Calcification responses of subtropical corals to ocean acidification: a case study from Sesoko Island, Okinawa, Japan

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Abstract We investigated the effect of five levels of pCO₂-adjusted seawater (300 μatm [pre-industrial] to 1200 μatm [near-future]) on calcification rates of six coral species: Acropora nasuta, A. tenuis, Montipora digitata, Pocillopora damicornis, Porites cylindrica, and Galaxea fascicularis, which are common species in the subtropical Ryukyu Archipelago, Japan. In most species, declines in calcification rates were pCO₂-dependent as previously reported. Responses to lower pH seawater significantly differed among species in common-garden tanks. Corals showed both linear and non-linear responses to a wide range of seawater pCO₂, which could be attributable to physiological differences in inter-species. Our results are consistent with previous studies and suggest that the responses of corals to ocean acidification can vary among species.

Keywords Corals, Calcification, Ocean acidification, Sesoko Island

Introduction

Anthropogenic CO₂ emissions contribute to increased atmospheric CO₂ partial pressure (pCO₂) and ocean acidification. This poses a threat to marine calcifying organisms because calcification is generally suppressed in lower pH that causes a decrease in carbonate ion concentrations [CO₃²⁻] thus reduction in aragonite saturation state (Ωₛₐₜ) (Orr et al. 2005; Kleypas et al. 2006; Hoegh-Guldberg et al. 2007). Atmospheric CO₂ is predicted to increase from 400 to 1000 μatm and surface-ocean pH to decrease by 0.3 units by the end of this century (Intergovernmental Panel on Climate Change [IPCC] 2019). It is often reported that reef-building corals, which are key organisms in coral-reef ecosystems, generally have lower calcification rates in lower pH seawater (Gattuso et al. 1998; Marubini et al. 2003, 2008; Kleypas et al. 2006; Hoegh-Guldberg et al. 2007; Anthony et al. 2008). However, several studies have reported more complex changes in calcification rate in lower pH seawater (Iglesias-Rodriguez et al. 2008; Ries et al. 2009; Price et al. 2011).

The effects of ocean acidification on corals remain controversial (Jury et al. 2010). Slowing of calcification is generally attributed to a decrease in carbonate ion (CO₃²⁻) availability in lower pH seawater (Kleypas et al. 2006; Hoegh-Guldberg et al. 2007), but the increase in pCO₂ also leads to an increase in bicarbonate ion concentration [HCO₃⁻], which is used by algal endosymbionts for photosynthesis (Marubini et al. 2008; Jury et al. 2010). This balance of carbonate and bicarbonate ions is explained by the stoichiometric dissociation constant of carbonic acid, H₂CO₃, and controlled by temperature, salinity, and surface pressure (Zeebe 2012). Marubini et al. (2008) reported that photosynthesis by symbiotic algae of the coral Stylophora pistillata was facilitated by an increase in [HCO₃⁻], which also led to an increase in coral calcifi-
cation, while they also reported that photosynthesis was insensitive to the increase in $pCO_2$. In a previous study we also observed that $S$. pistillata was insensitive to lower pH seawater (Nakamura et al. 2017). Thus, the effect of ocean acidification on corals is somewhat complicated because of the existence of endosymbionts.

