

Intraspecific variability in the response to ocean warming and acidification in the scleractinian coral *Acropora pulchra*

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Abstract Studies of coral colonies show that ocean acidification and temperature can affect calcification; however, less is known about the consequences for their populations. Understanding intraspecific variation in the response of corals to these conditions will be important for evaluating population-level consequences of environmental change. We examined intraspecific variability in the effects of elevated temperature and carbon dioxide levels on net calcification (G_n) in the coral *Acropora pulchra* in Moorea, French Polynesia. A common garden experiment showed that G_n in four colonies was affected negatively by high partial pressure of CO₂ (pCO₂) (~1000 μatm cf. ~400 μatm ambient conditions), whereas elevated temperature (30 °C cf. 27 °C) had a negative effect on one colony. Together, these results reveal intraspecific variation in the response of G_n to temperature but not to pCO₂. The fastest growing colonies under ambient temperature and ambient pCO₂ showed the greatest decline in G_n at high temperature and elevated pCO₂. For reef corals, effects of temperature and pCO₂ on calcification that depend on the intrinsic growth rate have potentially important consequences, because they imply that coral colonies contributing the most to population-level calcification will be disproportionately affected by changing environmental conditions.

Introduction

Increasing carbon dioxide in the ocean causes changes in seawater carbonate chemistry, collectively referred to as ocean acidification. These changes include an increase in seawater partial pressure of CO₂ (pCO₂), and a decrease in pH and the saturation state of calcium carbonate (Ω) (Feely et al. 2004). Ocean acidification is predicted to cause declines in calcification of important coral reef calcifiers, as well as increased dissolution rates on reefs, potentially threatening the long-term persistence of the coral reef calcium carbonate (CaCO₃) framework (Andersson and Glehdhill 2013; Comeau et al. 2016). Experimental exposure of corals to high pCO₂, representing future ocean acidification scenarios, generally [although not always (e.g., Reynaud et al. 2003; Comeau et al. 2014b)] leads to a decline in net calcification rates (G_n) with increasing pCO₂ (Chan and Connolly 2013). While the magnitude of these effects is variable, meta-analyses suggest they amount on average to a 15 % decline in coral calcification for each unit decline in Ω (Chan and Connolly 2013).

Ocean acidification is occurring in conjunction with ocean warming and numerous local-scale effects (Hoegh-Guldberg et al. 2007), and therefore, coral performance is being affected simultaneously by multiple environmental disturbances. Calcification rates in scleractinian corals can vary in direct response to seawater temperature (Marshall and Clode 2004; Pratchett et al. 2015), and indirectly through the consequences of bleaching, which can lead to large reductions in calcification and increased mortality (e.g., Glynn 1996). Laboratory studies in which multiple species of coral have been tested for their responses to physicochemical conditions have been invaluable in identifying key physiological processes (e.g., calcification) that will likely be sensitive to global change, but

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these studies also have identified a high degree of variation in their responses (Chan and Connolly 2013; Comeau et al. 2014b). This variation involves both interspecific and intraspecific variation. Interspecific variation in the sensitivity to environmental changes (Chan and Connolly 2013; Comeau et al. 2014c) suggests there will be shifts in species composition in the future (Fabricius et al. 2011), while intraspecific variation shows that colonies of a single species will vary in their responses to perturbed conditions (Marubini et al. 2003; Iguchi et al. 2012; Kavousi et al. 2015, 2016a, b), potentially providing a source of variation subject to natural selection (Kavousi et al. 2016b).

Intraspecific variability in the response of corals to future global change is less well understood than interspecific variation, particularly in response to ocean acidification (Ohki et al. 2013; Kavousi et al. 2015, 2016a, b). Differences among individuals in different environments can result from both acclimatization and from genetic differences, and both means of response can function in synergy and involve one or more of the coral host, its symbiotic *Symbiodinium* spp., or the other microbes with which they are integrally associated (Reusch 2014). To isolate the genetic component driving phenotypic variation in performance, through which natural selection could occur, common garden experimental approaches can be used (Whitham et al. 2006; Carroll et al. 2007). In these approaches, individuals are kept at the same site under the same environmental conditions, and after a period of time under such conditions it is assumed that remaining differences among organisms reflect only the intrinsic consequences of the genome (Whitham et al. 2006; Sanford and Kelly 2011).

