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RESEARCH ARTICLE

Emergent properties of branching morphologies modulate the sensitivity of coral calcification to high P_{CO_2}

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ABSTRACT

Experiments with coral fragments (i.e. nubbins) have shown that net calcification is depressed by elevated P_{CO_2}. Evaluating the implications of this finding requires scaling of results from nubbins to colonies, yet the experiments to codify this process have not been carried out. Building from our previous research demonstrating that net calcification of *Pocillopora verrucosa* (2–13 cm diameter) was unaffected by P_{CO_2} (400 and 1000 µatm) and temperature (26.5 and 29.7°C), we sought generality to this outcome by testing how colony size modulates P_{CO_2} and temperature sensitivity in a branching acroporid. Together, these taxa represent two of the dominant lineages of branching corals on Indo-Pacific coral reefs. Two trials conducted over 2 years tested the hypothesis that the seasonal range in seawater temperature (26.5 and 29.2°C) and a future P_{CO_2} (1062 µatm versus an ambient level of 461 µatm) affect net calcification of an ecologically relevant size range (5–20 cm diameter) of colonies of *Acropora hyacinthus*. As for *P. verrucosa*, the effects of temperature and P_{CO_2} on net calcification (mg day^{-1}) of *A. verrucosa* were not statistically detectable. These results support the generality of a null outcome on net calcification of exposing intact colonies of branching corals to environmental conditions contrasting seasonal variation in temperature and predicted future variation in P_{CO_2}. While there is a need to expand beyond an experimental culture relying on coral nubbins as tractable replicates, rigorously responding to this need poses substantial ethical and logistical challenges.

KEY WORDS: Scleractinia, Ocean acidification, Allometry

INTRODUCTION

Together with climate change (i.e. rising temperature), ocean acidification (OA) threatens tropical reef corals (Hoegh-Guldberg et al., 2007), with effects that augment other anthropogenic disturbances (e.g. Miller, 2015). Understanding the threats posed to net calcification of corals by OA is based on research conducted with coral fragments (i.e. nubbins; Birkeland, 1976) that lend themselves to experiments in aquaria. Such studies have found added relevance to reef restoration efforts that rely on coral 'fragging' to increase population sizes (Barton et al., 2017). As fragging produces numerous coral nubbins, this nascent field can benefit from what has been learned through scientific studies conducted with nubbins. Yet, a coral reef is more than countless nubbins growing on hard surfaces.

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Received 21 October 2019; Accepted 10 March 2020

Most tropical scleractinians are colonial organisms growing through iterations of a modular design (i.e. polyps) to create colonies with emergent properties attributed to corallum morphology (Patterson, 1992a,b; Enríquez et al., 2017; Stocking et al., 2018; Burgess et al., 2017; Dornelas et al., 2017). This body plan was long thought to circumvent allometric constraints on size (Sebens, 1987; Hughes, 2005), thereby providing a convenient rationale for using analyses of coral nubbins to support inferences about larger coral colonies (Spencer Davies, 1984; Shafir et al., 2003). However, there is now evidence that traits such as calcification, respiration and photosynthesis will vary in proportionately dissimilar ways with colony size in corals (Chamberlain and Graus, 1975; Jokiel and Morrissey, 1986; Patterson, 1992a,b; Edmunds and Burgess, 2016; Dornelas et al., 2017). Allometric scaling arises from intrinsic constraints caused by the covariation of organism area and volume (Schmidt-Nielsen, 1984), but it can also arise from extrinsic effects of organism design (i.e. morphology), such as within-colony variation in tissue thickness (Brown et al., 1999; Fitt et al., 2000; Thornhill et al., 2011), and its interactions with the environment (Patterson, 1992a,b; Ong et al., 2017; Burgess et al., 2017).

For more than a decade, nubbins have been used as experimental replicates to study the effects of OA on corals. While many such studies have revealed negative effects on calcification (Chan and Connolly, 2013; Kroeker et al., 2013; Comeau et al., 2014), greater ecological relevance in OA experiments has been sought (Gaylord et al., 2015; Barner et al., 2018), in part, by increasing the size of coral colonies studied. One test of these properties explored the impacts of elevated P_{CO_2} (400 and 1000 µatm) and temperature (26.4 and 29.7°C) on net calcification of *Pocillopora verrucosa* (identified based on morphological features after Veron and Pichon, 1976) varying in diameter from 2.6 to 12.0 cm (Edmunds and Burgess, 2016). The study revealed size-dependent effects, but net calcification was unaffected by P_{CO_2} or its interaction with temperature. These results contrasted with the inhibition of net calcification by high P_{CO_2} in nubbins of *P. verrucosa* (Comeau et al., 2014), and Edmunds and Burgess (2016) (see also Burgess et al., 2017) speculated that the different outcome was a product of the emergent properties of intact colonies.

In this study, we describe an experiment designed to assess the generality of our previous result, and expand the taxonomic breadth of our analyses (Edmunds and Burgess, 2016, 2018) by working with the common Indo-Pacific coral *Acropora hyacinthus* (Dana 1846). The colonies of *A. hyacinthus* also varied in diameter, but unlike *P. verrucosa*, they had a table-like morphology composed of many small and thin branches, among which the flow of seawater, solute concentrations, metabolite fluxes and zooplankton capture are likely to vary. We focused on an acroporid to test for generality of results for two of the most important functional groups of corals on Indo-Pacific fore reefs (Done, 1983; Veron, 2000; Vercelloni et al., 2019), and used *A. hyacinthus* because of its high abundance on the fore reefs of Mo'orea when the experiment was conducted (P.J.E., unpublished data).

