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INTERACTIONS BETWEEN OCEAN ACIDIFICATION AND WARMING ON THE MORTALITY AND DISSOLUTION OF CORALLINE ALGAE[1]

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Coralline algae are among the most sensitive calcifying organisms to ocean acidification as a result of increased atmospheric carbon dioxide (pCO_2). Little is known, however, about the combined impacts of increased pCO_2, ocean acidification, and sea surface temperature on tissue mortality and skeletal dissolution of coralline algae. To address this issue, we conducted factorial manipulative experiments of elevated CO_2 and temperature and examined the consequences on tissue survival and skeletal dissolution of the crustose coralline alga (CCA) *Porolithon* (=*Hydrolithon*) *onkodes* (Heydr.) Foslie (Corallinaceae, Rhodophyta) on the southern Great Barrier Reef (GBR), Australia. We observed that warming amplified the negative effects of high pCO_2 on the health of the algae: rates of advanced partial mortality of CCA increased from <1% to 9% under high CO_2 (from 400 to 1,100 ppm) and exacerbated to 15% under warming conditions (from 26°C to 29°C). Furthermore, the effect of pCO_2 on skeletal dissolution strongly depended on temperature. Dissolution of *P. onkodes* only occurred in the high-pCO_2 treatment and was greater in the warm treatment. Enhanced skeletal dissolution was also associated with a significant increase in the abundance of endolithic algae. Our results demonstrate that *P. onkodes* is particularly sensitive to ocean acidification under warm conditions, suggesting that previous experiments focused on ocean acidification alone have underestimated the impact of future conditions on coralline algae. Given the central role that coralline algae play within coral reefs, these conclusions have serious ramifications for the integrity of coral-reef ecosystems.

Key index words: **calcification; carbon dioxide; carbonate dissolution; climate change; coral reefs; coralline algae; global warming; greenhouse effect; ocean acidification; temperature effects**

Abbreviations: **CCA, crustose coralline alga; GBR, Great Barrier Reef; IPCC, Intergovernmental Panel on Climate Change; pCO_2, carbon dioxide partial pressure**

Coralline red algae play fundamental roles in the ecology of tropical and temperate reefs. For example, coralline algae contribute to the construction of the reef frameworks by depositing calcium carbonate in the form of high-magnesium calcite (Mg calcite) and binding adjacent substrata together (Littler and Littler 1984, Payri 1997, Adey 1998). Coralline algae also play important roles by inducing the settlement of coral larvae (Harrington et al. 2004, Diaz-Pulido et al. 2010) and other invertebrates, many of which are economically important (e.g., abalone and sea urchins; Hayakawa et al. 2008). By stimulating the settlement of organisms such as corals, they are instrumental in the recovery of damaged reefs after disturbances (Birrell et al. 2008). In light of the ecological importance of coralline algae, their vulnerability to anthropogenic climate change and ocean acidification (Diaz-Pulido et al. 2007, Kuffner et al. 2008) is a critical issue for coral reefs.

The world's oceans have absorbed approximately one-third of the atmospheric carbon dioxide (CO_2) produced by humans in the past 200 years, which has led to a reduction in the pH of surface water of 0.1 units and significant changes in the water carbonate chemistry (Sabine et al. 2004, Pelejero et al. 2010). Ocean acidification due to increased pCO_2 and consequent shifts in the marine carbon chemistry reduces calcification of a large range of calcareous marine organisms (Raven 2005), including corals (Kleypas et al. 1999, Langdon and Atkinson 2005, Hoegh-Guldberg et al. 2007), foraminiferans, coccolithophores (Riebesell et al. 2000, Hallegraeff 2010), and some coralline algae (Gao et al. 1993, Jokiel et al. 2008, Martin and Gattuso 2009). Recent experimental studies have also demonstrated that the survivorship of coralline algae is severely reduced under

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carbon chemistry conditions representative of projected carbon paths by the Intergovernmental Panel on Climate Change (IPCC) (Anthony et al. 2008, Hall-Spencer et al. 2008, Martin and Gattuso 2009). Because the high-Mg-calcite skeleton of coralline algae is more soluble than the aragonitic skeleton of corals and calcareous green algae or the low-Mg-calcitic skeletons of many foraminifera, coralline algae are believed to be the first responders to ocean acidification (Morse et al. 2006).

