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CORAL CALCIFICATION IN THE SOUTHERN PART OF VIETNAM, STUDIED WITH A NEW METHOD

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ABSTRACT: Ocean and coastal acidification has been recognized as one of emerging concerns due to the challenges for marine ecosystems. However, very few researches have been done on this issue in Vietnam. This study aims to (1) present an overview of acidification situation (spatial and temporal variation) at coral reefs, and (2) initially assess the possible influence on calcification and growth rate of coral. High accuracy methods were used to determine acidification related parameters, and a new method, which lets coral grow naturally, was tried and developed for coral calcification and growth rate estimation. The result showed that the highest pH mean value was 8.1223 ± 0.0944 (Phu Quy islands area), and the highest aragonite saturation state (Ω) mean value was 4.02 ±0.38 (Binh Thuan skerries) among offshore reefs. For coastal reef, the highest values of pH and Ω were 8.1298 ± 0.0539 and 3.35 ± 0.27 at Nha Trang bay area. On the other hand, all Ω values at offshore Southern reefs were lower than 3, which level would make coral and other calcareous organisms stressed. Temporal changes of pH and Ω values did not show a significant trend during Feb 2019–May 2020, although the study site was in a strong upwelling area. Growth rate in length of coral in this research did not seem to have a clear trend and neither relative to the pH and Ω conditions. However, this study gave some suggestions for method improvement via practical experiments.

Keywords: Ocean and coastal acidification, aragonite saturation state (Ω), pH value, calcification, coral growth rate

INTRODUCTION

Our oceans have absorbed about one-third of the discharged CO_2 since the industrial revolution. This huge amount of CO_2 has caused global changes in the chemistry of seawater, especially in the carbonate buffer system (Feely *et al*. 2004). According to geological data, this acidification has been happening at an unprecedented rate for at least 50 million years (Gattuso and Hansson 2011). The increased acidity may harm or help individual marine species in different ways, *i.e*., some organisms are likely to become more abundant, and others less so (EPA 2017). For calcareous organisms (calcifiers) such as corals, molluscs, *etc*., lowered pH in seawater makes it more difficult for them to synthesize calcium, and the existing calcium carbonate structure becomes susceptible to breakdown (Gattuso *et al*. 1998). It is predicted that ocean biodiversity will face two major challenges in the near future: temperature rising and acidification (Doney *et al*. 2009). At different levels, climate change, including acidification, will affect the adaptation of marine ecosystems, which supports the hypothesis that reducing biodiversity reduces stability and adaptation, and increases the risk of imbalances of marine ecosystems (Worm *et al*. 2006).

Changes of temperature, salinity, and acidification related parameters (especially pH) will lead to the changes of aragonite saturation state (Ω) of seawater. Under normal conditions, calcite and aragonite are stable in surface waters since the carbonate ion is at supersaturating concentrations. However, as pH falls, the concentration of carbonate ions required for saturation to occur increases, and when carbonate becomes unsaturated, structures made of calcium carbonate are vulnerable to dissolution. Therefore, even if there is no change in the rate of calcification, the rate of dissolution of calcareous material increases (Nienhuis *et al*. 2010). It is strongly pointed out that coral calcification responds to ambient Ω (Kleypas *et al*. 1999). Corals and other calcifiers are more likely to survive and reproduce when the Ω value is greater than 3. When this value decreases to below 3, these organisms become stressed, and when it is less than 1, shells and other aragonite structures begin to dissolve (SOS/NOAA). When Ω value was larger than 4, the coral samples calcified during night time, indicating an evidence of dark calcification (Ohde and Hossain 2004). Moreover, acidification and lower Ω affect not only coral calcification but also other biological functions (Doney *et al*. 2009; Erez *et al*. 2011; Chan and Connolly 2013; Comeau *et al*. 2014; Liu *et al*. 2018)

It is clear that ocean and coastal acidification has been recognized as one of emerging concerns due to the challenges for marine ecosystems. It was predicted that by 2050, the number of coral reefs would decline to 50%, and only 25% by 2100 in the South China Sea (named Bien Dong in Vietnam) (Blue Sea Vietnam). Unfortunately, there have been very few researches on this issue in Vietnam; most of the recent monitoring data of related parameters seem not to be comparable to existing acidification research systems due to lack of methods and approaches. Until 2011, there was still no publication (MoNRE 2011) and no improvement seemed to be forthcoming in the near future (Toan 2011). Hence, this study was carried out to (1) present an overview of the acidification situation (spatial and temporal variation) at coral reefs of South Vietnam, and (2) initially assess the possible influence on calcification of coral.