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MATERIALS AND METHODS

The two trials were completed during two field seasons (April to May in 2017 and 2018) at the Richard B. Gump South Pacific Research Station on Mo'orea (17°32'S 149°50'W). Both trials were completed in a mesocosm consisting of 150 l tanks that were independently heated, chilled, illuminated and supplied with CO_2 gas. The objective of working with large coral colonies prevented more than one colony of each size class being placed in each tank, because replicate colonies caused the total alkalinity (A_T) of the seawater to be drawn down through calcification. It was not possible to compensate for depressed A_T through higher rates of seawater turnover in each tank, as this destabilized treatment conditions for P_{CO_2} and temperature. Trial 1 was conducted in 2017 with 8 small (~6 cm diameter) and 8 large (~17 cm diameter) colonies of *A. hyacinthus* that were incubated for 23 days in 8 tanks crossing two temperatures (26.5 and 29.3°C) and two P_{CO_2} levels (~471 and 1062 µatm). Trial 2 was completed in 2018, and was implemented to replicate the first trial as closely as possible (i.e. 26.4 versus 29.1°C, and 450 versus 1062 µatm P_{CO_2}). Trial 2 included 1 small and 1 large coral in each of 7 tanks; an eighth tank was included in an orthogonal design, but was dropped from the analysis following an equipment malfunction.

Net calcification was used as a response variable and was measured by buoyant weighing (Spencer Davies, 1989), and expressed by colony as a function of colony size (tissue surface area). In both experiments, sample sizes were selected based on previous experiments in which statistical power was adequate to test the hypotheses of interest, but the final design was a compromise among the needs for experimental replicates, the importance of statistical independence, and the capacity for high precision in treatment conditions. All coral colonies were allocated randomly to treatment conditions.

Colonies of *A. hyacinthus* were haphazardly collected from 10 m depth on the fore reef of Mo'orea. Collection targeted small (~6 cm diameter) and large (~17 cm diameter) colonies that were bagged individually, and returned to the lab immersed in seawater and shaded from sunlight. Corals for trial 1 were collected on 11 April 2017, and those for trial 2 on 9 April 2018, and in both cases they were kept in a 1000 l tank for 4 days to adjust to laboratory conditions. This tank was maintained at ambient seawater temperature when the experiments were conducted (28.3±0.1°C in 2017 and 29.3±0.1°C in 2018, mean±s.e., N=5 and 26, respectively), supplied with fresh seawater pumped from 14 m depth in Cook's Bay (at 10 l min^{-1}), and illuminated at 452±12 and 618±11 µmol photons m^{-2} s^{-1} (mean± s.e., N=5 and 7, respectively) with LED lamps (Aquaillumination SOL white). Light was measured as photon flux density (PFD in the range of photosynthetically active radiation, PAR) using a quantum sensor (LiCor LI 193), and PFD was adjusted to approximate the values recorded at the collection depth on the fore reef of Mo'orea in April (ca. 645 µmol photons m^{-2} s^{-1} at noon). The corals were located on a table within the tank that rotated at 2 revolutions day^{-1} to remove position effects. Following lab adjustment, the corals were buoyant weighted (±1 mg for small corals and ±10 mg for large corals) and placed into the treatment tanks for the 23 day experiment.

One small and one large coral were randomly assigned to each 150 l tank in the mesocosm, with 8 tanks used to create orthogonal contrasts of two temperatures and two P_{CO_2} regimes. Two tanks were nested in each combination of conditions in both trials, but equipment malfunction reduced trial 2 to 7 tanks. Coral colonies were supported upright in the tanks by placing them in PVC cups, and each was identified with a numbered tag. The mesocosm (including lights and P_{CO_2} manipulations) is described elsewhere (Edmunds and Burgess, 2016), and here was used to target treatments of ~26.5 and ~29.0°C, which created a contrast of two temperatures within the current annual range of seawater temperatures in Mo'orea (Edmunds, 2017). The P_{CO_2} treatment contrasted present-day (i.e. ~400 µatm P_{CO_2}, ambient) and future P_{CO_2} conditions (~1000 µatm P_{CO_2}). The high P_{CO_2} is a possible outcome by the end of the current century under the pessimistic RCP 8.5 scenario (IPCC, 2014). The temperature treatments tested for the effects on net calcification of ecologically relevant temperatures occurring within a year, but the high value was also selected to reduce the possibility of thermal bleaching. In Mo'orea, coral bleaching occurs when seawater temperature exceeds about 29.8°C (Wyatt et al., 2019), and the use of an upper temperature below this value allowed us to focus on the effects on calcification rather than bleaching.

Both trials lasted 23 days, during which the physical and chemical conditions in the tanks were monitored, and the instantaneous values were used to adjust the control systems to maintain treatment conditions. Seawater temperature was recorded at least daily [using a certified thermometer (±0.05°C), model 15-077, Fisher Scientific, Pittsburgh, PA, USA]. Daily measurements were also taken for salinity (using a conductivity meter, YSI 3100, YSI Inc., Yellow Springs, OH, USA), pH (using a hand-held meter, Orion 3 star meter, Mettler-Toledo, LLC, Columbus, OH, USA, fitted with a Mettler DG115-SC probe) and light (Li Cor, LI-193 sensor attached to a Li Cor LI-1400 meter). Seawater samples (50 ml) were collected every 2–3 days for the analysis of A_T using potentiometric titrations (after SOP3b; Dickson et al., 2007) conducted using an open cell, automatic titrator (Model T50, Mettler-Toledo) fitted with a pH probe (Mettler DG115-SC) that was calibrated on the total scale using Tris/HCl buffers (Dickson et al., 2007). The accuracy and precision of the titrations was determined using certified reference materials (CRMs, batches 151, 158 and 172) from A. Dickson (Scripps Institution of Oceanography). The accuracy of the pH measurements obtained with the hand-held meter was determined by periodic comparison with values determined using *m*-Cresol Purple dye (no. 211761, Sigma-Aldrich, St Louis, MO, USA) according to SOP7 of Dickson et al. (2007). The measurements of seawater pH and A_T were used to calculate dissolved inorganic carbon (DIC) parameters using Seacarb (Gattuso et al., 2015) running in the R software environment (v3.6.1; R Foundation for Statistical Computing; http://www.R-project.org/).

Coral colonies were haphazardly moved within the tanks daily to avoid position effects, and periodically were inspected for night-time polyp expansion as an indicator of normal biological function. At the conclusion of the trials (8 May in 2017 and 7 May in 2018), the corals were buoyant weighed, and based on the change in buoyant weight, an empirical determination of seawater density and an aragonite density (Ω_{arag}) of 2.93 g cm^{-3}, the mass increment representing net calcification (in grams) was calculated. The surface area of live coral tissue was calculated by wax dipping (Stimson and Kinzie, 1991) and used as a measure of colony size.