The differing responses of corals to ocean acidification could be attributed to different experimental conditions such as experimental duration (Marubini et al. 2008), different $pCO_2$ range (Comeau et al. 2013; Comeau et al. 2019), temperature range (Prada et al. 2017; Guillermic et al. 2021), light intensity level (Venn et al. 2019) and/or feeding and nutrition (Edmunds 2011). Salinity range and the recovery time after coral sample collection are not always consistent among these studies simulating ocean acidification. Also, physiological differences among different coral species (Hikami et al. 2011; Price et al. 2011), and growth variation among colonies (Marubini et al. 2003; Iguchi et al. 2012; Sekizawa et al. 2017) seem to cause different response to ocean acidification. Comeau et al. (2013) reported that methodology differences to calculate calcification rates changed the best-fit model between growth rate and $pCO_2$. Also, different experimental conditions of $pCO_2$ range may lead to over simplified diagnoses of the responses of calcifying organisms to ocean acidification. If organisms show non-linear responses over a wide range of acidification, as reported by Ries et al. (2009), a simple comparison between calcification rates in control seawater and one acidified condition could be misleading and result in overestimation of the magnitude of responses to ocean acidification. In this study, we investigated the responses of six common subtropical corals ($Acropora nasuta$, $A. tenuis$, $Montipora digitata$, $Pocillopora damicornis$, $Porites cylindrica$, and $Galaxea fascicularis$) collected from a fringing reef of Sesoko Island, Okinawa, Japan, to $pCO_2$-adjusted seawater. We selected a wide range of $pCO_2$ treatments (from 300 $\mu$atm [pre-industrial] to 1200 $\mu$atm [near-future]) under uniform experimental conditions (nutrients, temperature, salinity, and light) in common-garden tanks. Our results were analyzed using Bayesian methods, which offer some advantages over conventional statistical techniques for analyzing experimental data (Wilkinson 2007). We applied four statistical models to find the best fit and avoid an oversimplified conclusion regarding the response of coral calcification to lower pH seawater.

## Materials and methods

### Coral rearing experiment

We prepared seawater under five $pCO_2$ levels for our experiment: approximately 320, 440, 750, 990, and 1210 $\mu$atm (Table 1). Precise and stable $pCO_2$ conditions ($pCO_2$ was maintained to within 5% of the target level during the 44-d experimental period) were achieved by using a high-precision $pCO_2$ control system called AICAL (Fig. 1; Fujita et al. 2011). This system has built in $pCO_2$ analyzer (non-dispersive infrared (NDIR) analyzer) and recorded $pCO_2$ every 90 minutes. Seawater was filtered using an inline filter system (1 $\mu$m pore size). We set up two aquaria for each $pCO_2$ level (total of ten aquaria as shown in Fig. 1). As nubbins from six species could not fit into one aquarium, we deployed three species in one

### Table 1  Summary of physical and chemical conditions in each experimental aquarium. The parameters are shown as mean values ± standard deviations.

| Aquarium | °C  | pH  | $pCO_2$ ($\mu$atm) | $HCO_3^-$ (μmol/kg) | $CO_2^-$ (μmol/kg) | $\Omega_{sat}$ | No. of measures |
|----------|-----|-----|-------------------|---------------------|-------------------|---------------|-----------------|
| Series A | 27.2±0.1  | 8.11±0.01 | 322±11 | 1647±12 | 247±5  | 3.97±0.1 | 693 |
| Series B | 27.4±0.1  | 8.11±0.01 | 324±12 | 1647±12 | 247±5  | 3.97±0.1 | 662 |
| Series A | 27.4±0.3  | 8.00±0.02 | 441±23 | 1751±15 | 205±6 | 3.30±0.1 | 689 |
| Series B | 27.4±0.4  | 8.00±0.02 | 444±23 | 1753±15 | 204±6 | 3.29±0.1 | 661 |
| Series A | 27.4±0.1  | 7.81±0.01 | 748±23 | 1905±8  | 143±3 | 2.30±0.0 | 691 |
| Series B | 27.7±0.2  | 7.80±0.01 | 757±23 | 1905±7  | 143±3 | 2.30±0.0 | 664 |
| Series A | 27.4±0.8  | 7.70±0.02 | 990±39 | 1971±6  | 116±2 | 1.86±0.0 | 692 |
| Series B | 27.4±0.1  | 7.70±0.01 | 992±27 | 1972±6  | 115±2 | 1.86±0.0 | 663 |
| Series A | 27.3±0.2  | 7.62±0.01 | 1216±39 | 2014±6 | 98±3  | 1.58±0.0 | 638 |
| Series B | 27.3±0.2  | 7.62±0.1 | 1216±40 | 2015±6 | 98±3  | 1.58±0.0 | 662 |
aquarium (series A), and the other three species in the other aquarium (series B). Two aquaria (12 L), series A and B, were filled with seawater adjusted to each pCO₂ value, and each aquarium was maintained as a flow-through system with a flow rate of 150 mL min⁻¹. During the experiment, the seawater temperature was maintained at approximately 27°C, which is the ambient water temperature during the coral spawning season in Okinawa (Table 1). The seawater temperature in each aquarium was maintained with a thermostat and heater, and was recorded every 30 min by data loggers (Water Temp Pro; Onset, MA, USA). The photoperiod was set to 12 h:12 h light:dark (180–200 μmol m⁻² s⁻¹) under metal-halide lamps (Funnel2 150W; Kamihata, Hyogo, Japan). The seawater within each aquarium was circulated at approximately 5 cm s⁻¹ with a water-jet pump (Kotobuki mini box; Kotobuki, Japan). Total alkalinity was measured with an automated titrator (ATT-05, KIMOTO ELECTRIC CO. LTD, Osaka, Japan) using 0.1 mol/L HCl once a week. The chemical and physical conditions of each treatment are summarized in Table 1. The pH, [HCO₃⁻], [CO₃²⁻], and Ωₛₐ₉ were estimated from pCO₂, temperature, total alkalinity (2255 ± 33 μmol kg⁻¹; mean ± standard deviation), and salinity (34.5) with the computer program CO2SYS (Lewis and Wallace 1998). In this program, CO₂ constant was selected from Mehrabach et al. (1973) refitted by Dickson and Millero (1987).

