

# Ocean acidification does not affect the physiology of the tropical coral *Acropora digitifera* during a 5-week experiment

A. Takahashi · H. Kurihara

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**Abstract** The increase in atmospheric CO<sub>2</sub> concentration, which has resulted from the burning of fossil fuels, is being absorbed by the oceans and is causing ocean acidification. Ocean acidification involves the decrease of both the pH and the calcium carbonate saturation state. Ocean acidification is predicted to impact the physiology of marine organisms and reduce the calcification rates of corals. In the present study, we measured the rates of calcification, respiration, photosynthesis, and zooxanthellae density of the tropical coral *Acropora digitifera* under near-natural summertime temperature and sunlight for a 5-week period. We found that these key physiological parameters were not affected by both mid-CO<sub>2</sub> (pCO<sub>2</sub> = 744 ± 38, pH = 7.97 ± 0.02, Ω<sub>arag</sub> = 2.6 ± 0.1) and high-CO<sub>2</sub> conditions (pCO<sub>2</sub> = 2,142 ± 205, pH = 7.56 ± 0.04, Ω<sub>arag</sub> = 1.1 ± 0.2) throughout the 35 days experimental period. Additionally, there was no significant correlation between calcification rate and seawater aragonite saturation (Ω<sub>arag</sub>). These results suggest that the impacts of ocean acidification on corals physiology may be more complex than have been previously proposed.

**Keywords** Ocean acidification · Coral · Calcification · Photosynthesis

## Introduction

The increase in atmospheric CO<sub>2</sub> is quickly acidifying oceans and is affecting oceanic carbonate chemistry (Caldeira and Wickett 2003, 2005). According to the IPCC (2007) models, atmospheric CO<sub>2</sub> is predicted to rise to 540–970 μatm by the end of this century and reach a maximum of approximately 1,900 μatm when the world's fossil fuel reserves are fully exploited. Atmospheric CO<sub>2</sub> dissolves into seawater and leads to a decrease in the seawater pH and a decrease in the calcium carbonate saturation state (Ω), together referred to as ocean acidification (Orr et al. 2005).

Until now, a substantial number of laboratory studies have suggested a decline in coral calcification with a rise in seawater pCO<sub>2</sub> (Gattuso et al. 1998; Marubini et al. 2001, 2003; Reynaud et al. 2003; Langdon and Atkinson 2005; Kleypas et al. 2006; Hoegh-Guldberg et al. 2007; Anthony et al. 2011; Pandolfi et al. 2011). Field observations of calcium carbonate (CaCO<sub>3</sub>) precipitation in coral lagoons have been linked to aragonite saturation state (Ω<sub>arg</sub>; aragonite is the calcium carbonate mineral deposited by corals) (Broecker and Takahashi 1966; Broecker et al. 2001; Silverman et al. 2007; Kleypas et al. 2011), although Shamberger et al. (2011) demonstrated that the relationship between Ω<sub>arg</sub> and coral reef communities is variable among reefs. Recent laboratory and field studies have suggested that the net calcification rates of corals have already decreased by 17–35 % compared with calcification rates prior to industrial revolution (Kleypas et al. 1999). Calcification rates have been also projected to decrease further by a further 65 % by the end of this century (Langdon et al. 2000; Reynaud et al. 2003).

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Recent studies, however, have postulated that the sensitivity of corals to elevated levels of CO<sub>2</sub> is potentially more diverse than previously considered (Rodolfo-Metalpa et al. 2011; Fabricius et al. 2011; Pandolfi et al. 2011). The variance in the response to high CO<sub>2</sub> may be attributed not only to physiological differences among coral species, but also to the limitation of experimental methodology (e.g., addition of strong acid vs. CO<sub>2</sub>), the experimental duration (several hours to days vs. several weeks to months), and the experimental setup (indoor vs. outdoor, closed system vs. flow-through system) (Jokiel et al. 2008; Jury et al. 2010; De Putron et al. 2011). Previously, most of the ocean acidification experiments have been conducted over the short-term (days), utilizing indoor, closed system conditions. Additionally, several studies have used strong acids instead of CO<sub>2</sub> to control seawater carbonate chemistry. Strong acids decrease total alkalinity (TA) while maintaining constant dissolved inorganic carbon (DIC), whereas CO<sub>2</sub> bubbling increases DIC while maintaining constant TA (Gattuso and Lavigne 2009). To our knowledge, there have been only a few studies that have evaluated the medium-term (weeks) and long-term (months) effects of CO<sub>2</sub>-induced ocean acidification on corals under near-natural temperature, light and continuous water flow conditions.

Anthony et al. (2008) demonstrated that calcification and production of coral *Acropora intermedia* cultured under natural light and controlled seawater conditions (28–29 °C) was suppressed by high CO<sub>2</sub> (1,000–1,300 μatm), but not by mid-CO<sub>2</sub> (520–705 μatm) treatments. Elevated CO<sub>2</sub> treatments (700 μatm) had no significant effect on the calcification and respiration rates of the temperate coral *Cladocora caespitosa* cultured for 1 year under seminatural conditions (Rodolfo-Metalpa et al. 2010). Holcomb et al. (2010) reported that the calcification rate of the temperate coral colonies *Astrangia poculata* was negatively affected by 780 μatm CO<sub>2</sub> when corals were reared under artificial light (40–115 μmol quanta m<sup>-2</sup> s<sup>-1</sup>), at a temperature of 25.8 °C, and subjected to ambient nutrient conditions for 6 months. These corals were not affected, however, when subjected to the same treatment but under elevated nutrient conditions. Edmunds (2011) reported that when the massive *Porites* spp., under modest light intensity (600 μmol quanta m<sup>-2</sup> s<sup>-1</sup>) and natural field temperature (29.3 °C), were fed zooplankton, they had the capacity to resist high CO<sub>2</sub> (1,000 μatm) treatment for 1 month.