Statistical analyses

The experiment was analyzed using linear mixed effects models in the nlme package in R. Colony size (surface area in cm^2), temperature and P_{CO_2} were modeled as fixed effects. Years and tanks were modeled as random effects. The response variable was net calcification, measured as the average daily change in skeletal mass (mg) over 23 days (i.e. mg day^{-1}). Heteroscedasticity of residuals was modeled as a power variance function with respect to colony size. Analysis of variance was performed on the linear mixed

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effects model using conditional *F*-tests, although it should be noted that the *P*-values are large-sample approximations and are anti-conservative.

RESULTS

The physical and chemical conditions during trials 1 and 2 were stable and differed between treatments for temperature and P_{CO_2} (Table 1). Trial 1 contrasted 26.5±<0.1 versus 29.3±<0.1°C, and 471±26 versus 1062±9 µatm P_{CO_2}. Trial 2 contrasted 26.4±0.1 versus 29.1±0.1°C, and 450±9 versus 1062±13 µatm P_{CO_2} (mean± s.e., N=3–4). Mean (±s.e.) A_T of the incubation seawater remained close to ambient seawater in Mo'orea (~2300 µmol kg^{-1}; Doo et al., 2019) during trial 1 (2304±3 µmol kg^{-1}, N=8) and trial 2 (2285±4 µmol kg^{-1}, N=7), indicating that the corals were not depressing A_T through calcification. All corals in both trials remained normally colored relative to *A. hyacinthus* at 10 m depth on the outer reef when the study was completed, although one small colony in trial 1 became pale (Fig. 1C). Overall, there were no signs of tissue loss in either trial, and polyps routinely were seen expanded and feeding at night.

At the start of trial 1, small corals ranged in diameter from 5 to 8 cm, and large corals from 13 to 20 cm; at the start of trial 2, small corals ranged from 5 to 8 cm, and large corals from 16 to 20 cm. At the end of the experiment, the tissue area of trial 1 corals ranged from 93 to 1222 cm^2, and for trial 2 from 149 to 1076 cm^2. All corals regardless of treatment, tank or trial increased in mass over 23 days, with increments varying from 123 to 1472 mg day^{-1} in trial 1, and from 59 to 1476 mg day^{-1} in trial 2 (Fig. 1). Net calcification increased by 1.063 mg day^{-1} (0.694–1.433 mg day^{-1}, 95% confidence interval, CI) for every cm^2 increase in colony surface area at 26.5°C and ambient P_{CO_2}, but was unaffected by increases in temperature or P_{CO_2} (Tables 2 and 3, Fig. 1).

DISCUSSION

After nearly two decades of experimentation, the effects of high P_{CO_2} and temperature on the calcification of reef corals has been tested in numerous studies (Chan and Connolly, 2013; Kornder et al., 2018). The overall outcome of most of these studies is that high P_{CO_2} (relative to ~400 µatm) depresses net calcification (Chan and Connolly, 2013; Kroeker et al., 2013; Comeau et al., 2014). There are exceptions to this negative trend (Reynaud et al., 2003; Castillo et al., 2014; Strahl et al., 2015; Wall et al., 2017) and indications that its intensity can be attenuated by incubation conditions and nutritional status of the coral (Edmunds, 2011; Dufault et al., 2013; Vogel et al., 2015). In at least two cases, temperature and high P_{CO_2} have been found to act in positive synergy to affect coral calcification, but one of these contrasted temperatures within a normal range for tropical reefs (25.0 versus 28°C; Reynaud et al., 2003) and the other contrasted a naturally occurring temperature with a likely future temperature (26 versus 32°C; Langdon et al., 2018). More commonly, however, temperature and P_{CO_2} have been shown to affect coral calcification independently (Horvath et al., 2016; Okazaki et al., 2017; Kroeker et al., 2013; Anderson et al., 2019). On its own, temperature affects net calcification of corals with a non-linear relationship characterized by a thermal threshold at ~28°C (Edmunds, 2005; Castillo et al., 2014; Pratchett et al., 2015; Silbiger et al., 2019). Meta-analyses of the effects of high P_{CO_2} (and reduced seawater pH) show negative effects on coral calcification: for 25 studies predating 2011, Chan and Connolly (2013) reported a ~15% decline for each unit reduction in Ω_{arag} of seawater over the range of 2<Ω_{arag}<4, and for 41 experiments published before 1

Table 1. Physical and chemical conditions during two trials conducted in 2017 and 2018

