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Response of Corals *Acropora pharaonis* and *Porites lutea* to Changes in pH and Temperature in the Gulf

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Abstract: Coral reefs are harboring a large part of the marine biodiversity and are important ecosystems for the equilibrium of the oceans. As a consequence of anthropogenic CO₂ emission, a drop in pH and an increase in seawater temperature is observed in the Gulf coastal waters that potentially threaten coral assemblages. An experimental study was conducted on two species of corals to assess the effect of ocean warming and ocean acidification on the net calcification rate. Two pH conditions 8.2 and 7.5 and three temperatures, 22.5, 27.5 and 32.5 °C, were considered. Net calcification rates were measured using ⁴⁵Ca radiotracer. Both temperature and pH had a significant effect on net calcification rates following a similar pattern for both species. The highest calcification rate was observed at low temperature and high pH. Increased temperature and decreased pH led to a decrease in net calcification rates. An interactive effect was observed as the effect of pH decreased with increasing temperature. However, the two species of coral were able to calcify in all the tested combination of temperature and pH suggesting that they are adapted to short term changes in temperature and pH. Ability to calcify even at a high temperature of 32.5 °C that is identical to the summertime Gulf seawater temperature under both the ambient and low pH condition with no mortalities, raises a question: are these corals adapted to high seawater temperatures and low pH? More in-depth assessments will be required to confirm if this is an adaptation to higher temperatures in Persian Gulf corals.

Keywords: CO₂ emission; ocean warming; ocean acidification

1. Introduction

Coral reefs are crucial ecosystems for the equilibrium of the oceans. Reefs harbor large marine biodiversity and are environmentally and economically significant to most of the countries that contain them. A large number of studies have investigated the physiological response of calcifying organisms to environmental changes and have highlighted how these extremely delicate systems (coral reefs) are affected by anthropogenic activities [1] such as warming and acidification [2].

Persian or Arabian Gulf experiences extreme temperature and salinities due to its subtropical location and hyper-arid climate [3–5]. In this study, the term Gulf will be used for the area. In recent years a drop in the seawater pH and increase in seawater temperature was observed in the Gulf [5]. Increased water temperature hampers the ability of coral to maintain their symbiosis [6]. Seasonal and reversible bleaching of corals is observed in Kuwait [7] and it is likely that with further increase in temperature and decrease in pH, these bleaching events would increase and may become irreversible.

The change in the seawater carbonate chemistry associated with ocean acidification is leading to mounting concern about the potential impact on tropical reef-building corals to maintain calcium carbonate (CaCO_3) skeletons and the diverse species that depend upon them [8]. Calcification is only one of the physiological processes that might be affected by acidification [9]; however, it has been the most studied. It has been documented that a decrease in pH from 8.07 to end-of-century projected levels (7.82) might result in a 20–40% decline in coral calcification [10]. This pH level already exists during short durations in the Gulf.

Studies show a consistent decline in coral and coralline algae calcification rates as a result of increasing ocean acidification [11]. Temperature and salinity extremes in many areas do not allow corals to form extensive frameworks of enough coral density that could be cemented into solid reefs [12,13]. The most important reef-building coral in the Gulf is *Acropora*, and it dominates the shallowest areas. *Porites* are the next most dominant corals in the Gulf.

This study was designed to test the effect of decreased pH and increased temperature on calcification rates of two dominant coral species in the Gulf. Experiments were carried out to test the impact of pH and temperature on survival and calcification of *Acropora pharaonis* and *Porites lutea*. The corals were cultured under three different temperatures (22.5, 27.5 and 32.5 °C) and two different pH conditions (8.2 and 7.5). These temperatures cover the current summer temperature range. The average pH observed in the Gulf is 8.2, while 7.5 correspond to a 0.4 pH unit decrease from the minimum pH value recorded in the Gulf.