In the present study, we investigated the effect of CO<sub>2</sub>-induced ocean acidification on the tropical scleractinian coral *Acropora digitifera* under near-natural conditions. Terminal branches of the coral (i.e., nubbins) were cultured for 35 days (5 weeks) in an outdoor flow-through CO<sub>2</sub> system, at control (343 ± 23 μatm; mean ± SD), medium (744 ± 38 μatm), and high (2,142 ± 205 μatm) CO<sub>2</sub> conditions, under natural summertime light and temperature conditions. We studied the effects of these CO<sub>2</sub>

treatments on the several physiological responses including calcification, photosynthesis, respiration, photosynthetic efficiency, and zooxanthellae density.

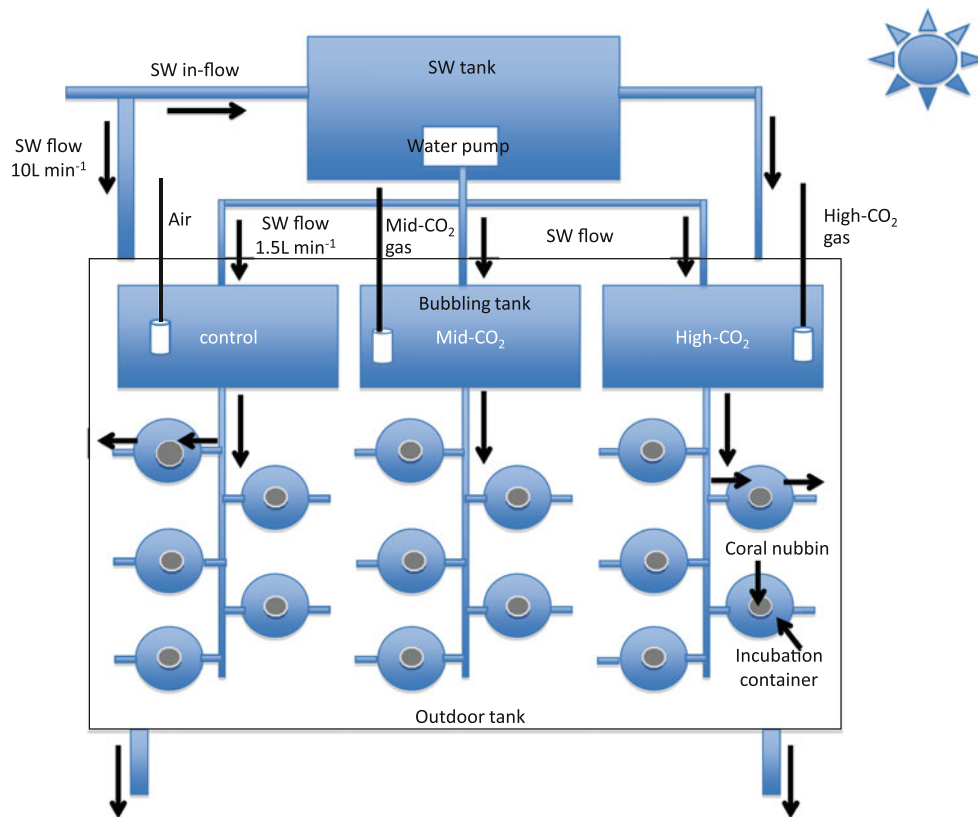
## Materials and methods

### Coral collection

Five colonies of the coral *Acropora digitifera* were collected in June 2010 from inshore patch reefs (ca. 0.5–1 m depth) in front of the Sesoko Station, University of the Ryukyus, Okinawa Island, Japan (26°38′11.64″ N, 127°51′57.08″ E). *Acropora digitifera* is a dominant reef-building coral in the Indo-Pacific and is commonly found at shallow depth reef margins (Veron 2000). Three nubbins from each of the five colonies were collected 5 weeks prior to the experiment. All nubbins (ca. 2 cm long) were placed, for 2 weeks, in an outdoor tank (1.2 m × 0.7 m × 0.2 m, volume = 187 L) supplied continuously with seawater (1.5 L min<sup>-1</sup>) pumped from 4–5 m depth in front of the station. Thereafter, all nubbins were placed individually in incubation containers (diameter = 10 cm; height = 20 cm; volume = 450 ml), which were kept in an outdoor tank (Fig. 1). Each container was continuously supplied with seawater (300 ml min<sup>-1</sup>) aerated with air and nubbins kept an additional another 3 weeks under these conditions.

### Experimental setup

One nubbin from each of the five colonies was cultured under the control (343 ± 23 μatm; mean ± SD), medium (744 ± 38 μatm), and high (2,142 ± 205 μatm) CO<sub>2</sub> conditions, respectively, for 35 days (5 weeks) (Fig. 1; Table 1). Seawater pCO<sub>2</sub> was adjusted by continuously bubbling the flowing seawater (1.5 L min<sup>-1</sup>) in the bubbling tank with air, or mid- or high-CO<sub>2</sub> concentration gas at a rate of 3.0 L min<sup>-1</sup> made by mixing air and pure CO<sub>2</sub> using a mass-flow controller (HORIBASTEC, SEC-E40, Japan). The gas mixtures were supplied to each incubator container at the flow rate of 300 ml min<sup>-1</sup>. Seawater pH and temperature of the bubbling tank were measured daily in the morning (0800–1000 hours) using a temperature-compensated pH electrode (Mettler Toledo, MP125, USA). The pH electrode was calibrated every day before measurement using NBS scale buffer solutions. From pre-experimental studies, we verified that the temperature and pH of each bubbling tanks were similar with the downstream incubation containers (temperature and pH standard deviation between tanks were less than ±0.3 and ±0.03, respectively). Each experimental setup was placed outdoors under natural irradiance conditions, and seawater temperature was adjusted to the field temperature by running seawater (10 L min<sup>-1</sup>)



