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ORIGINAL PAPER

Intraspecific variation in the response of the scleractinian coral *Acropora digitifera* to ocean acidification

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Abstract

To examine the possible variation in responses of corals to ocean acidification (OA) among populations, we compared the sensitivity of two Okinawan populations (Sesoko and Bise) of the scleractinian coral *Acropora digitifera* to high pCO_2. We found that both light and dark calcification rates of Sesoko corals did not change with an increase in seawater pCO_2, while the calcification rates of Bise corals significantly decreased. Additionally, calcification rate of Sesoko corals was significantly lower than Bise corals at control conditions. Expressions of two putative calcification-related genes (BAT: bicarbonate transporter and galaxin) were up-regulated at high CO_2 compared to the control and expression of the BAT gene was significantly higher in Sesoko compared to Bise corals. Consequently, differences in the calcification rate between populations and differences in the expression of genes related to inorganic carbon transport regulation could be reasons that explain the difference in the response to OA between the two populations. Furthermore, taking into account that Sesoko corals were located in relatively nearshore areas where the environmental conditions are more variable, while Bise corals were located in the forereef which shows more stable conditions, plasticity for coral calcification in response to different environmental conditions and/or acclimation response to changes such as seawater pCO_2 may lead to differences in sensitivity between the two populations to high seawater pCO_2. Studies considering the potential variability in corals sensitivity to OA among local populations from different habitats are important to predict the potential effects of climate change on reef ecosystems.

Introduction

Ocean acidification (OA) caused by the increasing atmospheric CO_2 is predicted to pose a significant threat to scleractinian corals and reef ecosystems (Kleypas et al. 2006; Hoegh-Guldberg et al. 2007). When seawater pH decreases, the concentration of carbonate ions also declines, resulting in a reduction in seawater aragonite saturation (Ω_{arag}). From field observations, it has been reported that net reef calcification shows a strong correlation with Ω_{arag}, and a number of experimental studies have demonstrated that net calcification rates of most corals decline with Ω_{arag} (Langdon and Atkinson 2005; Chan and Connolly 2013). Presently, the negative effect on calcification is one of the most robust predictions regarding the potential impact of OA on marine organisms. However, recent studies have revealed that some coral species can be relatively more tolerant than other species to OA (Jokiel et al. 2008; Jury et al. 2010; De Putron et al. 2011), suggesting interspecific variations in the response of corals to OA (Pandolfi et al. 2011; Comeau et al. 2014a).

Identification of the interspecific variations in the response to OA is essential information for evaluating how OA will affect organisms at community levels. If the tolerance capacity differs among species, changes in community structure can be predicted, tending to become dominated by species that show higher tolerance to OA. A tank experiment study evaluating the sensitivity of eight different functional coral species to OA demonstrated that the responses significantly differed among species (Comeau

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et al. 2014a). Additionally, studies of CO_2-vent associated assemblages in reef ecosystems have demonstrated that these coral communities can dramatically change to potentially more OA-tolerant coral species such as massive *Porites* or soft-corals, resulting in the loss of biodiversity (Fabricius et al. 2013; Inoue et al. 2013).

Meanwhile, identification of intraspecific variations in response to OA could give useful information for evaluating the adaptation or acclimation potential of individual species to OA (Sunday et al. 2011). Although studies evaluating intraspecific variations are still limited, some studies have demonstrated that sensitivity to OA could differ among populations within the same species. For example, Parker et al. (2011) demonstrated that selectively bred populations of the oyster *Crassostrea gigas* are more resilient to elevated pCO_2 than wild populations. Additionally, a blue mussel *Mytilus edulis* population living in a Fjord where seawater pCO_2 highly fluctuates in response to upwelling can maintain similar shell growth rate to controls when reared at high-CO_2 (1400 µatm) seawater (Thomsen et al. 2010). Differences in the response to OA among different local populations were also documented in the mussel *Mytilus galloprovincialis* on the Mediterranean Sea coast (Range et al. 2014). These results suggest that the OA sensitivity of the organisms can differ among local populations experiencing different environmental conditions. For corals, one study has evaluated the possible differences in the resistance of the corals *Pocillopora damicornis* and massive *Porites* resistance to OA between populations from different regions (Okinawa, Moorea and Hawaii); however, both coral species from all locations were insensitive to OA (Comeau et al. 2014b). Another study using a common garden experiment in the coral *Acropora pulchra* in Moorea, French Polynesia, revealed intraspecific variation on calcification rate to elevated temperature but not to OA (Shaw et al. 2016). Meanwhile, studies that have investigated the effect of OA among colonies of the coral *Acropora digitifera*, *Montipora digitata* and *Porites cylindrica* indicated that the response of corals to OA could vary among individuals within the same population (Kavousi et al. 2015, 2016). Additionally, in our previous study, we identified that the coral *Acropora digitifera* in Okinawa did not show a significant correlation between calcification rate and seawater Ω_{arag} (Takahashi and Kurihara 2013), suggesting that this species is potentially resilient to OA, which differed from other studies suggesting high vulnerability of fast-growing corals such as *Acropora* spp. (e.g. Comeau et al. 2014a, Shaw et al. 2016). However, when the same experiment was repeated using *A. digitifera* collected from another location, we found a strong correlation between calcification rate and seawater Ω_{arag}. Consequently, we hypothesized that the OA sensitivity of the coral *A. digitifera* may show variations among different sites.

Here, to examine the possible variation in responses of corals to OA among populations, we collected the coral *A. digitifera* from two locations around Okinawa, one collection site being the same location where *A. digitifera* were collected for the previous study (Takahashi and Kurihara 2013), and evaluated the effect of high pCO_2 on coral net calcification. Effect of high pCO_2 on the rate of light and dark calcification rate, photosynthesis rate, respiration rate, photosynthesis efficiency, zooxanthellae density, and protein content was compared between the corals from the two sites. In addition, we examined the gene expression of four genes with putative roles in the calcification process of corals to evaluate the potential mechanism for OA sensitivity variation between sites.