Five species of branching corals (Acropora nasuta, A. tenuis, Montipora digitata, Pocillopora damicornis, Porites cylindrica), and one species of massive coral (Galaxea fascicularis) were collected from a fringing reef of Sesoko Island, Okinawa, Japan. The samplings were permitted by Okinawa Prefecture (permit no. 23–25). One mother colony was used for each species. Collected colonies were maintained in a tank with running seawater under natural light conditions at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. From the five branching corals, similar-sized branches, hereinafter called nubbins (around 2–3 cm long), were cut from the parental colonies with end cutting pliers and attached to plastic bolts with superglue. Corallites of G. fascicularis were detached from the base of the coenosteum, and single corallites were attached to plastic bolts with superglue. The nubbins were allowed to recover in a tank with running seawater for around one to two weeks under natural light conditions until coral tissues started to spread on the surface of the plastic bolts.

Seven (P. damicornis) or eight nubbins (other species) from a single mother colony for each species were maintained in each aquarium to which pCO₂-adjusted seawater was supplied in a flow-through manner. Nubbins of A. nasuta, G. fascicularis, and P. cylindrica were placed in aquaria for Series A, and those of the other three species in Series B. Coral skeletal weight (mg) was measured as buoyant weight as described by Iguchi et al. (2012). The net calcification rate was calculated as the percentage change in skeletal weight relative to the initial weight during the experiment (4 weeks). Nubbins of M. digitata in 440-μatm seawater suffered from an unknown disease.
and showed lower calcification rates, and thus these nubbins were excluded from the further analyses.

**Statistical analysis**

We applied a Bayesian statistical approach to avoid artifacts resulting from fluctuations in $pCO_2$. All Bayesian estimations were conducted using the statistical software R 2.15.1 (R Development Core Team 2012) and WinBUGS 1.4.3 (Lunn et al. 2000). It should be noted that WinBUGS is a software made for Windows and BUGS means Bayesian inference Using Gibbs Sampling. The $pCO_2$ in each aquarium was assumed to follow a normal distribution, and the observed mean $pCO_2$ and standard deviation (Table 1) were used as the prior distribution. Furthermore, we used the following regression models with normal error distributions to analyze the effect of $pCO_2$ on net calcification rates of the six coral species:

\[
\begin{align*}
\text{Constant model: Net calcification rate} &= \beta_0 \\
\text{Linear model: Net calcification rate} &= \beta_0 + \beta_1 pCO_2 \\
\text{Quadratic model: Net calcification rate} &= \beta_0 + \beta_1 pCO_2 + \beta_2 pCO_2^2 \\
\text{Exponential model: Net calcification rate} &= \exp(\beta_0 + \beta_1 pCO_2)
\end{align*}
\]