The majority of studies on the effects of ocean acidification on coralline algae have focused on calcification responses (Anthony et al. 2008, Semesi et al. 2009), and only few have examined population-level consequences, such as rates of mortality or recruitment (Jokiel et al. 2008, Kuffner et al. 2008). Furthermore, and more importantly, most studies investigating the impacts of rising pCO_2 on coralline red algae have mainly explored the effects of changing biogeochemistry without formally considering its interaction with increasing temperature that inevitably will come in parallel with ocean acidification (but see Anthony et al. 2008, Martin and Gattuso 2009, and reviewed in Hurd et al. 2009, Nelson 2009). Here, we examine the independent and combined effects of ocean acidification and warming on mortality responses of a CCA, *P.* (=*Hydrolithon*) *onkodes*, on the southern GBR, Australia. Furthermore, since CCA harbor abundant endolithic algal populations that play important roles in the bioerosion of their skeletons (Tribollet and Payri 2001), we also explore the relationship between rates of skeletal dissolution and abundance of endolithic algae under multiple acidification and temperature experiments. This work was part of a multifactorial CO_2-dosing and temperature-control experiment on Heron Island (GBR), using a 30-tank flow-through aquarium system (Anthony et al. 2008).

MATERIALS AND METHODS

General approach and collecting site. We compared partial tissue mortality and rates of dissolution of adult *P. onkodes* across three levels of CO_2 and two levels of temperature in aquarium experiments at Heron Island Research Station (HIRS), southern GBR. *P. onkodes* is widely distributed throughout the Indo-Pacific and is one of the most abundant and ecologically important CCA in shallow coral reef habitats (Littler and Doty 1975, Adey et al. 1982, Chisholm 2000, Ringeltaube and Harvey 2000). Individuals of *P. onkodes* were collected from the reef crest and shallow upper reef slopes of nearby Wistari Reef (23°27′ S, 151°52′ E, mean depth of 1–4 m). We used CCA chips of $\sim 3 \times 3$ cm, which were collected by breaking off fragments of the alga attached to the reef substrate using a chisel and hammer. Only healthy, pink fragments were selected, cut to the size needed using pliers, and cleaned of epiphytes and cryptic invertebrates (e.g., sponges and worms). The CCA were handled carefully to avoid tissue damage and acclimated in aquaria with running seawater for a week in the outdoor facilities at the HIRS prior to the experiment. The CCA surfaces were cleaned from epiphytic algae at 2–3 d intervals using a soft tooth brush.

Experimental setup—pCO_2 and temperature experiments and saturation state of Mg calcite. To explore the potentially interactive effects of ocean acidification and warming, we used a CO_2 control system in an outdoor flow-through experimental aquarium system at HIRS. The control system (Campbell Scientific, Townsville, Qld, Australia) used computer-operated solenoid valves (Dupla, Gelsdorf, Germany) to regulate the amount of pure CO_2 (analytical grade) bubbled into seawater mixing tanks (200 L), which was regulated based on pH levels measured automatically every 30 s in the mixing tanks (one mixing tank per CO_2 treatment; details in Anthony et al. 2008). The CO_2 treatment included three CO_2 levels: (i) high (estimated pCO_2 between 1,000 and 1,300 ppm, pH target value 7.60–7.70); (ii) intermediate (520–700 ppm, pH target value 7.85–7.95); and (iii) control (present-day pCO_2 without dosing, 130–465 ppm, pH ranged from 8.0 to 8.4). High and intermediate levels represent the high-end (above category VI) and high category IV of the CO_2 stabilization scenarios predicted by the IPCC (Meehl et al. 2007) (Table 1). Two temperature levels were used: (i) low, 25°C–26°C, and (ii) high, 28°C–29°C, representing low and high average summer temperatures for the southern GBR. Industrial heater-chillers (TC800; Teco, Ravenna, Italy) maintained temperatures within a 1°C–2°C window during the course of the experiment (8 weeks, February–March 2007). Each treatment combination had five, 20 L replicate aquaria (total of 30), which continuously received CO_2-treated and temperature-controlled seawater from the mixing tanks. Each aquarium contained three experimental *P. onkodes* chips placed at the bottom. Small powerheads were used in each aquarium for water circulation. Light levels in the experimental CCA ranged from 700 to 1,200 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$.

