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Effect of increased CO2 concentration on the growth rate of *Isopora palifera* and *Acropora hyacinthus* from different cross-shelf reef zones

Effect of increased CO2 concentration on the growth rate of *Isopora palifera* and *Acropora hyacinthus* from different cross-shelf reef zones

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Abstract. The rise in atmospheric carbon dioxide (CO2) concentration due to emissions associated with economic development is altering ocean chemistry, a process known as ocean acidification. The resultant decrease in oceanic pH will affect marine organisms, in particular those which build their skeletons through calcification such as scleractinian corals. The aim of this research was to analyse the likely impact of changing CO2 concentrations and thus seawater pH on the growth rate of two common corals, *Isopora palifera* and *Acropora hyacinthus*. This research was conducted at the Marine, Coastal and Small Island Research Centre, Universitas Hasanuddin, Indonesia. Samples of *Isopora palifera* and *Acropora hyacinthus* were collected along an inshore-offshore cross-shelf gradient from 3 sites: Pulau Karanrang (inner zone), Pulau Badi (intermediate zone), and Pulau Kapoposang (outer zone). A fully randomised research design was used with three replicates for each of three CO2 treatments: 390 ppm (control), 550 ppm (2030 prediction), 1000 ppm (2050 prediction). The samples were weighed weekly for 1 month (digital balance, accuracy 0.1 mg). ANOVA analysis with post hoc Tukey Test showed a significant ($p < 0.05$) between treatment difference in growth rate for both *Isopora palifera* and *Acropora hyacinthus* (P<0,05). The corals from all three zones exhibited positive growth at 390 ppm CO2, and negative growth at CO2 concentrations of 550 ppm and 1000 ppm.

1. Introduction

The phenomenon of climate change is currently a popular topic for research around the world. Increased human economic activities derived from the use of fossil fuels, such as oil and coal, have an impact on increasing greenhouse gas emissions, in particular carbon dioxide (CO2). These emissions are causing an increase in atmospheric CO2 concentrations. The scientific consensus estimate is that the average carbon dioxide partial pressure concentration (pCO2) in the atmosphere has increased rapidly from around 280 ppm in pre-industrial times to 390 ppm in 2005 [1]. It has been predicted that by 2030 pCO2 concentrations would likely reach 450 ppm [2], and are predicted to reach 900 ppm by the end of the 21st century [3].

In the natural carbon cycle, atmospheric carbon dioxide gas levels are determined by the balance of reactions in the atmosphere, land and ocean. Much of the CO2 gas in the atmosphere is absorbed by plants on land and some is dissolved into the ocean and is utilized by marine organisms [1]. Atmospheric carbon dioxide (CO2) absorbed by the oceans reacts with sea water (H2O) to form carbonic acid (H2CO3) which dissolves rapidly to form ionised hydrogen (H^+) ions (an acid) and bicarbonate, $HCO3^-$ (a base) [4]. The increasing abundance of hydrogen ions in the ocean reduces the pH of seawater, a phenomenon known as ocean acidification [5,6,7].

The results of previous research [8,9,10] show that acidification of sea water due to increased carbon dioxide concentration can affect the survival of marine organisms which calcify during some or all life cycle stages, including calcareous macroalgae, shellfish, and coral reefs. In calcareous macroalgae, decreasing water pH levels can interfere with the calcification process of *Halimeda* sp.[8]. Calcite formation in shellfish can become harder due to ocean acidification, and can interfere with the biomineralisation process [9]. Ocean acidification can also reduce the availability of carbonate needed by coral animals to form calcium carbonate skeletons, so that growth can be inhibited [2,10,11,12].

The Spermonde Islands in the southern Makassar Strait, off the southwest coast of Sulawesi Island, have extensive coral reefs, influenced by the Makassar Strait water mass [13]. The Spermonde Islands can be divided into four zones, starting from the inner zone to the outer zone [14]. Coral biodiversity is fairly high (78 genera with a total of 262 species recorded), of which around 80-87% are in the outer reef areas. However, in 1996 [15] it was noted that, compared to data recorded in the early 1980's some of the same locations [14], live coral cover and diversity had been reduced by around 20% within 12 years. This appeared to be the result of high levels of sedimentation and eutrophication originating from anthropogenic activities.