Intraspecific genetic variation in response to environmental conditions is a critical factor determining how populations will respond to global change (Parkinson and Baums 2014). Individuals are acted upon by natural selection, and thus, genetic variability within a population will be important in determining adaptive capacity to future conditions (Parkinson and Baums 2014). Scleractinians exhibit strong, intraspecific genetic variation in their response to a range of in situ environmental conditions (e.g., irradiance, flow rate, sedimentation) (Foster 1977; Budd Foster 1979; Bruno and Edmunds 1997), including thermal stress caused by high seawater temperature (Edmunds 1994; D’Croz and Maté 2004; Barshis et al. 2013). Recently Kavousi et al. (2015) measured the response of multiple physiological traits (e.g., calcification, maximum photochemical efficiency) of several colonies of the common Indo-Pacific corals *Acropora digitifera*, *Montipora digitata*, and *Porites cylindrica* to temperature and pCO₂ and found the response of conspecifics to differ from the mean response of the respective species (see also Kavousi et al. 2016a, b). Evidence of an intraspecific host genetic component to the response of corals to elevated temperature and pCO₂, together with

intraspecific variation in growth rates (Pratchett et al. 2015), suggests that this level of variation might be a key feature mediating the ways in which coral populations will respond over the long term to ocean acidification and warming. Such intraspecific variability will influence future reef calcification beyond what typically is reported in studies that only consider mean responses of individual species.

This study explored intraspecific variability in the response of calcification in the scleractinian coral *Acropora pulchra* to ocean acidification and warming. *A. pulchra* was selected for study because it is representative of an important functional group of corals on reefs throughout the Indo-Pacific (Veron 2000), and is common on the shallow reefs of Moorea, French Polynesia, where this study was completed.

Methods

In the first experiment, a common garden approach was used to cultivate clonal replicates of four colonies of *Acropora pulchra* and expose them to treatments crossing ambient and high pCO₂ with low and elevated temperature. In this configuration, among-colony variation measures the extent to which intraspecific variation in the coral holobiont could drive phenotypic variation of potential selective value. It was beyond the scope of this study to genetically identify the coral host or their *Symbiodinium*, and therefore, it is unknown whether variation among colonies reflects plasticity among clone-mates, differences among host genotypes, effects due to genetic variants of *Symbiodinium*, or a combination of all three effects. However, the likelihood that variation among colonies was caused by host genotypes was increased by the sampling regime employed to collect colonies, and the known *Symbiodinium* diversity in this species from Moorea (Putnam et al. 2012). The four colonies were collected from the back reef with ≥ 1.25 km separation between all pairs of colonies, and therefore, given sexual reproduction by broadcast spawning of *A. pulchra* in Moorea (Carroll et al. 2006), it is likely that the spatially separated colonies were the product of settlement by genetically unique larvae. Asexual reproduction by fragmentation is well known in *Acropora* (Neigel and Avise 1983), but it is unlikely that branch fragments would be scattered large distances along the shore of Moorea given the strong cross-reef transport arising from wave-driven circulation (Hench et al. 2008). Although variation in the performance of the coral holobiont also can be driven by genetic variation in the *Symbiodinium* they contain (Sampayo et al. 2008, Howells et al. 2012), the *Symbiodinium* found in *A. pulchra* from Moorea represent two clades (A and D) with low haplotype diversity, and some colonies harbor only one *Symbiodinium* clade (Putnam et al. 2012).

Therefore, while we cannot exclude the possibility that the variation reported herein is a result of plasticity among clone-mates or variation in *Symbiodinium* complement, the biology and ecology of *Acropora pulchra* in Moorea make these possibilities unlikely.

The ability to infer from the common garden experiment that among-colony variation reflected host genotype effects was strengthened by a second experiment in which 68 colonies (sampled with 1 nubbin colony⁻¹) of *A. pulchra* were collected from two shores and over ~22 km of the back reef. These corals primarily were used to test the hypothesis that intraspecific variability in the response to ocean acidification and warming was related to colony growth rate, but the results of this experiment also strengthened the generality of intraspecific variation in this species. The second experiment was motivated by the initial study, which suggested that overall growth rate was related positively to the sensitivity of growth to ocean acidification [described below, see also Comeau et al. (2014c)].

Experiment 1

Sample collection

One colony of *A. pulchra* was collected from each of four back reef locations (<1 m depth) and grown together in a common garden (~5 m depth) in the back reef on the north shore. Each location was >1.25 km from any other sampling location, thereby increasing the likelihood that they represent unique host genotypes (named colonies A–D). The four colonies were grown in the common garden for 10–15 months to remove physiological effects attributed to variation in physical conditions at each collection location. Twenty-four branches (all of similar shape and length of ~5 cm) were harvested from each colony on the common garden on July 9, 2015, transported to the Richard B. Gump South Pacific Research Station, and prepared as nubbins (Birkeland 1976) by attaching them to plastic bases using Z-Spar A788 epoxy. Nubbins were left in a shallow tank supplied with flowing seawater for 1 day before being placed into the mesocosms at ambient pCO₂ and 27 °C. Field logistics prevented a longer recovery time following preparation, but we reasoned that any limitations of this constraint would be equally distributed among treatments and unlikely to bias the experimental outcome. Temperature and pCO₂ of half of the treatment mesocosms were increased over a 2-day acclimation period to ~30 °C and ~1000 μatm pCO₂.