Materials and methods

Coral collection

Five colonies of the coral *Acropora digitifera* were collected in June 2011 from each of two locations around Okinawa, Japan: inshore patch reefs (ca. 0.5–1.0 m depth) at Sesoko (26°38′11.64″N, 127°51′57.08″E) and at edge of reef crest towards to the forereef zone (ca. 0.5–1.0 m depth) at Bise (26°42′40.0″N, 127°52′53.3″E). The distance between the two Bise and Sesoko sites was less than 8 km. Light sensor (DEFI2-L, JFE-Advantech, Japan) and multi-parameter water quality sonde (6820 V2, YSI, USA) were placed at the two sites for 3 days to measure light intensity, seawater temperature, dissolved oxygen, salinity, and pH (calibrated with NBS scale buffer). Several seawater samples were also collected for total alkalinity (TA) and dissolved inorganic carbon (DIC) measurement during the 3 days. Three pieces with three branches from each of the five colonies were collected 4 weeks prior to the experiment. After sampling, all coral pieces were immediately transferred to the Sesoko Marine Laboratory Station of University of the Ryukyus and placed for 2 weeks in an outdoor tank (1.2 m × 0.7 m × 0.2 m, volume = 187 L) under natural light condition and supplied continuously with seawater (1.5 L min^{-1}) pumped from 4–5 m depth in front of the station. Thereafter, all coral pieces were placed individually in incubation chambers (volume = 450 mL), which were kept in an indoor water bath supplied continuously with seawater pumped from the front of the station to control temperature. The corals were set under artificial light provided by a 400 W metal halide lamp (light intensity: 200 µmol photons m^{-2} s^{-1} $L{:}D$ = 14 h:10 h). The light intensity was slightly lower but within the range of the mean June daytime light intensity measured at Sesoko (mean light intensity: 280 µmol photons m^{-2} s^{-1}, range

70–430 µmol photons m^{-2} s^{-1}) and cultured for another 2 weeks for acclimation.

Experimental setup

One piece with three branches from each of the five colonies and two locations (Sesoko and Bise) was incubated individually under control (420 ± 40 µatm; mean ± SD), mid-CO_2 (1030 ± 48 µatm) and high-CO_2 (2381 ± 156 µatm) conditions for 5 weeks. Seawater was bubbled in a bubbling tank (18 L) using air, or air mixed with CO_2 controlled by mass flow controllers (SEC-E40, Horiba Stec, Japan), and supplied to each incubator chamber at a flow rate of ca. 150 mL min^{-1}. Seawater pH and temperature of the bubbling tank were measured daily using a temperature-compensated pH electrode (MP125, Mettler Toledo, USA) calibrated using NBS scale buffer solutions. Temperature and pH of each bubbling tanks were verified as being same to the downstream incubation chambers. Salinity (34 ± 0) and total alkalinity (TA) were measured every week using a refractometer (100-S, Atago, Japan) and autoburette titrator (ATT-05, Kimoto, Japan), respectively. Seawater pCO_2, HCO_3^-, CO_3^{2-} and Ω_{arag} were calculated based on pH, temperature, TA and salinity using the CO2SYS program of Lewis and Wallace (1998), with dissociation constants K_1 and K_2 from Mehrbach et al. (1973) and an aragonite solubility of Mucci (1983) (Table 1).

Coral physiology

The net calcification rates (G) of all coral pieces were measured by the buoyant weight technique (Davis 1989) using an electronic balance (0.1 mg precision HR-200, A&D, Japan) before and after the 5 weeks of experimental culture. Skeletal dry weight was calculated based on aragonite density (2.94 g cm^{-3}) and the increase in the skeletal dry weight was normalized to the surface area measured by covering the surface of the coral pieces with aluminum foil and the area of the foil was analyzed using image J (Image J, U.S.).

Light calcification (light G), dark calcification (dark G), net photosynthesis (nP) and dark respiration (R) rates of all coral pieces were measured by the dissolved inorganic carbon (DIC)–total alkalinity (TA) technique (Smith 1973; Smith and Key 1975) at the end of the 5-week incubations. Light G and nP were measured during the daytime under light conditions (light intensity: 200 µmol photons m^{-2} $s^{-1)}$ and dark G and R during the nighttime under dark conditions. Initial seawater samples (ca. 100 mL) were collected from each airtight glass chamber (450 mL), and immediately after the chambers were re-filled with new flowing seawater, all chambers were sealed without a head space. All experimental chambers 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 by adding 100 µl saturated mercuric chloride immediately after sampling. DIC and TA were measured using closed cell titration (ATT-05, Kimoto, Japan) using 0.1 N HCl. Accuracy and precision of the determinations were evaluated by analyzing reference material Batch AG (TA = 2295 ± 0.55 µmol kg^{-1}, DIC = 2032.8 ± 0.72 µmol kg^{-1}, from Kanso Technos, Japan) which was verified against certified reference material (CRM, Batch 95, from A. Dickson Laboratory). Light and dark G were calculated using the following equation:

$$\text{Light, dark } G \text{ (µmol } CaCO_3 \text{ cm}^{-2} \text{ h}^{-1}) = \Delta TA \times V \times D_{sw}(2 \times SA \times t)^{-1},$$

where ΔTA is the change in TA measured at the beginning and end of the day and night time incubations, V is the seawater volume of each chamber minus the displacement volume of the coral piece, D_{sw} is seawater density, SA is the surface area of each coral piece, and t is the duration of culture. nP and R were calculated by the organic production rate during day and night time incubations and calculated by the following equation:

$$\text{nP, } R \text{ (µmol C cm}^{-2}\text{h}^{-1}) = (\Delta DIC - 0.5\Delta TA) \times V \times D_{sw}(SA \times t)^{-1},$$

where ΔDIC and ΔTA for nP and R are the DIC and TA change during the light and dark incubations, respectively. Gross photosynthesis (gP) was calculated by summing nP and R.

