~ 200 y, more extreme P_{CO_2} regimes could produce different results. For instance, wound healing in massive *Porites* spp. was slowed in two of three studies in which P_{CO_2} regimes extended from 1,381–4,000 μatm (reductions in Horwitz and Fine [2014] and Renegar et al. [2008], but not in Hall et al. [2015]). Therefore, in microhabitats where seawater P_{CO_2} already exceeds 1,000 μatm (e.g., natural CO_2 seeps [Fabricius et al., 2011]), or further than ~ 100 y into the future under pessimistic predictions of human CO_2 emissions (van Vuuren et al., 2011), it remains possible that coral tissue regeneration will be impaired in massive *Porites* spp. Finally, our results draw attention to the need to know more about the capacity for *Porites* spp. to calcify in wounded areas where only a small fraction of the original host tissue remains, and the speed with which normal rates of calcification resume in such areas as tissue healing progresses.

Overall, the responses to ocean acidification described herein supports evidence of the physiological robustness of massive *Porites* spp. to P_{CO_2} as high as $\sim 2,000 \mu\text{atm}$ (Fabricius et al., 2011; Comeau et al., 2014b), by revealing that members of this taxon can recover equally well from modest damage at both ambient and elevated P_{CO_2} ($\sim 1,000 \mu\text{atm}$). As three other coral species and massive *Porites* spp. show depressed tissue regeneration at $1,800 \mu\text{atm}$ (versus ambient) (Horwitz and Fine, 2014), as does *P. porites* (but not *P. astreoides*) at pH 7.6 (ca. $2,105 \mu\text{atm } P_{\text{CO}_2}$, see Hall et al., 2012) (Hall et al., 2015), and *P. astreoides* at $1,381 \mu\text{atm } P_{\text{CO}_2}$ (Renegar et al., 2008), the resilience of wound healing in massive *Porites* spp. may have a threshold around $1,000 \mu\text{atm } P_{\text{CO}_2}$, beyond which impairment occurs. Together, the present results suggest that damaged massive *Porites* spp. will be able to maintain tissue regeneration and perhaps calcification at P_{CO_2} values expected by the end of the current century.

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Figures

Figure 1. Effects of manipulative experiments testing the effects of damage on calcification and biomass of massive *Porites* spp.: (A) area-normalized calcification for Experiment 1 (19 d, $n = 18 \text{ treatment}^{-1}$), (B) area-normalized calcification for Experiment 2 (20 d, $n = 20 \text{ treatment}^{-1}$), (C) biomass for Experiment 2 ($n = 20 \text{ treatment}^{-1}$), and (D) biomass-normalized calcification for Experiment 2 ($n = 20 \text{ treatment}^{-1}$). Values displayed are means $\pm$ SE.

Figure 2. Percent of the original lesion area remaining on damaged corals as a function of time and treatment (P_{CO_2} regime) in Experiment 2. Lesions were $2.56 \pm 0.08 \text{ cm}^2$ when first administered. Mean $\pm$ SE shown ($n = 20 \text{ measurement}^{-1}$)

Figure 1

Figure 2

Table 1. Summary of the tank parameters for Experiments 1 (19 d) and Experiment 2 (20 d). Mean $\pm$ SE (n) shown.

Experiment	Treatment	Tank	Temperature (°C)	Irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	pH	P_{CO_2} (μatm)	TA ($\mu\text{mol kg}^{-1}$)	Ω_{arag}
1	Ambient	1	28.4 ± 0.1 (41)	362 ± 5 (3)	8.06 ± 0.01 (61)	401 ± 8 (41)	2297 ± 3 (17)	3.74 ± 0.01 (41)
		3	28.2 ± 0.1 (41)	377 ± 12 (3)	8.06 ± 0.01 (61)	384 ± 6 (41)	2289 ± 4 (19)	3.80 ± 0.01 (41)
		8	28.4 ± 0.1 (41)	373 ± 13 (3)	8.07 ± 0.01 (61)	380 ± 6 (41)	2288 ± 3 (18)	3.85 ± 0.01 (41)
	Elevated	4	28.4 ± 0.1 (41)	365 ± 25 (3)	7.74 ± 0.01 (61)	976 ± 38 (41)	2289 ± 3 (21)	2.09 ± 0.01 (41)
		7	28.3 ± 0.1 (41)	376 ± 20 (3)	7.73 ± 0.01 (61)	978 ± 31 (41)	2298 ± 2 (18)	2.07 ± 0.01 (41)
		9	28.1 ± 0.1 (41)	379 ± 9 (3)	7.74 ± 0.01 (61)	956 ± 33 (41)	2288 ± 3 (21)	2.09 ± 0.01 (41)
2	Ambient	1	28.8 ± 0.1 (41)	636 ± 14 (21)	8.01 ± 0.01 (21)	422 ± 17 (21)	2210 ± 17 (21)	3.36 ± 0.07 (21)
		6	28.9 ± 0.1 (40)	647 ± 19 (21)	8.02 ± 0.01 (21)	418 ± 12 (21)	2268 ± 32 (7)	3.58 ± 0.09 (21)
	Elevated	2	28.8 ± 0.1 (40)	621 ± 18 (21)	7.67 ± 0.01 (21)	1091 ± 29 (23)	2262 ± 14 (7)	1.83 ± 0.05 (21)
		7	29.0 ± 0.1 (40)	604 ± 14 (21)	7.70 ± 0.01 (21)	1010 ± 26 (23)	2283 ± 15 (7)	2.00 ± 0.04 (21)