Trial	Tank no.	Treatment	pH_T	A_T (µmol kg^{-1})	C_T (µmol kg^{-1})	P_{CO_2} (µatm)	Ω_{arag}	T (°C)	Salinity	Light (µmol m^{-2} s^{-1})
2017	1	HTHC	7.68±* (24)	2316±3 (24)	2171±3 (24)	1083±12 (24)	1.99±0.02 (24)	29.3±0.1 (24)	35.8±0.1 (24)	624±4 (10)
	3	HTHC	7.69±0.01 (24)	2314±7 (24)	2165±7 (24)	1057±15 (24)	2.02±0.02 (24)	29.2±0.1 (24)	35.8±* (24)	647±7 (10)
	2	LTHC	7.69±* (24)	2303±5 (24)	2171±3 (24)	1042±12 (24)	1.81±0.02 (24)	26.5±0.1 (24)	35.7±0.1 (24)	654±6 (10)
	9	LTHC	7.68±0.01 (24)	2300±9 (24)	2165±7 (24)	1065±17 (24)	1.79±0.02 (24)	26.5±* (24)	35.9±* (24)	630±19 (10)
	5	HTAC	7.94±0.02 (24)	2303±7 (24)	2020±11 (24)	537±25 (24)	3.28±0.08 (24)	29.3±0.1 (24)	35.9±* (24)	658±11 (10)
	4	HTHC	7.97±0.01 (24)	2295±11 (24)	1996±9 (24)	487±12 (24)	3.43±0.06 (24)	29.3±0.1 (24)	35.8±0.1 (24)	652±7 (10)
	8	LTAC	8.02±0.01 (24)	2309±3 (24)	2007±6 (24)	430±12 (24)	3.44±0.05 (24)	26.5±* (24)	35.9±* (24)	626±11 (10
	10	LTAC	8.01±0.01 (24)	2291±13 (24)	1986±11 (24)	431±9 (24)	3.37±0.06 (24)	26.5±* (24)	35.9±* (24)	646±9 (10)
2018	12	HTAC	7.98±* (28)	2294±7 (14)	1997±5 (28)	469±7 (24)	3.49±0.03 (28)	29.2±* (68)	35.2±* (14)	633±6 (27)
	3	HTHC	7.68±0.02 (28)	2267±8 (14)	2129±10 (28)	1037±17 (24)	1.98±0.07 (28)	29.0±* (68)	35.3±0.1 (14)	596±7 (27)
	10	HTHC	7.65±0.03 (28)	2284±8 (14)	2156±14 (28)	1083±14 (24)	1.88±0.06 (28)	29.0±0.1 (68)	35.3±* (14)	609±7 (22)
	4	LTAC	8.01±0.01 (28)	2288±5 (14)	1998±4 (28)	437±7 (24)	3.38±0.04 (28)	26.6±* (68)	35.2±* (14)	639±7 (27)
	11	LTAC	8.01±0.01 (28)	2273±8 (14)	1989±4 (28)	443±7 (24)	3.32±0.04 (28)	26.5±* (68)	35.2±* (14)	644±5 (27)
	5	LTHC	7.66±0.02 (28)	2291±5 (14)	2172±11 (28)	1043±20 (24)	1.74±0.05 (28)	26.4±* (68)	35.2±* (14)	644±7 (23)
	9	LTHC	7.65±0.04 (28)	2297±4 (14)	2181±18 (28)	1085±17 (24)	1.76±0.08 (28)	26.6±* (68)	35.2±* (14)	655±5 (22)

In both trials, temperatures targeted 26.5°C (low, LT) and 29.0°C (high, HT), and P_{CO_2} targeted ~400 µatm (ambient, AC) and ~1000 µatm (high, HC). The concentration of dissolved inorganic carbon (C_T), partial pressure of CO_2 (P_{CO_2}) and saturation state of aragonite (Ω_{arag}) were calculated from measured pH_T, total alkalinity (A_T), temperature (T) and salinity using the R package Seacarb. Values are means±s.e. [N=sample size*values ≤0.01 (pH), ≤0.1°C (temperature) or <0.1 (salinity)].

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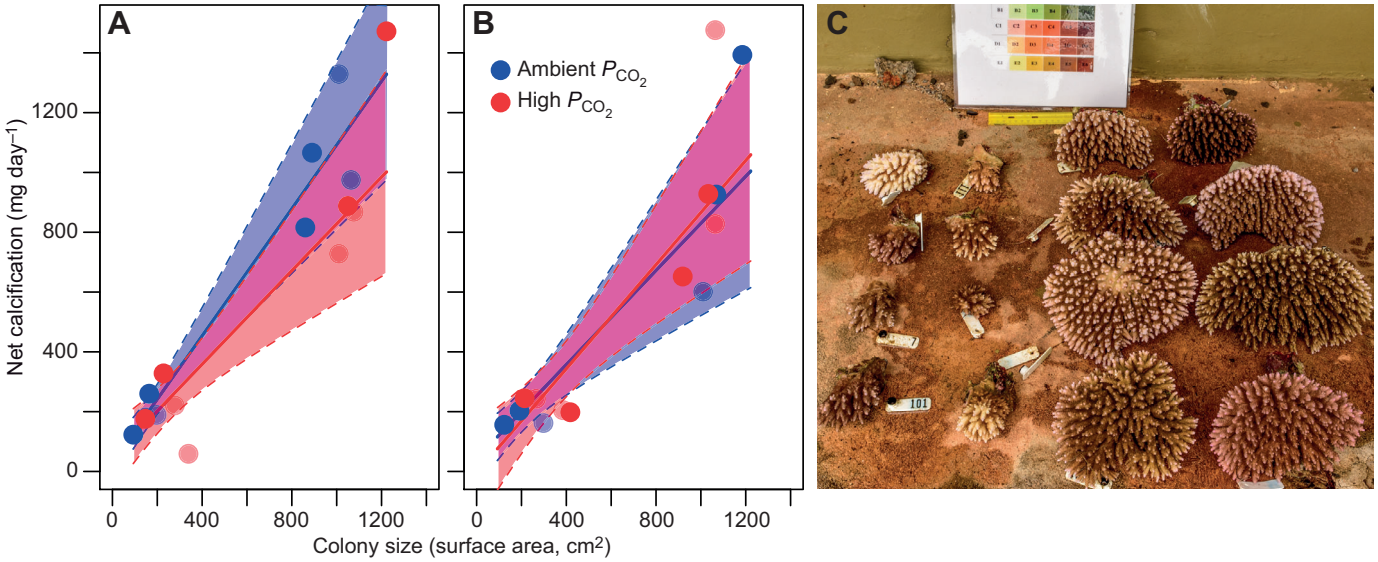

Fig. 1. Net calcification of *Acropora hyacinthus* under the experimental conditions. *Acropora hyacinthus* (5–20 cm diameter) were collected from 10 m depth on the fore reef of Mo'orea and incubated under two temperatures (A, 26.5°C; and B, 29.1°C) and two P_{CO_2} regimes (ambient and elevated). Experiments were conducted in two trials, one from April to May 2017 (light tone), and one from April to May 2018 (dark tone). Lines show the fit (solid lines) and 95% confidence intervals (dashed lines and shading, showing fixed effects uncertainty) estimated from linear mixed-effects models. At 26.5°C, *N*=8 at ambient P_{CO_2} and high P_{CO_2}; at 29.1°C, *N*=6 at ambient P_{CO_2} and *N*=8 at high P_{CO_2}. (C) Photograph of corals at the conclusion of the 2017 experiment.