MATERIALS AND METHODS

Water samples were collected from 2018 to 2020 in three coastal areas (Nha Trang Bay, Ninh Thuan waters, and coastal Phu Quoc Islands), and six offshore or areas with less influence from freshwater runoff (Spratly Islands, Phu Quy Islands, Binh Thuan waters, Con Dao Islands, offshore Phu Quoc Islands, and Nam Du - Tho Chu area (Fig. 1). Totally, 55 water samples (30 offshore) at 28 sites (18 offshore) were analysed.

The pH of seawater was measured by a high accuracy method, using the indicator dye m-cresol purple according to the WESTPAC Standard Operation Procedures (SOPs) for ocean acidification research and monitoring (WESTPAC 2016; Dickson *et al*. 2007).

Temperature and salinity parameters were in situ measured by Seabird CTD (SBE 19 + V2) or YSI Multiparameter Meter (ProDSS). Aragonite saturation values (Ω) were calculated by the use of CO2sys software according to WESTPAC (2016)

Coral growth and calcification rate were investigated by a new method developed by us. The studied site is at Bai Nho in Ninh Thuan waters (Fig. 1C). Data from 8 months in 2019 (Feb, Jun, Jul, Aug, and Sep) and 2020 (Mar, Apr, and May) were used to assess the relationship between coral calcification and related acidification parameters. Corals of the species *Acropora muricata* (Linnaeus, 1758) and *Acropora robusta* (Dana, 1846) in nature were marked, measured, left for continued growth, and sampled. The method is as follows:

[1] Coral branches in nature were measured using a tube ruler and marked with a plastic drawstring at 5 cm length from the tip of the branch. Coral branches from healthy colonies were selected. They must be straight and without signs of branching or sprouting. The total number of branches per sampling should be 12–20 in order to cover the natural variation and allow for the detection of differences.

[2] After 30 days, the marked branches were collected and the new length measured (see Fig. 2). The difference is the growth in length of coral. Similar branches were collected as control samples for calcification accretion analyzing.

[3] After measuring and cutting, each coral branch was mashed, thoroughly mixed and dissolved in 20% HCl solution. Calcium content is titrated by EDTA method, following APHA (2017). Based on this, the calcium carbonate ($CaCO_3$) content was calculated.

[4] The calcification rate (mg/day) was calculated by subtracting the weight of calcium carbonate at the sampling from the initiation, and dividing by the number of days ($MCaCO_3$ after - $MCaCO_3$ before) / days.

Pearson correlation analysis tool of Microsoft Excel (2007 version) was used to test for correlation between coral calcification and related acidification parameters.