Photosynthetic efficiency, zooxanthellae density, and lipid content

Photochemical efficiency of photosynthesis II (F_v F_m^{-1}) of zooxanthellae was measured using a pulse amplitude

Table 1 Seawater carbon chemistry during the 5 weeks of coral culture

	pCO_2 (µatm)	pH (NBS scale)	TA (µmol/kg)	DIC (µmol/kg)	Ω_{arag}	Temperature
Control	420 ± 40	8.16 ± 0.03	2236 ± 22	1931 ± 22	3.51 ± 0.25	28.9 ± 0.9
Mid-CO_2	1027 ± 48	7.83 ± 0.02	2233 ± 33	2091 ± 8	1.87 ± 0.08	28.9 ± 0.9
High-CO_2	2374 ± 155	7.50 ± 0.03	2227 ± 27	2205 ± 8	0.93 ± 0.05	28.9 ± 0.9

pCO_2, dissolved inorganic carbon (DIC) and Ω_{arag} were calculated with CO2SYS program by daily measured pH, temperature and weekly measured total alkalinity (TA) and salinity. Mean ± SD

fluorescence system (MINI-PAM, Walz Effeltrich, Germany) according to Schreiber et al. (1986). Maximum fluorescence of each coral piece was measured by a 0.8 s saturation light pulse (8000 µmol photon m^{-2} s^{-1}). Measurements were conducted at night under dark conditions after the 5 weeks of incubation.

After all physiological measurements, one branch from each coral piece was cut and the coral tissue was removed with a waterpik and homogenized with a hand-held homogenizer in filtered seawater. The sample was centrifuged (4 min at 2400 rpm), and after removing the supernatant, the zooxanthellae pellet was re-suspended with filtered seawater and the number of zooxanthellae was determined by counting four replicates using a hematocytometer and standardized against surface area.

For analysis of the total lipid amount, extraction was performed according to the method by Yamashiro et al. (1999). A second branch was cut from each coral piece, freeze-dried and then transferred into a chloroform–methanol solution (CM, 2:1 volume) and kept at 40 °C for 30 min to extract lipids. Extraction was conducted three times for each branch, the CM extracts were centrifuged, and the supernatant was dried up and weighed using an electronic balance. After the lipid extraction, the coral piece was decalcified in 10% formaldehyde containing 20% acetic acid and then dried. The dry tissue was weighed and lipid content (%) was calculated as lipid weight divided by tissue dry weight.

Gene expression

The third branch from each coral piece was frozen with liquid nitrogen and kept frozen in the deep freezer at − 80 °C until molecular analysis. Total RNA from frozen coral tissues was isolated using Qiazol lysis reagent (Qiagen) following product specifications. Coral branches were homogenized, and frozen coral powder was transferred to Qiazol lysis reagent (Qiagen) for RNA extraction. RNA pellets were re-dissolved in nuclease-free water and cleaned further with RNeasy Mini columns (Qiagen). RNA quantity and integrity were assessed with a NanoDrop ND-1000 spectrophotometer and an Agilent 2100 Bioanalyzer, respectively. cDNA was synthesized from 300 ng of DNase-treated RNA samples using the SuperScript III system for RT-PCR (Invitrogen). For the gene expression analysis, only samples of control and high-CO_2 condition were used.

Here, we selected four *Acropora digitifera* genes with putative roles in calcification as molecular markers: a plasma membrane calcium-ATPase (PMCA)(aug_v2a.22365.t1), a bicarbonate transporter (BAT)(aug_v2a.09009.t1), a carbonic anhydrase II (CAII)(aug_v2a.24568.t1) and the structural organic matrix protein galaxin (aug_v2a.18631.t1) (Grasso et al. 2008; Reyes-Bermudez et al. 2009; Hayward et al. 2011; Zoccola et al. 2004). The specific forward and reverse primers for the genes were designed (100 bp amplicon length, 55% *G–C* content and T_m of 65 °C) using primer-express®v3 software (Applied Biosciences). The GAPDH primers used in this experiment were previously used and designed for *A. millepora* by Souter et al. (2010) and were able to amplify the corresponding *A. digitifera* GAPDH ortholog. Amplicons were checked by conventional gel electrophoresis and primer efficiencies were determined using standard curve analysis with a twofold dilution series from pooled control and treated cDNA samples. Primer efficiencies ranged between 1.9 and 2.2. Primer sequences can be found in Table S1 (Supplementary information). Quantitative real-time PCR was performed on an ABI-7500 platform (Applied Biosciences) using SYBR green chemistry. Briefly, samples were run in triplicate in 20 µl reactions consisting of 10 µl SYBR green mix, 15 ng of template and 500 nM of each primer. Thermo-cycling conditions included a 3 min initial activation stage at 95 °C followed by 40 cycles of 15 s at 95 °C, 1 min at 60 °C and a final disassociation step (60–95 °C) to confirm the absence of either non-specific products or primer dimers. Threshold cycle differences (ΔC_t) were obtained by manually locating the threshold immediately above the background in the exponential amplification phase. C_t values were analyzed by relative quantification using the Pfaffl (2001) method.

Statistical analysis

Effect of CO_2 on net calcification, light and dark calcification, photosynthesis, respiration, $F_v F_m^{-1}$, zooxanthella density, lipid content and expression of the four studied genes between sites were evaluated using mixed-effect-model ANOVA with CO_2 and sites as fixed effects and colony as a random effect based on the restricted maximum likelihood (REML) method that automatically corrected variances across interaction effects. Model assumptions of normality and homoscedasticity were checked by examining model residuals. Differences between CO_2 and sites were assessed using Tukey's honestly significant difference (HSD) multiple comparison tests. All analyses were performed using JMP (JMP 7; SAS Inc.)

