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Intraspecific variations in responses to ocean acidification in two branching coral species

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ABSTRACT

Ocean acidification is widely recognised to have a negative impact on marine calcifying organisms by reducing calcifications, but controversy remains over whether such organisms could cope with ocean acidification within a range of phenotypic plasticity and/or adapt to future acidifying ocean. We performed a laboratory rearing experiment using clonal fragments of the common branching corals *Montipora digitata* and *Porites cylindrica* under control and acidified seawater (lower pH) conditions (approximately 400 and 900 µatm pCO_2, respectively) and evaluated the intraspecific variations in their responses to ocean acidification. Intra- and interspecific variations in calcification and photosynthetic efficiency were evident according to both pCO_2 conditions and colony, indicating that responses to acidification may be individually variable at the colony level. Our results suggest that some corals may cope with ocean acidification within their present genotypic composition by adaptation through phenotypic plasticity, while others may be placed under selective pressures resulting in population alteration.

1. Introduction

Decreased ocean pH due to atmospheric CO_2 is expected to have negative effects on marine calcifying organisms, including reef-building corals (Orr et al., 2005; Kleypas et al., 2006; Hoegh-Guldberg et al., 2007). Phenotypic plasticity and potential for evolutionary adaptation (change of genotypic composition within populations through several generations) to lower pH conditions have been suggested as possible outcomes for such organisms (Munday et al., 2013; Sunday et al., 2014). The main reason is because life-history traits such as growth rate and stress tolerance are variable within natural populations and can be influenced by conditions such as environmental pH (Reusch, 2014). Such variation among genotypes has also been suggested in some organisms, including coccolithophores (Langer et al., 2009), bryozoans (Pistevos et al., 2011), oysters (Parker et al., 2012), and polychaetes (Calosi et al., 2013).

In corals, phenotypic plasticity and potential for adaptation to ocean acidification has not yet been fully evaluated (Császár et al., 2010). One reason for this is that the reproduction of marine organisms in multigenerational experiments is difficult under laboratory conditions. Long duration for life stages in corals (at least a few years) also prevents from performing laboratory experiments. On the other hand, in the case of clonal organisms such as corals, all variation in physiological traits such as growth and reproduction within a clonal colony is attributed to phenotypic plasticity caused by environmental factors. Thus, it can be assumed that variation among colonies is partially because of genetic differences. This allows for additional straightforward experiments that can evaluate the adaptive potential of corals against environmental changes (Császár et al., 2010; Hayashi et al., 2013).

In this study, we aimed to evaluate the intraspecific variations in branching corals' responses to ocean acidification, using laboratory-reared clonal fragments from 12 different colonies of each species. We studied two branching coral species, *Montipora digitata* and *Porites cylindrica*, which are common species throughout the Ryukyu Archipelago

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in Japan. We examined calcification and photosynthetic efficiencies among colonies at two different pCO_2 levels in order to predict their likely responses to ocean acidification.

2. Methods

Coral nubbins were collected from haphazardly selected colonies spaced at least 10 m apart. All nubbins from the same species were collected on the same date. We collected 10 clonal nubbins from each of 12 colonies of *Montipora digitata* (120 nubbins in total) from the shallow reef lagoon at Bise, Okinawa, Japan, and 10 clonal nubbins from each of 12 colonies of *Porites cylindrica* collected from the fringing reef at Sesoko Island, Okinawa, Japan (120 nubbins in total). All samplings were performed with permission from Okinawa Prefecture. Coral nubbins were prepared as per Ohki et al. (2013). Similarly sized nubbins were used for each species, with initial skeletal weights of 0.279 ± 0.075 mg (mean ± S.D.) for *M. digitata* and 0.768 ± 0.275 mg for *P. cylindrica*. Nubbins were acclimated for one week prior to experimentation in an outdoor aquarium with running seawater under natural light conditions at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, before starting experiments.

Two levels of CO_2 atmospheric pressure were used to induce variable acidic pH conditions, including a control pCO_2 at approximately 400 µatm and a high pCO_2 at approximately 900 µatm (Table 1), by using an AICAL high-precision pCO_2 control system. This system monitors pCO_2 using a non-dispersive infrared absorption (NDIR) system with a LI-COR 840 detector (LI-COR Biosciences Co., Lincoln, NE, USA) (Iguchi et al., 2014). Two 12 L tanks were filled with seawater adjusted to each pCO_2 value, and each tank was maintained as a closed system with an exchange flow rate of around 150 mL/min. Seawater temperature was maintained at around 27 °C, and the tanks were lit by using metal-halide lamps at around 190–220 µmol/m^2/s with a 12 h:12 h light:dark cycle (Funnel2 150W, Kamihata, Japan) (Table 1).

Unfiltered seawater samples of 100 mL were collected from each tank and fixed by immediately adding a saturated solution of $HgCl_2$. Total alkalinity in the sample was determined in aliquots of 50 mL using potentiometric acid titration method with a Radiometer automated burette (Model ABU91) at 25 °C (Kawahata et al., 2000). Primary standardisation of the instrument was performed using reference material solutions prepared by KANSO TECHNOS Co. Ltd. (Osaka, Japan) using a similar procedure of the Certified Reference Material (CRM) preparation (Dickson et al., 2003). The chemical and physical conditions of each treatment are summarised in Table 1 with the pH, HCO_3^-, CO_3^{2-}, Ω_{arg} estimated from pCO_2, temperature, mean total alkalinity (2253 ± 5 (mean ± S.D.; n = 3) and 2293 ± 12 (n = 3) µmol/kg, in *M. digitata* and *P. cylindrica* experiments, respectively), and salinity of 34.5, which is the average value of the same laboratory, by using the computer program CO2SYS (Lewis and Wallace, 1998). To confirm chemical conditions in tanks, we also measured pH values in each tank manually.