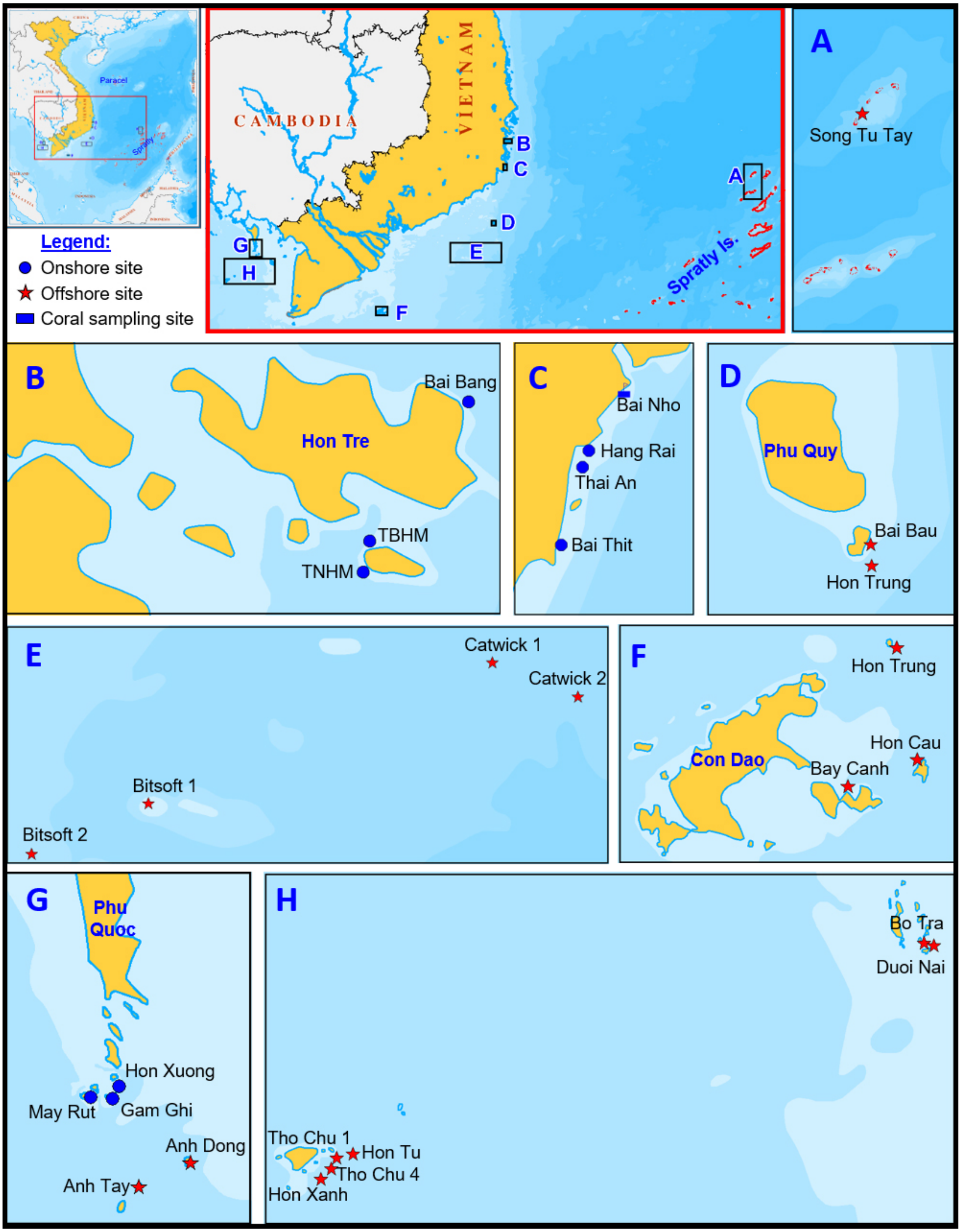

Figure 1. Sampling sites in the southern region of Vietnam (A: Spratly Islands; B: Nha Trang Bay; C: Ninh Thuan Waters; D: Phu Quy Islands; E: Binh Thuan skerries; F: Con Dao Islands; G: Phu Quoc Islands; H: Nam Du – Tho Chu Area)

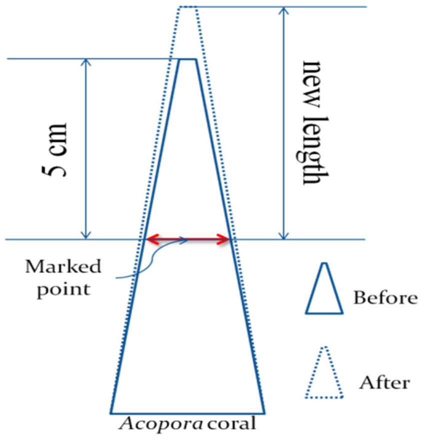

Figure 2. Schematic drawing of field growth experiment set-up. The solid triangle represents a tip of a coral branch, and the dotted line is the tip at the end of the experiment.