**Fig. 1** Experimental setup that was used to regulate seawater pCO<sub>2</sub> of the cultured *Acropora digitifera* corals

pumped from the ocean to the outdoor tank (Fig. 1). All incubation containers were cleaned every week. Seawater temperature of the outdoor tank was also logged each 30 min using a data logger (Hobo U22-001, Onset Corp., USA). Irradiance was logged each 1 min by quantum light meter (MDS-MkV/L, JFE, Japan) placed inside of the incubator container. Seawater samples were drawn from the bubbling tanks, and salinity and total alkalinity (TA) were measured every week using a refractometer (Atago, 100-S, Japan) and autoburette titrator (Kimoto, ATT-05, Japan). Seawater pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and Ω<sub>arag</sub> were calculated by pH, temperature, TA, and salinity using CO2SYS program of Lewis and Wallace (1998), with dissociation constants K1 and K2 from Mehrbach et al. (1973) and the aragonite solubility of Mucci (1983) (Table 1).

#### Calcification, dissolution, photosynthesis, and respiration

The calcification rates (*G*) of corals were measured using two different techniques. The alkalinity anomaly technique was used to measure light and dark net calcification rates, at a timescale of hours (Smith and Key 1975). The buoyant weight technique was used to measure the net calcification rate, at a timescale of weeks (Davies 1989). Net photosynthesis (*P*) and respiration (*R*) rates were calculated

using dissolved inorganic carbon (DIC)–total alkalinity (TA) technique (Smith 1973; Smith and Key 1975; Fujimura et al. 2008). TA and DIC measurements were taken during 2-h culture at midday (1200–1400 hours; light condition) and night (2100–2300 hours; dark condition) at 7, 14, and 35 days after the start of experimental culture. Seawater samples (ca. 100 ml) for the initial DIC and TA measurement were collected from each container, and again immediately after the chambers were refilled with new flowing seawater (ca. 30 s after sampling), all experimental containers were sealed without head space. The initial seawater pCO<sub>2</sub>, pH, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and Ω<sub>arag</sub> were calculated by measured TA and DIC and salinity using CO2SYS program (Table 2), although it is evident that the seawater pCO<sub>2</sub> calculated from TA and DIC tend to be underestimated at high CO<sub>2</sub> levels (Hoppe et al. 2012). All experimental containers were well mixed with a magnetic stirrer (450 rpm) during culture, and seawater was sampled at the end of 2-h culture. The seawater was poisoned with mercuric chloride immediately after sampling. Dissolved inorganic carbon and TA were measured using closed cell titration (Kimoto, ATT-05, Japan), and the precision of the determinations was evaluated by analyzing reference material Batch AG (TA = 2,259 ± 0.45 μmol kg<sup>-1</sup>, DIC = 2,035.4 ± 0.64 μmol kg<sup>-1</sup>, from KANSO TECHNOS, JAPAN), which was traced with certified reference

**Table 1** Carbonate chemistry of seawater in the three conditions

CO <sub>2</sub> condition	pCO <sub>2</sub> (µatm)	pH (NBS scale)	Alkalinity (µmol/kg)	DIC (µmol/kg)	HCO <sub>3</sub> <sup>-</sup> (µmol/kg)	CO <sub>3</sub> <sup>2-</sup> (µmol/kg)	Ω aragonite	Temp (°C)	Salinity
Control	343.79 ± 23.7	8.25 ± 0.02	2,323.5 ± 16.1	1,953.5 ± 18.7	1,684 ± 30	261.2 ± 12.1	4.26 ± 0.2	29.34 ± 0.89	34.2 ± 0.29
Mid-CO <sub>2</sub>	744.16 ± 38.3	7.97 ± 0.02	2,324.6 ± 11.8	2,113.7 ± 11.5	1,935 ± 18	159.3 ± 7.3	2.60 ± 0.13	29.29 ± 0.92	34.2 ± 0.29
High-CO <sub>2</sub>	2,142.6 ± 205.2	7.56 ± 0.04	2,323.7 ± 13.3	2,278.9 ± 11.7	2,155 ± 12	68.93 ± 4.9	1.12 ± 0.1	29.29 ± 0.92	34.2 ± 0.29

All parameters were calculated from measured pH, temperature, alkalinity, and salinity using the CO<sub>2</sub>SYS program. Temperature is the mean value for the data measured daily with pH, and alkalinity and salinity are the mean value for three measurements in each CO<sub>2</sub> conditions

material (CRM, Batch 95, from A. Dickson Laboratory). The measurement reproducibility of TA and DIC was  $\pm 4.8$  and  $\pm 3.0 \mu\text{mol kg}^{-1}$ , respectively. Seawater temperature and irradiance were measured and logged during measurement. Net calcification rates ( $G$ ) were calculated by the following equations:

$$G = \Delta\text{TA} (2V D_{\text{sw}} t)^{-1}$$

where  $\Delta\text{TA}$  is the TA measured at the end of culture subtracted from the initial TA and  $t$  is the duration of culture.  $V$  is the seawater volume of each container minus the displacement volume of the coral nubbin.

Respiration ( $R$ ) and net photosynthesis ( $P$ ) were estimated by the organic production rate during day and night culture and calculated by the following equations:

$$R = (\Delta\text{DIC} - 0.5\Delta\text{TA}) (V D_{\text{sw}} t)$$

$$P = (\Delta\text{DIC} - 0.5\Delta\text{TA}) (V D_{\text{sw}} t) - R$$

where  $\Delta\text{DIC}$  is the DIC measured at the end of culture subtracted from the initial DIC.

