Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 3

Effects of ocean acidification and increased temperature on skeletal growth of two scleractinian corals, *Pocillopora meandrina* and *Porites rus*

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Abstract
For tropical corals, the projected decreases in seawater pH due to increasing atmospheric pCO_2 are predicted to reduce calcification by 8% to 40%, but little is known of the interactions with other physical conditions, or the effects on other biological processes. This study tested for the interactive effects of pH and temperature on the photosynthesis and calcification of corals using experiments in which pH was reduced (7.8), simulating year 2100 pCO_2 levels, and was crossed with two temperatures (27 ºC and 29 ºC). In 14-day incubations with *Pocillopora meandrina* and *Porites rus*, calcification was depressed ≥ 50% at 27 ºC and 700 ppm CO_2, compared to 27 ºC and 395 ppm CO_2, but was not depressed by high pCO_2 at 29 ºC; dark-adapted maximum quantum yield of PSII (F_v/F_m) was unaffected by the same treatments of temperature and pCO_2. Thus, while F_v/F_m in *P. meandrina* and *P. rus* is resilient to slight increases in temperature and high pCO_2, calcification shows temperature-dependent sensitivity to high pCO_2, notably with high temperature apparently conferring resistance against the short-term effects of high pCO_2.

Key words: Scleractinia, Calcification, Climate change, Photosynthesis, Temperature,

Introduction

The foundations of tropical reefs rely on the prodigious capacity of symbiotic scleractinians to calcify and to fix large amounts of carbon (Hoegh-Guldberg 2005). Rapid calcification by corals plays a critical role in coral reef function, because it leads to the formation of a massive, wave resistant framework that provides habitat for many taxa (Idjadi and Edmunds 2006). Understanding the factors controlling coral calcification is critical to project coral community structure into the a future strongly impacted by global climate change (GCC), and to elucidate the implications of global climate change (GCC) for the taxa that rely on coral structure as habitat.

Already, many coral reefs are in a state of decline (Hoegh-Guldberg et al. 2007) due in large part to the effects of multiple anthropogenic disturbances acting locally (Jackson et al. 2001) as well as regionally (Hoegh-Guldberg et al. 2007). Predictions of the biotic effects of GCC have identified temperature and partial pressure of atmospheric CO_2 (pCO_2) as factors with serious implications for marine ecosystems, specifically through increasing acidity of seawater (i.e., ocean acidification, or OA), and rising ocean temperatures (IPCC 2007). Current pCO_2 levels of ≈350-380 ppm are predicted to exceed 700 ppm by the year 2100, if "business-as-usual" CO_2 emissions are maintained (IPCC 2007). Such a dramatic increase will cause substantial declines in the pH, aragonite saturation state (Ω_a), and carbonate (CO_3^{2-}) concentrations of seawater, which in turn, will depress coral calcification (Hoegh-Guldberg et al. 2007). Multiple studies have shown a strong and positive relationship between coral calcification and Ω_a (Langdon 2000), with declines in Ω_a depressing coral calcification by 20-40% for a doubling of pCO_2 from current levels (Hoegh-Guldberg 2005).

In addition to the interest in the consequences of OA for corals, the effects of rising temperature have also attracted attention, with the majority of this effort focusing on thermal bleaching (Brown 1997). Surprisingly however, few studies have simultaneously tested the effects of increases in temperature and declining pH (OA) on coral calcification, and in those studies that have, conflicting results have been obtained. For instance, one study found that high temperature and low pH acted in positive synergy to further depress coral calcification (Reynaud et al. 2003), while another reported that coral calcification under summer temperatures (27 ºC) was less affected by pH than under winter (23 ºC) temperatures (Langdon and Atkinson 2005). Potentially, some of the complexity

in the synergistic effects of temperature and OA on coral calcification is a result of the tight association between calcification and photosynthesis (McConnaughey and Whelan 1997), such that the effects of temperature and OA on calcification might not be independent of the effects on photosynthesis (Gattuso et al. 1999).