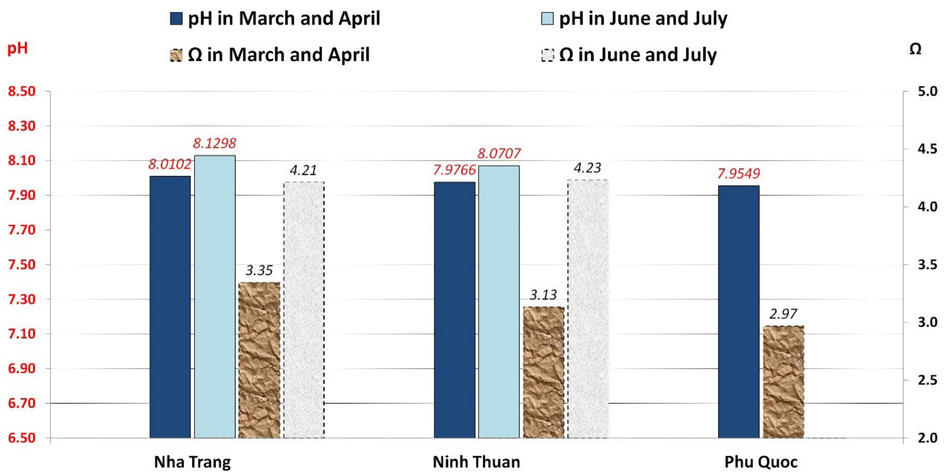

Figure 3. pH and Ω values variation at coastal reefs in late Northeast monsoon (March and April) and early Southwest Monsoon (June and July).

RESULTS

Spatial variation of pH values and acidification related parameters

Offshore areas: A total of 30 samples from 18 sites at 6 areas were collected in early South-West monsoon (in June 2018, and May 2019). From the results, it can be realized that there are not much difference between values at each area; standard deviations and min/max differences are quite low (Table 1).

At the southern offshore areas such as Con Dao, Phu Quoc, and Nam Du - Tho Chu (ND-TC), the pH (7.98–8.01) and Ω values (2.56–2.80) were slightly low. At the central areas, similar parameters seem better for coral and calcifiers, all Ω values in these areas were higher than 3 (only 1 minimum value at Phu Quy was 2.98 ~ 3).

***Coastal coral reefs*:** we collected 12 samples during late Northeast monsoon (March and April) in three areas Nha Trang bay (three sites), Ninh Thuan waters (three sites), and Phu Quoc coastal islands (three sites) (Fig. 1); and 13 samples during early Southwest Monsoon (June and July) in two areas Nha Trang and Ninh Thuan. A total amount of 25 samples was collected in 9 sites in such areas in 2018 and 2019.

Table 1. pH values and acidification related parameters at offshore coral reefs.

Area	*No. of sites*	*n*	Value	Temp. (°C)	Sal. (‰)	pH	Ω
Con Dao	*3*	*3*	**Mean**	**28.23**	**30.57**	**8.0135**	**2.80**
			std	0.05	0.02	0.0064	0.04
			min	28.20	30.55	8.0083	2.75
			max	28.30	30.60	8.0207	2.83
Offshore	*2*	*3*	**Mean**	**28.20**	**29.26**	**7.9832**	**2.56**
Phu Quoc	2	3	*Mean*	28.20	29.26	7.9832	2.56
			std	0.17	0.10	0.0196	0.09
			min	28.10	29.19	7.9612	2.46
			max	**28.40**	**29.38**	**7.9990**	**2.63**
Nam Du -			*std*	0.40	0.40	0.0461	0.24
Tho Chu	6	9	*Mean*	28.76	30.20	7.9952	2.71
			std	0.40	0.40	0.0461	0.24
			min	**28.00**	**29.48**	**7.8915**	**2.13**
			max	29.20	30.45	8.0672	2.91
Binh Thuan			*min*	29.80	31.50	8.0657	3.62
skerries	*4*	*8*	*Mean*	30.10	31.92	8.1191	4.02
			std	**0.20**	**0.40**	**0.0489**	**0.38**
			min	29.80	31.50	8.0657	3.62
			max	30.40	32.54	8.1926	4.60
Phu Quy	*2*	*5*	*Mean*	28.41	32.62	8.1223	3.60
			std	**2.29**	**0.96**	**0.0944**	**0.61**
			min	25.01	31.93	8.0163	2.98
			max	30.10	33.96	8.2615	4.61
Spratly	*1*	*2*	*Mean*	28.22	32.93	8.0598	3.87
			min	28.00	32.87	8.0344	3.37
			max	28.50	32.99	8.1081	4.29