To measure calcification by buoyant weighing, the skeletal weight was measured at the day before the start of the experiment (day 0) and 7, 14, 21, and 35 days after the start of experimental culture by weighing each nubbin with an electronic balance (0.1 mg precision HR-200, A&D, Japan). Measurement was taken 2 times for each nubbin and verified that the measurement difference was less than 0.05 %. The skeletal weight was calculated using the following formula:

$$W_{\text{air}} = W_{\text{sw}} [1 - (D_{\text{sw}} D_{\text{sk}}^{-1})]^{-1}$$

where  $W_{\text{air}}$  and  $W_{\text{sw}}$  are the weight of the coral nubbin in air and in seawater, and  $D_{\text{sw}}$  and  $D_{\text{sk}}$  are the seawater and aragonite density ( $2.94 \text{ g cm}^{-3}$ ), respectively. Seawater density was measured considering the seawater temperature during measurements. The initial skeletal weight of the nubbins was  $1.45 \pm 0.39 \text{ g}$  ( $n = 15$ ). Calcification rate was calculated using the following formula:

$$G = [(W_b - W_a) W_a^{-1} n^{-1}] \times 100$$

where  $W_a$  and  $W_b$  are the skeletal dry weight of the coral nubbin measured at time  $a$  and  $b$ , respectively, and  $n$  is the time (days) between time  $a$  and  $b$ .

#### Photosynthetic efficiency

The photochemical efficiency of photosynthesis II ( $F_v F_m^{-1}$ ) of zooxanthellae was investigated using a pulse amplitude fluorescence system (miniPAM Walz Effeltrich, Germany) according to Schreiber et al. (1986). Nubbins of *A. digitifera* were removed from the experimental chamber,

**Table 2** Carbonate chemistry of the initial seawater taken at midday (1200 hours) and night (2100 hours), at day 7th, 14th, and 35th after the start of experimental culture to evaluate calcification, photosynthesis, and respiration rate

CO <sub>2</sub> condition	Time	pCO <sub>2</sub> (µatm)	pH (calculated)	Alkalinity (µmol/kg)	DIC (µmol/kg)	HCO <sub>3</sub> <sup>-</sup> (µmol/kg)	CO <sub>3</sub> <sup>2-</sup> (µmol/kg)	Ω (arag)	Salinity	
Control	Day	7th	356.8 ± 8.0	8.16 ± 0.001	2,306.3 ± 8.5	1,987.1 ± 2.7	1,750 ± 3	226.6 ± 4.4	3.6 ± 0.1	34 ± 0
		14th	367.0 ± 6.1	8.14 ± 0.00	2,325.8 ± 4.3	2,008.8 ± 2.0	1,772 ± 4	225.9 ± 2.9	3.6 ± 0.0	34 ± 0
		35th	347.1 ± 17.9	8.23 ± 0.01	2,338.3 ± 5.4	2,001.8 ± 6.5	1,759 ± 13	235.6 ± 8.7	3.8 ± 0.1	34 ± 0
Mid-CO <sub>2</sub>	Night	7th	377.1 ± 13.1	8.11 ± 0.01	2,307.5 ± 3.8	1,999.6 ± 7.5	1,770 ± 11	219.3 ± 4.7	3.5 ± 0.1	34 ± 0
		14th	383.6 ± 6.8	8.09 ± 0.00	2,314.1 ± 6.7	2,008.7 ± 2.9	1,780 ± 3	217.9 ± 3.4	3.5 ± 0.1	34 ± 0
		35th	378.6 ± 5.5	8.13 ± 0.01	2,333.4 ± 6.1	2,021.6 ± 7.7	1,788 ± 8	222.8 ± 1.3	3.6 ± 0.0	34 ± 0
High-CO <sub>2</sub>	Day	7th	722.8 ± 21.9	7.92 ± 0.01	2,312.9 ± 8.4	2,128.6 ± 9.1	1,967 ± 10	141.3 ± 3.4	2.3 ± 0.1	34 ± 0
		14th	815.6 ± 20.5	7.88 ± 0.02	2,324.4 ± 14.5	2,159.1 ± 8.7	2,006 ± 5	130.2 ± 3.9	2.1 ± 0.1	34 ± 0
		35th	805.9 ± 11.2	7.96 ± 0.01	2,336.5 ± 4.8	2,167.8 ± 4.7	2,012 ± 5	132.6 ± 1.5	2.1 ± 0.0	34 ± 0
High-CO <sub>2</sub>	Night	7th	810.8 ± 14.9	7.85 ± 0.01	2,301.8 ± 5.3	2,138.3 ± 6.8	1,987 ± 7	128.5 ± 1.6	2.1 ± 0.0	34 ± 0
		14th	836.5 ± 46.9	7.82 ± 0.00	2,336.2 ± 22.2	2,173.4 ± 10.4	2,020 ± 5	129.2 ± 8.1	2.1 ± 0.1	34 ± 0
		35th	880.3 ± 32.0	7.78 ± 0.01	2,339.0 ± 12.7	2,184.5 ± 6.1	2,035 ± 4	124.3 ± 4.6	2.0 ± 0.1	34 ± 0
High-CO <sub>2</sub>	Day	7th	2,112.5 ± 105.2	7.57 ± 0.02	2,315.0 ± 4.4	2,289.3 ± 5.8	2,170 ± 5	59.0 ± 2.7	0.9 ± 0.0	34 ± 0
		14th	2,011.2 ± 69.0	7.63 ± 0.02	2,317.0 ± 4.3	2,284.7 ± 1.5	2,166 ± 2	61.6 ± 2.1	1.0 ± 0.0	34 ± 0
		35th	2,012.2 ± 78.3	7.70 ± 0.03	2,338.9 ± 6.9	2,305.3 ± 7.5	2,185 ± 7	62.7 ± 2.2	1.0 ± 0.0	34 ± 0
High-CO <sub>2</sub>	Night	7th	2,346.9 ± 49.4	7.54 ± 0.02	2,312.5 ± 5.0	2,301.4 ± 3.8	2,181 ± 4	53.5 ± 1.2	0.9 ± 0.0	34 ± 0
		14th	2,201.2 ± 69.9	7.54 ± 0.00	2,315.8 ± 11.3	2,295.8 ± 12.0	2,176 ± 11	56.8 ± 1.7	0.9 ± 0.0	34 ± 0
		35th	2,180.6 ± 55.8	7.38 ± 0.02	2,335.2 ± 7.6	2,312.8 ± 28.4	2,193 ± 7	58.2 ± 1.4	0.9 ± 0.0	34 ± 0