The objective of this study was to explore the interactive effects of high temperatures and high CO_2 on the calcification and photosynthesis of corals. We selected *Porites rus* and *Pocillopora meandrina* for this analysis because these species are common in the lagoon of Moorea, French Polynesia, where they form branching colonies that are tractable to manipulation. A microcosm system was used to expose the corals to either ≈27.3 ºC or ≈29.4 ºC at pH levels of 7.8 or 8.2, with the low temperature falling close to the ambient temperature (28.4 ºC) when the experiment was conducted (April 2007), and the high temperature being close to maximum temperature occurring in the lagoon (29.9 ºC); the two Ω_a levels represent present day conditions and those expected within ≈100 years (IPCC 2007).

Methods

Incubations were conducted for 2 weeks in April 2007 using a design in which corals were placed into one of four tanks (each 135 l) that created two crossed levels of temperature and Ω_a. The outcomes of the incubations were assessed through calcification and photosynthetic efficiency (maximum dark-adapted quantum yield, F_v/F_m), and were analyzed with a Model I, two way ANOVA. The statistical replicates were coral branches collected from *Porites rus* and *Pocillopora meandrina* growing at 1-2 m depth in the lagoon of Moorea. For each species, one branch (3.5–6 cm long) was collected from each of 44 colonies that were separated by 3-5 m to increase the likelihood that they were unique genotypes. Following collection, the fragments were transported to the lab and prepared as nubbins by attaching them to plastic bases with epoxy (Z-Spar A788). Nubbins were then placed in flowing seawater for 12 h and monitored visually for signs of damage. Healthy nubbins from each species were assigned randomly to one of the four incubation tanks, such that every tank contained equal numbers (n = 11) of *P. rus* and *P. meandrina*. Nubbins were acclimated to tank conditions for 24 h at a mean temperature of 28.4 ± 0.5 ºC (± SE, n = 54), a mean pH of 8.2 ± 0.1 (± SE, n = 76), and a constant mean light level of 480 ± 87 µmol photons m^{-2} s^{-1} (n = 12) provided with lamps (described below).

During treatments, nubbins were maintained in tanks located indoors, with each fitted with a chiller, heater, and pump that mixed and aerated the seawater. The seawater was replaced partially each day (20 % d^{-1}) with unfiltered seawater collected from the lagoon. Two 1000 W metal halide lamps (Sylvania BT37, Metalarc) were suspended 0.5 m above the tanks to provide light intensities of 797 ± 14 µmol photons m^{-2} s^{-1} (± SE, n = 148, measured with a Li-Cor LI 193SA) on a 12:12 light:dark cycle. The light intensity was selected to be less than the intensity at noon (1,720 ± 370 µmol photons m^{-2} s^{-1}, n = 17) at the collection depth when the experiment was conducted. Each tank was fitted with a microprocessor-controlled regulator (Neptune Systems) that operated the heaters, chillers, and pH regulation system to maintained temperatures with a resolution of ± 0.2 ºC, and pH with a resolution of ± 0.1 unit. Using this system, the two tanks at the control temperature were maintained at 27.2 ± 0.4 ºC and 27.5 ± 0.3 ºC (± SE, n = 236 and 246, respectively), and the two at the high temperature were maintained at 29.6 ± 0.2 ºC and 29.2 ± 0.4 ºC (mean ± SE, n = 290 and 249, respectively).

The pH of the tanks was controlled by adding dilute HCL or NaOH, using pumps operated by the regulators. The pH was logged using pH electrodes (Orion 9156 BN-WP, calibrated every 12 h using NBS buffers) attached to the regulators, and the output of the electrodes was used indirectly to operate the pumps adding acid or alkali. Using this system, the tanks at control pH were maintained at mean levels of 8.17 ± 0.01 (±SE, n > 278), and the treatment tanks were maintained at mean levels of 7.81 ± 0.05 (± SE, n = 587), and 7.82 ± 0.06 (± SE, n = 587). In addition to monitoring the pH, DIC parameters (Table 1) were assessed every 2-3 d by Gran titrations that were used to calculate total alkalinity (TA); TA and pH were used to calculate Ω_a using the CO2SYS program (Lewis and Wallis 1998) and the NBS buffer scale. The accuracy of the TA values was assessed using Certified Reference Materials (from Scripps Institute of Oceanography), which revealed downward discrepancies of 6% for TA and 11% for total DIC.