Water carbonate chemistry parameters (including pCO_2, bicarbonate, and carbonate ions) were estimated for all treatments using the program CO2SYS (Lewis and Wallace 1998) and were based on measurements of pH (made on the seawater scale using polarographic sensors, InPro4501VP; Mettler-Toledo, Urdorf, Switzerland), temperature, total alkalinity (using the Gran alkalinity method on a Mettler-Toledo T50 automated titrator using 0.1 M HCl for 130 g seawater samples), and salinity (measured using a Bellingham Stanley refractometer, Tubridge Wells, Kent, UK) (Table 1). Saturation state of the seawater with respect to high-Mg calcite was calculated for 14% mol Mg calcite. We assumed this value in our samples based on average concentrations reported for tropical species of *Porolithon*. For example, Milliman et al. (1971) reported 14 and 16 mole% Mg calcite for two species of *Porolithon* and Kuffner et al. (2008) found 13 mole% Mg calcite in CCA from Hawaii. Since concentrations of Mg in coralline algae may vary, for example, among species, latitudes, and temperature regimes (e.g., Chave and Wheeler 1965), saturation states for our experimental samples are preliminary but still more realistic than calculations for low-Mg calcites. Mg calcite saturation state was calculated using stoichiometric solubility products based on the biogenic minimally prepared solubility curve of Plummer and Mackenzie (1974) and following equation (8) described in Morse et al. (2006). The Plummer and Mackenzie (1974) solubility curve was chosen instead of the biogenic extensively cleaned (best fit) or synthetic Mg-calcite solubility curves because the former has been suggested to possibly best describe how biogenic Mg calcites react in nature (Bischoff et al. 1987, see also discussions in Morse et al. 2006, Andersson et al. 2008). Saturation state was calculated using total calcium, magnesium, and carbonate activity coefficients (Millero and Pierrot 1998). Carbonate ion concentrations were derived from CO2SYS, using the K1, K2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987). Calculations of saturation states of high-Mg calcite were conducted by Andreas Andersson (Bermuda Institute of Ocean Sciences).

TABLE 1. Summary of values for water chemistry parameters for CO_2 and temperature treatments.

Treatments	pH	T (°C)	TA ($\mu mol \cdot kg^{-1}$)	pCO_2 (ppm)	HCO_3 ($mmol \cdot kg^{-1}$)	CO_3 ($mmol \cdot kg^{-1}$)	$\Omega_{\text{High-Mg calcite}}$
Ambient	8.00–8.40	25–26	2,375–2,450	135–460	1,390–1,930	207–415	1.2–2.3
Ambient	8.00–8.40	28–29	2,375–2,450	130–465	1,325–1,885	225–440	1.3–2.5
Medium	7.85–7.95	25–26	2,375–2,450	520–705	1,900–2,050	155–200	0.8–1.1
Medium	7.85–7.95	28–29	2,375–2,450	520–705	1,860–2,020	170–220	0.9–1.2
High	7.60–7.70	25–26	2,375–2,450	1,010–1,350	2,080–2,210	95–125	0.5–0.7
High	7.60–7.70	28–29	2,375–2,450	1,020–1,360	2,020–2,190	105–135	0.6–0.8

TA, total alkalinity; $\Omega_{\text{High-Mg calcite}}$, high-magnesium calcite saturation state.

Response variables and data analyses. Response variables included rates of partial mortality and carbonate dissolution. To calculate mortality rates of *P. onkodes*, experimental chips of the algae were photographed at the beginning and end (8 weeks) of the experiment, and the tissue area showing mortality and paling, as well as the healthy tissue, was estimated using the software UTHSCSA ImageTool (San Antonio, TX, USA). Tissue assessments were conducted visually based on underwater color charts (Amphibico Inc., Montreal, QC, Canada, and Siebeck et al. 2006). Partial mortality included two categories reflecting different stages of endolithic algal colonization on the coralline skeleton: (i) dead bleached areas (recently dead corallines with white skeleton and no visible superficial colonization by endolithic algae) and (ii) dead green areas (older mortality with green skeleton heavily colonized by endolithic algae, Fig. 1). Tissue paling was defined as the tissue being pale pink or yellow, but not bleached—and without endolithic algal blooms on the surface. Healthy corallines included pink to dark pink tissue areas (Fig. 1).