Results

Coral physiology

The net calcification rate (*G*) of *Acropora digitifera* over the 5-week incubation was significantly different among pCO_2 conditions but not between Sesoko and Bise corals (REML, CO_2: $F_{(2,16)} = 15.9$, $p = 0.0002$, locations: $F_{(1,8)} = 2.03$, $p = 0.19$) without interaction ($CO_2 \times$ location: $F_{(2,16)} = 1.93$, $p = 0.17$, Fig. 1). The calcification rate of control Bise corals

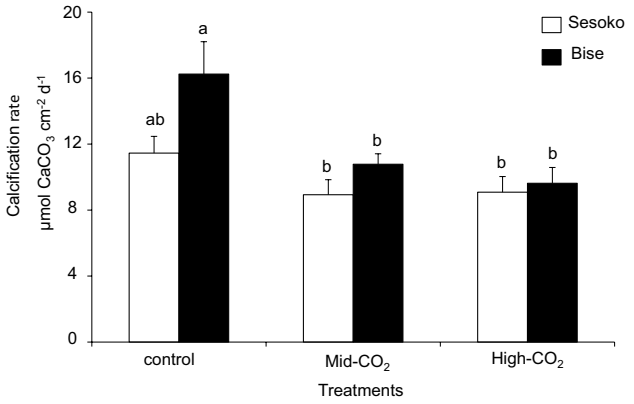

Fig. 1 Percentage change in skeletal dry weight of the corals from Sesoko and Bise during 5 weeks of culture under three different CO_2 conditions. Mean ± SE of five colonies. Significant differences among CO_2 conditions and corals from the two sites are indicated by lower case letters (Tukey HSD test $p < 0.05$)

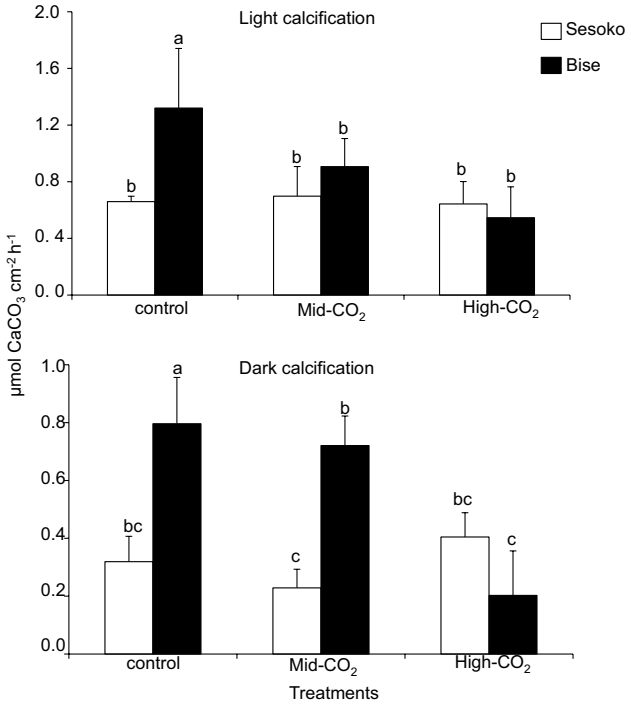

Fig. 2 Light and dark calcification of the corals from Sesoko and Bise after 5 weeks of culture under three different CO_2 conditions. Mean ± SE of five colonies. Significant differences among CO_2 conditions and corals from the two sites, are indicated by lower case letters (Tukey HSD test $p < 0.05$)

(16.2 ± 1.9 μmol $CaCO_3$ cm^{-2} day^{-1}) was significantly higher compared to mid- (10.7 ± 0.6 μmol $CaCO_3$ cm^{-2} day^{-1}), and high-CO_2 corals (9.6 ± 0.9 μmol $CaCO_3$ cm^{-2} day^{-1}, $p < 0.05$, Tukey HSD), while the calcification rate of Sesoko corals did not differ among all three CO_2 conditions (control = 11.4 ± 1.0 μmol $CaCO_3$ cm^{-2} d^{-1}, mid-CO_2 = 8.9 ± 0.9 μmol $CaCO_3$ cm^{-2} day^{-1} and high CO_2 = 9.0 ± 0.9 μmol $CaCO_3$ cm^{-2} day^{-1}, Tukey HSD, Fig. 1).

The light calcification rate (light *G*) was significantly different among pCO_2 conditions, but not between locations (REML, CO_2: $F_{(2,16)} = 19.7$, $p < 0.0001$, locations: $F_{(1,8)} = 0.66$, $p = 0.44$) and there was a significant interaction (CO_2 × location: $F_{(2,16)} = 75.25$, $p = 0.01$). Light *G* of control Bise corals (1.32 ± 0.42 μmol $CaCO_3$ cm^{-2} h^{-1}) was significantly higher than Sesoko corals (0.65 ± 0.03 μmol $CaCO_3$ cm^{-2} h^{-1}, $p < 0.05$, Tukey HSD, Fig. 2), but there was no significant difference at high-CO_2 between Bise (0.54 ± 0.21 μmol $CaCO_3$ cm^{-2} h^{-1}) and Sesoko corals (0.64 ± 0.15 μmol $CaCO_3$ cm^{-2} h^{-1}). Additionally, light *G* of control Bise corals was significantly higher than mid-CO_2 (0.90 ± 0.19 μmol $CaCO_3$ cm^{-2} h^{-1}) and high-CO_2 (0.54 ± 0.21 μmol $CaCO_3$ cm^{-2} h^{-1}, $p < 0.05$, Tukey HSD, Fig. 2), while there was no significant difference of light *G* of Sesoko corals among all CO_2 conditions.

Dark calcification rate (dark *G*) was significantly different among CO_2 conditions and between Sesoko and Bise corals (REML, CO_2: $F_{(2,16)} = 40.3$, $p < 0.0001$, locations: $F_{(1,8)} = 11.8$, $p = 0.0088$) with interaction (CO_2 × location: $F_{(2,16)} = 37.8$, $p < 0.0001$). Dark *G* of control (0.79 ± 0.15 μmol $CaCO_3$ cm^{-2} h^{-1}) and mid-CO_2 (0.72 ± 0.10 μmol $CaCO_3$ cm^{-2} h^{-1}) Bise corals were significantly higher than Sesoko control (0.31 ± 0.08 μmol $CaCO_3$ cm^{-2} h^{-1}) and mid-CO_2 (0.22 ± 0.06 μmol $CaCO_3$ cm^{-2} h^{-1}) corals ($p < 0.05$, Tukey HSD), respectively, while there was no significant difference between high-CO_2 Bise (0.20 ± 0.15 μmol $CaCO_3$ cm^{-2} h^{-1}) and Sesoko (0.40 ± 0.08 μmol $CaCO_3$ cm^{-2} h^{-1}) corals (Fig. 2). Additionally, dark *G* of control Bise corals was significantly higher than both mid-CO_2 and high-CO_2 corals, while there was no significant effect of CO_2 on dark *G* of Sesoko corals.