Calcification was assessed as the change in mass of the carbonate skeleton as determined by buoyant weighing, and was normalized to the coral area (µg mm^{-2} d^{-1}) determined by wax dipping (Stimson and Kinzie 1991). The maximum dark-adapted quantum yield of PSII (F_v/F_m) was measured before and after incubation for *Pocillopora meandrina* and *Porites rus* using a pulse-amplitude-modulation fluorometer (Diving PAM, Walz, GmbH) that was maintained at constant instrument settings for each species. Based on the results from previous studies, corals were dark adapted for ≥ 3 h prior to measuring F_v/F_m.

The results were analyzed using a two-way ANOVA, in which temperature and pH were fixed factors, and nubbins were statistical replicates. To

gain insight into the variation in calcification between each pH level within each temperature level (i.e., where a significant interaction was detected), a Student's t-test was subsequently applied, but as this represented an unplanned post hoc analysis, the results should be interpreted with caution. The statistical assumptions of normality and homoscedascity were tested through graphical analyses of the residuals, and all statistical tests were accomplished using JMP software (Version 7, SAS Institute Inc) running in a Windows environment.

Results

Of the 44 *Porites rus* nubbins used in the experiment, all appeared healthy throughout the incubations and, although slight paling did occur in all tanks, this was not associated with significant changes in F_v/F_m as compared to freshly collected corals ($p > 0.90$). *Pocillopora meandrina* appeared to be more sensitive to the incubation conditions, with some nubbins showed paling in the control and treatment for both pH and temperature conditions.

Calcification rates

For *Porites rus*, mass increased by 33 to 110 µg mm^{-2} over the 14 d incubation in all treatments (Fig. 1), and growth was affected significantly by a temperature x pH interaction ($F = 17.554$, df = 1,39, $p < 0.001$), as well as pH ($p < 0.001$), but not temperature ($p = 0.150$). At 27 °C, mean calcification rates were reduced 70% from 7.9 ± 0.8 µg mm^{-2} d^{-1} (± SE, n = 10) at pH 8.18, to 2.4 ± 0.7 µg mm^{-2} d^{-1} (± SE, n = 11) at pH 7.80, and this difference was significant ($t = 6.768$, df = 19, $p < 0.001$). At 29 °C, mean calcification rates were unaffected by pH ($t = -0.219$, df = 20, $p = 0.828$), and they changed only slightly from 6.0 ± 0.6 µg mm^{-2} d^{-1} (mean ± SE, n = 11) at pH 8.18, to 6.3 ± 0.9 µg mm^{-2} d^{-1} (mean ± SE, n = 11) at pH 7.80.

For *Pocillopora meandrina*, four nubbins (9% of the total) were removed from the analyses of calcification rate because they lost weight and displayed low values of F_v/F_m (< 0.500) that are indicative of poor health. Calcification rates for this species were also affected significantly by a temperature x pH interaction ($F = 4.819$, df = 1,36, $p = 0.034$), but neither of the main effects were significant alone ($p \geq 0.805$). At 27 °C, mean calcification rates were reduced 50% from 1.8 ± 0.3 µg mm^{-2} d^{-1} (± SE, n = 11) at pH 8.18, to 1.0 ± 0.1 µg mm^{-2} d^{-1} (± SE, n = 10) at pH 7.80, but this trend was not significant ($p > 0.05$). At 29 °C, the direction of the pH effect on calcification was reversed, with mean rates doubling from 1.0 ± 0.6 µg mm^{-2} d^{-1} (± SE, n = 9) at pH 8.18, to 2.0 ± 0.5 µg mm^{-2} d^{-1} (± SE, n = 11) at pH 7.80, although this trend was also not significant ($p > 0.05$).