As for the offshore data, the values were almost identical in each area, and pH as well as Ω values were higher at central areas (Nha Trang, Ninh Thuan), lower at southern area (Phu Quoc) (Fig. 3). In terms of seasonal variation, the late Northeast monsoon months have slightly lower pH, and Ω values were lower than during the Southwest one.

Temporal changes

pH and Ω values variation over months

During the period from Feb 2019 to May 2020, there had been no significant trend of pH and Ω values. These values changed from 2.84 to 3.19 in Ω parameter and 7.91–8.01 for pH (Fig. 4).

Coral growth rate in length

Due to objective conditions (bad weather, broken samples), enough samples could only be collected on eight months during the study. Although we were able to measure and estimate the length growth rate (mm/day), there did not seem to be a clear trend at all.

In addition, the coral growth rate was neither correlated to the pH nor Ω conditions. There was only a remarkable point that in Dec 2018, in 44 days *A. muricata* did not grow (0 mm) while *A. robusta* had a good growth rate (0.104 mm/day). Generally, *A. muricata* grew much better than *A. robusta* during this study (except Dec 2018) (Fig. 5).

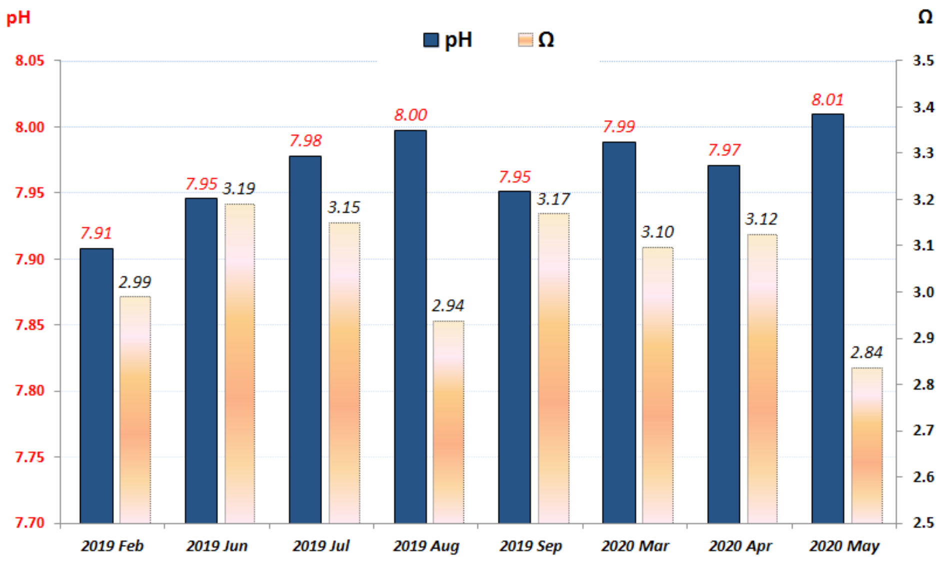

Figure 4. pH and Ω values changes from Feb 2019 to May 2020.