Rate of skeletal dissolution was calculated as the change in buoyant weight (Spencer-Davies 1989) between the beginning and the end of the experiment. Negative changes indicated dissolution, while positive changes indicated calcification. Daily rates of calcification and dissolution data were expressed as milligrams (from buoyant weight) normalized to crust surface area.

Mortality and calcification-dissolution data were analyzed using a factorial, nested analysis of variance (ANOVA), with CO_2 and temperature as fixed factors and three plants (= chips) nested within tanks. Post hoc comparisons were conducted using Tukey's tests. As there was no effect of tanks in the analyses (data not shown), tanks were pooled and a two-way ANOVA run with 15 replicates per treatment combination. Data were checked for homogeneity of variance (Cochran's test) and for normality of residuals (graphically). Data were arc-sin transformed. When significant interactions occurred, data were tested within treatment combinations. Regression analyses were conducted using the least squares method to explore the relationship between dissolution rates and % coralline algal mortality and colonization by endolithic algae. Regression analyses were checked for leverage by potential outliers in the data set.

RESULTS

Partial tissue mortality (% dead white, and green areas colonized by endolithic algae) and % partial tissue paleness of *P. onkodes* increased consistently and significantly with increasing pCO_2 (Fig. 2). Mortality rates were significantly higher in the high-pCO_2 treatment than in the medium and control CO_2 levels (Fig. 2, A and B; Table 2). Accordingly,

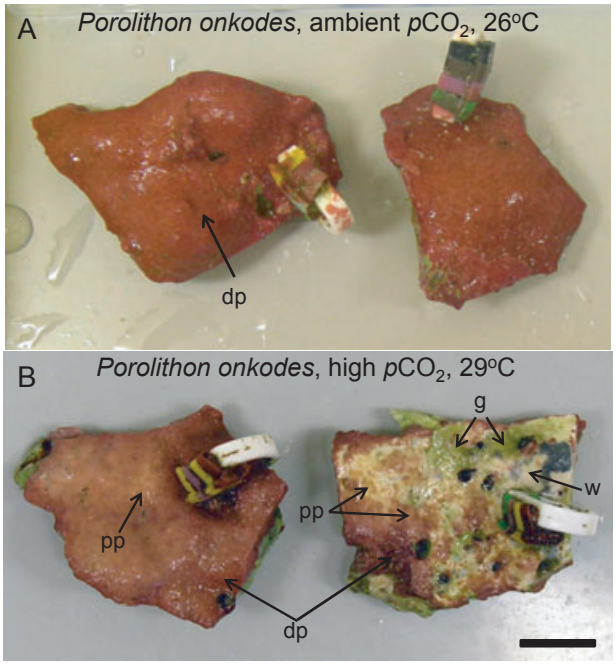

FIG. 1. Responses of *Porolithon onkodes* to elevated CO_2 concentrations and temperature. (A) *P. onkodes* growing under ambient, control conditions at the end of the experiment, 8 weeks. Tissue looks healthy (pink to dark pink, dp) with no signs of partial paleness or partial mortality. (B) *P. onkodes* growing under elevated pCO_2 and 29°C at the end of the experiment. Tissue shows different degrees of partial mortality; skeleton shows different levels of endolithic algal colonization, from white uncolonized (w) to green and densely colonized calcium carbonate (g). pp, partial paleness. Scale bar, 1 cm.

the amount of healthy, pink algal tissue was dramatically (up to 71%) reduced with increasing pCO_2 (Fig. 2D). Importantly, the effect of CO_2 on the % tissue mortality and % healthy tissue was exacerbated by warming. Specifically, in the highest pCO_2 treatment, the amount of recently dead tissue (white skeleton, Fig. 2A), dead tissue colonized by endolithic algae (green skeleton, Fig. 2B), and pale tissue (Fig. 2C) increased by 933, 67, and 69%, respectively, in warm conditions. Mortality rates were higher in the high-pCO_2 treatment than in the medium and control CO_2 levels (Fig. 2, A and B; Table 2). The proportion of healthy, pink tissue in

the high-pCO_2 treatment was reduced by 53% in the high temperature level compared to that of the low temperature. Interactions between the effects of CO_2 and temperature on the amount of healthy CCA tissue were statistically significant (Table 2), indicating that the increase in temperature acts synergistically with CO_2.