All parameters were calculated from measured alkalinity, DIC, and salinity using the CO2SYS program. Temperature was fixed to 25 °C during alkalinity and DIC measurement. Values are mean ± SD (*n* = 5)

placed in a box field with each experimental condition's seawater, and dark adapted for 15 min prior to the measurement. The light-pulse fiber was placed at a fixed distance (10 mm) from the coral tissue. Maximum fluorescence of the corals was measured by a 0.8 s saturation light pulse ( $8,000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ). All measurements were taken at 1000 hours at the day before start of the experiment (day 0) and 7, 14, 21, and 35 days after the start of experimental culture.

#### Zooxanthella density

After 35 days incubation under each different  $\text{CO}_2$  conditions, coral tissue was removed with a Waterpik and homogenized with a homogenizer. A subsample was centrifuged (4 min at 2,400 rpm) to isolate the algae. The algal pellet was washed and re-suspended and the number of zooxanthellae was counted and standardized by the surface area measured using the aluminum foil technique (Marsh 1970).

#### Statistical analysis

REML model was performed using JMP (JMP 7; SAS Inc.) to compare the rates of calcification, photosynthesis, respiration, and photosynthetic efficiency between different  $\text{CO}_2$  conditions. Differences in zooxanthella density among  $\text{CO}_2$  treatments were evaluated using a one-way ANOVA. Multiple linear regression analyses of the calcification rate versus light intensity and  $\Omega_{\text{arag}}$  were applied, respectively.

### Results

The average seawater temperature was  $29^\circ\text{C}$ , and fluctuated between 28 and  $31^\circ\text{C}$ , reflecting the natural diurnal cycle of the inshore reef in summer (Table 1, Electronic Supplemental Material, ESM Fig. S1). Light intensity was highly variable between sunny and cloudy days and noon irradiance varied from 152 to  $3,789 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  (average day light:  $336 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ , ESM Fig. S1). Seawater  $\text{pCO}_2$  showed diurnal change and the difference between day and night values of the three treatments (control, mid- $\text{CO}_2$ , and high- $\text{CO}_2$ ) was 16–31  $\mu\text{atm}$ , 20–88  $\mu\text{atm}$  and 168–234  $\mu\text{atm}$ , respectively (Table 2). Positive net calcification was observed in all  $\text{CO}_2$  conditions throughout the experiment during both day and night, except for a slight negative net calcification (=dissolution) for the night measurements in the mid- $\text{CO}_2$  condition conducted at Day 7 (Fig. 2). There was no significant difference in calcification during both day (REML,  $F_{(2, 12)} = 0.86$   $p = 0.45$ ) and night measurements (REML,  $F_{(2, 12)} = 0.56$   $p = 0.58$ ) among treatments, throughout the 35 days

(5 weeks) experiment (Fig. 2). Aggregated data of daytime calcification rates throughout the experiment did not show a significant correlation with  $\Omega_{\text{arag}}$  between  $1.1 < \Omega_{\text{arag}} < 4.2$  (Fig. 3,  $R^2 = 0.01$ ,  $p > 0.05$ ). A positive correlation between calcification rate and irradiance was observed in all  $\text{CO}_2$  conditions (Fig. 4). The calcification rate measured by buoyant weighing varied over time; however, there was no significant difference among the  $\text{CO}_2$  treatments (REML,  $F_{(2, 12)} = 1.25$ ,  $p = 0.32$ , Fig. 5) and over time (REML,  $F_{(3, 36)} = 1.23$ ,  $p = 0.29$ , Fig. 5).