Experimental design

Incubations were completed over 3 weeks in July 2015. Calcification rates of the four colonies of *A. pulchra* were

compared under ambient (~400 μatm) and high (~1000 μatm) pCO₂ at ~27 and ~30 °C in an orthogonal design. Eight mesocosms (each 150 L in volume) were used in the experiment, with each of the four temperature–pCO₂ combinations replicated in two randomly assigned tanks. Three replicate nubbins of each colony were allocated randomly to each tank and were exposed to the elevated conditions in half the tanks following a 2-day ramping period. In this design, the undesirable effects of pseudoreplication of the nubbins in each tank (Hurlbert 1984) were unavoidable given the logistical challenges of creating precise and ecologically relevant conditions, but insights into the potential implications of these effects were provided by the duplicate tanks within each treatment combination. In the absence of a statistically significant effect of tank nested in each treatment, it is reasonable to infer that the overall results are unlikely to be biased by the implications of pseudoreplication.

In this experiment, the 27 °C treatments corresponded to the mean monthly seawater temperature on the back reef in Moorea during July (Leichter 2015). The 30 °C treatment is ~1 °C warmer than the mean monthly temperatures during the warmest months of the year (March–April) and within the range of temperatures observed on the warmest days of the year (Leichter 2015). The temperature treatments were implemented to provide stable values representative of average conditions found in the back reef habitat (from which the corals were collected) and did not duplicate the typically diurnal variation of seawater temperature found in this habitat [~1 °C (Edmunds et al. 2010)]. The high pCO₂ treatment (~1000 μatm) corresponds to the atmospheric level expected by the end of the current century under a high emissions (RCP 8.5) scenario (Riahi et al. 2007).

Incubation and preparation conditions

Each mesocosm was supplied with fresh seawater at ~214 mL min⁻¹, which was pumped directly from 14 m depth in Cook's Bay and filtered through a sand filter. In each mesocosm, seawater was mixed with a pump (Danner Mag-Drive, 2, 650 L h⁻¹), and irradiance, temperature, and pCO₂ were controlled individually. Irradiances of ~500 μmol quanta m⁻² s⁻¹ (measured with a 4π LI-193 quantum sensor and a LiCor LI-1400 m) were provided over 12:12 h light/dark photoperiod by 75 W LED modules (Sol White LED Module, Aqua Illumination). Temperatures of each tank were measured daily using a certified digital thermometer (Fisher Scientific, 15-007-8, ±0.05 °C) and were maintained close to the target values (Table 1). Previous experiments in which temperatures were logged every 15 min each tank showed that the daily temperature range was ~0.8 °C around the mean value (PJ Edmunds unpublished data), with the variation attributed to the sensitivity of the solenoids controlling the heater and chiller.

Table 1 Mean (SE) ($n = 21$) of temperature and carbonate chemistry conditions of the eight mesocosms over the 21-day experimental period during Experiment 1

Treatment: Temperature, pCO ₂ (replicate)	Temp. (°C)	pH	A _T (μmol kg ⁻¹)	DIC (μmol kg ⁻¹)	pCO ₂ (μatm)	Ω _{ar}
27 °C, Ambient (1)	27.5 (0.1)	8.04 (0.01)	2311 (14)	1979 (28)	395 (45)	3.7 (0.3)
27 °C, Ambient (2)	27.2 (0.2)	8.06 (0.03)	2319 (12)	1979 (23)	380 (34)	3.8 (0.2)
30 °C, Ambient (1)	30.1 (0.6)	8.01 (0.03)	2314 (17)	1982 (25)	437 (41)	3.8 (0.2)
30 °C, Ambient (2)	30.1 (0.3)	8.01 (0.03)	2320 (12)	1983 (21)	431 (32)	3.8 (0.2)
27 °C, 1000 μatm (1)	27.2 (0.5)	7.70 (0.01)	2316 (15)	2164 (17)	1000 (21)	1.9 (<0.1)
27 °C, 1000 μatm (2)	27.3 (0.5)	7.70 (0.01)	2312 (20)	2161 (22)	1001 (36)	1.9 (0.1)
30 °C, 1000 μatm (1)	30.2 (0.4)	7.68 (0.01)	2319 (10)	2161 (12)	1076 (20)	2.1 (<0.1)
30 °C, 1000 μatm (2)	29.9 (0.6)	7.68 (0.02)	2317 (16)	2158 (17)	1056 (42)	2.1 (0.1)

The carbonate chemistry of the high pCO₂ treatment was modified by bubbling CO₂ gas into the seawater. The activation of CO₂ bubbling was controlled through a solenoid that was regulated by an input controlled by a pH electrode attached to a meter (Aquacontroller, Neptune systems, USA). Ambient air was bubbled continuously into all tanks, and there was no control of carbonate chemistry in the ambient pCO₂ tanks. The position of nubbins within the tanks was changed randomly every 3 days to account for position effects.