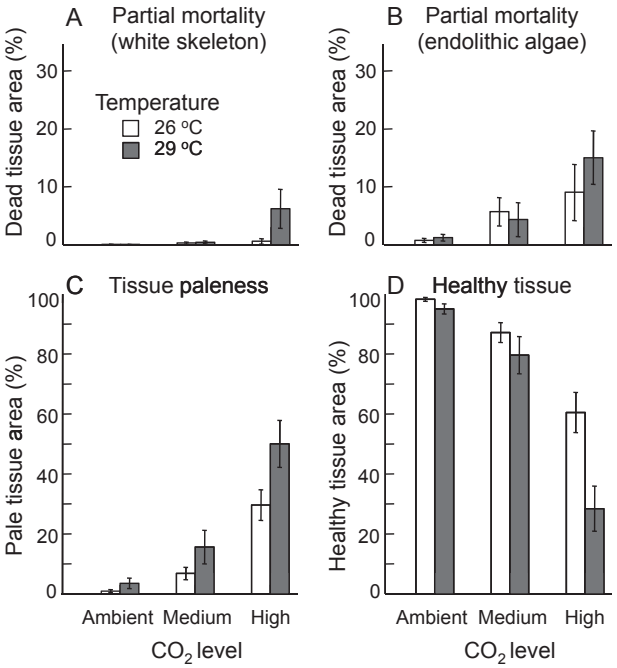

FIG. 2. Effects of CO_2 levels and temperature on the coralline alga *Porolithon onkodes*. (A) Partial tissue mortality expressed as % area of white skeleton (w). (B) Partial tissue mortality expressed as % area of green skeleton colonized by endolithic algae (g). (C) % area of pale tissue (partial paleness, pp). (D) % area of healthy, pink to dark pink tissue (dp). Ambient: pCO_2 135–460 ppm; medium: pCO_2 520–705 ppm; high: pCO_2 1,010–1,350 ppm. Data are means of $n = 15 \pm$ SEM.

Partial mortality (both white and green skeleton) in the intermediate pCO_2 treatment was low compared to that in the high-CO_2 treatment and was not significantly higher than mortality under ambient, control pCO_2. Furthermore, there was no effect of temperature on partial mortality in either the intermediate- or ambient-pCO_2 treatments. This finding suggests that mortality thresholds for *P. onkodes* are above CO_2 concentrations of 520–700 ppm CO_2.

Rates of CCA calcification decreased significantly with increasing CO_2 levels, but dissolution of the calcium carbonate skeleton mainly occurred in the highest-pCO_2 treatment (Fig. 3). In this treatment, increased temperature dramatically enhanced the dissolution of the skeleton (Fig. 3; see also Anthony et al. 2008). Rates of skeletal dissolution were proportionally related to the amount of dead tissue colonized by endolithic algae (mainly the cyanobacteria *Mastigocoleus testarum* Lagerheim ex Bornet et Falhault) (multiple $R = 0.62$; $R^2 = 0.384$, $P < 0.001$), particularly in the high-temperature and high-CO_2 combination treatment (multiple $R = 0.84$; $R^2 = 0.706$, $P < 0.001$; Fig. 3).

DISCUSSION

Effects on mortality. The results of our study demonstrate that *P. onkodes* is highly sensitive to the impacts of increased pCO_2 and consequent ocean acidification. However, the processes of warming and ocean acidification interact in such a way that the combination of both causes a much greater

TABLE 2. Factorial analyses of variance (ANOVAs) for the effects of CO_2 levels and temperature on *Porolithon onkodes*.

Source of variation	df	MS	*F*	*P*	Conclusion—Tukey test
Partial mortality (W)					
CO_2	2	0.056	5.319	0.007	Within 29°C: H > A = M
Temperature	1	0.041	3.901	0.052	Within high CO_2: 29 > 26
$CO_2 \times$ temp	2	0.039	3.703	0.029	
Error	78	0.011			
Partial mortality (G)					
CO_2	2	0.306	8.235	0.001	H > A = M
Temperature	1	0.007	0.190	0.664	Ns
$CO_2 \times$ temp	2	0.046	1.235	0.296	Ns
Error	78	0.037			
Partial paleness					
CO_2	2	2.476	38.992	<0.001	H > M > A
Temperature	1	0.331	5.216	0.025	29 > 26
$CO_2 \times$ temp	2	0.087	1.373	0.259	Ns
Error	78	0.063			
% Healthy tissue					
CO_2	2	4.060	57.917	<0.001	Within 26°C: H < M < A. 29°C: H < M = A
Temperature	1	0.683	9.748	0.003	Within high CO_2: 26 > 29
$CO_2 \times$ temp	2	0.325	4.640	0.012	
Error	78	0.070			