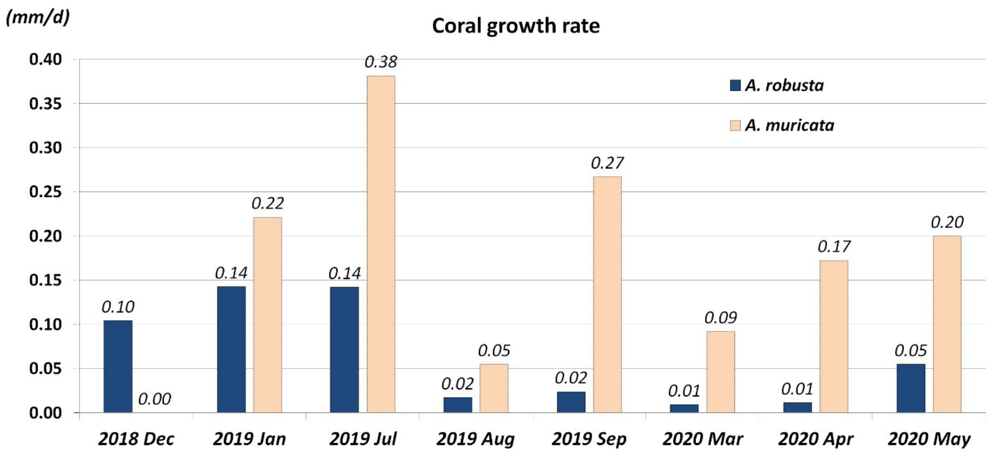

Figure 5. Coral growth rate in length over time.

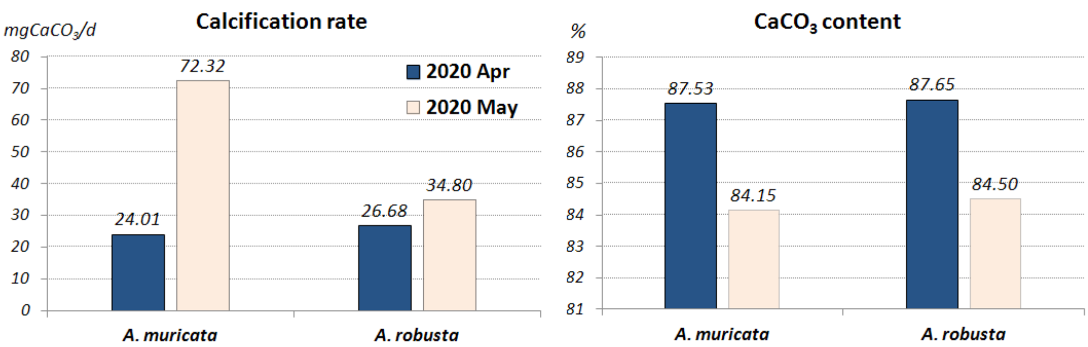

Figure 6. Calcification rate and $CaCO_3$ content of coral.

Table 2. Correlation analysis of $\%CaCO_3$ and Growth of coral to acidification parameters. (Numbers in boldface are significant).

		$\%CaCO_3$	*TA*	*pH*	*CO2*	*pCO2*	Ω	*Temp.*	*Sal.*
A. muricata	Ω	**0.922**	0.262	-0.390	-0.091	0.290			
	Temp.	0.169	0.054	-0.125	-0.086	0.248	0.486		
	Sal.	0.482	0.261	-0.179	-0.620	-0.950	-0.102	-0.270	
	Growth	-0.112	0.000	0.680	0.078	0.244	0.212	0.457	-0.285
A. robusta	Ω	**0.908**	0.262	-0.390	-0.091	0.290			
	Temp.	0.556	0.054	-0.125	-0.086	0.248	0.486		
	Sal.	0.456	0.261	-0.179	-0.620	-0.950	-0.102	-0.270	
	Growth	-0.350	-0.166	0.571	0.394	0.607	0.164	-0.073	-0.585
Both	Ω	**0.875**	0.262	-0.390	-0.091	0.290			
	Temp.	0.383	0.054	-0.125	-0.086	0.248	0.486		
	Sal.	0.433	0.261	-0.179	-0.620	-0.950	-0.102	-0.270	
	Growth	-0.278	-0.043	0.466	0.138	0.271	0.142	0.195	-0.285

We used the Pearson correlation analysis to try to find any relationship between coral growth and acidification related parameters, thence predict the potential impacts. However, although 100 *A. robusta* and 118 *A. muricata* measurements were used, the only significant relationship was between $CaCO_3$ content and Ω values (Table 2).

Coral calcification and percentage of CaCO3:

Data from two periods 03–04/2020 and 04 /05/2020 were selected to estimate the calcification of the two coral species. Besides, the $CaCO_3$ content (% of weight) was also calculated to estimate the density of coral. It was a strange result that when corals had better calcification rate, the $CaCO_3$ content became lower, meaning lower density of coral (Fig. 6).