Carbonate chemistry

Determinations of seawater pH on the total scale were completed daily using a Mettler DGi-115 pH electrode, which was calibrated with TRIS buffers [SOP 6a (Dickson et al. 2007)]. Total alkalinity (A_T) was measured every 3 days using the open cell method of acidimetric titration [SOP 3b, Dickson et al. (2007)]. Accuracy of A_T analyses was determined through analysis of certified reference materials (CRMs; from A. Dickson Laboratory, Scripps Institution of Oceanography). The mean (±SE) difference between measured and certified values of CRMs (i.e., accuracy) was 1.3 ± 0.3 μmol kg⁻¹ ($n = 34$), and the precision was 1.8 ± 0.1 μmol kg⁻¹ ($n = 475$).

pH was reported on the total hydrogen ion scale. The full carbonate system of parameters was calculated using measured pH, A_T, temperature, and salinity with the R package seacarb (Lavigne and Gattuso 2010). Calculations were done using the carbonic acid dissociation constants of Lueker et al. (2000), the K_{SO4} for the bisulfate ion from Dickson (1990), and the K_f constant of Perez and Fraga (1987).

Net calcification measurements

The buoyant weight of each nubbin was measured at the start and end of the experiment (Davies 1989), and the difference between the two values was used to calculate the

increase in dry skeletal weight (i.e., net calcification, G_n) using the density of aragonite [2.93 g cm⁻² (Jokiel et al. 1978)]. Net calcification (G_n) was normalized to the surface area of each nubbin, with area determined at the end of the experiment using a double wax dipping procedure (Stimson and Kinzie 1991; Veal et al. 2010), which assumes that the increase in area is negligible during short incubations.

Statistical analysis

To test whether there was a tank effect on G_n , a four-way, mixed model ANOVA was used in which tank was a random factor nested within treatment, and colony, temperature, and pCO₂ were fixed effects. The effect of tank was not significant at $P = 0.25$ (described in “Results”) and, therefore, was dropped from the statistical model (Quinn and Keough 2002) to create a model I three-way ANOVA; interactions were dropped from this model when not significant at $P = 0.25$. In this model, the nubbins from each colony were treated as functionally independent replicates of their respective colonies, even though they were pseudoreplicated in each tank. Colony was treated as a fixed factor because the experimental objective was to explicitly compare the four colonies from the common garden, as opposed to making inference regarding all colonies on the reef. Differences in G_n among colonies were identified with post hoc comparisons using Tukey’s HSD (in the case of significant interactions least significant difference was used). Assumptions of ANOVAs were confirmed through graphical analyses of standardized residuals. Statistical analyses were performed in SPSS (version 23), with a significance level (α) of 0.05.

Experiment 2

As described in Results, Experiment 1 revealed differences among colonies in the response of G_n to the treatment conditions. Although intraspecific variability in the

Table 2 Mean (SE) of temperature and carbonate chemistry conditions during Experiment 2 ($n = 7$ for “ambient” and 6 for “high”)

Treatment	Temp. (°C)	pH	A_T ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	pCO_2 (μatm)	Ω_{ar}
Ambient	27.2 (0.03)	8.11 (0.07)	2335 (2)	1961 (4)	327 (7)	4.2 (0.05)
High	29.4 (0.2)	7.69 (0.004)	2355 (3)	2198 (5)	1064 (13)	2.1 (0.02)

response of corals to ocean acidification has rarely been studied, there is evidence that the response to high pCO_2 is related to the growth rate under ambient pCO_2 (Movilla et al. 2012; Comeau et al. 2014c; Kavousi et al. 2016b). To test whether growth rate contributed to the intraspecific variability observed in *A. pulchra* (Experiment 1), the study was extended to sample a greater number of colonies than initially studied. To test whether calcification of individual corals under ambient pCO_2 and temperature was related to the change in calcification rate at elevated pCO_2 and high temperature, *A. pulchra* nubbins from 68 colonies were exposed simultaneously to high temperature and elevated pCO_2 . In this experiment, a single branch of *A. pulchra* was sampled from each of the 68 colonies located at 1 m depth and scattered haphazardly along 22 km of Moorea back reef along both the northern and western shores. Samples were collected on November 17, 2015, and were prepared as nubbins as described above for Experiment 1. All nubbins were transferred to one 420-L tank to provide a common exposure for 3 days to identical conditions. Light was the same as for the previous experiment and the tank rotated (~ 4 rotations day^{-1}) to allow uniform lighting among all nubbins. Prior to the start of the experiment, all nubbins were buoyant weighed following the procedure described above.

Experiment 2 ran for 42 days and was split into two 21-day periods. During the first 21 days, nubbins were kept under constant pCO_2 and temperature conditions that approximated ambient conditions for the back reef of Moorea when the experiment was conducted. pCO_2 was not altered from the incoming seawater (flow rate ~ 300 mL min^{-1}), and temperature was kept at 27.2 ± 0.03 °C (Table 2), which approximates the in situ seawater temperatures in the back reef during October–November when the experiment was conducted. At the end of the first 21 days, nubbins were buoyant weighed and returned to the same tank that was gradually warmed to the high-temperature treatment within 1 day. For the second 21 days, nubbins were exposed to high temperature (29.4 ± 0.2 °C) and elevated pCO_2 (1064 ± 13 μatm), to compare G_n under both ambient and high treatments for the same nubbins. The high pCO_2 treatment was created by bubbling CO_2 gas into the seawater as described in Experiment 1. At the end of the second 21 days, nubbins were buoyant weighed and their tissue surface area was determined using wax dipping (Stimson and Kinzie 1991).