$\beta_i$ $(i=0-2)$ are parameters of linear predictors to be estimated. Posterior distributions of $\beta_i$ and $pCO_2$ were estimated using Markov chain Monte Carlo (MCMC) simulations employing Gibbs sampling. MCMC uses the previous sample value to generate the next sample value, creating a Markov chain. An important characteristic of this chain is that each new sample only depends on the sample that preceded it. Gibbs sampling is a simulation tool for obtaining samples from a non-normalized joint density function (Gelfand 2000). Bayesian estimations of $\beta_i$ were conducted using a normal distribution (mean, 0; variance, 100) as the nearly non-informative prior distribution. MCMC requires four values to be defined before running: (1) the number of iterations (draws), (2) the number of burn-in iterations, (3) the number of chains, and (4) the thinning (subsampling) interval. We obtained estimates from 1,000,000 MCMC iterations after a 100,000-iteration burn-in, three chains to confirm that estimated values were independent of initial parameters, and thinning at intervals of 900. This will supplement our experimental data as we used the single mother colony (one genotype) for each coral species. We verified that R-hat values ranged between 1.00 and 1.01, which indicates convergence (Gelman et al. 2004). We used the deviance information criterion (DIC) and DIC model weights $(W)$ to rank models (Burnham and Anderson 2002). Model selection was conducted among exponential, quadratic, linear, and constant models by maximizing the goodness of fit given by the DIC. The $W_i$ of a model is used as a measure of the strength of the model among the candidate models and indicates the probability that the model is the actual best model.

**Results**

Overall, the net calcification rates of coral nubbins decreased with an increase in seawater $pCO_2$ (Fig. 2). Coral nubbins showed positive calcification rates in all treatments, although nubbins of *Pocillopora damicornis* showed the lowest calcification rates (below 5%) in all $pCO_2$ conditions. The best-fit regression models and posterior distributions of parameter estimates varied by species (Tables 2 and 3). None of the 95% credible intervals of posterior means for any of the parameters or species contained zero (i.e., all parameters were significant) (Table 3). Five species other than *P. damicornis* showed non-linear responses to $pCO_2$-adjusted seawater. The model weights ($W_i$) of *Acropora tenuis* and *Porites cylindrica* were the highest in the quadratic model (Table 2). The response of *A. tenuis* was positively parabolic whereas that of *P. cylindrica* was negatively parabolic (Fig. 2 and Table 3). Although the quadratic model was selected as the best fit for these species, there were no apparent changes in calcification rates for *A. tenuis* between a $pCO_2$ of 750 and 1200 $\mu$atm. On the other hand, it is noteworthy that *P. cylindrica* had higher calcification rates at intermediate $pCO_2$ levels. The responses of *Acropora nasuta*, *Galaxea fascicularis*, and *Montipora digitata* were consistent with the exponential model, although the model weights ($W_i$) were only slightly higher than those of the linear model (Table 2). Similarly, the best-fit model for *P. damicornis* was linear, but there was also a high model weight assigned to the exponential model.
Fig. 2  Calcification rates of branching and massive corals (A: *Acropora nasuta*; B: *A. tenuis*, C: *Galaxea fascicularis*, D: *Montipora digitata*, E: *Pocillopora damicornis*, F: *Porites cylindrica*) in 5 pCO2 conditions (4 pCO2 for *M. digitata* by the end of the experiment). Solid lines and shaded gray indicate posterior means and 95% credible intervals of the best-fit regression models, respectively. pCO2 intervals outside of 200–1200 μatm levels (shaded in light gray) have greater uncertainty in the best fit model based on DIC as the data are distant from measured values shown with black circles.
Table 2  Ranking of regression models for explaining calcification rates of six coral species among exponential (Exp: \( \text{calcification rate}=\exp (\beta_0 + \beta_1 pCO_2) \)), quadratic (Quad: \( \text{calcification rate}=\beta_0 + \beta_1 pCO_2 + \beta_2 pCO_2^2 \)), linear (Line: \( \text{calcification rate}=\beta_0 \)) and constant (Const: \( \text{calcification rate}=\beta_0 \)) models. Models are arranged in order of increasing deviance information criterion (DIC). The best model is shown in bold. \( \Delta \text{DIC} \) is the difference in DIC from that of the best model. Models were ranked by the DIC model weight (\( W_i \)) after Burnham and Anderson (2002).