Although an increase in the concentration of carbon dioxide gas in the atmosphere has been detected world-wide, there was a lack of scientific information on the impact of increasing carbon dioxide concentrations on coral condition in the Spermonde Islands. It was therefore considered important to evaluate the impact of increased carbon dioxide concentrations on the genus *Acropora*, which has been used as a reference for coral health in different zones within the Spermonde waters.

The purpose of this study was to examine the effect of increasing carbon dioxide, at different concentrations, on the growth rate of *Isopora palifera* and *Acropora hyacinthus*. The research was planned on a laboratory scale, and compared the growth rates of *Isopora palifera* and *Acropora hyacinthus* from each Spermonde zone under different carbon dioxide concentrations.

2. Materials and methods

2.1. Time and place

This study was carried out in April - May 2018. Acropora coral samples were collected from sampling stations on several islands across the Spermonde Archipelago (Kapoposang... Karanrang, Badi, and Kapoposang Islands). The experimental research was conducted at the Research Center for Marine, Coastal and Small Islands, Hasanuddin University, Makassar.

2.2. Setting up the experimental CO2 system

The CO2 system is a tool that was assembled from a number of components, including supporting equipment such as a CO2 supply, an O2 compressor and a mass flow controller (MFC) (Figure 1). This system functions as a regulator of carbon dioxide (CO2) concentration in the water. Carbon dioxide gas from CO2 gas cylinders and oxygen from the O2 compressors both enter the mass flow controller. The MFC regulates the flow rate and has CO2 meters with digital displays so that the rate of carbon dioxide concentration flowing into the aquaria can be measured and regulated. For this research, the mass flow controller was set for two concentrations: (i) 550 ppm pCO2 (CO2 gas flow rate range 7.95 - 8 mL/min and O2 range 2:49 to 2:55 L/min) and (ii) 1000 ppm pCO2 (CO2 gas flow rate range 9.95 - 10 mL/min and O2 range 1 - 1:10 L/ min)..

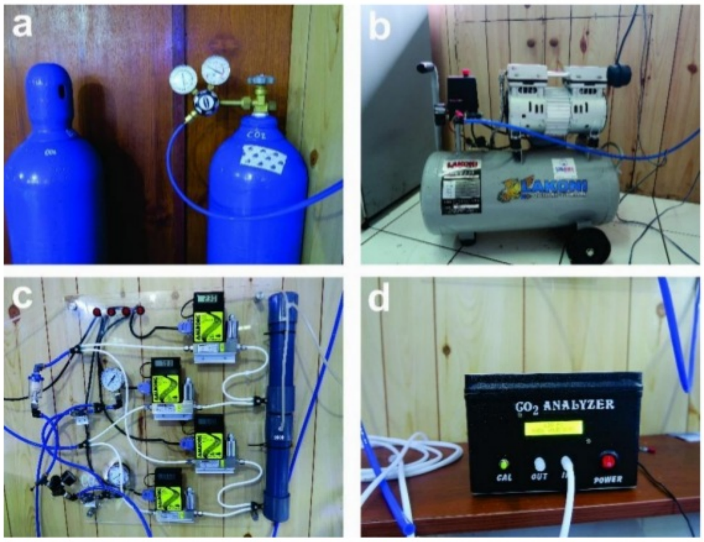

Figure 1. CO2 system components:
(a) CO2 gas cylinders
(b) O2 compressor
(c) Mass flow controller
(d) CO2 meter

2.3. Research Procedures

2.3.1. Collection and acclimatization of coral samples. Coral sampling sites in the Spermonde Islands were selected to represent the cross-shelf zones. Karanrang Island represents the inner zone, Badi Island represents the middle zone, and Kapoposang Island the outer zone. Stations at each sampling site were selected at a depth of 3 - 5 meters, at which Acropora colonies are typically most abundant. The weight of each sample (fragment) collected from Acropora hyacinthus and Isopora palifera coral colonies was <100 grams. The fragments were taken using pliers, then transplanted (using glue) on a mica substrate and placed at the same depth as the 5-month sampling location, so that the transplanted samples (fragments) could adjust (recover) before being used in the experiment.