January 2012, Kroeker et al. (2013) reported a 32% decline for a ≤0.5 reduction of seawater pH, as is predicted to occur by ∼2100.

Relative to the aforementioned expectations, our previous discovery that net calcification of *P. verrucosa* ranging in size from 2.0 to 13.1 cm diameter was unaffected by temperature (26.5 versus 29.7°C) and P_{CO_2} (∼400 and 1000 µatm) (Edmunds and Burgess, 2016) was unexpected (cf. Chan and Connolly, 2013; Kroeker et al., 2013; Comeau et al., 2014; Pratchett et al., 2015). Here, we show that a similar outcome resulted from incubating intact colonies of *A. hyacinthus* ranging in size from 4.6 to 19.9 cm diameter under treatment conditions equivalent to those applied to *P. verrucosa* (Edmunds and Burgess, 2016). Together, our results make a case for the generalized outcome of our earlier results. For branching corals, net calcification of intact colonies of ecologically relevant sizes is resistant to the effects of high P_{CO_2} interacting with high and a low seawater temperatures within the current seasonal range for the study location.

This conclusion has three important caveats. First, like all experiments testing the effect of temperature and P_{CO_2} on corals, the results are only relevant to the range of treatment conditions and incubation durations employed. While the physical and chemical conditions in our experiment are commensurate with those employed in many other studies of the same topic (e.g. Comeau et al., 2014; Kroeker et al., 2013), from which strong inferences have been made (e.g. Comeau et al., 2014), we do not know whether our results are consistent across a wider range of treatment conditions or over lengthier incubations. Meta-analyses of the biological effects of ocean acidification on calcification, however, downplay the role of incubation duration in affecting the results of the experiment (Kroeker et al., 2013).

Second, while we explicitly sampled the size range of *A. hyacinthus* colonies found at 10 m depth on the fore reef of Mo'orea when the experiments were conducted, our experiment did not include small fragments or single branches (i.e. nubbins). Instead, we focused on intact colonies. To support the interpretation that it is notable to have null effects of high P_{CO_2} acting across a range of normally occurring temperatures on colonies of *A. hyacinthus*, we infer that nubbins of this species would have been negatively affected by high P_{CO_2} as in Anderson et al. (2019), who worked with <3 cm diameter fragments of the same species, Comeau et al. (2014) who worked with 5 cm tall branch tips of the congener *Acropora pulchra*, and the numerous studies compiled in the meta-analyses of Chan and Connolly (2013) and Kroeker et al. (2013).

Finally, as the 95% CIs on net calcification (mg day^{-1}) as a function of colony size (cm^2) reveal (Fig. 1), the ability to detect a size-specific treatment effect declines with colony size (i.e. the risks of Type II error increase as the effect size in terms of mg day^{-1} becomes smaller). A larger sample size (i.e. more colonies) would provide a means to resolve two interpretations of the present results: (1) treatment effects were absent because the corals did not respond to the temperature and P_{CO_2} regimes employed (which we posit is a reasonable assertion given the consensus of contemporary literature), or (2) they were obscured by the risks of Type II error. Increasing sample sizes was not possible, however, because there are ethical objections to collecting numerous large coral colonies, and logistical challenges to accommodating them in tanks. The challenge of tank experiments is ensuring independence among corals while manipulating P_{CO_2} without allowing coral calcification to deplete seawater A_T. The ethical objections to much-needed experimentation with large coral colonies are unlikely to change,

Table 2. Analysis of variance for the effects of temperature (low, 26.5°C; or high, 29.2°C) and P_{CO_2} (ambient, 462 µatm; or high, 1062 µatm) on net calcification of *Acropora hyacinthus*

Term	d.f.	*F*-value	*P*-value
Area	1,11	85.63	<0.001
Temperature	1,10	0.77	0.40
P_{CO_2}	1,10	0.80	0.39
Area×temperature	1,11	0.34	0.57
Area×P_{CO_2}	1,11	0.48	0.50
Temperature×P_{CO_2}	1,10	0.11	0.75
Area×temperature×P_{CO_2}	1,11	1.00	0.34

Note the *P*-values from conditional *F*-tests in linear mixed effects models are large-sample approximations and are anti-conservative.

Table 3. Parameter estimates from linear mixed-effects models fitted to whole-colony calcification rate in *A. hyacinthus*

Parameter	Interpretation	Estimate (95% CI)
α	Response of corals in LTAC at the intercept (i.e. size 0 cm^2)	28.964 (−50.177–108.104)
β_1	Slope of calcification versus coral surface area (cm^2) in LTAC	**1.063 (0.649–1.433)**
β_2	Difference in calcification of corals in HTAC compared with LTAC at the intercept (i.e. size 0 cm^2)	13.595 (−126.389–153.579)
β_3	Difference in calcification of corals in LTHC compared with LTAC at size 0 cm^2	16.426 (−136.098–168.950)
β_4	Difference in slope of coral surface area (cm^2) versus calcification in HTAC compared with LTAC	−0.276 (−0.836–0.283)
β_5	Difference in slope of coral surface area (cm^2) versus calcification in LTHC compared with LTAC	−0.281 (−0.815–0.253)
β_6	Difference in calcification of corals in HTHC compared with LTAC at the intercept (i.e. size 0 cm^2)	−63.991 (−333.162–205.179)
β_7	Marginal difference in slope of coral surface area (cm^2) versus calcification in HTHC compared with LTAC	0.365 (−0.447–1.177)
σ_j	Standard deviation in calcification among tanks within years	0.425
σ_k	Standard deviation in calcification among years	0.147
σ_i	Residual standard deviation in calcification of corals within each tank	0.658

Net calcification (mg day^{-1}) was estimated as the difference in buoyant weight (measured in grams) at the start and end of the experiment (23 days later), converted to dry weight using the density of aragonite. Colonies were incubated at low (LT; 26.5°C) or high (HT; 29.2°C) temperature, and ambient (AC; 462 µatm) or high (HC; 1062 µatm) P_{CO_2}. The area of each coral is the surface area of tissue estimated by wax dipping. The fitted model was: $y_i \sim \alpha_{LTAC_i} + \beta_1 area_{LTAC_i} + \beta_{2HTAC_i} + \beta_{3LTHC_i} + \beta_4 area_{HTAC_i} + \beta_5 area_{LTHC_i} + \beta_{6HTHC_i} + \beta_7 area_{HTHC_i} + \sigma_j + \sigma_k + \sigma_i$. Values in bold indicate 95% confidence intervals (CI) that do not overlap zero.

and greater use of *in situ* chambers (e.g. Camp et al., 2015) and FOCE technology (e.g. Doo et al., 2019) may be required to address the issues highlighted in the present study.