A, ambient pCO_2; M, medium pCO_2; H, high pCO_2; ns, not significant; W, white skeleton; G, green skeleton with endolithic algae.

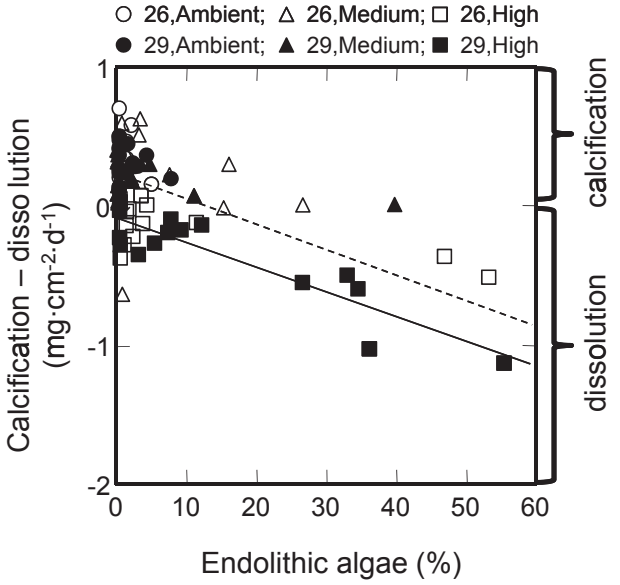

FIG. 3. Relationship between amount of endolithic algal colonization (as surface area colonized by endolithic algae) and rates of calcification/dissolution of the skeleton of *Porolithon onkodes* under different experimental conditions of pCO_2 and temperature. Positive values indicate calcification, and negative values skeletal dissolution. Linear regression analyses are plotted for the entire data set (dashed line, multiple $R = 0.62$; $R^2 = 0.384$, $P < 0.001$) and for data of the high-pCO_2 and high-temperature combination treatment (solid line, multiple $R = 0.84$; $R^2 = 0.706$, $P < 0.001$). CO_2 levels: ambient: pCO_2 135–460 ppm; medium: pCO_2 520–705 ppm; high: pCO_2 1,010–1,350 ppm. Temperature levels: 26°C and 29°C.

effect on coralline algal mortality than when the impacts of each process are considered independently. Previous studies have reported lowered abundance and recruitment of tropical (Kuffner et al. 2008) and temperate (Hall-Spencer et al. 2008, Martin and Gattuso 2009, Russell et al. 2009) species of coralline algae with rising CO_2 levels. However, only Martin and Gattuso (2009) have analyzed the simultaneous effects of these processes on the mortality of CCA. Using a Mediterranean species (*Lithophyllum cabiochae*), they observed a strong interaction between processes, where the increase in pCO_2 (700 ppm) caused algal necrosis and mortality only under elevated temperature. Similarly, in our experiment, recent mortality was mainly observed under the combination of high pCO_2 and high temperature. A stronger synergistic interaction occurred when considering the amount of healthy, pink tissue. Here, healthy tissue declined with increased pCO_2 at both levels of temperature, although the magnitude of the damaging effect of CO_2 was much higher in warm conditions. Furthermore, contrary to Martin and Gattuso (2009), we found increased paleness and tissue necrosis at intermediate pCO_2 and ambient temperature. This higher level of sublethal stress of the algae in our experiment may simply reflect different susceptibilities between species to increased pCO_2/lower pH, differences between tropical and temperate taxa, or different experimental conditions (e.g., irradiance levels). The irradiance levels used in our experiment were high, but representative of shallow reef habitats, such as reef crests. The extent to which high irradiance levels contributed to the bleaching and mortality observed in combination with the elevated CO_2 and temperature cannot be determined from our experiment. However, recent experiments have shown that solar UV radiation can exacerbate the negative effects of CO_2 on photosynthetic pigments of coralline algae (Gao and Zheng 2010), and therefore bleaching and mortality are less likely in lower-light environments. It is interesting to consider that some CCA can recover following light-induced bleaching once stress conditions have been reduced (Figueiredo et al. 2000). In our experiment, however, bleached CCA were rapidly colonized by endolithic algae suggesting limited potential for recovery. Although the mechanics of the interactions between warming and ocean acidification in the tropical and Mediterranean example are variable, in both instances, multiple climate change stressors interact to affect the survival of coralline algae.