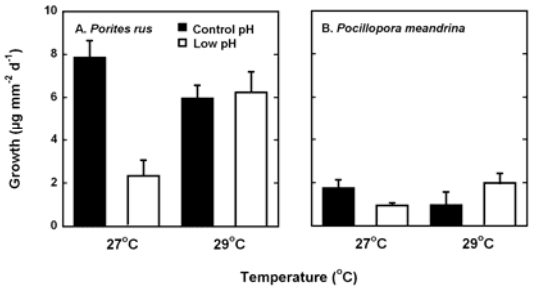

Figure 1. Effect of temperature and pH on the growth (µg mm^{-2} d^{-1}) of (A) *Porites rus,* and (B) *Pocillopora meandrina* during a 14 d incubation under combinations of control pH (8.2) and low pH (7.8); values shown are mean ± SE (n = 9-11 for each temperature and pH combination). Calcification was affected significantly by an interaction between temperature and pH in *P. rus* ($p < 0.001$) and *P. meandrina* ($p = 0.034$), largely because it was depressed by low pH at 27 °C, but not 29 °C.

Maximum dark-adapted quantum yield

The mean F_v/F_m values for all corals before treatment for *Porites rus* (0.573 ± 0.005, mean ± SE) and *Pocillopora meandrina* (0.631± 0.004, mean ± SE) were not significantly different ($p > 0.05$) from the average values in the control tanks after treatment (0.585 ± 0.012 and 0.640 ± 0.011, respectively, mean ± SE, Fig. 2), nor were any treatment combinations of temperature and pH significantly different from each other ($p > 0.05$). For *P. rus*, F_v/F_m was unaffected by pH ($F = 0.971$, df = 1,40, $p = 0.330$), and temperature ($F = 0.428$, df = 1,40, $p = 0.516$), and there was no interaction between the two ($F = 0.587$, df = 1,40, $p = 0.447$). A similar result was obtained for *P. meandrina,* with F_v/F_m again unaffected by pH ($F = 0.022$, df = 1,38, $p = 0.881$), temperature ($F = 0.278$, df = 1,38, $p = 0.600$), or the interaction between the two ($F = 1.108$, df = 1,38, $p = 0.299$).

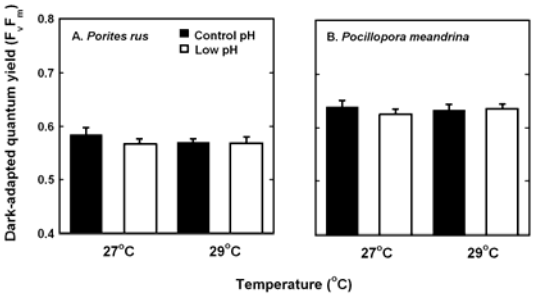

Figure 2. Maximum dark-adapted quantum yield of PSII (F_v/F_m) of (A) *Porites rus,* and (B) *Pocillopora meandrina* incubated for 14 d at control pH (8.2) and low pH (7.8); values shown are means ± SE, n=10-11 nubbins for each treatment combination. For both species, F_v/F_m was unaffected by pH ($p > 0.330$), temperature ($p > 0.516$), and the interaction between the two ($p > 0.299$).

Parameter	Control pH	Treatment pH
pH (NBS)	8.18 ± 0.01	7.80 ± 0.01
Alkalinity (μEquiv kg^{-1})	2143 ± 68	1638 ± 66
pCO_2 (μatm)	395 ± 40	720 ± 205
Total Carbon (μmol kg^{-1})	2010 ± 41	1692 ± 40
CO^{2-}_3 (μmol kg^{-1})	237 ± 11	126 ± 47
$\Omega_{aragonite}$	3.8 ± 0.2	2.0 ± 0.7
	n=8	n=6

Table 1. Characteristics of the seawater in the four tanks over the 14 d experiment; all values are means ± SE.

Discussion

The effects of high temperature and OA on coral reefs are predicted to compromise calcium carbonate accretion, reduce coral diversity, and weaken the carbonate reef framework (Hoegh-Guldberg 2005). The likelihood of such outcomes is increased by the lack of evidence demonstrating that corals can acclimatize to the effects of OA (Langdon et al. 2000). Nevertheless, there is good reason to suppose that corals exhibit the same kinds of abilities to acclimatize as most eukaryotes (Gates and Edmunds 1999), and for rising temperature there is evidence that some corals can acclimatize to warmer conditions (Jones et al. 2008). Thus while there is a consensus regarding the negative implications for corals of increasing CO_2 and rising temperature (Hoegh-Guldberg et al. 2007), at least when the factors act in isolation, there is less certainty over their interactive effects (Kleypas and Langdon 2006). In order to make accurate predictions of the responses of coral reefs to GCC, experimental manipulations on short time scales are required to understand the potential for interactive effects of CO_2 and temperature.