G_n based on the dry skeletal increment over each 21-day period was calculated and in both cases was normalized to the final surface area with the assumption that changes in nubbin area were small over the experiment. This assumption was necessary because it was not possible to measure tissue area nondestructively in the middle of the experiment. Following calcification (i.e., growth), however, the surface area of nubbins will be greater after 42 versus 21 days and, therefore, calcification after 21 days that is normalized to the area of nubbins at 42 days will be downwardly biased. Calculations of the changes in shape of a hypothetical (but ecologically relevant) cylindrical nubbins 5 cm tall and 1.5 cm diameter that grew at 2.9 mg cm^{-2} day^{-1} (i.e., like the nubbins of *A. pulchra*), and produced a skeleton with a density of 1.47 g cm^{-3} (i.e., an aragonite skeleton with 50 % porosity), suggest that the area after 21 days would be ~ 5 % lower than after 42 days. Evaluating the implications of this discrepancy on the test of association between growth rate and response to elevated pCO_2 and high temperature is described in Statistical analysis.

Carbonate chemistry and temperature were measured throughout the experiment using the same procedures as described in Experiment 1. The mean accuracy and precision of A_T analyses during Experiment 2 were 2.7 ± 0.9 $\mu\text{mol kg}^{-1}$ ($n = 7$) and 1.8 ± 0.1 $\mu\text{mol kg}^{-1}$ ($n = 141$), respectively.

Statistical analysis

To test for an effect of the treatment sequence of the consecutive 21-day periods, G_n for each coral in both periods was compared using the Wilcoxon signed-rank test. A nonparametric procedure was used because the differences in G_n between periods were not normally distributed. Pearson's correlation was used to test for an association between the change in G_n between periods and initial G_n . This analysis was primarily conducted with area-normalized G_n as described above, but a secondary test of the same hypothesis was conducted using absolute dry skeletal increment (i.e., mg day^{-1}) for each 21-day period. In designing Experiment 2, a control for the treatment sequence was not employed in favor of supporting the analysis of 68 colonies using a single large tank in which an unambiguous treatment sequence could be administered. As with Experiment 1, Experiment 2 was pseudoreplicated (Hurlbert 1984), but this approach was rationalized by the primary objective

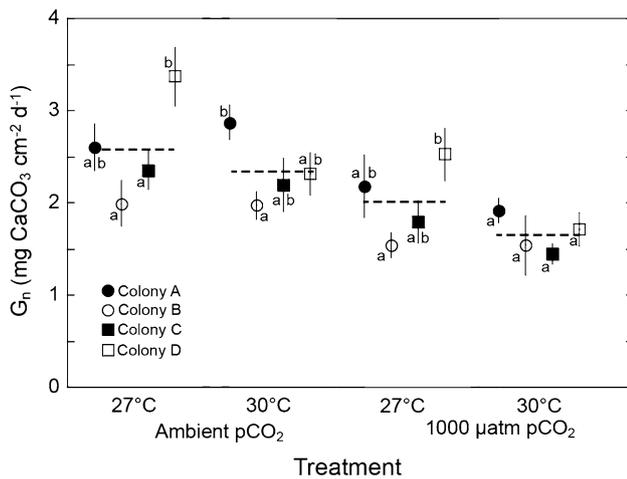


Fig. 1 Mean (\pm standard error, $n = 6$ nubbins colony⁻¹) of surface area-normalized net calcification rates (G_n) of four colonies (A–D) of *Acropora pulchra* under four pCO₂/temperature treatments. Horizontal dashed lines show mean response at each pCO₂/temperature combination. Letters by each symbols show results of Tukey's HSD tests within each treatment group using the 3-way model MSE; colonies with the same letters in each treatment group do not differ

of exploring the relationship between the magnitude of change in calcification as a function of initial calcification rate. Moreover, it was motivated by Experiment 1 that provided a statistically rigorous analysis of the effects of temperature and pCO₂. The design of Experiment 2 does not allow a distinction between treatment effects (i.e., temperature and pCO₂) and uncontrolled random effects in causing calcification to vary, but this limitation does not impede the test of the primary objective (comparing among colonies). All statistical analyses were performed in SPSS (version 23), with a significance level (α) of 0.05.