The mechanisms responsible for the relationship between elevated pCO_2 and enhanced partial tissue mortality are not known (Martin and Gattuso 2009, Russell et al. 2009) but could involve (i) direct effects of acidosis (Anthony et al. 2008), (ii) disruption of photoprotective mechanisms (particularly under high irradiance, such as that used in our experiment; Crawley et al. 2010, Gao and Zheng et al. 2010), or (iii) indirect effects mediated by associated microorganisms. Coralline algae harbor diverse and abundant epilithic and endolithic microbial communities dominated by bacteria, cyanobacteria, and green and red algae (Johnson et al. 1991, Tribollet and Payri 2001). The growth rate and metabolism of these microorganisms may be stimulated by elevated pCO_2 (Levitan et al. 2007, Tribollet et al. 2009). Indeed, in our experiment, increased abundance of endolithic algae was positively associated with increasing levels of CO_2 (Figs. 1 and 3). Furthermore, enhanced abundance of microbial communities may lead to an additional overall rise in pCO_2 (due to increased respiration) and microbial metabolic production in the boundary layers, potentially creating hypoxic and toxic microenvironments promoting coralline algal morality. Moreover, N-fixing cyanobacteria (e.g., *Mastigocoleus*) are abundant within the skeletons of *P.* spp. in the GBR (G. Diaz-Pulido, pers. obs.), and there is evidence that rates of N fixation by cyanobacteria are stimulated by high CO_2 levels (Levitan et al. 2007). This can potentially enhance N availability for growth of other endolithic and epilithic algae, further promoting coralline algal mortality. Elevated temperatures can also enhance microbial metabolic

processes (Johnk et al. 2008, Paerl and Huisman 2008) exacerbating the mortality of coralline algal tissue. Martin and Gattuso (2009) suggested that increased temperature caused thermal stress in coralline algae, which could have facilitated infection by pathogens in their acidification experiment. However, we did not observe evidence of coralline algae diseases (such as the coralline lethal orange disease [CLOD]; Littler and Littler 1995, Diaz-Pulido 2002) in our experimental algae. Kuffner et al. (2008) suggested that the decline in percentage cover and recruitment of coralline algae due to ocean acidification was related to an increase in abundance of diatoms and filamentous algae. However, it is unclear whether the occurrence of filamentous algae was a cause or a consequence of declined CCA abundance. Further studies are needed to elucidate the potential direct or indirect mechanisms involved in coralline algae mortality in response to ocean acidification.

Effects on calcification/dissolution. As we have shown in a previous investigation (Anthony et al. 2008), rates of CCA calcification diminish with increasing pCO_2/reduced pH, consistent with the results of other studies (Gao et al. 1993, Jokiel et al. 2008, Semesi et al. 2009, Gao and Zheng 2010). High sensitivity of coralline algae calcification to ocean acidification is due to the high Mg content in the calcitic skeleton. High Mg increases the solubility of $CaCO_3$ in seawater (e.g., Feely et al. 2004). Reduced carbonate ion concentration [CO_3^{2-}] in the water due to ocean acidification decreases the saturation state of high-Mg calcite ($\Omega_{Mg\ calcite}$) reducing the deposition of $CaCO_3$ in the skeleton (Morse et al. 2006). A recent study using the coralline alga *Neogoniolithon* sp., however, showed enhanced rates of calcification at intermediate levels of ocean acidification (606 and 903 ppm pCO_2) (Ries et al. 2009), which was presumably associated with enhanced photosynthesis under increased pCO_2 in the water. Rates of photosynthesis in our experimental *P. onkodes* (Anthony et al. 2008) were, however, not enhanced by CO_2. Different mineralogies (e.g., $MgCO_3$ content) among species may be responsible for the observed discrepancies in calcification rates.