2.3.2. Experimental Design. This research used a factorial design with 5 factors: CO2 concentration treatment, sampling zone, species, fragments, and replicates. There were three pCO2 concentration levels (390 ppm, 550 ppm, and 1000 ppm), each with three replicates (Figure 2).

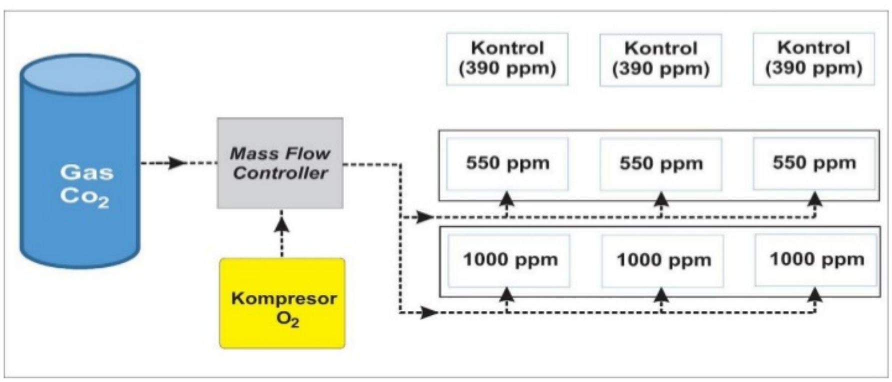

Figure 2. Research Layout

The 390 ppm concentration is the current ambient pCO2 concentration [16], while 550 ppm and 1000 ppm are the predicted pCO2 concentration in 2030 and 2100 [1,2,3]. This concentration

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difference assumes that an increase in CO2 results in a decrease in seawater pH [5,6,7]. The three sampling zones were: inner zone (Karanrang Island), middle zone (Badi Island), and outer zone (Kapoposang Island). The two species were *Isopora palifera* and *Acropora hyacinthus*. The fragment factor comprised fragments A and B. Thus, the total number of experimental units (fragments) in this study design is 3x3x3x2x2 = 108 coral fragments. During the study, water quality parameters were also recorded and water changed once a week. Temperature, salinity, dissolved oxygen, and pH were measured using water quality meters, alkalinity using a Mini Alkalinity Titrator.

2.3.3. Sample weight and growth rate. Test organisms (Acropora coral fragments) were placed into an aquarium (90 cm x 39 cm x 35 cm) for 1 month. Before being placed in the aquarium, each fragment was weighed to obtain the initial weight (t_0) using analytical digital scales with an accuracy of 0.1 mg (0.0001 g). The fragments were then weighed weekly, so that the rate of weight change could be calculated using the formula:

$$C = t_n - t_0$$

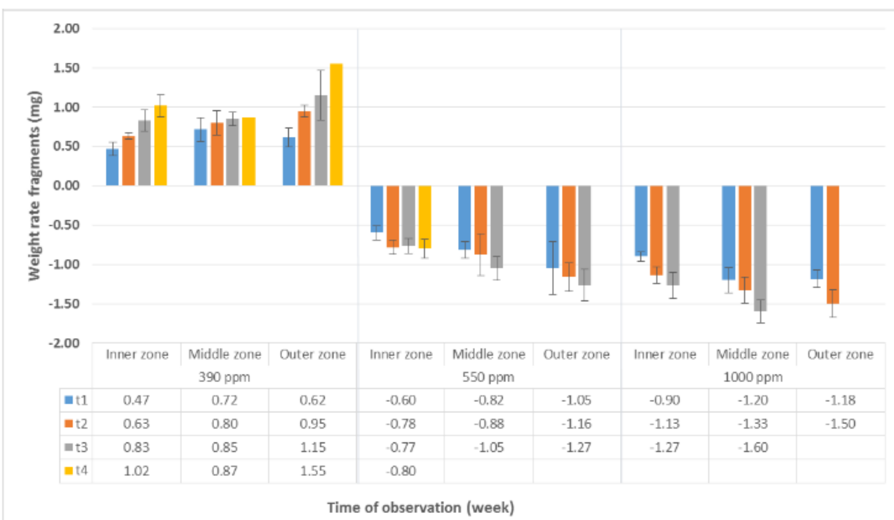
Where: C = weight change (mg); t_n = weight in week-n (mg); t_o = initial weight (mg)

2.4. Data Analysis
The weight change data were analysed descriptively, tabulated and presented in graphic (histogram) form. Prior to conducting statistic tests, the data on growth rates of *Isopora palifera* and *Acropora hyacinthus* from different zones were first tested for normality. If the data were normally distributed, an Analysis of variance (Factorial ANOVA was applied, otherwise the non-parametric Kurskall-Wallis test was applied. Data analysis was performed in SPSS v.18.