The common features of the present and our previous work (Edmunds and Burgess, 2016) are the use of intact colonies, collected to reflect the range of colony sizes naturally occurring, and the reliance on net calcification as a response variable. The two taxa we have studied, however, are not morphologically identical, and other aspects of their response to environmental conditions are unlikely to be the same. For example, the contrast of corallum structure between our species might explain why area-normalized calcification declined with colony size in *P. verrucosa* (Edmunds and Burgess, 2016), but remained ca. 0.96 mg cm^{-2} day^{-1} across colonies between 93 and 1222 cm^2 for *A. hyacinthus*. In general, it is quite likely that multiple physiological traits will respond in dissimilar ways to OA and temperature (Kroeker et al., 2013)

Although the cause(s) of the discordant results between the present study and the expected outcomes (cited above) remains unclear, there are several hypotheses that could account for these effects. For example, turbulent flow speed could mediate colony size dependency of physiological performance in branching corals (Edmunds and Burgess, 2018). Additionally, interference among closely spaced branches could affect the flux of metabolites (Patterson, 1982a,b; Burgess et al., 2017), zooplanktivory (Sebens et al., 1997) or the concentration of solutes in interstitial seawater between branches that could determine tissue–surface seawater pH (Chan et al., 2016). As larger colonies of branching corals have lower area-normalized calcification than smaller colonies (Edmunds and Burgess, 2016), and the sensitivity of net calcification to high P_{CO_2} is dependent on the absolute rate of calcification (Comeau et al., 2014), it is also possible that increasing colony sizes has a negative feedback on the sensitivity of calcification to high P_{CO_2}. Regardless of the causal basis of the results described herein, they have profound implications for the field of experimental coral biology that is built on a foundation of research heavily dominated by analyses of coral nubbins. Large colonies of branching corals probably are not functionally equal to their detached and isolated branches with respect to their response to high P_{CO_2} and elevated temperature.

Acknowledgements
We thank S. Ginther for technical support, A. Potter, A. Widrick and S. Zimmermann for field assistance, and the staff of the UC Berkeley Richard B. Gump South Pacific Research Station for making our visits to Mo'orea productive and enjoyable. Research was completed under permits issued by the French Polynesian Government (Délégation à la Recherche) and the Haut-Commissariat de la République en Polynésie Française (DTRT) (Protocole d'Accueil 2017-2018).

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: P.J.E., S.C.B.; Methodology: P.J.E., S.C.B.; Formal analysis: S.C.B.; Resources: P.J.E.; Data curation: P.J.E.; Writing - original draft: P.J.E., S.C.B.; Writing - review & editing: P.J.E., S.C.B.; Project administration: P.J.E.; Funding acquisition: P.J.E.

Funding
This research was funded by the US National Science Foundation through the Mo'orea Coral Reef (MCR) LTER (OCE 12-36905 and OCE 16-37396) and grants for research on coral biology (to P.J.E.: OCE 14-15268 and OCE 18-29898; and to S.C.B.: OCE 18-29867). This is a product of the MCR-LTER and is contribution number 302 of the Marine Biology Program of California State University, Northridge.

Data availability
Data are available from the EDI Data Portal: https://doi.org/10.6073/pasta/c4490637abcbbd54899e28c7d4f74ae7

References
Anderson, K., Cantin, N. E., Casey, J. M. and Pratchett, M. S. (2019). Independent effects of ocean warming versus acidification on the growth, survivorship and physiology of two *Acropora* corals. *Coral Reefs* **28**, 1225-1240. doi:10.1007/s00338-019-01864-y

Barner, A. K., Chan, F., Hettinger, A., Hacker, S. D., Marshall, K. and Menge, B. A. (2018). Generality in multispecies responses to ocean acidification revealed through multiple hypothesis testing. *Glob. Chang. Biol.* **24**, 4464-4477. doi:10.1111/gcb.14372

Barton, J. A., Willis, B. L. and Hutson, K. S. (2017). Coral propagation: a review of techniques for ornamental trade and reef restoration. *Rev. Aquac.* **9**, 238-256. doi:10.1111/raq.12135

Birkeland, C. (1976). An experimental method of studying corals during early stages of growth. *Micronesica* **12**, 319-322.

Brown, B. E., Dunne, R. P., Ambarsari, I., Le Tissier, M. D. A. and Satapoomin, U. (1999). Seasonal fluctuations in environmental factors and variations in symbiotic algae and chlorophyll pigments in four Indo-Pacific coral species. *Mar. Ecol. Prog. Ser.* **191**, 53-69. doi:10.3354/meps191053

Burgess, S. C., Ryan, W. H., Blackstone, N. W., Edmunds, P. J., Hoogenboom, M. O., Levitan, D. R. and Wulff, J. L. (2017). Metabolic scaling in modular animals. *Invertebr. Biol.* **136**, 456-472. doi:10.1111/ivb.12199

Camp, E. F., Krause, S.-L., Santos, L. M. F., Naumann, M. S., Kikuchi, R. K. P., Smith, D. J., Wild, C. and Suggett, D. J. (2015). The "flexi-chamber": a novel cost-effective in situ respirometry chamber for coral physiological measurements. *PLoS ONE* **10**, e0138800.