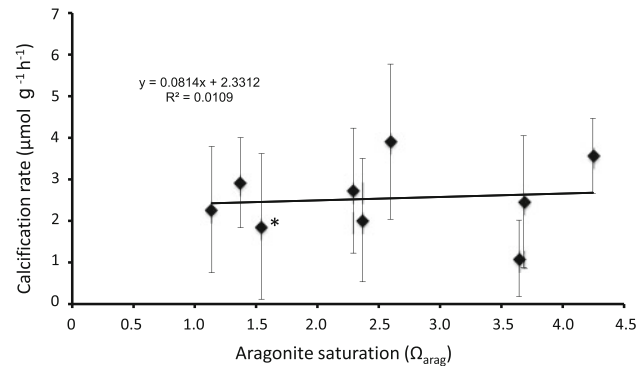
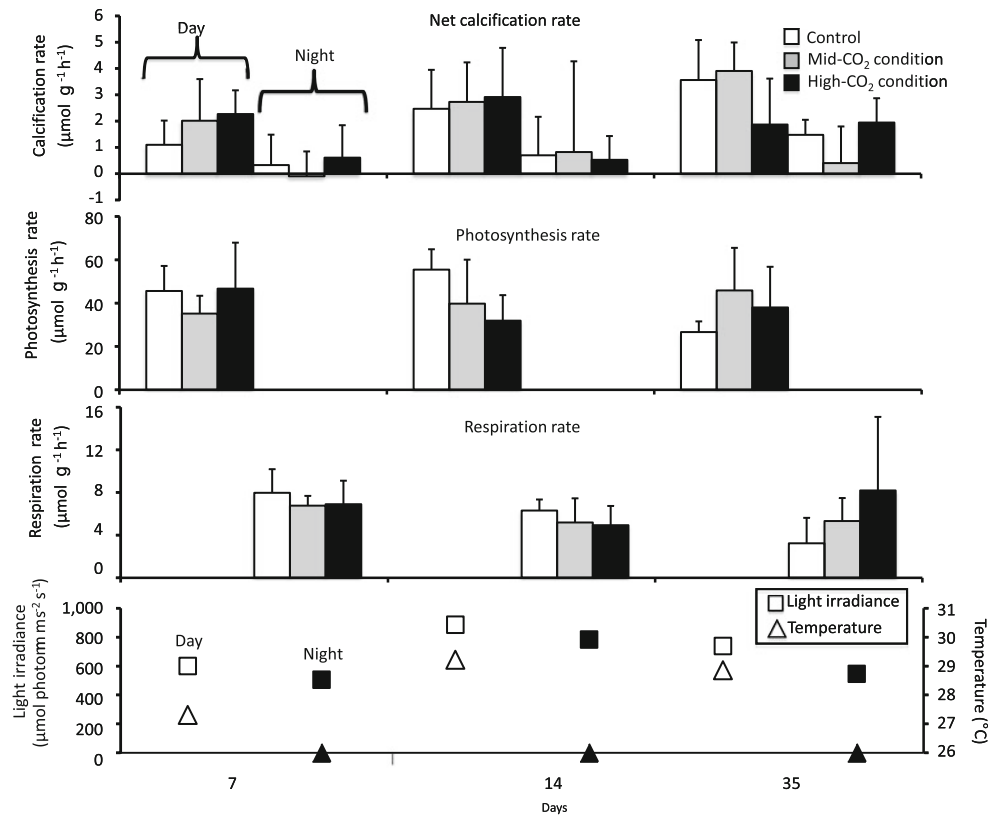
No significant response was observed either in the photosynthesis rate (REML,  $F_{(2, 12)} = 0.09$   $p = 0.91$ ) or in the respiration rate (REML,  $F_{(2, 12)} = 0.28$ ,  $p = 0.75$ ) among the  $\text{CO}_2$  treatments (Fig. 2). The photosynthetic efficiency ( $F_v F_m^{-1}$ ) of zooxanthellae was approximately 0.70 for all three conditions throughout the experiment and there was no significant difference among the  $\text{CO}_2$  treatments (REML,  $F_{(2, 12)} = 0.225$ ,  $p = 0.801$ , Fig. 6). Although not significant, a slight increase in zooxanthella density per surface area was observed in the mid- $\text{CO}_2$  treatment ( $8.19 \times 10^6 \text{ cell m}^{-2}$ ) compared with the control ( $5.75 \times 10^6 \text{ cell m}^{-2}$ ) and high- $\text{CO}_2$  ( $5.65 \times 10^6 \text{ cell m}^{-2}$ ) treatment (one-way ANOVA, Fig. 7).

### Discussion

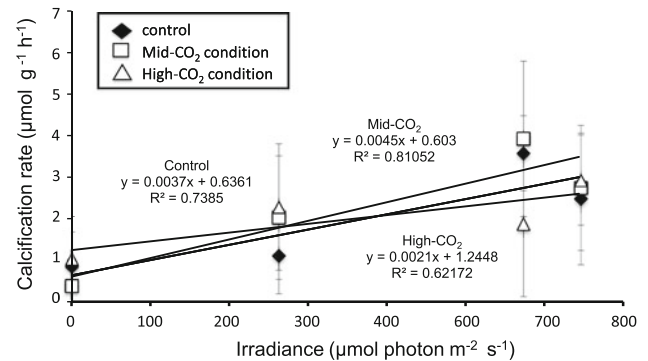
In the present study on *Acropora digitifera*, we did not detect any significant effects of elevated  $\text{CO}_2$  on either photosynthesis rate, respiration rate, photosynthetic efficiency, zooxanthella density, or calcification rate (measured by both the alkalinity anomaly and the buoyant weight technique). Our  $\text{CO}_2$  treatments ranged from mid- $\text{CO}_2$  ( $\text{pCO}_2 = 744 \pm 38$ ,  $\text{pH} = 7.97 \pm 0.02$ ,  $\Omega_{\text{arag}} = 2.6 \pm 0.1$ ) to a high- $\text{CO}_2$  condition ( $\text{pCO}_2 = 2,142 \pm 205$ ,  $\text{pH} = 7.56 \pm 0.04$ ,  $\Omega_{\text{arag}} = 1.1 \pm 0.1$ ), under summertime irradiance and temperature conditions for 35 days (5 week) experimental period.

Several studies have shown that the rate of coral photosynthesis is unaffected by elevated  $\text{CO}_2$ , probably because zooxanthellae mainly use external  $\text{HCO}_3^-$  (Goiran et al. 1996; Gattuso et al. 1999; Schneider and Erez 2006). Similarly, Rodolfo-Metalpa et al. (2010) demonstrated that elevated  $\text{CO}_2$  (700  $\mu\text{atm}$ ) does not affect several other physiological parameters, including photosynthesis, respiration, photosynthetic efficiency ( $F_v F_m^{-1}$ ), and zooxanthella density in *Cladocora caespitosa*. By contrast, Iguchi et al. (2012) reported that the photosynthetic efficiency of the massive coral *Porites australiensis*, reared under 120–140  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , was reduced at high  $\text{CO}_2$  (1,175–1,439 and 1,801–2,193  $\mu\text{atm}$ ), although zooxanthella density was not affected. Recently, Anthony et al. (2008) reported that high  $\text{CO}_2$  levels (1,000–1,300  $\mu\text{atm}$ ) induced productivity loss and bleaching of *A. intermedia*