Results

Experiment 1

All nubbins survived and had positive G_n over the 21-day experiment, regardless of treatment. Mean G_n for each colony by treatment is shown in Fig. 1. Tank effects were not significant [ANOVA, $F(4, 88) = 0.963$, $P = 0.963$] and were dropped from the statistical model. There was an effect of pCO₂ [ANOVA, $F(1, 87) = 29.423$, $P < 0.001$], with a higher mean G_n under ambient conditions, and an interaction between colony and temperature [ANOVA, $F(3, 87) = 3.622$, $P = 0.016$]; both main effects of colony and

temperature were significant ($P \leq 0.011$), but all other 2- and 3-way interactions were not significant ($P \geq 0.619$) and were dropped from the model. The interaction of colony and temperature reflected a lower G_n for colony D at 30 versus 27 °C, but not for any other colony. Overall, Tukey's HSD post hoc comparisons showed that colony A grew faster than colony B ($P = 0.001$) and C ($P = 0.038$), colony B grew slower than colony D ($P < 0.001$), and colony C grew slower than colony D ($P = 0.008$); no other contrasts differed ($P \geq 0.668$).

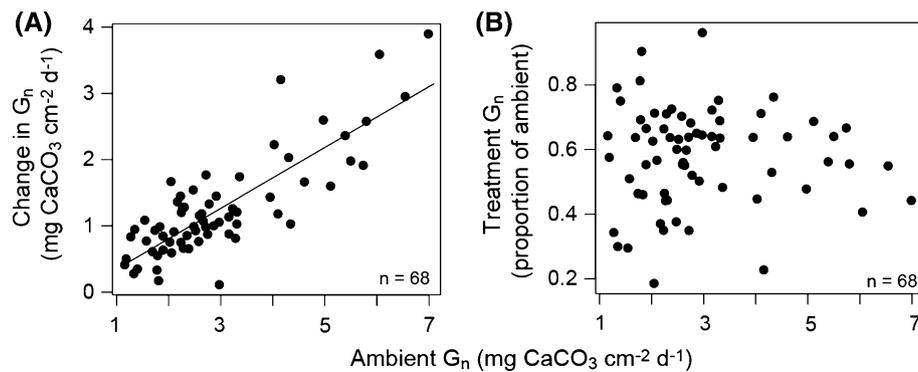
The difference in G_n among colonies was greatest under ambient conditions, where colony D had the highest G_n (3.37 ± 0.32 mg CaCO₃ cm⁻² day⁻¹), which was greater than the G_n of colony B, which had the lowest G_n (1.99 ± 0.25 mg CaCO₃ cm⁻² day⁻¹) (Table 3; Fig. 1). Although colony D had the highest G_n under control conditions, it also experienced the greatest decline (49 %) from control to high temperature and high pCO₂ (Table 3). This led to reduced variation in G_n among colonies under high temperature and elevated pCO₂ relative to control conditions, with no difference in G_n among colonies under treatment conditions (Fig. 1).

Experiment 2

Similarly to Experiment 1, there was a decrease (Wilcoxon signed-rank test, $Z = -7.167$, $N = 68$, $P < 0.001$) in G_n between ambient pCO₂ and temperature and high pCO₂ and temperature, with G_n declining from a mean (\pm SE, $n = 68$) of 2.96 ± 0.17 mg CaCO₃ cm⁻² day⁻¹ to 1.71 ± 0.10 mg CaCO₃ cm⁻² day⁻¹. The absolute change in G_n from ambient to high pCO₂ and high temperature was correlated with G_n under ambient conditions ($r = 0.825$, $N = 68$, $P < 0.001$), with faster-growing colonies experiencing a greater decline in G_n in response to exposure to high temperature and pCO₂ (Fig. 2a). This result largely was unchanged when calcification was expressed as an overall increment by coral nubbin (i.e., mg day⁻¹) ($r = 0.677$, $N = 68$, $P < 0.001$, data not shown). However, the proportional change in G_n due to the treatment conditions (relative to ambient) was unrelated to G_n under ambient conditions ($r = 0.016$, $N = 68$, $P = 0.895$), with G_n declining to a mean of 58 % of the ambient rate under high temperature and elevated pCO₂ (Fig. 2b). Also consistent with Experiment 1, in which the difference in G_n among colonies was higher in ambient temperature and pCO₂ than high temperature and elevated pCO₂ (Fig. 1), the variance in G_n in Experiment 2 was higher (Levene's test, $P < 0.01$) under ambient ($\sigma^2 = 1.91$) than treatment conditions ($\sigma^2 = 0.75$).

Table 3 Mean surface area-normalized net calcification (G_n) rates for each colony under each treatment condition and the rate expressed as a percentage of the rate under control ($\sim 27^\circ\text{C}$, ambient pCO_2) conditions ($n = 6$)

Colony	Treatment (temp. ($^\circ\text{C}$), pCO_2)	Mean G_n (SE) ($\text{mg CaCO}_3 \text{ cm}^{-2} \text{ day}^{-1}$)	% of control G_n
A	27, Ambient	2.60 (0.25)	100
	30, Ambient	2.87 (0.19)	111
	27, High	2.18 (0.34)	84
	30, High	1.92 (0.13)	74
B	27, Ambient	1.99 (0.25)	100
	30, Ambient	1.97 (0.15)	99
	27, High	1.54 (0.13)	77
	30, High	1.54 (0.32)	77
C	27, Ambient	2.35 (0.21)	100
	30, Ambient	2.20 (0.29)	93
	27, High	1.80 (0.23)	76
	30, High	1.45 (0.11)	61
D	27, Ambient	3.37 (0.32)	100
	30, Ambient	2.32 (0.23)	69
	27, High	2.53 (0.28)	75
	30, High	1.71 (0.18)	51