DISCUSSION

Influencing factors of pH and Ω values

Both pH and Ω values were low at the southern offshore areas when compared to some other offshore coral reef areas ((Langdon and Atkinson 2005; Feely *et al*. 2009; Zhang *et al*. 2013; Hossain *et al*. 2015; Cotovicz *et al*. 2020). Notably, all Ω values here were lower than 3, which would make marine organisms stressed (SOS/NOAA; Cohen and Holcomb 2009). These low pH and Ω values might be due to the influence of Mekong-Gulf of Thailand water masses, which have high temperature, low salinity and pH (Kremf *et al*. 1947; Lanh 1995). However, this issue needs more data from these areas to be confirmed.

Four sites among Binh Thuan skerries and Phu Quy islands areas had all Ω values higher than 4, which is ideal for coral growth and recruitment (Ohde and Hossain 2004; Cohen and Holcomb 2009). It is noted that Phu Quy and Binh Thuan are in a strong upwelling area, which might explain the ideal Ω values. According to Bai and Lanh (1997) and Duong *et al*. (2019), this area usually has water masses/layers with low temperature and high nutrient concentration, which may influence the Ω value.

Comparing to other coral reef dominated waters (Ω ~ 3.49 ± 0.07) (Cotovicz *et al*. 2020), the Ω values of Nha Trang, Ninh Thuan were almost similar, while Phu Quoc was a bit lower. It seems that the environmental conditions in central waters, both coastal and offshore, are much better than southern waters for coral and other calcifiers survival and reproduction (in term of acidification). However, given the fact that there are many big healthy coral reefs in Con Dao, Phu Quoc areas (Tuan *et al*. 2008) with quite high resilience and rehabilitation (Vo Si Tuan, unpublished data), it is necessary to research on comprehensive environmental conditions and accumulated impacts on resilience of marine life in the future.

Temporal changes

It was expected the pH and Ω values would change during the upwelling time (about June to August in Ninh Thuan waters) due to the water masses characteristics (low pH, low temperature, high salinity and nutrients) (Duong *et al*. 2019). However, this hypothesis was not supported by the results of pH and Ω values variation over months. This might be explained if there was no upwelling phenomenon during the study period, or the selection of sampling sites and times were not properly designed, or maybe longer periods of sampling are needed to catch the right time and place for such phenomena.

Coral calcification rate changes seasonally due to changes of temperature, salinity, and Ω values (Roik *et al*., 2018), and longer term monitoring and researches might be required to identify more specific factors that influence coral density and calcification. However, the present practical experiments are a preliminary step towards developing a new method for determining coral growth and calcification. Below are some suggestions for improvement in future experiments:

Beside coral shape and length selection, sample size is also quite important. We suggest that the position of the marking point should be from 7–10 mm for *A. muricata*, and 8–12 mm for *A. robusta*. By applying this position, the coral samples would be healthy and long enough for marking, and not too old to sprout after we marked.

The proper duration before sampling should be 25–35 days after marking. Shorter duration will not let coral grow enough for analyzing, while longer duration will increase the risk of sprouting and breaking.

CONCLUSIONS

The coral reefs at central sites were in better acidification situation with the highest pH at Phu Quy area (8.1223 ± 0.0944) and highest Ω value at Binh Thuan skerries (4.02 ± 0.38) among off-shore reefs; highest pH and Ω value were at Nha Trang (8.1298 ± 0.0539, and 3.35 ± 0.27) among near shore reefs.

All Ω values at offshore southern reefs were lower than 3, which level would make coral and other marine calcifiers stressed. The coastal reefs at Phu Quoc were somewhat better, but the mean value of Ω was still below the stressed threshold (2.97 ± 0.10).

Temporal variation of pH and Ω values at studied site in Vinh Hy bay, Ninh Thuan did not show significant trend although this place is in a strong upwelling area.

Growth rate in length of coral in this research did not seem to have a clear trend, neither in relation to pH nor to Ω conditions.

This research could not assess any clear impacts from acidification related parameters to coral calcification and density. However, we did develop and try a new method for such assessment. Thence we gave some suggestions for future improvement.

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