3. Results and Discussion

3.1. Results

3.1.1. Acropora hyacinthus weight change. The weight change of Acropora hyacinthus under each treatment (Figure 3) shows that under the 390 ppm pCO2 treatment, Acropora hyacinthus from all three zones increased in weight.



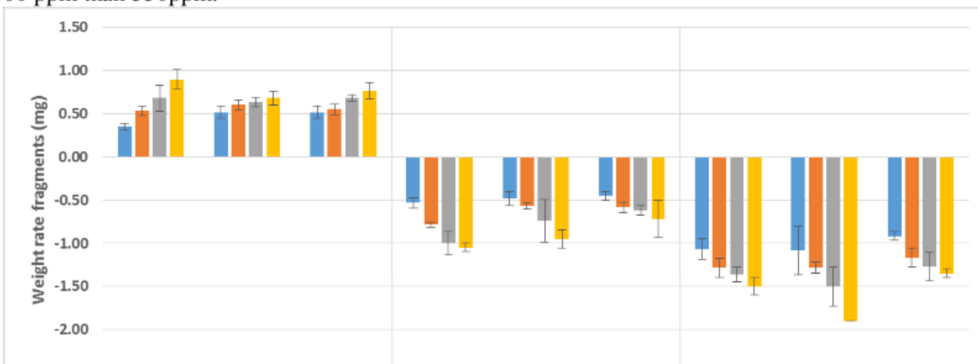
	Inner zone (390 ppm)	Middle zone (390 ppm)	Outer zone (390 ppm)	Inner zone (550 ppm)	Middle zone (550 ppm)	Outer zone (550 ppm)	Inner zone (1000 ppm)	Middle zone (1000 ppm)	Outer zone (1000 ppm)
t1	0.47	0.72	0.62	-0.60	-0.82	-1.05	-0.90	-1.20	-1.18
t2	0.63	0.80	0.95	-0.78	-0.88	-1.16	-1.13	-1.33	-1.50
t3	0.83	0.85	1.15	-0.77	-1.05	-1.27	-1.27	-1.60	
t4	1.02	0.87	1.55	-0.80					

Figure 3. Weight change of *Acropora hyacinthus* fragments under the three pCO2 treatments

At 550 ppm pCO2, the coral fragments decreased in weight every week, with inner zone fragments having the lowest decrease and outer reef fragments the highest. Fragments from the middle and outer zones experienced bleaching in the third week. At 1000 ppm pCO2 concentration the coral fragments from all coral reef distribution zones in Spermonde waters experienced a greater decrease in weight rate than under the 550 ppm treatment, and bleached more rapidly, after 2 to 3 weeks. Weight loss was higher and bleaching occurred more rapidly in outer than inner zone fragments.

A One-Way Anova with Tukey post hoc test showed the weight change of *Acropora hyacinthus* fragments was significantly different (P <0.05) between the 390 ppm, 550 ppm, and 1000 ppm carbon dioxide concentration treatments. However the Kruskall Wallis test showed the weight changes were not significantly different (P> 0.05) between zones

3.1.2. Isopora palifera weight change. The weight changes Isopora palifera fragments (Figure 4) show that under the 390 ppm pCO2 treatment Isopora palifera coral fragments from all three zones increased in weight during the experiment. At 550 ppm and 1000 ppm pCO2, Isopora palifera from all zones in Spermonde waters experienced a decrease in weight every week, with a higher weight loss at 100 ppm than 550ppm.