Dissolution of calcium carbonate of coralline algae occurred only in the highest-pCO_2 treatment and was associated with conditions involving an undersaturated state of high-Mg calcite and endolithic algae. It has been shown that dissolution rates of Mg calcites increase with increasing seawater pCO_2 and decreasing carbonate saturation state (Morse et al. 2006, Andersson et al. 2009). The saturation state with respect to a 14 mol% Mg-calcite (a % reported for tropical *Porolithon*; Milliman et al. 1971) in the highest experimental pCO_2 treatment was <1 ($\Omega_{Mg\text{-}calcite}$ 0.5–0.8; Table 1), indicating undersaturation for that particular carbonate mineral and in part explaining the dissolution of the Mg-calcite observed in our experiment.

It is not clear why increasing temperature led to increased rates of skeletal dissolution in our experiment. However, enhanced temperature is known to proportionally increase the %mol $MgCO_3$ in the coralline skeletons, even at monthly temporal scales (Chave and Wheeler 1965, Kamenos et al. 2008), and calcites with higher Mg content are more susceptible to dissolution than low-Mg calcites (Morse et al. 2006), particularly with increasing pCO_2 (Burton and Walter 1991). Therefore, it is likely that the corallines under the high-pCO_2 and high-temperature treatments were undergoing higher rates of dissolution due to variability in the $MgCO_3$ content. Saturation states for Mg calcite in the high-pCO_2 treatment, however, were not consistent with enhanced dissolution under high-temperature conditions (Table 1), as warming enhanced Mg-calcite saturation state (although the effects of temperature on saturation state are minor compared to the effects of increased CO_2, as discussed by Andersson et al. 2008). Unfortunately, we were not able to examine the Mg content of the skeletons because of an unexpected fire that destroyed the samples.

Dissolution rates were related not only to undersaturated states of Mg calcite in the water, but also to increased abundance of endolithic algae. It is not clear whether increased abundance of endolithic algae was the cause of coralline algae mortality (as discussed before) or a consequence of tissue necrosis and subsequent colonization and bloom. However, it is likely that endolithic algal blooms and microbial decomposition of coralline algal tissue under elevated pCO_2, and particularly under warming conditions (Fig. 1), could have driven significantly undersaturated seawater (primarily at night) with respect to Mg calcite via respiration (Morse et al. 2006), exacerbating the process of dissolution of the skeletons of coralline algae. Rates of dissolution of coral carbonates by endolithic microorganisms have been shown to increase with increasing pCO_2 (Tribollet et al. 2009). It is likely that low saturation states (both in the surface water and skeletal porewater) and endolithic algal blooms (affecting interstitial carbon chemistry) operated simultaneously in dissolving algal skeletons.

The interactions between different impacts (specifically here ocean acidification and warming) associated with rising greenhouse gases pose a significant challenge for coralline algal populations in the future. Despite coralline algae radiated during geological periods, which had higher atmospheric CO_2 concentrations, such as the Eocene (Steneck 1983, Aguirre et al. 2000, Royer 2006, Diaz-Pulido et al. 2007), the rate of change at which the increase in pCO_2 is occurring in the last 100 years (Hoegh-Guldberg et al. 2007, Hoegh-Guldberg and Bruno 2010, Pelejero et al. 2010) questions the potential for adaptation of these algae to climate change. Potential mechanisms of adaptation to changes in water chemistry exist for some coralline

algae (e.g., by changing skeletal mineralogies; Stanley et al. 2002, Ries 2006); however, there is very little information on the potential of coralline algae to adapt to climate change. Reduced coralline algal populations may lead to reduced coral settlement success and early growth (Harrington et al. 2004, Price 2010), potentially reducing the overall resilience of coral-reef ecosystems. Furthermore, enhanced dissolution of coralline algae predicted for 2100 under the A1FI scenario (future emission scenario described in the *Special Report on Emissions Scenarios* [SRES] prepared by the IPCC, representing a future with fossil-fuel intensive and rapid economic growth) may have important negative consequences for reef consolidation and growth. Given the important ecological functions of CCA on coral reefs, a deeper understanding of CCA susceptibility will be key to understanding the ecological consequences of ocean acidification and warming for coral reef ecology in a high-CO_2 world.

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