| Coral species         | Model | DIC | \( \Delta \text{DIC} \) | \( W_i \) |
|-----------------------|-------|-----|------------------------|---------|
| A) Acropora nasuta    | Exp   | 145.9 | 0.0 | 0.402 |
|                       | Line  | 146.4 | 0.5 | 0.313 |
|                       | Quad  | 146.6 | 0.7 | 0.284 |
|                       | Const | 157.7 | 11.8| 0.001 |
| B) Acropora tenuis    | Quad  | 201.5 | 0.0 | 0.997 |
|                       | Exp   | 213.3 | 11.8| 0.003 |
|                       | Line  | 216.4 | 14.9| 0.001 |
|                       | Const | 236.6 | 35.1| 0.000 |
| C) Galaxea fascicularis | Exp   | 218.8 | 0.0 | 0.390 |
|                       | Line  | 218.8 | <0.1| 0.389 |
|                       | Quad  | 220.5 | 1.7 | 0.166 |
|                       | Const | 222.7 | 3.9 | 0.056 |
| D) Montipora digitata | Exp   | 191.5 | 0.0 | 0.401 |
|                       | Line  | 191.6 | 0.1 | 0.380 |
|                       | Quad  | 192.9 | 1.4 | 0.199 |
|                       | Const | 197.5 | 6.0 | 0.020 |
| E) Pocillopora damicornis | Line     | 154.0 | 0.0 | 0.383 |
|                       | Exp   | 154.6 | 0.6 | 0.288 |
|                       | Quad  | 154.9 | 0.8 | 0.252 |
|                       | Const | 157.2 | 3.2 | 0.077 |
| F) Porites cylindrica | Quad  | 220.3 | 0.0 | 0.970 |
|                       | Line  | 229.4 | 9.1 | 0.010 |
|                       | Const | 229.6 | 9.2 | 0.010 |
|                       | Exp   | 229.6 | 9.2 | 0.010 |

Table 3  Posterior distributions of parameter estimates in the best-fit regression models for explaining calcification rate of each coral species by \( pCO_2 \) level. A posterior mean in bold indicates that zero is not included within the 95% credible interval. Abbreviations of model are shown in Table 2.

| Coral species         | Best-fit model | Parameter | Posterior mean | 95% credible interval (lower, upper) |
|-----------------------|----------------|-----------|----------------|-------------------------------------|
| A) Acropora nasuta    | Exp            | Intercept | 2.57           | (2.47, 2.67)                        |
|                       |                | \( pCO_2 \) | \(-2.51 \times 10^{-4}\) | \((-3.87 \times 10^{-4}, -1.21 \times 10^{-4})\) |
| B) Acropora tenuis    | Quad           | Intercept | 33.4           | (27.7, 39.3)                        |
|                       |                | \( pCO_2 \) | \(-4.63 \times 10^{-2}\) | \((-6.50 \times 10^{-2}, -2.79 \times 10^{-2})\) |
|                       |                | \( pCO_2^2 \) | \(2.47 \times 10^{-5}\) | \((1.25 \times 10^{-5}, 3.71 \times 10^{-5})\) |
| C) Galaxea fascicularis | Exp            | Intercept | 2.87           | (2.67, 3.05)                        |
|                       |                | \( pCO_2 \) | \(-3.11 \times 10^{-4}\) | \((-5.83 \times 10^{-4}, -5.52 \times 10^{-4})\) |
| D) Montipora digitata | Exp            | Intercept | 3.13           | (2.89, 3.35)                        |
|                       |                | \( pCO_2 \) | \(-4.50 \times 10^{-4}\) | \((-7.51 \times 10^{-4}, -1.57 \times 10^{-4})\) |
| E) Pocillopora damicornis | Line     | Intercept | 6.75           | (4.93, 8.66)                        |
|                       |                | \( pCO_2 \) | \(-2.63 \times 10^{-3}\) | \((-4.82 \times 10^{-3}, -2.03 \times 10^{-3})\) |
| F) Porites cylindrica | Quad           | Intercept | 7.41           | (0.090, 14.9)                       |
|                       |                | \( pCO_2 \) | \(3.35 \times 10^{-5}\) | \((1.03 \times 10^{-5}, 5.59 \times 10^{-5})\) |
|                       |                | \( pCO_2^2 \) | \(-2.38 \times 10^{-3}\) | \((-3.85 \times 10^{-3}, -8.92 \times 10^{-3})\) |
Discussion