**Fig. 2** Relationship between the net calcification rate (G_n) of *Acropora pulchra* under ambient temperature and pCO_2 conditions and **a** the change in G_n from ambient to high temperature and pCO_2 and **b** the G_n under high temperature and pCO_2 expressed as a proportion of

the ambient value. *Line in a* shows a Model I regression line for the purpose of prediction of change in G_n from G_n under ambient conditions; n shows the number of colonies

Discussion

Effects of temperature and pCO_2 on G_n in *Acropora pulchra*

Previous studies of *A. pulchra* in Moorea have shown that G_n varies in response to elevated pCO_2 and high temperature. Working in January/February 2013, Comeau et al. (2014a) found lower G_n for *A. pulchra* at $\sim 30^\circ\text{C}$ and $\sim 1100 \mu\text{atm}$ pCO_2 , compared with ambient ($\sim 27^\circ\text{C}$ and $400 \mu\text{atm}$ pCO_2) conditions, whereas Brown and Edmunds (2016), working in April/May 2011, found no significant difference in G_n in response to temperature (~ 28 vs $\sim 30^\circ\text{C}$) or pCO_2 (~ 400 and $\sim 900 \mu\text{atm}$). In the present study conducted in July 2015 (Experiment 1) and November 2015 (Experiment 2), treatment conditions (27 vs ~ 29 – 30°C crossed

with ~ 300 – 400 vs ~ 1000 – $1100 \mu\text{atm}$ pCO_2) were similar to those of Comeau et al. (2014a) and Brown and Edmunds (2016), but G_n was negatively affected by high temperature and elevated pCO_2 . However, Experiment 1 revealed differences among colonies, with colony D showing an effect of temperature on G_n . Thus, two non-exclusive possible explanations for the different responses observed by Comeau et al. (2014a) and Brown and Edmunds (2016) versus the current study are that they sampled colonies with different susceptibility to temperature and pCO_2 treatments or that there are strong temporal (i.e., among seasons) differences in the calcification response of *A. pulchra* to temperature and pCO_2 .

In contrast to temperature, pCO_2 had a significant effect on G_n in all four colonies. From control ambient pCO_2 to $\sim 1000 \mu\text{atm}$ pCO_2 at $\sim 27^\circ\text{C}$, Ω_{ar} declined by ~ 1.8 units

(Table 1). In a meta-analysis examining the response of scleractinians to high pCO₂, the mean decline in G_n of corals exposed to high pCO₂ is 15 % per unit Ω_{ar} (Chan and Connolly 2013), which equates to a 27 % decline over the 1.8 unit reduction in Ω_{ar} created in the present study. The declines in G_n observed here for each colony varied from 16 to 25 % (Table 3), suggesting a slightly a lower-than-average sensitivity in this study compared with other corals.

Intraspecific variability in the response of corals to environmental change

Coral conspecifics can vary greatly from one another in growth and morphology in natural environments (reviewed in Pratchett et al. 2015), and this level of variation is important in refining projections of ecological dynamics under future environmental condition (Bolnick et al. 2011). In particular, it is of interest to consider whether phenotypic buffering [i.e., maintenance of a functional phenotype under environmental stress (Reusch 2014)] can allow for key processes (e.g., growth) in corals to be maintained under future conditions of seawater warming and ocean acidification. Previous studies of scleractinians have shown strong intraspecific variability in the response to environmental conditions (e.g., Edmunds 1994; Sampayo et al. 2008; Csaszar et al. 2010; Howells et al. 2012; Kavousi et al. 2016a,b) that represents variation on which natural selection could act (Parkinson et al. 2015).

To date, relatively few studies exposing organisms from any taxon to future ocean acidification conditions have considered intraspecific variability (Kelly and Hofmann 2013). In the few taxa and small number of response variables studied thus far, some studies have detected intraspecific variability in the trait of interest, whereas others have not. For example, intraspecific variability in calcification response to high pCO₂ occurs in the mussel *Mytilus chilensis* (Duarte et al. 2015), the fertilization success of the sea urchin *Heliocidaris erythrogramma* (Schlegel et al. 2012), and microalgal growth rates (Kremp et al. 2012). However, among-genotype variation was not observed in infection success of the trematode parasite *Maritrema novaezealandiae* to ocean acidification (Harland et al. 2016). The studies that have tested for intraspecific variation in the response of corals to ocean acidification (Reynaud et al. 2003; Movilla et al. 2012; Ohki et al. 2013; Kavousi et al. 2015, 2016a, b) have reported strong intraspecific variability that could modulate how populations will respond to future seawater carbonate chemistry conditions (Kavousi et al. 2016b).