The gross photosynthesis rate (gP) was significantly different among CO_2 conditions but not between Sesoko and Bise corals (REML, CO_2: $F_{(2,16)} = 34.3$, $p < 0.0001$, locations: $F_{(1,16)} = 0.08$, $p = 0.78$) without interaction (CO_2 × location: $F_{(2,16)} = 0.16$, $p = 0.85$). gP of control corals (Bise: 3.24 ± 0.46 μmol C cm^{-2} h^{-1}, Sesoko: 3.19 ± 0.26 μmol C cm^{-2} h^{-1}) was significantly higher compared to mid- and high-CO_2 corals in both Bise and Sesoko corals ($p < 0.05$, Tukey HSD, Fig. 3).

The respiration rate (*R*) was significantly different among CO_2 conditions (REML, CO_2: $F_{(2,16)} = 21.0$, $p < 0.0001$) but not between Sesoko and Bise corals (locations: $F_{(1,8)} = 5.04$, $p = 0.05$) with interaction (CO_2 × location: $F_{(2,16)} = 8.63$, $p = 0.0029$, Fig. 3). Average F_v F_m^{-1} of Sesoko and Bise corals for all CO_2 conditions ranged from 0.67 to 0.71 and there was no significant difference among CO_2 conditions and between Sesoko and Bise corals. Average zooxanthella density of Sesoko and Bise corals for all

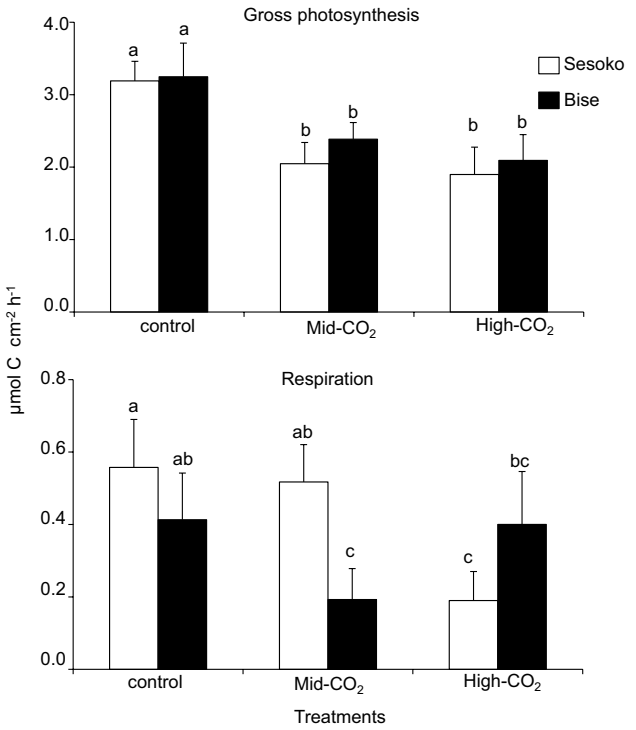

Fig. 3 Gross photosynthesis and respiration of the corals from Sesoko and Bise sites after 5 weeks of culture under three different CO_2 conditions. Mean ± SE of five colonies. Significant differences among CO_2 conditions and corals from the two sites are indicated by lower case letters (Tukey HSD test $p < 0.05$)

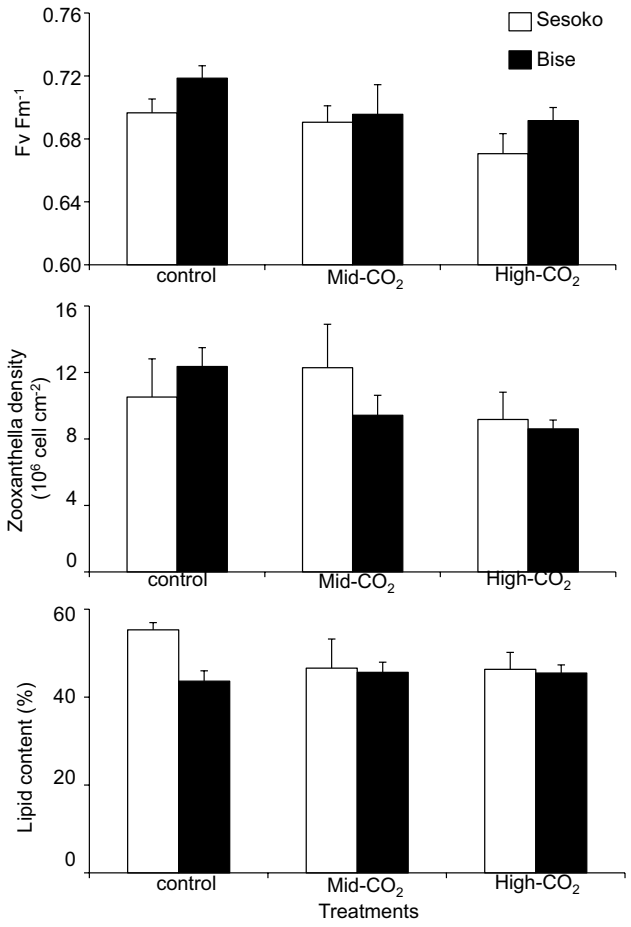

Fig. 4 Photosynthesis efficiency, zooxanthella density and lipid content of corals from Sesoko and Bise after 5 weeks of culture under three different CO_2 conditions. Mean ± SE of five colonies

CO_2 conditions ranged from 8.6 to 12.3 × 10^6 cell cm^{-2}. No significant differences were found in $F_v F_m^{-1}$, zooxanthella density or lipid content among pCO_2 conditions and between Sesoko and Bise corals (Fig. 4).