**Fig. 2** Day and night time calcification, photosynthesis, and respiration rate of *A. digitifera* cultured for 35 days under the 3 different CO<sub>2</sub> conditions; white, gray, and black bars show control, mid-, and high-CO<sub>2</sub> conditions, respectively. Calcification rates were calculated by the alkalinity anomaly technique, and photosynthesis and respiration by dissolved inorganic carbon (DIC)–total alkalinity (TA) technique, respectively. Measurements were taken during midday and night at 7, 14, and 35 days after the start of culture. Data are mean ± SD of *n* = 5 nubbins for each CO<sub>2</sub> condition, except for the calcification and photosynthesis rate at 35 days high-CO<sub>2</sub> condition (*n* = 4). Light (open triangle) and temperature (open square) conditions during day (open symbol) and night (closed symbol) measurement were also shown



**Fig. 3** Calcification rate (measured by TA anomaly technique) of *A. digitifera* with respect to aragonite saturation (Ω<sub>arag</sub>). Data are mean ± SD of *n* = 5 nubbins, except for the data with asterisk (*n* = 4)

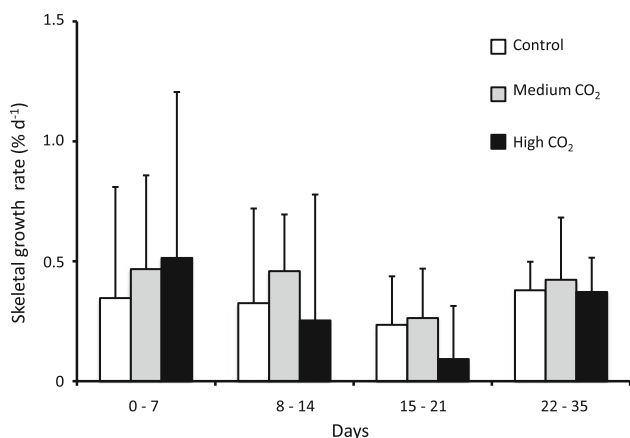


**Fig. 4** Calcification rate (measured by TA anomaly technique) and irradiance relationship of *A. digitifera* cultured under control (filled diamond), mid- (open square), and high- (open triangle) CO<sub>2</sub> conditions and the best fit straight line to each data set. Data are mean ± SD of *n* = 5 nubbins for each CO<sub>2</sub> condition

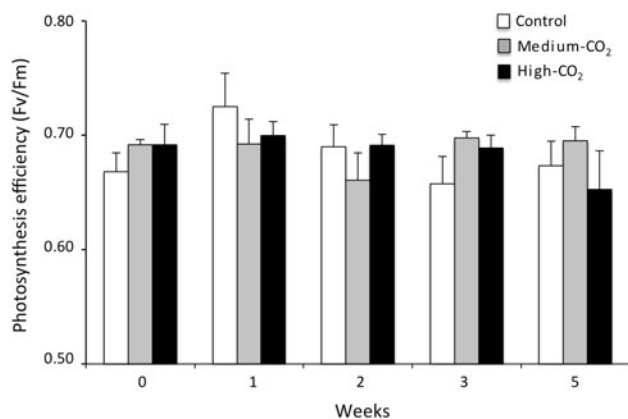
and *P. lobata* reared under natural light (700–1,200 μmol m<sup>-2</sup> s<sup>-1</sup> at noon) and summertime temperature (28–29 °C). Although other studies have not found an effect of increased CO<sub>2</sub> on coral bleaching and productivity, most of previous studies have been conducted under moderate seawater temperatures and under low to mid-light conditions (15–350 μmol m<sup>-2</sup> s<sup>-1</sup>). However, in this study, we found no evidence of CO<sub>2</sub> impact on bleaching, productivity, zooxanthella density, or photosynthetic efficiency of *A. digitifera* reared summertime seawater (28–31 °C) and irradiance conditions (152–3,789 μmol

photon m<sup>-2</sup> s<sup>-1</sup> at noon). These results suggest that the effect of high CO<sub>2</sub> on coral productivity and bleaching may differ among species, although further studies are necessary that evaluate the interaction between irradiance and temperature.

In general, studies show reduced calcification rates with lower Ω<sub>arag</sub>, although some recent studies have shown resistance, or insensitivity, to elevated CO<sub>2</sub>. Ries et al. (2009) demonstrated that the calcification rate of the temperate coral *Oculina arbuscula* showed a threshold

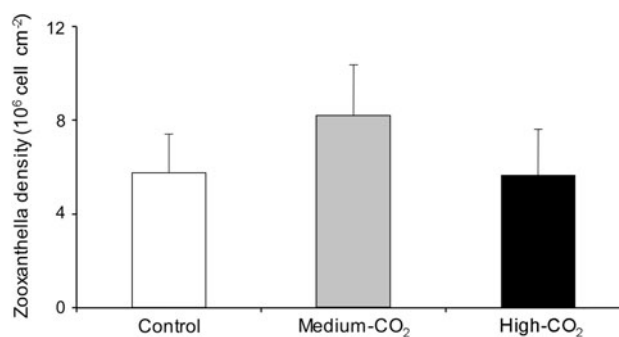


**Fig. 5** Calcification rate of *A. digitifera* measured by buoyant weight technique during the first (0–7 days), second (8–14 days), third (15–21 days), and fourth to fifth (22–35 days) weeks culture under the 3 different CO<sub>2</sub> conditions. *White, gray, and black bars* show the control, mid-, and high-CO<sub>2</sub> conditions, respectively. Data are mean  $\pm$  SD of  $n = 5$  nubbins for each CO<sub>2</sub> condition



