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Negative effects of ocean acidification on two crustose coralline species using genetically homogeneous samples

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ABSTRACT

We evaluated acidification effects on two crustose coralline algal species common to Pacific coral reefs, *Lithophyllum kotschyanum* and *Hydrolithon samoense*. We used genetically homogeneous samples of both species to eliminate misidentification of species. The growth rates and percent calcification of the walls of the epithallial cells (thallus surface cells) of both species decreased with increasing pCO_2. However, elevated pCO_2 more strongly inhibited the growth of *L. kotschyanum* versus *H. samoense*. The trend of decreasing percent calcification of the cell wall did not differ between these species, although intercellular calcification of the epithallial cells in *L. kotschyanum* was apparently reduced at elevated pCO_2, a result that might indicate that there are differences in the solubility or density of the calcite skeletons of these two species. These results can provide knowledge fundamental to future studies of the physiological and genetic mechanisms that underlie the response of crustose coralline algae to environmental stresses.

1. Introduction

Coralline algae have recently been the subjects of numerous investigations because of their perceived vulnerability to the potential impacts of ocean acidification (OA), the reduction of pH, and calcium carbonate saturation state caused by rising concentrations of anthropogenic carbon dioxide (Koch et al., 2013). Many studies have reported adverse effects of elevated partial pressures of carbon dioxide (pCO_2) on the growth of coralline algae (e.g., Anthony et al., 2008; Hofmann et al., 2012; Diaz-Pulido et al., 2012). Coralline algae are one of the largest calcifying macroalgal groups and are important components of benthic marine communities; they contribute to the formation of coral reef structures and provide habitats for other organisms from cold, temperate to warm, tropical shores (e.g., Kamenos et al., 2004; Tierney and Johnson, 2012). They also play roles as inducers of the settlement and morphogenesis of marine invertebrates around the world (e.g., Nelson, 2009). A decline in the abundance of coralline algae may therefore affect reef accretion and cementation as well as the recruitment of invertebrates to the reef (Anthony et al., 2008; Kleypas and Yates, 2009). However, in addition to reports of the adverse effects caused by elevated pCO_2 on coralline algae, there have also been reports of no significant effects and even positive effects. In laboratory-based studies, elevated pCO_2 has increased the photosynthetic rates of *Hydrolithon* sp. (Semesi et al., 2009) and net calcification by *Neogoniolithon* sp. (Ries et al., 2009). Even in the field, Porzio et al. (2011) found *Hydrolithon cruciatum* to be more abundant at volcanic CO_2 vent sites, although they observed a significant reduction in the abundance of other coralline algae.

One of the causes of these variable responses might be related to the solubility of coralline skeletons. A reduction in the calcification of cell walls caused by high pCO_2 has been reported for *Corallina officinalis* (Hofmann et al., 2012) and *Lithothamnion glaciale* (Ragazzola et al., 2012). Coralline algae contain calcium carbonate

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Table 1
Summary of carbonate chemistry parameters and water temperature in the experimental treatments. Values are presented as averages. Numbers in parentheses are standard deviations. TA, $\Omega_{calcite}$ and $\Omega_{aragonite}$ indicate total alkalinity, saturation state of seawater with respect to calcite and aragonite, respectively.

Temperature (°C)	pH_T	TA (μmol kg^{-1})	pCO_2 (μatm)	HCO_3^- (μmol kg^{-1})	CO_3^{-2} (μmol kg^{-1})	CO_2 (μmol kg^{-1})	$\Omega_{calcite}$	$\Omega_{aragonite}$
27.3 ± 0.2	8.18 ± 0.06	2279 ± 12	263 ± 45	1585 ± 61	282 ± 27	7.1 ± 1	6.9 ± 0.7	4.6 ± 0.4
27.3 ± 0.1	8.04 ± 0.03	2279 ± 12	399 ± 37	1737 ± 34	221 ± 16	11 ± 1	5.4 ± 0.4	3.6 ± 0.3
27.2 ± 0.2	7.71 ± 0.06	2279 ± 12	960 ± 143	1983 ± 35	121 ± 16	26 ± 4	2.9 ± 0.4	2.0 ± 0.3

($CaCO_3$), which accounts for up to 95% of their mass (Bilan and Usov, 2001; Kamenos et al., 2008b). Coralline algae deposit high-magnesium calcite, the Mg content of which varies mainly as a function of taxonomic position (Smith et al., 2012), temperature (Halfar et al., 2000; Kamenos et al., 2008a), and ambient seawater chemistry (Ries, 2010). The greater the Mg content of the calcite, the more it tends to be soluble and vulnerable to dissolution or recrystallization (Morse et al., 2006; Andersson et al., 2008). However, biogenic Mg-calcites with similar Mg contents exhibit a wide range of solubility. The solubility may therefore depend on organism-specific factors such as structural disorder and impurities rather than the Mg content (Morse et al., 2006). Nonetheless, there have been few comparative studies of multiple coralline species subjected to the same experimental conditions (e.g., Comeau et al., 2013b; Noisette et al., 2013).