Gene expression

Bicarbonate transporter (BAT) gene expression was significantly up-regulated at high-CO_2 compared to control conditions (REML, CO_2: $F_{(1,12)} = 6.02$, $p = 0.03$) and was significantly different between Sesoko and Bise corals (locations: $F_{(1,12)} = 7.52$, $p = 0.01$) without interaction (CO_2 × location: $F_{(1,12)} = 0.15$, $p = 0.70$, Fig. 5). Expression of galaxin was also up-regulated at high-CO_2 compared to control conditions (REML, CO_2: $F_{(1,12)} = 26.5$, $p = 0.0002$), and there was a significant difference between Sesoko and Bise corals (REML, CO_2: $F_{(1,12)} = 5.85$, $p = 0.03$) and with interaction among CO_2 and sites (CO_2 × location: $F_{(1,12)} = 7.09$, $p = 0.02$, Fig. 5). Plasma membrane calcium-ATPase (PMCA) and carbonic anhydrase II (CAII) expression did not change significantly between CO_2 conditions and Sesoko and Bise corals (Fig. 5).

Discussion

Physiology

In the present study, the first finding of note is that the coral *Acropora digitifera* collected from two different sites shows significantly different responses to seawater pCO_2 change. Similar to our previous study (Takahashi and Kurihara 2013), all net calcification (G), light calcification (light G) and dark calcification (dark G) rates of the *A. digitifera* colonies collected from Sesoko site did not differ when incubated at control (pCO_2 = 420 µatm) or high-CO_2 conditions (pCO_2 = 2374 µatm), in which seawater Ω_{arag} was under-saturated ($\Omega_{arag} = 0.93$). Meanwhile, *A. digitifera* collected from the Bise site showed high sensitivity to elevated pCO_2 and both light and dark G significantly declined with the increasing seawater pCO_2. Light and dark G of Bise corals was estimated to decrease at a rate of 2.24 and 1.87 µmol $CaCO_3$ per Ω_{arag}, respectively. These results suggest that the coral *Acropora*

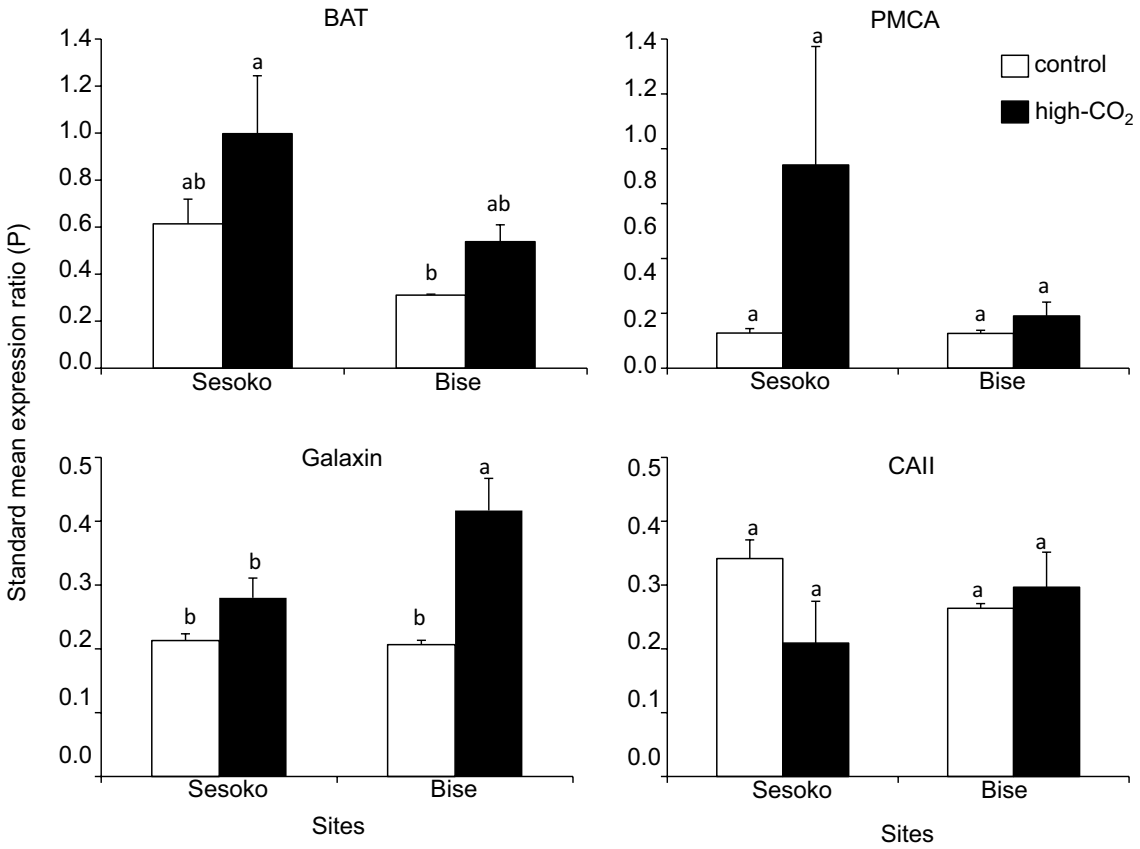

Fig. 5 Gene expression of the four putative calcification-related genes (*BAT* bicarbonate transporter, *PMCA* plasma membrane calcium ATPase, *galaxin and CAII* carbonic anhydrase II) in the control corals from Sesoko and Bise after 5 weeks culture under three different CO_2 conditions. Mean ± SE of five colonies

digitifera shows intraspecific variability of calcification to high pCO_2 among populations.