Twenty clonal nubbins, which were cut from a parent colony and attached to plastic bolts with superglue, were prepared (240 nubbins per species). Ten clonal nubbins of each colony were randomly allocated into duplicated tanks for each treatment. The seawater in each tank was circulated using a water-jet pump (approx. 5 cm s^{-1}). The calcification rate of each nubbin was calculated as percentage change in skeletal weight relative to the initial weight as described in previous studies (Marubini et al., 2001; Anthony et al., 2008; Iguchi et al., 2012). Photosynthetic efficiencies of coral nubbins were evaluated using a Diving-PAM Underwater Fluorometer (Walz, Germany) at the end of experiments, as per Iguchi et al. (2012). We used dark-adapted *Fv/Fm*, a reliable parameter of the photochemical efficiency of *Psi*I (Demmig and Bjorkman, 1987), as it has been previously applied as an indicator for monitoring photodamage (or photoinhibition) during photosynthesis (Franklin et al., 1992; Takahashi et al., 2004). The rearing experiment was carried out for 28 days.

Statistical analyses were carried out using General Linear Mixed Model (GLMM) fitted with a Gaussian distribution to analyse calcification rates and *Fv/Fm* values. To check the effect of tank allocation, we compared two models as follows. Model 1 considered the level of acidification to be the explanatory variable and colony to be a random-effect (colony nested within level of acidification), and Model 2 considered the level of acidification to be the explanatory variable and both level of acidification and tank allocation to be random-effects (tank allocation and colony nested within level of acidification, respectively). To check the effect of the acidification treatment, we made two further comparisons. Model 3 was used as a null model with random-effects, with colony nested within treatment. This was compared to Model 1. When tank effect was found to be significant, we compared Model 4, a null model with random-effects of tank and colony (tank allocation and colony nested within level of acidification, respectively) to Model 2. In the GLMM analyses above, likelihood ratio tests (LRTs) were used to check the statistical significance of the inclusion of each explanatory variable (Zuur et al., 2009). Pearson's product-moment correlation tests between growth rates and *Fv/Fm* values were performed. These analyses were performed by using lmer function in R (R Core Team, 2015). Assumptions of normality and equality of variance were checked using graphical analyses.

3. Results

Positive calcification rates were observed in 11 out of 12 colonies of *M. digitata*, and 6 out of 12 colonies of *P. cylindrica*. The following analyses includes only data from positively grown colonies, as the majority of the negatively grown nubbins appeared to be dead and covered by filamentous algae, possibly due to the insufficient removal of mucus and short acclimation to experimental conditions especially for *P. cylindrica*.

The effect of tank allocation was significant on calcification rate in *M. digitata* (LRT, p = 0.03). Thus, we considered tank allocation as a random effect in the model. No significant difference in *P. cylindrica* calcification rate was detected between duplicated tanks (LRT, p > 0.1). Thus, the data from the replicated tanks for each pCO_2 treatment could be pooled.

Coral calcification rates tended to decrease under acidified seawater

Table 1
Summary of physical and chemical conditions in each experimental aquarium. All values are shown as mean ± S.D. (n = 345 in *Montipora digitata*, 697 in *Porites cylindrica*, respectively).

Species	pCO_2	Aquarium	°C	pH_T	pCO_2 (µatm)	HCO_3^- (µmol/kg)	CO_3^{2-} (µmol/kg)	Ω_{arg}
Montipora digitata	Control	1	27.3 ± 0.1	8.05 ± 0.06	391 ± 56	1706 ± 63	222 ± 25	3.58 ± 0.41
Montipora digitata	Control	2	27.3 ± 0.3	8.05 ± 0.06	390 ± 55	1706 ± 63	222 ± 25	3.58 ± 0.41
Montipora digitata	High	1	27.2 ± 0.1	7.74 ± 0.14	922 ± 198	1936 ± 120	129 ± 48	2.08 ± 0.78
Montipora digitata	High	2	27.3 ± 0.5	7.74 ± 0.14	925 ± 198	1936 ± 120	129 ± 48	2.08 ± 0.78
Porites cylindrica	Control	1	27.4 ± 0.3	8.07 ± 0.07	378 ± 59	1716 ± 81	235 ± 33	3.79 ± 0.53
Porites cylindrica	Control	2	27.0 ± 0.3	8.07 ± 0.07	373 ± 59	1717 ± 81	235 ± 32	3.77 ± 0.52
Porites cylindrica	High	1	27.0 ± 0.1	7.75 ± 0.13	908 ± 168	1969 ± 120	132 ± 48	2.13 ± 0.78
Porites cylindrica	High	2	27.0 ± 0.4	7.75 ± 0.13	910 ± 170	1969 ± 119	132 ± 48	2.13 ± 0.77