2. Materials and Methods

Coral samples were collected from south of the Kuwait territorial waters during August 2016. Two species of corals *Acropora pharaonis* and *Porites lutea* were collected and transported in seawater to the laboratory. These corals were transplanted into 1000 L tank and acclimatized for two weeks, where the average seawater temperature was 28.0 °C and an average pH of 8.18. Small healthy fragments were collected (20–25 polyps) and hung using fishing line into the experimental tanks and exposed to different experimental conditions. The net coral calcification (calcification – dissolution) rates were measured using ^{45}Ca [14]. Six fragments of live coral for each treatment and a fragment of dead coral was added to each 55 L tank, with two replicates of each treatment. Eight fluorescent tubes (40 W) were used following a 12 h light-dark cycle mimicking a typical daytime in the region during most of the year. The temperature was increased using a Schego titanium tube 50-watt heater in each tank. The pH was controlled using the IKS aquastar system. pH was decreased by bubbling CO_2 into the aquaria. Carbonate was added to the high pH aquariums to maintain the pH constant at 8.2. The pH, alkalinity, salinity, and temperature were measured twice a day in each aquarium. pH was measured using Metrohm 914 pH meter calibrated on the nbs scale, and alkalinity using Hanna HI84531-01 alkalinity titrator and Lachat 8700 Flow injection analyzer. Other parameters (pCO_2 , saturation states for calcite (Ω_{calc}) and aragonite (Ω_{ar})) were calculated using pH, total alkalinity, temperature and salinity using CO_2sys [15,16].

The net coral calcification was measured using the ^{45}Ca technique [14]. All aquaria were spiked with $^{45}\text{CaCl}_2$ (with the activity of 50 Bq mL^{-1}) for 72-h. After exposure, each sample was immersed for 30 s in 1 L of filtered seawater, then rinsed five times with 5 mL of ice-cold glycine. Corals were dissolved using 1–5 mL of IM NaOH at 90 °C for 30 min to separate the zooxanthellae from the skeleton. The NaOH soluble fraction was collected, and the skeleton was rinsed with 1 mL of distilled water and dried, before addition to NaOH solution and later with filtered seawater five times. The 37% HCl was added to the skeleton sample in increments of 0.5 mL until the sample was completely dissolved. The sample was evaporated at <90 °C until completely dry. Distilled water was added to the sample and 10 mL Ultima Gold-XR cocktail. The sample was kept for an hour before the amount of ^{45}Ca deposited was measured using Quantulus 1220 LSC. The dead specimen was similarly measured to assess the adsorption of ^{45}Ca , and the amount of ^{45}Ca uptake by dead specimens (covered with tissue) was subtracted from the amount measured in intact (live) specimens to obtain the net calcification.

Net calcification was calculated from the activity recorded in skeleton (digest) and seawater control samples and normalized to $\mu\text{mol CaCO}_3$ per skeleton dry weight using the formula:

$$\text{Calcification } (\mu\text{mol CaCO}_3\text{g}^{-1}\text{d}^{-1} \text{ dry skeleton}) = \frac{\text{Activity}_{\text{sample}} \times \frac{1.17}{\text{Activity}_{\text{seawater}}}}{\text{Weight} \times \text{Time}} \quad (1)$$

where $\text{Activity}_{\text{sample}}$ is the total counts per minute (CPM) in the dissolved skeleton sample; $\text{Activity}_{\text{seawater}}$ is the total CPM in 100 mL seawater sample (control); 1.17 is the amount of Ca^{2+} in 100 mL ambient seawater (in μmol); Weight is dry skeleton weight (in grams) and Time is the incubation duration (in days).

Each mean calcification value is expressed with its standard error of the mean (mean \pm SEM). Three-way analysis of variance (ANOVA) was used for each species to test the effect of pH and temperature as fixed factors, and tanks (replicates) as a random factor nested within fixed factors on calcification rates and carbonate chemistry parameters (pH, $p\text{CO}_2$ and saturation states). As interactions between temperature and pH were observed for calcification rates, post-doc Scheffe tests were performed to test the difference between temperature for each tested species and pH treatment. A Shapiro-Wilk test was used to confirm that the data were normally distributed. All the data were analyzed using SAS/STAT software, version 9.2 (SAS Institute, Cary, NC, USA).

3. Results

The carbonate chemistry in the experimental system is summarized in Table 1. pH was the controlled parameter and was kept constant using IKS aquastar computers as well as the addition of carbonates in controls. As a consequence, there was a significant difference between the nominal pH values, but no difference between temperatures, their interaction or replicates. For the calculated parameters ($p\text{CO}_2$, Ω_{calc} , and Ω_{ar}) significant differences were also observed between the nominal pH, but there were also significant effects of temperature and the interaction between pH and temperature. These effects can be explained by the impact of temperature on CO_2 solubility and the slight difference in alkalinity between the two tested pH (High pH: salinity 43.6; alkalinity 2792 ± 20 ; Low pH: salinity 43.4; alkalinity 2582 ± 10). These differences between temperatures within pH values are rather small compared to the difference between pH and shall not translate into a significant difference in calcification rates. There were no significant difference between replicates.