Other causes of the observed variable responses may well have a genetic basis. Corals and coccolithophores depend on both photosynthesis and calcification to grow, and within or between species of these organisms simulated responses to the effects of OA are highly variable (Marubini et al., 2003; Fabry, 2008; Langer et al., 2009; Iguchi et al., 2012). Crustose coralline algae and other calcareous red algae (e.g., Peyssonneliaceae) can be easily misidentified at higher than the species level if nothing more than gross morphology is used. Moreover, morphologically almost indistinguishable species often exhibit interspecific genetic differences (Kato et al., 2013; Dixon and Saunders, 2013). *Porolithon onkodes*, a reef-building coralline species that is frequently used for experiments related to OA effects, comprises at least two lineages (species) even in just the subtropical region of Japan (Kato et al., 2011). In this study, we investigated species-level effects of simulated OA by using genetically homogeneous samples to eliminate misidentification and genetic influences. The two crustose coralline species we examined were *Lithophyllum kotschyanum* and *Hydrolithon samoense*, which are commonly found in the shallow water of Pacific coral reefs. We used a high-precision pCO_2 control system to evaluate the simulated effects of ongoing OA on rates of growth and calcification under pre-Industrial Revolution (281 μatm), present (418 μatm), and possible near-future pCO_2 conditions (1019 μatm).

2. Materials and methods

2.1. Sample preparation

Specimens of two crustose species, *L. kotschyanum* and *H. samoense*, were collected from the upper sublittoral zone around Sesoko Island in Okinawa, Japan, in October 2010. We identified the species by using the descriptions by Yoshida and Baba (1998) and Harvey et al. (2006). Sixty fragments (less than 100 mg) were cut from a single parent thallus and mounted to acrylic bolts with superglue to prevent exposure of the cut surface of the fragments. The fragments from each thallus were treated as clonal samples, the intention being to eliminate misidentification and genetic differences. The fragments were maintained in a flow-through aquarium for two weeks before the start of the experiment under a modulated light intensity that we judged to be similar to the intensity in a shallow coral reef environment at a water depth of ~3 m.

2.2. Experimental set-up

Samples of *L. kotschyanum* and *H. samoense* were cultured in seawater at Sesoko Station, University of the Ryukyus, Okinawa, Japan. The pCO_2 of the seawater was maintained at three levels (Table 1): 281 μatm (conditions prior to the Industrial Revolution), 418 μatm (present conditions), and 1019 μatm [concentration projected by the Intergovernmental Panel on Climate Change (IPCC, 2007) for the end of this century]. A precise pCO_2 control system (Fujita et al., 2011) supplied pCO_2^- adjusted seawater to flow-through (150 ml min^{-1}) aquaria (12 L). Two replicate aquaria containing 10 samples of each species were used for each CO_2 treatment. The seawater temperature was maintained at 27 °C, and the algae were grown on a 12:12 h light:dark cycle throughout all treatments. During the photoperiod an irradiance of 40–60 μmol m^{-2} s^{-1} was provided by metal-halide lamps (Funnel 2 150 W, Kamihata, Japan). Seawater temperature, pH, and light intensity were confirmed twice each week. We measured pH (total hydrogen ion scale) and total alkalinity according to the protocols of Ushie et al. (2010). Carbonate chemistry (pCO_2, HCO_3^-, CO_3^{2-}, and CO_2), and calcium carbonate saturation state ($\Omega_{calcite}$ and $\Omega_{aragonite}$) were calculated from the pH (total scale) and total alkalinity at the in situ temperature and salinity (34.1) by using CO2calc software (Robbins et al., 2010) and the carbonate dissociation constants of Mehrbach et al. (1973), refit by Dickson and Millero, (1987) (Table 1).

2.3. Growth rate and calcification analysis

Growth rates of samples were determined by calculating the percentage increase of fresh weight during the experiment (8 weeks) relative to the initial weight. Fresh weights were measured

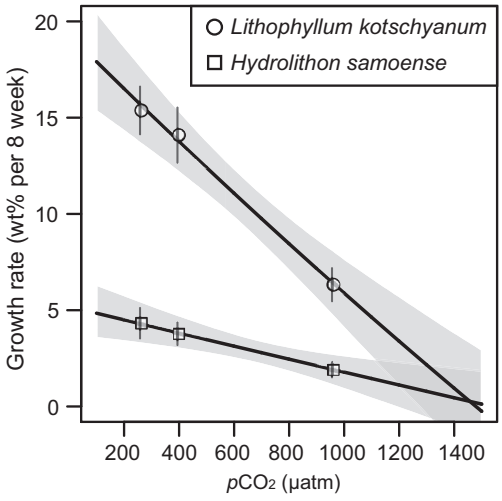

Fig. 1. Growth rate (mean ± standard error) of *Lithophyllum kotschyanum* and *Hydrolithon samoense*, shown on a percent scale. The points pooled between aquaria ($n = 20$). The regression curves and bootstrapped 95% confidence intervals are shown in solid lines and in gray bands, respectively.