Coral calcification is known to be a process with a high energy demand to control the pH and Ω_{arag} in the calicoblastic fluid where calcification occurs, and the required energy is suggested to be mainly supplied by photosynthesis (Al-Horani et al. 2003; Holcomb et al. 2014). Hence, if high pCO_2 increases the photosynthesis rate of the coral, the negative effect of high pCO_2 on calcification may be compensated by the additional energy. Indeed, the corals *Acropora millepora* collected from a CO_2 vent site was found to show increased net photosynthesis rate at high-pCO_2 condition and the light *G* was not affected by the high CO_2, while dark *G* decreased in high pCO_2 (Strahl et al. 2015). However, in the present study, although light *G* of Sesoko corals was not affected by high pCO_2, the gross photosynthesis rate (gP) of Sesoko corals was found to decrease with high-CO_2 conditions. Additionally, dark *G* was also not affected by high CO_2, suggesting that the insensitivity of net *G* in Sesoko corals is not related to the effect of CO_2 on their photosynthesis rate. Meanwhile, Bise corals showed a strong correlation among light *G* and gP. Decrease in gP and suppression of metabolism gene at high pCO_2 was found for *A. millepora* (Kaniewska et al. 2012), while photosynthesis rates were reported not to be effected by CO_2 on other corals such as *Galaxea fascicularis*, *Acropora eurystoma* and *Cladocora caespitosa* (Goiran et al. 1996; Schneider and Erez 2006; Rodolfo-Metalpa et al. 2010). These results suggest that high-seawater pCO_2 condition can affect both the calcification and photosynthesis rates, although this effect could differ among coral species and populations. Additionally, another study reported a positive correlation between calcification and photochemical efficiency of photosynthesis II ($F_v F_m^{-1}$ for the massive coral *Porites australiensis*, and authors suggested that differences in coral calcification sensitivity to high pCO_2 among individuals could be related with the difference in the $F_v F_m^{-1}$ among colonies (Iguchi et al. 2012). However, in the present study, we did not find differences in $F_v F_m^{-1}$ between the corals from the two sites and also there was no interaction between $F_v F_m^{-1}$ and calcification rate. Since lipid content was suggested to be a proxy of energy storage and physical conditions (Stimson 1987; Anthony 2006), here we also measured the lipid content of the corals to evaluate the possibility that energy storage and

physical condition can be attributed to the difference in the sensitivity of corals to high CO_2. However, lipid content did not differ between Sesoko and Bise corals.

One notable result, however, was that Sesoko corals demonstrated significantly lower calcification rates than Bise corals at control pCO_2 conditions. While the calcification rate of Bise corals was negatively affected by an increase in seawater pCO_2, Sesoko corals showed constant low calcification rate at all pCO_2 concentrations in both light and dark calcification rates. Movilla et al. (2012) studied the effect of high pCO_2 on two temperate corals, *Cladocora caespitosa* and *Oculina patagonica*, and found that although both species show negative responses in calcification rate, faster growing colonies of both species were more affected by high pCO_2. Similar findings were reported by Shaw et al. (2016) using coral *Acropora pulchra* and Kavousi et al. (2016) using *Montipora digitata*. Additionally, Comeau et al. (2014a) compared the sensitivity of eight coral species to OA and reported that tolerance of the corals to OA was correlated with their growth rate. Fast-growing corals are suggested to need higher amounts of energy to extract protons from the calicoblastic fluid compared to slow-growing corals (Tambutté et al. 2011; Comeau et al. 2014a). Consequently, it can be speculated that slow-growing Sesoko corals are more efficient to maintain the pH in the calicoblastic fluid at high-CO_2 condition than Bise corals, and hence they are more resistant to high CO_2. Indeed when the calcifying fluid pH of Sesoko *A. digitifera* corals was measured using boron isotopes ($^{11}\delta B$), it was found that the pH was maintained at 8.3, and Ω_{arag} was higher than 5, even when the corals were reared at seawater, pH 7.4 (Tanaka et al. 2015).

Reasons that could explain the differences in calcification rate of the corals between the two sites are unclear. One possible reason could be due to the difference in the local scale habitat that the corals were collected; colonies collected from the Bise site were at edge of reef crest towards to the forereef, while corals collected from Sesoko site were located in a relatively more inner reef area and close to nearshore. Although there were no significant differences in the mean values of pH, temperature, salinity, oxygen concentration and light intensity measured over a 3-day period in those two sites, Sesoko site showed slightly higher seawater pCO_2, lower pH and lower Ω_{arag} and higher variability in environmental conditions compared to the Bise site (Table 2). The study that has evaluated the skeletal extension change of the coral *Siderastrea sidera* colonies from forereef, backreef and nearshore in Belize since the early 1900s found that colonies from forereef and backreef sites show significantly faster growth rate than nearshore; however, the annual skeletal extension has significantly decreased in the forereef corals over the last century, while no change was found in the backreef and nearshore colonies (Castillo et al. 2011). Castillo et al. (2011) suggested that these differences could be attributed to historical differences of the environmental stress; less anthropogenic impact at forereef allowed higher calcification rates compared to nearshore colonies in the past, while forereef coral are more susceptible to environmental change than nearshore colonies which are more acclimatized and/or adapted to environmental change (e.g. more variable environmental conditions, higher sedimentation, etc.). Phenotypic plasticity for calcification among habitat was also reported in the coral *Porites lobata*, and reciprocal transplant experiment demonstrated that the calcification of colonies transplanted to the backreef always shows higher calcification than forereef colonies (Smith et al. 2007). Therefore, one possibility could be that the corals from the two sites show different sensitivities to OA due to the difference in calcification rate caused by the difference of local environment found in forereef (Bise site) and nearshore (Sesoko site). Alternatively, Sesoko coral could be acclimatized to more variable pCO_2 and pH environmental conditions found in nearshore, and hence show higher tolerance to high-CO_2 conditions.

In the present study, calcification rate of the corals from the two sites was significantly different even after they were acclimated for 1 month under the same condition. However, because the distance between the Bise and Sesoko sites was less than 8 km, and previous work, evaluating the genetic connectivity of these broadcast spawning *A. digitifera* populations around Okinawa, reported that this species shows high connectivity among populations within the 1000 km long Ryukyu Archipelago, including Okinawa (Nakajima et al. 2010), genetic variations seem unlikely to be the mechanisms that could explain the variation in tolerance

Table 2 Seawater field environment in Sesoko and Bise sites

	pH (NBS scale)	Temperature (°C)	Salinity	DO (mg L^{-1})	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	pCO_2 (μatm)	Ω_{arag}	Light (μmol photon m^{-2} s^{-1})
Sesoko	8.15 ± 0.10	27.3 ± 0.69	34.2 ± 0.09	6.37 ± 1.66	2265 ± 20	1975 ± 61	456 ± 121	3.35 ± 0.64	864 ± 570
Bise	8.17 ± 0.06	26.9 ± 0.43	34.6 ± 0.02	6.61 ± 0.66	2227 ± 5	1926 ± 39	403 ± 64	3.41 ± 0.41	771 ± 488