Table 1. Carbonate chemistry—measured pH and calculated $p\text{CO}_2$, saturation state for calcite (Ω_{calc}) and aragonite (Ω_{ar}) (means \pm standard error of the mean (SEM)) using pH, temperature, salinity, and alkalinity (see Methods). Results of a three-way analysis of variance (ANOVA 3) testing the impact of nominal pH, temperature, their interaction and replicates.

Nominal pH	Temperature [°C]	pH _{nbs}	$p\text{CO}_2$ [atm]	Ω_{calc}	Ω_{ar}
8.2	22.5	8.18 \pm 0.003	451 \pm 6	6.48 \pm 0.05	4.24 \pm 0.04
	27.5		437 \pm 5	7.21 \pm 0.04	4.80 \pm 0.03
	32.5		447 \pm 6	7.81 \pm 0.06	5.29 \pm 0.04
7.5	22.5	7.50 \pm 0.002	2453 \pm 16	1.54 \pm 0.01	1.01 \pm 0.01
	27.5		2525 \pm 28	1.75 \pm 0.02	1.17 \pm 0.01
	32.5		2688 \pm 18	2.00 \pm 0.01	1.36 \pm 0.01
ANOVA 3					
Model (F _{11,83} , p)		4015.9	2422.5	2751.1	2763.4
pH (F ₁ , p)		<0.0001	<0.0001	<0.0001	<0.0001
Temperature (F ₂ , p)		44157.0	26529.3	29578.1	29454.0
pH x temperature (F ₂ , p)		<0.0001	<0.0001	<0.0001	<0.0001
Replicate (F ₆ , p)		1.39	28.38	272.2	372.2
		0.26	<0.0001	<0.0001	<0.0001
		0.63	28.95	65.52	95.0
		0.54	<0.0001	<0.0001	<0.0001
		0.38	0.47	1.47	1.45
		0.89	0.83	0.20	0.21

No mortality or bleaching was observed over the course of the experiment. However, temperature and pH had the same effect on the calcification of the two species of corals. The highest net calcification was observed at high pH and low temperature (Figure 1). For both species, there was a significant effect of pH, temperature and their interactions, but no difference between replicates (Table 2). At high pH values, the net calcification rate decreased linearly with increasing temperature with a ~80% decrease at 27.5 °C and a ~60% decrease at 32.5 °C as compared to 22 °C. The effect of decreased pH was the strongest at 22.5 °C (~64% decrease in net calcification rate) as compared to other temperatures (~85% decrease in net calcification rate). For both species, Scheffe's tests revealed that all temperature treatments were significantly ($p < 0.01$) different from each other at pH 8.2. However, no significant difference was observed between 22.5 and 27.5 °C at pH 7.5, while calcification rates at 22.5 and 27.5 °C were significantly higher than at 32.5 °C.

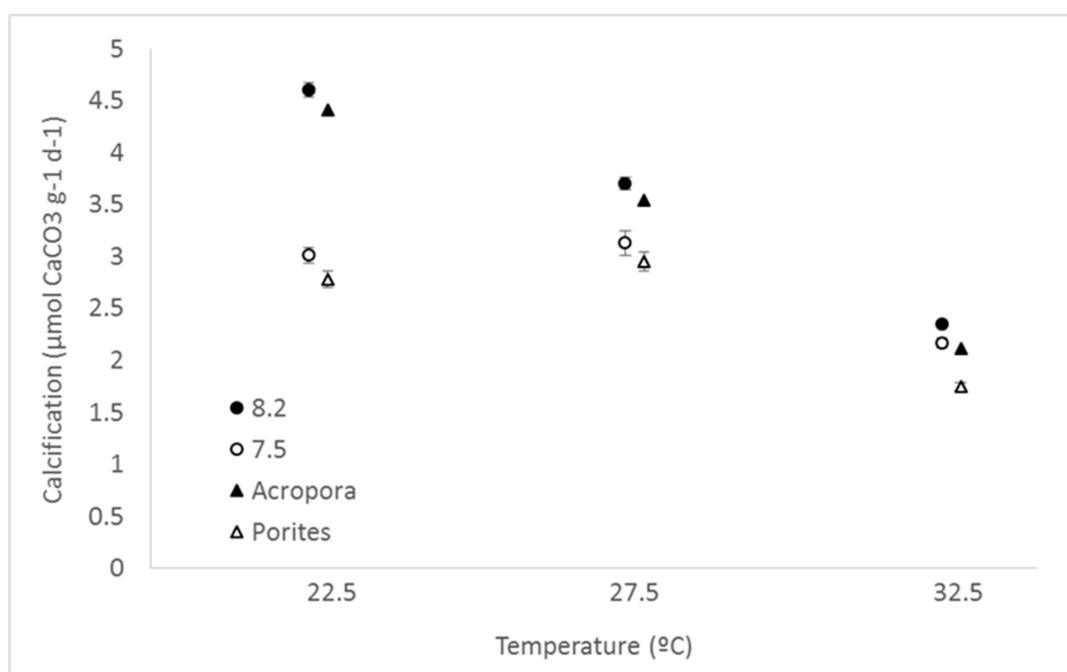


Figure 1. Effect of temperature and pH on net calcification rates ($\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ d}^{-1}$) in two coral species (*Acropora pharaonis* and *Porites lutea*).