In general, calcification rates of corals decreased with increasing seawater $p$CO$_2$; however, the patterns of responses were different among species (Fig. 2). By having five levels of $p$CO$_2$ and applying four different statistical models, we succeeded in capturing detailed patterns of variation among the six species. Considering that we reared corals under almost uniform experimental conditions and used clonal fragments from a single colony of each species, we believe that these differences can be attributed to variations in physiology among coral species or colonies.

Coral skeletons are a mixture of aragonite crystals and organic materials (Allemand et al. 1998). Coral calcification is therefore performed not only by inorganic precipitation of calcium carbonate, but also by biogenic processes including the production of an organic matrix, which is a prerequisite step in coral skeletogenesis (Allemand et al. 1998). Thus, coral calcification should be affected by the energetic status of corals, which is facilitated by photosynthetic activity of symbiotic algae (Gattuso et al. 1999; Yellowlees et al. 2008) and feeding by the coral host (Anthony and Fabricius 2000; Edmunds 2011). It has already been suggested that the recovery of corals from bleaching would differ among species according to how their energy intake depends on heterotrophy or autotrophy (Grottoli et al. 2006; Conti-Jerpe et al. 2020). Edmunds (2011) reported that coral heterotrophic feeding (zooplanktivory) could reduce the negative impacts of lower pH seawater on coral calcification; thus, variations in tolerance to ocean acidification among coral species would be related to their relative dependence on heterotrophy or autotrophy. In our experiment, however, we excluded plankton from the seawater by using 1-$\mu$m filters; therefore, the differences in responses to lower pH seawater among coral species were likely caused by other characteristics of coral physiology and/or dependency of heterotrophic feeding as our experiment was starvation study. Another thing to consider for coral physiology is that corals utilize their energy to maintain a high saturation state at Centers of Calcification (COC). McCulloch et al. (2017) stated that corals can regulate pH at COC largely independent from changes in seawater chemistry. Cohen and Holcomb (2009) also reported that corals are able to convert [HCO$_3^-$] to [CO$_3^{2-}$] at COC although this efficient system can be hampered by rising $p$CO$_2$.

The effects of ocean acidification on corals are complicated because ocean acidification affects both coral calcification and the photosynthesis of symbiotic algae through a decrease in [CO$_3^{2-}$] and an increase in [HCO$_3^-$]. The reduction of calcification in calcifying organisms including corals is generally attributed to a decrease in [CO$_3^{2-}$] (Kleypas et al. 2006; Hoegh-Guldberg et al. 2007). However, Jury et al. (2010) reported that changes in the concentration of HCO$_3^-$ have a greater influence on coral calcification than changes in CO$_3^{2-}$ concentration, $\Omega_{arag}$, or pH. This suggests that increased $p$CO$_2$ may play a role in fertilization of photosynthesis (Marubini et al. 2008; Ries et al. 2009). Also Herfort et al (2008) reported that increases in [HCO$_3^-$] caused stimulation in both photosynthesis and calcification simultaneously suggesting that negative impacts were not always linked to higher $p$CO$_2$.