Castillo, K. D., Ries, J. B., Bruno, J. F. and Westfield, I. T. (2014). The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean

acidification and warming. *Proc. R. Soc. B* **281**, 20141856. doi:10.1098/rspb.2014.1856
Chamberlain, J. A., Jr., and Graus, R. R. (1975). Water flow and hydromechanical adaptations of branched reef corals. *Bull. Mar. Sci.* **25**, 112-125.
Chan, N. C. S. and Connolly, S. R. (2013). Sensitivity of coral calcification to ocean acidification: a meta-analysis. *Glob. Chang. Biol.* **19**, 282-290. doi:10.1111/gcb.12011
Chan, N. C. S., Wangpraseurt, D., Kühl, M. and Connolly, S. R. (2016). Flow and coral morphology control coral surface pH: implications for the effects of ocean acidification. *Front. Mar. Sci.* **3**, 1-11.
Comeau, S., Edmunds, P. J., Spindel, N. B. and Carpenter, R. C. (2014). Fast coral reef calcifiers are more sensitive to ocean acidification in short-term laboratory incubations. *Limnol. Oceanogr.* **59**, 1081-1091. doi:10.4319/lo.2014.59.3.1081
Dickson, A. G., Sabine, C. L. and Christian, J. R. (Eds.) (2007). Guide to best practices for ocean CO2 measurements. *PICES Special Publication* 3, 191.
Done, T. J. (1983). Coral zonation, its nature and significance. In *Perspectives on Coral Reefs* (ed. D. J. Barnes and B. Clouston), pp. 107-147. Australia, Australian Institute of Marine Science.
Doo, S. S., Edmunds, P. J. and Carpenter, R. C. (2019). Ocean acidification effects on *in situ* coral reef metabolism. *Sci. Rep.* **9**, 1-8. doi:10.1038/s41598-018-37186-2
Dornelas, M., Madin, J. S., Baird, A. H. and Connolly, S. R. (2017). Allometric growth in reef-building corals. *Proc. R. Soc. B.* **284**, 20170053. doi:10.1098/rspb.2017.0053
Dufault, A. M., Ninokawa, A., Bramanti, L., Cumbo, V. R., Fan, T.-Y. and Edmunds, P. J. (2013). The role of light in mediating the effects of ocean acidification on coral calcification. *J. Exp. Biol.* **216**, 1570-1577. doi:10.1242/jeb.080549
Edmunds, P. J. (2005). The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Great Barrier Reef. *Oecologia* **146**, 350-364. doi:10.1007/s00442-005-0210-5
Edmunds, P. J. (2011). Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. *Limnol. Oceanogr.* **56**, 2402-2410. doi:10.4319/lo.2011.56.6.2402
Edmunds, P. J. (2017). Unusually high coral recruitment during the 2016 El Niño in Moorea, French Polynesia. *PLoS ONE* **12**, e0185167. doi:10.1371/journal.pone.0185167
Edmunds, P. J. and Burgess, S. C. (2016). Size-dependent physiological responses of the branching coral *Pocillopora verrucosa* to elevated temperature and P_{CO2}. *J. Exp. Biol.* **219**, 3896-3906. doi:10.1242/jeb.146381
Edmunds, P. J. and Burgess, S. C. (2018). Colony size and turbulent flow speed modulate the calcification response of the coral *Pocillopora verrucosa* to temperature. *Mar. Biol.* **165**, 338. doi:10.1007/s00227-017-3257-z
Enríquez, S., Méndez, E. R., Hoegh-Guldberg, O. and Iglesias-Prieto, R. (2017). Key functional role of the optical properties of coral skeletons in coral ecology and evolution. *Proc. R. Soc. B.* **284**, 20161667. doi:10.1098/rspb.2016.1667
Fitt, W. K., McFarland, F. K., Warner, M. E. and Chilcoat, G. C. (2000). Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol. Oceanogr.* **45**, 677-685. doi:10.4319/lo.2000.45.3.0677
Gattuso, J.-P., Epitalon, J.-M. and Lavigne, H. (2015). Seacarb: Seawater carbonate chemistry, R package version 3.0.6., available at: http: //CRAN.R project.org/package=seacarb 2015.
Gaylord, B., Kroeker, K. J., Sunday, J. M., Anderson, K. M., Barry, J. P., Brown, N. E., Connell, S. D., Dupont, S., Fabricius, K. E., Hall-Spencer, J. M. et al. (2015). Ocean acidification through the lens of ecological theory. *Ecology* **96**, 3-15. doi:10.1890/14-0802.1
Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., Harvell, C. D., Sale, P. F., Edwards, A. J., Caldeira, K. et al. (2007). Coral reefs under rapid climate change and ocean acidification. *Science* **318**, 1737-1742. doi:10.1126/science.1152509
Horvath, K. M., Castillo, K. D., Armstrong, P., Westfield, I. T., Courtney, T. Ries, R. B. (2016). Next-century ocean-acidification and warming both reduces calcification rate, but only acidification alters skeletal morphology of reef-building coral *Siderastrea siderea*. *Sci. Rep.* **6**, 29613. doi:10.1038/srep29613
Hughes, R. N. (2005). Lessons in modularity: the evolutionary ecology of colonial invertebrates. *Sci. Mar.* **69**, 169-179. doi:10.3989/scimar.2005.69s1169
IPCC (2014). Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. In *Climate Change 2014: Synthesis Report* (ed. Core Writing Team, R. K. Pachauri, L. and A. Meyer), p. 151. Geneva, Switzerland: IPCC.
Jokiel, P. L. and Morrissey, J. I. (1986). Influence of size on primary production in the reef coral *Pocillopora damicornis* and the macroalga *Acanthophora spicifera*. *Mar. Biol.* **91**, 15-26. doi:10.1007/BF00397566
Kornder, N. A., Riegl, B. M. and Figueiredo, J. (2018). Thresholds and drivers of coral calcification responses to climate change. *Glob. Chang. Biol.* **24**, 5084-5095. doi:10.1111/gcb.14431
Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. and Gattuso, J.-P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Chang. Biol.* **19**, 1884-1896. doi:10.1111/gcb.12179