**Fig. 6** Photosynthetic efficiency (Fv Fm<sup>-1</sup>) of *A. digitifera* cultured for 35 days under the 3 different CO<sub>2</sub> conditions; *white, gray, and black bars* show control, mid-, and high-CO<sub>2</sub> conditions, respectively. Measurements were taken day before start of the experiment (day 0) and 7, 14, 21, and 35 days after the start of experimental culture. Data are mean  $\pm$  SD of  $n = 5$  nubbins for each CO<sub>2</sub> condition

response to  $\Omega_{\text{arag}}$ , but showed resistance to calcification declines when the aragonite saturation state was between  $1.5 < \Omega_{\text{arag}} < 2.5$ . Similarly, the net calcification rate of the temperate coral *Cladocora caespitosa* exposed to 700  $\mu\text{atm}$  CO<sub>2</sub> (pH 7.87), for 1 year, under seasonally changing seawater temperature and irradiance, was not lower than the control (Rodolfo-Metalpa et al. 2010). The authors suggested that temperate or cold-water species possibly lack sensitivity to elevated CO<sub>2</sub> because of their slow growth and the effect of low  $\Omega_{\text{arag}}$  on calcification, which is more pronounced in fast-growing tropical corals. In the present study, however, we found that both light and dark calcification rate in mid- and high-CO<sub>2</sub> conditions,



**Fig. 7** Zooxanthella density of *A. digitifera* cultured for 35 days under the 3 different CO<sub>2</sub> conditions; *white, gray, and black bars* show control, mid-, and high-CO<sub>2</sub> conditions, respectively. Data are mean  $\pm$  SD of  $n = 5$  nubbins for each CO<sub>2</sub> condition

measured by alkalinity anomaly technique, did not show significant difference compared with controls. Additionally, the calcification rate measured by the buoyant weight technique was also not affected by CO<sub>2</sub> over time. These results suggest that the sensitivity of corals to ocean acidification is highly species-specific, and that some tropical corals are potentially highly resistant to high CO<sub>2</sub>. Though, in the present study, it should be noted that the standard deviation of calcification rate was very high, suggesting high biological variance, and therefore detection power was low. Meanwhile, pooled data through the experiment also did not show a significant correlation with  $\Omega_{\text{arag}}$  between  $1.1 < \Omega_{\text{arag}} < 4.2$  and calcification of the coral *A. digitifera* (Fig. 3). Although previous studies suggest a strong linear relationship between coral calcification with  $\Omega_{\text{arag}}$ , reaching zero when  $\Omega_{\text{arag}} = 1$  (see Pandolfi et al. 2011), the present results suggest that the relationship between calcification rate and  $\Omega_{\text{arag}}$  is most likely more variable than previously predicted.

Pandolfi et al. (2011) have recently reviewed the possible reasons for the high variability of physiological responses of corals to changing CO<sub>2</sub> among studies. The most obvious differences among responses were likely the response of corals to different treatments using different techniques and methodologies. Pandolfi et al. (2011) further pointed out that the sensitivity of the calcification rate measurements tended to decrease when studies are conducted over weeks or months compared with studies conducted over hours to days. Additionally, Jury et al. (2010) reported that coral calcification responded strongly to variation in [HCO<sub>3</sub><sup>-</sup>] (calcification rate exponentially increase with [HCO<sub>3</sub><sup>-</sup>]), but did not consistently respond to [CO<sub>3</sub><sup>2-</sup>],  $\Omega_{\text{arag}}$  or pH. Since ocean acidification causes a slight increase in [HCO<sub>3</sub><sup>-</sup>], this increase might partially explain the lack of correlation between  $\Omega_{\text{arag}}$  and calcification rate of *A. digitifera* observed in this study. The authors also suggest that the reason that several studies have observed a correlation between calcification and  $\Omega_{\text{arag}}$



may be partially due to the usage of HCl instead of CO<sub>2</sub> in treatment experiments.

Other environmental factors such as nutrient, temperature, and irradiance might also possibly interact with coral physiological responses to ocean acidification. Anthony et al. (2008) demonstrated that the calcification in *A. intermedia* decreased at 500–700  $\mu\text{atm CO}_2$  at low temperature (25–26 °C) but not at high temperature (28–29 °C), whereas net productivity was not affected at low temperature but increased at high temperature compared with controls (135–460  $\mu\text{atm CO}_2$ ). Nutrient enrichment, or zooplanktivory, has been demonstrated to ameliorate the effect of ocean acidification on the corals (Langdon and Atkinson 2005; Chauvin et al. 2011; Edmunds 2011). Calcification rate of *Astrangia poculata* was negatively impacted by CO<sub>2</sub> (780  $\mu\text{atm CO}_2$ ) at low nutrient concentrations, but not at high nutrient concentrations (Holocomb et al. 2010). Irradiance is also suggested to affect the effect of ocean acidification on coral physiology. The effect of increasing irradiance on calcification is well documented for scleractinian corals (reviewed by Barnes and Chalker 1990; Gattuso et al. 1999), and several models have been hypothesized to explain the mechanism for light-enhancement calcification rates in corals, including (1) increase in coelomic pH by the uptake of CO<sub>2</sub> for photosynthesis (Goreau 1959), (2) dehydration of HCO<sub>3</sub><sup>−</sup> by calcification-dependent H<sup>+</sup> secretion (McConnaughey et al. 1997), or (3) neutralization of H<sup>+</sup> by light-dependent HCO<sub>3</sub><sup>−</sup> secretion (Furla et al. 2000; Allemand et al. 2004). In our present study, a positive increase in calcification with irradiance was observed in all CO<sub>2</sub> conditions (Fig. 4). However, most physiological studies that have evaluated the effect of ocean acidification on corals have been conducted under low, artificial light conditions. In conclusion, the results of the present study suggest that the impacts of ocean acidification on coral physiology are more complex than have been previously suggested. We highlight that there is considerable variability among species, and that results will be influenced by temperature, irradiance, and the incubation system.

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