The responses of corals to ocean acidification are known to differ among coral species (Marubini et al. 2003; Anthony et al. 2008; Hii et al. 2009; Comeau et al. 2014). Marubini et al. (2003) reported that calcification rates of four coral species uniformly decreased in lower pH seawater, but the magnitude of the response in terms of microstructure crystallization differed between species. In their observations, crystallization in Acropora verweyi clearly changed in lower pH seawater, but that in Turbinaria reniformis did not. Anthony et al. (2008) reported that the net photosynthesis of Porites lobata decreased in both temperature ranges examined (25–26°C, 28–29°C) in accordance with the increase in $p$CO$_2$, whereas that of Acropora intermedia increased in moderately lower pH seawater (520–705 $\mu$atm) and dramatically decreased in the lowest pH seawater (1020–1360 $\mu$atm) at 25–26°C. An increase of net photosynthesis in moderately low pH seawater (600–790 $\mu$atm) has also been reported for A. formosa (taxonomically A. muricata; Crawley et al. 2009), and Hii et al. (2009) reported different physiological responses between Galaxea fascicularis and Porites cylindrica in lower pH seawater. Therefore it is widely accepted that OA resistance varies depending on species. Traits that can explain these phenomena can be categorized as the following; (i) dependency of host coral on
symbiotic algae, (ii) difference between perforate and imperforate species, (iii) whether fast or slow growth rate species, (iv) degree of organic growth, and/or (v) OA adaptation ability.

Regarding the first point, Cunning et al. (2017) modeled an interaction of host coral and symbiotic algae growth under light stress using available CO2 from the surrounding seawater and recycled CO2 from both host and the algae. Such a simulation incorporating pH stress helps in understanding the complex relationship between corals and symbiotic algae. Secondly, van Woesik et al. (2013) succeeded in culturing both perforate (Montipora sp.) and imperforate (Pectibia sp.) corals, and found out perforate corals will dissolve more rapidly and cause ~10.5 mm of vertical reduction of reef framework per year. In our experiment, only imperforate coral was P. damicornis, and we could not explain the difference in OA response only by the difference of perforate versus imperforate. As to third point, Comeau et al. (2014) reported that the response of calcification to lower pH seawater at several levels was unrelated to colony morphology or skeletal structure in corals, but fast-calcifying corals (Acropora pulchra, Porites rus, Psammocora profundacella, and Porites spp.) were more sensitive than slower ones (Porites irregularis, Pocillopora verrucosa, Pocillopora damicornis, and Pavona cactus). To address the fourth point, Kaniewska et al. (2012) stated that OA significantly regulated the gene expression of membrane cytoskeletal interactions and cytoskeletal remodeling. For example, they confirmed the reduction in Ca binding protein (Calmodulin) that serves as the organic material to support skeletal structure. Finally, Comeau et al. (2019) examined the long-term effects of lower pH seawater by culturing corals for one year to test adaptation ability and found that the calcification rates of Pocillopora verrucosa and Porites spp. were not affected by lower pH seawater, whereas those of Psammocora profundacella and Acropora pulchra were negatively affected.

Our study introduced a Bayesian statistical approach to analyze the results from coral culture experiments, which indicated that the responses of corals to lower pH seawater are diverse, as shown for other taxa, even under extremely uniform environmental conditions (Iglesias-Rodriguez et al. 2008; Ries et al. 2009; Fujita et al. 2011; Hikami et al. 2011; Price et al. 2011). The reasons for the different responses to ocean acidification among coral species cannot be fully determined at this stage. It should be noted that we used a single mother colony (one genotype) for each species due to collection restriction. In this study, we were able to infer the response of each species to pCO2, and more studies are needed to test the difference of individual mother colonies. Further research involving phylogenetically and physiologically different corals is also necessary to understand the relationship between calcification and photosynthesis in coral-algal holobionts which should be tested at different light levels. In addition, experiments to test dependency of heterotrophic feeding will be a future topic. For example, half of the nubbins can be fed and others should strictly depend on algal-holobionts. We should be also mindful that most coral reefs experience diel fluctuations of pCO2. For example, Ohde and van Woesik (1999) reported diel fluctuations of pH from 7.8 to 8.6 at a coral reef site located in the south of Okinawa. The results from here are flat line pCO2 conditions, and we will also need to conduct in situ monitoring of coral reefs. However, we believe that laboratory simulations are essential to predict how coral communities might shift under future seawater carbon chemistry as a result of varied responses to near-future ocean acidification.

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Compliance
Coral colonies were collected by snorkeling with the permission of Okinawa Prefecture (no. 23–25).
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