Using a common garden experiment, the present results reveal differences among colonies of *A. pulchra* in their calcification response to elevated temperature, although all colonies had lower G_n under high pCO₂ (Fig. 1). Given the spatial scale over which these colonies were sampled,

and limited genetic variability of their *Symbiodinium* (Putnam et al. 2012), it is reasonable to hypothesize that the “colony” effects represent differences among host genotypes, although it was beyond the scope of this study to test for these effects, or to evaluate the role of *Symbiodinium* in driving the colony-level variation. Nevertheless, our results suggest, albeit based on short-term incubations with a small number of colonies, that calcification in at least some *A. pulchra* colonies in Moorea (i.e., three out of four tested) shows phenotypic buffering to temperatures between 27 and ~30 °C. This variation may be important if it represents effects subject to natural selection and, therefore, which could promote the success of thermally tolerant individuals. Conversely, G_n in all colonies was sensitive to high pCO₂ (~1000 μ atm), indicating no ability for phenotypic buffering to this condition in the colonies used in this study.

Reduction in variance of calcification

Although there was intraspecific variability of G_n in *A. pulchra* exposed to ambient temperature and pCO₂ characteristic of the back reef of Moorea, this variability was reduced under elevated pCO₂ and high temperature (Fig. 1). This suggests that there may be less variability in G_n in the future on which selection can act. Expanding the analysis beyond the four colonies used in Experiment 1, to 68 colonies used in Experiment 2, the results again demonstrated a higher variance in G_n under ambient temperature and pCO₂ compared with high temperature and elevated pCO₂.

The discovery of reduced intraspecific variance in G_n under conditions predicted to occur during this century is in agreement with an analysis of the response of two Mediterranean corals (*Oculina patagonica* and *Cladocora caespitosa*) to high pCO₂ (i.e., 800 μ atm) (Movilla et al. 2012). Similar to the results of Experiment 2 in the present study, Movilla et al. (2012) found that the absolute decline in G_n under high pCO₂ was related positively to the rate of calcification in control conditions for both species. Likewise, Kavousi et al. (2016a) reported a positive relationship between the calcification of *Montipora digitata* at ambient pCO₂ (425 μ atm) and elevated pCO₂ (786 μ atm) (both at ~25–32 °C), with a slope of 0.64, indicating that the inhibitory effects of high pCO₂ were stronger for faster-growing corals. Together, these results underscore the likelihood that the sensitivity of calcification in corals to high pCO₂ is related to overall growth rates (Comeau et al. 2014c). The greater effect of ocean acidification treatments on fast-growing colonies and species has been attributed to the higher energy requirements of fast growers to export hydrogen ions to increase the carbonate ion concentration at the site of calcification (Jokiel 2011; Movilla et al. 2012; Comeau et al. 2014c).

Movilla et al. (2012) suggested that the intraspecific variability they observed in the sensitivity of calcification to high pCO₂ was likely a result of genetic variation rather than other possible causes of intraspecific variability, such as colony size or sex. Our common garden experiment and the subsequent experiment with 68 colonies of *A. pulchra* show the importance of intraspecific variation in the response to temperature and pCO₂ and indirectly support the role of host genotypes in driving this level of variation. However, although growing corals together in a common garden for 10–15 months likely limited the amount of phenotypic variation arising from historic effects, we cannot exclude the possibility that there was still persistent phenotypic variation arising from legacy conditions at the reef locations from which they were collected (Sanford and Kelly 2011). Ideally, common garden studies should raise individuals for at least two generations to minimize persistent and maternal effects; however, this can be logistically challenging for many marine species (Sanford and Kelly 2011), particularly *A. pulchra*, which only spawns annually.

Coral populations in a warmer, high CO₂ ocean

The results of the present study and other recent studies (Ohki et al. 2013; Movilla et al. 2012; Kavousi et al. 2015, 2016a, b) demonstrate that there is ecologically meaningful intraspecific variation in the response of corals to increasing temperature and ocean acidification. Identifying the role of genetic variability in these effects and collectively evaluating the role of intraspecific phenotypic variation are important steps in better understanding how coral populations will respond to climate change over evolutionary time (Parkinson and Baums 2014). For ocean acidification research in particular, where little is known about underlying variable responses on which selection could act, there is a need to expand studies to other species. We show that variation around the mean response for a single coral species is not just residual variance, but rather inter-genotype variation that is masked by pooling the responses of genotypes together.

Although there was significant intraspecific variability of G_n in *A. pulchra*, there were no colonies capable of maintaining ambient growth rates under high temperature and elevated pCO₂ likely to occur by the end of the present century under a high emissions (RCP 8.5) scenario. Therefore, for this species to persist in the future and calcify at rates similar to those on present-day reefs, there would likely need to be acclimatization to increasing temperatures and pCO₂ over the next century to a greater extent than was evident in our short-term experiment. Adaptation through selection of more resistant genotypes may also play an important role in future population dynamics of corals

(Coles and Brown 2003), as will interactions with other species in the community that may be affected either positively or negatively under future change.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal rights statement All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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