Langdon, C., Albright, R., Baker, A. C. and Jones, P. (2018). Two threatened Caribbean coral species have contrasting responses to combined temperature and acidification stress. *Limnol. Oceanogr.* **63**, 2450-2464. doi:10.1002/lno.10952
Miller, M. W. (2015). Coral disturbance and recovery in a changing world. In *Coral Reefs in the Anthropocene* (ed. C. Birkeland), pp. 217-230. Dordrecht: Springer.
Okazaki, R. R., Towle, E. K., van Hooidonk, R., Mor, C., Winter, R. N., Piggot, A. M., Cunning, R., Baker, A. C., Klaus, J. S., Swart, P. K. et al. (2017). Species-specific responses to climate change and community composition determine future calcification rates of Florida Keys reefs. *Glob. Change Biol.* **23**, 1023-1035. doi:10.1111/gcb.13481
Ong, R. H., King, A. J. C., Kaandorp, J. A., Mullins, B. J. and Julian Caley, M. (2017). The effect of allometric scaling in coral thermal microenvironments. *PLoS ONE* **12**, e0184214. doi:10.1371/journal.pone.0184214
Patterson, M. R. (1992a). A chemical engineering view of cnidarian symbioses. *Am. Zool.* **32**, 566-582. doi:10.1093/icb/32.4.566
Patterson, M. R. (1992b). A mass transfer explanation of metabolic scaling relations in some aquatic invertebrates and algae. *Science* **255**, 1421-1423. doi:10.1126/science.255.5050.1421
Pratchett, M. S., Anderson, K. D., Hoogenboom, M. O., Widman, E., Baird, A. H., Pandolfi, J. M., Edmunds, P. J. and Lough, J. M. (2015). Spatial, temporal and taxonomic variation in coral growth - implications for the structure and function of coral reef ecosystems. *Oceanogr. Mar. Biol. Ann. Rev.* **53**, 215-295. doi:10.1201/b18733-7
Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pagès, C., Jaubert, J. and Gattuso, J.-P. (2003). Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Glob. Chang. Biol.* **9**, 1660-1668. doi:10.1046/j.1365-2486.2003.00678.x
Schmidt-Nielsen, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge University Press.
Sebens, K. P. (1987). The ecology of indeterminate growth in animals. *Annu. Rev. Ecol. Syst.* **18**, 371-407. doi:10.1146/annurev.es.18.110187.002103
Sebens, K. P., Witting, J. and Helmuth, B. (1997). Effects of water flow and branch spacing on particle capture by the reef coral *Madracis mirabilis* (Duchassaing and Michelotti). *J. Exp. Mar. Biol. Ecol.* **211**, 1-28. doi:10.1016/S0022-0981(96)02636-6
Shafir, S., Van Rijn, J. and Rinkevich, B. (2003). The use of coral nubbins in coral reef ecotoxicology testing. *Biomol. Eng.* **20**, 401-406. doi:10.1016/S1389-0344(03)00062-5
Silbiger, N. J., Goodbody-Gringley, G., Bruno, J. F. and Putnam, H. M. (2019). Comparative thermal performance of the reef–building coral *Orbicella franksi* at its latitudinal range limits. *Mar. Biol.* **166**, 126. doi:10.1007/s00227-019-3573-6
Spencer Davies, P. (1984). The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. *Coral Reefs* **2**, 181-186.
Spencer Davies, P. (1989). Short-term growth measurements of corals using an accurate buoyant weighing technique. *Mar. Biol.* **101**, 389-395. doi:10.1007/BF00428135
Stimson, J. and Kinzie, R. A., III (1991). The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Bio. Ecol.* **153**, 63-74. doi:10.1016/S0022-0981(05)80006-1
Stocking, J. B., Laforsch, C., Sigl, R. and Reidenbach, M. A. (2018). The role of turbulent hydrodynamics and surface morphology on heat and mass transfer in corals. *J. R. Soc. Interface* **15**, 20180448. doi:10.1098/rsif.2018.0448
Strahl, J., Stolz, I., Uthicke, S., Vogel, N., Noonan, S. H. C. and Fabricius, K. E. (2015). Physiological and ecological performance differs in four coral taxa at a volcanic carbon dioxide seep. *Comp. Biochem. Physiol. Part A* **184**, 179-186. doi:10.1016/j.cbpa.2015.02.018
Thornhill, D. J., Rotjan, R. D., Todd, B. D., Chilcoat, G. C., Iglesias-Prieto, R., Kemp, D. W., LaJeunesse, T. C., Reynolds, J. M., Schmidt, G. W., Shannon, T. et al. (2011). A connection between colony biomass and death in Caribbean reef-building corals. *PLoS ONE* **6**, e29535. doi:10.1371/journal.pone.0029535
Vercelloni, J., Kayal, M., Chancerelle, Y. and Planes, S. (2019). Exposure, vulnerability, and resiliency of French Polynesian coral reefs to environmental disturbances. *Sci. Rep.* **9**, 1027. doi:10.1038/s41598-018-38228-5
Veron, J. E. N. (2000). *Corals of the World*, Vol. 1-3. Townsville: Australian Institute of Marine Science.
Veron, J. E. N. and Pichon, M. (1976). Scleractinia of Eastern Australia. Part I. Families Thamnasteriidae, Astroceoniidae, Pocilloporidae. Australian Institute of Marine Science Monograph Series. Volume I.
Vogel, N., Meyer, F. W., Wild, C. and Uthicke, S. (2015). Decreased light availability can amplify negative impacts of ocean acidification on calcifying coral reef organisms. *Mar. Ecol. Prog. Ser.* **521**, 49-61. doi:10.3354/meps11088
Wall, C. B., Mason, R. A. B., Ellis, W. R., Cunning, R. and Gates, R. D. (2017). Elevated pCO_2 affects tissue biomass composition, but not calcification, in a reef coral under two light regimes. *R. Soc. Open Sci.* **4**, 170683. doi:10.1098/rsos.170683
Wyatt, A. S. J., Leichter, J. J., Toth, L. T., Miyajima, T., Aronson, R. B. and Nagata, T. (2019). Heat accumulation on coral reefs mitigated by internal waves. *Nat. Geosci.* **13**, 28-34. doi:10.1038/s41561-019-0486-4