Table 2. ANOVA 3 testing the impact of pH, temperature and replicates on net calcification rates ($\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ d}^{-1}$) in two coral species (*Acropora pharaonis* and *Porites lutea*).

	F	P
<i>Acropora pharaonis</i>		
Model	$F_{11,35} = 74.3$	<0.0001
pH	$F_1 = 190.0$	<0.0001
Temperature	$F_2 = 258.4$	<0.0001
pH \times Temperature	$F_2 = 51.0$	<0.0001
Replicate	$F_6 = 1.41$	0.25
<i>Porites lutea</i>		
Model	$F_{11,35} = 114.71$	<0.0001
pH	$F_1 = 298.4$	<0.0001
Temperature	$F_2 = 415.6$	<0.0001
pH \times Temperature	$F_2 = 61.83$	<0.0001
Replicate	$F_6 = 1.44$	0.24

4. Discussion

Our results show that net calcification was reduced under increased temperature and decreased pH in both tested coral species. The impact of pH is modulated by temperature, is strongest at low temperatures (~64% decrease in net calcification rate) and is less pronounced at a higher temperature (~85% decrease in net calcification rate).

The net calcification results from *Acropora pharaonis* and *Porites lutea* are similar to the response reported for *Siderastrea siderea*, where a parabolic response of temperature and acidification was observed for net calcification [17]. Several species of corals in Hawaii are reported to grow slower in winters and faster in summer when the temperature is around 27 °C [18,19] and a significant reduction in calcification rates is observed at a higher temperature. In the Gulf, the temperature during the summer month of August and September can reach ~33 °C in northern Gulf [1], with a variation between summer and winter temperature of about 20 °C [20,21].

Earlier workers have reported adaptation among *Acropora downingi*, *Acropora clathrata*, *Acropora valenciennesi*, *Cyphastrea microphthalma*, *Favia pallida*, and *Platygyra daedatea* to extreme environmental conditions in the southern Gulf [22]. Our results show that the two tested coral species showed a decreased net calcification with no mortalities even at the highest temperature and lower pH, simulating the year 2100 as per IPCC RCP 8.2 in the Gulf. This suggests local adaptation to the extreme environment among these corals in the northern Gulf similar to the southern Gulf corals. Adaptation to increased temperature in the Gulf corals has also been reported [23–25], the best growth in southern coral species was reported during the month of April, where the temperature is in the 21–23 °C range. The northern Gulf corals that we tested also showed the highest net calcification at a temperature of 22.5 °C, suggesting similarity within the Gulf coral temperature preference. *Acropora* species have suffered an extensive loss during the 1996–1998 warming [12] but recovered substantially in a decade [26]. During the 1998 coral bleaching, about 75–95% of the most dominant coral *Stylophora* at eight sites around Mirba, southern Oman was bleached [27]. The temperature during this event was between 29.5 and 31.5 °C [28] and upwelling conditions prevailed, which would have brought low CO₂ water. However, the experimental results from this study suggest that there was net calcification at all the three tested temperatures from 22.5 to 32.5 °C, even at low pH conditions, suggesting that the northern Gulf corals are probably more resilient to corals outside the Gulf.

Reduced survival and mortality of *Lithophyllon repanda* were reported as a result of temperature increase [29], whereas others have reported higher tolerance to temperature and ocean acidification in *Fungia fungites* and *Lithophyllon repanda* [30] larvae. It is interesting to note that these observations in the different response from different regions may be due to trans-generational exposure of these species to a specific set of environmental conditions.

The northern Gulf coral species that have been tested in this experiment also suggest resilience at higher temperatures and lower pH, probably due to their long exposure to the extreme temperature and fluctuating pH in coastal waters. However, additional work is needed to understand acclimation and adaptation in this population. Most of the experimental work carried out thus far have researched short term exposure and longer-term experiments are needed to capture acclimation and transgenerational effects.

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