Seawater pH, temperature, salinity, dissolved oxygen (DO) and light intensity was measured continuously for 3 days. Total alkalinity (TA) and dissolved inorganic carbon (DIC) were measured by sampling seawater, and pCO_2 and Ω_{arag} were calculated with CO2SYS program. Means ± SD

capacity between the Sesoko and Bise corals. Some studies have found that genetic differentiation among populations even within small-scale habitats (Magalon et al. 2005); however, data that have shown local adaptation for broadcast spawning organisms are highly restricted (Sanford and Kelly 2011). Interestingly, a study that evaluated the effect of OA on different colonies of *Acropora digitifera* sampled in the inner reef area of Bise has reported that there was no significant effect of high CO_2 (1000 atm) at all three studied colonies (Kavousi et al. 2015).

Gene expression

Expression of the bicarbonate transporter (BAT) gene was found to be significantly higher in the Sesoko population compared to the Bise population and being up-regulated under high-CO_2 conditions compared to the control in both Sesoko and Bise corals. Although the main source of dissolved inorganic carbon (DIC) used for coral calcification and photosynthesis is still under debate, using isotopes and BAT inhibitors Furla et al. (2000) showed that bicarbonate plays an important role in both mechanisms. Later, Bertucci et al. (2013) reviewed the movement of DIC within coral tissues and suggested the existence of BAT that transports bicarbonate from the oral ectoderm to endoderm cells, where bicarbonate is converted to CO_2 by carbonic anhydrase (CA) and supplied to the zooxanthellae. Additionally, BAT is suggested to transport bicarbonate from the aboral endoderm to calicoblastic cells and the calcifying medium, which is converted to carbonate and used for calcification. By immunostaining BAT in the coral *Stylophora pistillata*, it was determined that these transporters are indeed specifically localized in the endodermal and ectodermal cells of oral and aboral tissues (Zoccola et al. 2015). Similar to our study, the expression of the BAT gene was found to be up-regulated in the coral *Pocillopora damicornis* exposed to pH 7.4 compared to control (pH 8.1, Vidal-Dupiol et al. 2013). Although the calcification rate of *P. damicornis* was not studied by Vidal-Dupiol et al. (2013), other studies have demonstrated that this species is relatively robust to high CO_2 (Comeau et al. 2014b). From all these studies, up-regulation of the BAT gene can increase the availability of bicarbonate ions in the calcifying fluid, and to counteract the effect of low pH. Since we found higher expression of this gene in Sesoko corals, this could be one of the reasons for the insensitivity of Sesoko coral to the high-CO_2 condition. In the previous study using boron isotopes ($^{11}\delta B$), the calcifying fluid pH of Sesoko *A. digitifera* corals was calculated to be maintained at 8.3, and Ω_{arag} to be maintained at higher than 5, even when the corals were reared in seawater, pH 7.4 (Tanaka et al. 2015), which is in line with the present findings. Meanwhile, the reason for the higher expression of this gene in Sesoko corals, which show lower calcification rates compared to Bise corals under the control condition, is still open to question. Galaxin, which is the skeletal organic matrix protein identified in the coral (Fukuda et al. 2003), was found to be up-regulated under high CO_2 conditions only in Bise corals. Moya et al. (2012) evaluated the change in expression of genes encoding several organic matrices under high-CO_2 conditions in the juvenile coral *Acropora millepora*, and revealed that the expression of many of these genes was altered. For galaxins, 40% of the genes were up-regulated while about 20% were down-regulated at 1000 µatm CO_2. Similarly, up-regulation of this gene was also found in *P. damicornis.* Hence, these results suggest that the expression of galaxin could be up-regulated by an increase in CO_2; however, it is not clear if there is a direct relationship between the expression of this gene and sensitivity of coral calcification to OA. Calcium-ATPase (PMCA) is hypothesized to be another key transporter that removes protons from the calcifying medium to calicoblastic cells and transports calcium ions into the calcifying medium (McConnaughey and Falk 1991; Cohen and McConnaughey 2003; Allemand et al. 2011). However, there was no significant difference in the expression of PMCA between Sesoko and Bise corals, and among CO_2 conditions. Meanwhile, the expression of PMCA in high-CO_2 Sesoko corals showed extremely high variations, suggesting PMCA expression variations among colonies. Finally, carbonic anhydrase (CA) is the enzyme that regulates the inter-conversion of HCO_3^- to CO_2 and is suggested to convert CO_2 derived from metabolic respiration into bicarbonate, which is transported by the bicarbonate transporter genes to the calcifying fluid and used for calcification (Bertucci et al. 2013). Because CA inhibitor was found to reduce the calcification rate of corals by about 50–70%, CA is suggested to be another factor in coral calcification (Goreau 1959; Isa and Yamazato 1984). However, the expression of CA II did not differ between Sesoko and Bise corals, or among CO_2 conditions. These results contradict previous results showing down-regulation in *A. millepora* (Moya et al. 2012), and up-regulation in *P. damicornis* at low-pH conditions (Vidal-Dupiol et al. 2013), which suggest different response of CA to high CO_2 among coral species.

Conclusion

This study provides the first evidence that the calcification of the two populations of the coral *Acropora digitifera* shows significantly different responses to seawater pCO_2 change. The population that was highly resistant to OA exhibited a slower calcification rate and higher expression of the BAT gene, suggesting that colonies that have higher capability to maintain the pH of calicoblastic fluid could be more tolerant to OA. Additionally, taking into account that the resistant

populations were located at relatively nearshore where the environmental conditions are more variable, while the sensitive population was located in forereef with more stable conditions, plasticity for coral calcification in response to the environment and/or acclimation response to the change in environmental conditions such as seawater pCO_2 may lead to differences in sensitivity to high seawater pCO_2. Further studies considering the potential variability in coral sensitivity to OA among local populations from different habitat are necessary to predict the effect of climate change on reef ecosystems.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Ethical approval The manuscript has not been submitted to more than one journal for simultaneous consideration. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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