Utilizing mesocosms, the present study examined the effect of OA on calcification and photosynthesis of *Porites rus* and *Pocillopora meandrina* under two different temperature regimes predicted to occur in the next 100 years (IPCC 2007). An interesting outcome of these analyses is the demonstration that a decrease in pH interacts with small differences in temperature to decrease growth at 27.3 ^{o}C, but not at 29.4 ^{o}C; this effect was striking for *P. rus*, but was less developed in *P. meandrina*. These finding differ from those of Reynaud et al. (2003), who found that the inhibitory effect of elevated CO_2 (760 ppm versus 460 ppm) on the growth of *Stylophora pistillata* were accentuated at higher temperature (28 ºC versus 25 ºC). However, Reynaud et al. (2003) also found no inhibitory effect of high CO_2 at 25 ºC, which is inconsistent with previous studies that have reported inhibitory effects of high CO_2 on coral calcification over a range of temperatures (23 - 27 ºC) (Kleypas and Langdon 2006). However, Langdon and Atkinson (2005) reported findings that are similar to the present study, principally by showing indirectly that higher temperature may alleviate the effects of increased CO_2 on coral calcification. Importantly, they found that a reduction in pH of 0.22-0.28 depressed the calcification of *Montipora capitata* and *Porites compressa* by ≈80% during the cool winter, but by only ≈40% in the warm summer, although the net effect of temperature on the relationship between Ω_a and calcification was not significant (Langdon and Atkinson 2005). The present findings could have biological significance as they suggest that elevated temperature can mitigate the effects of rising CO_2, at least for some corals over a short period.

In the context of evaluating how temperature might alleviate the effects of high CO_2 on coral calcification, it is noteworthy that temperature has numerous biological effects that operate in a biphasic pattern, with rising temperature stimulating processes to a threshold value, and inhibiting them beyond this point. For corals, rising temperatures stimulate enzyme activity (Marshall and Clode 2004) and metabolic rate (Edmunds 2005), and drives the classic bell-shaped relationship between calcification and this physical factor (Marshall and Clode 2004) with typical threshold temperatures of 26-28 ºC (Buddemeier and Kinzie 1976). Together, these results suggest that the temperature manipulations used in this study might not have surpassed the threshold value for calcification in *Porites rus* and *Pocillopora meandrina* in the warm lagoon of Moorea, perhaps because acclimatization to local conditions (Jones et al. 2008) has lead to higher threshold temperatures in this location. With a high threshold temperature for calcification (e.g., > 29 ºC), the effects of the temperature increase in the present study may have been sufficient to offset the effects of carbonate limitation caused by low pH, at least temporarily.

The lack of a significant effect of temperature and pH on F_v/F_m indicates that the high temperature treatment (29 ºC) in this experiment did not reduce photosynthetic efficiency in photosystem II (PSII) when compared to the control temperature treatment (27 ºC). Although temperatures above 29 ºC can reduce photosynthesis in hermatypic corals (Coles and Jokiel 1977), there is considerable evidence demonstrating that the severity of thermal stress is best measured by its magnitude relative to the local seasonal maximum temperature (Jones et al. 2008), with increases of 1 to 2 ºC above this value typically serving as reliable predictors of photosynthetic damage (Hoegh-Guldberg et al. 2007). In the lagoon of Moorea, the maximum temperature recorded at the site of collection is 29-30 ºC, and therefore the high temperature used in the present study may have been too low to illicit a negative response in photosynthesis in *P. rus* and *P. meandrina*. In this context, the

absence of a pH effect might be construed as preliminary evidence that more acidic conditions do not enhance the sensitivity of F_v/F_m to high temperature.

Acknowledgement
This project was funded by grant OCE 04-17412 from the National Science Foundation, gifts from the Gordon and Betty Moore Foundation, and CSUN. This is a contribution of the Moorea Coral Reef LTER Site, and is contribution number xxx of the Marine Biology Program of CSUN.

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