	Zona dalam (390 ppm)	Zona tengah (390 ppm)	Zona luar (390 ppm)	Zona dalam (550 ppm)	Zona tengah (550 ppm)	Zona luar (550 ppm)	Karanrang (1000 ppm)	Badi (1000 ppm)	Kapoposang (1000 ppm)
t1	0.35	0.52	0.52	-0.53	-0.48	-0.45	-1.07	-1.08	-0.92
t2	0.53	0.60	0.55	-0.78	-0.57	-0.58	-1.28	-1.28	-1.17
t3	0.68	0.63	0.68	-1.00	-0.74	-0.62	-1.36	-1.50	-1.27
t4	0.90	0.68	0.77	-1.05	-0.95	-0.72	-1.50	-1.90	-1.35

Figure 4. Weight change of *Isopora palifera* coral fragments under the three pCO2 treatments

Based on the results of the One-Way Anova test with post hoc tests Tukey showed that the weight rate of coral fragments *Isopora palifera* between treatments of 390 ppm, 550 ppm, and 1000 ppm carbon dioxide concentration was significantly different (P <0.05). While the weight rate of coral fragments *Acropora palifera* was not significantly different (P> 0.05)between inner zones, middle zones and outer zones.

3.1.3. Water quality parameters. The water quality parameters for each experimental unit (aquarium) (Table 1) show that the pH values and other parameters measured were similar between replicates within each treatment. Parameters which varied significantly between treatments were DO, Alkalinity, and $CO_{2\ (aq)}$.

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Table 1. Water quality parameters by treatment and replicate

Parameter	Replicate	pCO$_2$ 390 ppm	550 ppm	1000 ppm
pH	I	8.03 ± 0.05	7.91 ± 0.04	7.76 ± 0.05
	II	8.02 ± 0.07	7.91 ± 0.04	7.75 ± 0.06
	III	8.02 ± 0.07	7.93 ± 0.04	7.75 ± 0.07
DO (ppm)	I	6.71 ± 0.19	4.11 ± 0.32	3.03 ± 0.25
	II	7.04 ± 0.40	4.18 ± 0.21	3.17 ± 0.24
	III	6.80 ± 0.33	4.01 ± 0.24	3.33 ± 0.22
Salinity (ppt)	I	31.61 ± 0.53	32.28 ± 0.83	31.66 ± 0.65
	II	31.60 ± 0.60	32.03 ± 0.49	31.59 ± 0.61
	III	32.25 ± 0.85	31.96 ± 0.92	31.51 ± 0.96
Temperature(°C)	I	29.68 ± 0.69	29.69 ± 0.59	29.53 ± 0.74
	II	29.54 ± 0.62	29.09 ± 0.65	29.07 ± 0.72
	III	30.09 ± 0.78	29.99 ± 0.63	29.71 ± 0.82
Alkalinity (ppm)	I	92.98 ± 0.65	83.48 ± 0.90	76.84 ± 0.94
	II	93.18 ± 0.72	84.37 ± 0.54	75.95 ± 1.08
	III	93.04 ± 0.57	83.75 ± 0.23	75.04 ± 0.90
$CO_{2\,(aq)}$ (ppm)	I	10.63 ± 0.34	16.15 ± 0.24	27.18 ± 0.15
	II	10.6 ± 0.32	16.28 ± 0.3	27.48 ± 0.25
	III	10.75 ± 0.54	16.43 ± 0.13	27.4 ± 0.22

3.2. Discussion

The results of this study show that under increased carbon dioxide (CO2) concentrations there was a decrease in the coral growth rate, and indeed negative growth, in both *Acropora hyacinthus* and *Isopora palifera* (Figure 3 and Figure 4). The 390 ppm (control/ambient) CO2 concentration treatment showed positive coral growth throughout the observation time; furthermore, growth rates varied with fragment size. Coral growth can be influenced by many factors including the age, shape and size of fragments or colonies [17]. Coral growth is also influenced by the reciprocal symbiotic relationship between polyps and zooxanthellae (*Symbiodinium* sp.), where zooxanthellae produce the oxygen and nutrients needed by polyps and polyps provide the zooxanthellae with living space and carbon dioxide for photosynthesis. The more polyps, the more zooxanthellae are found in the coral, so the calcification process should also be faster, resulting in increased coral growth rates. This hypothesis is supported by research showing that coral growth rate is influenced by the number of polyps[18]; furthermore, two and three polyps can use food more optimally compared to one polyp.

In the 550 and 1000 ppm pCO2 treatments (Figure 3 and Figure 4), coral growth was negative. This indicates that under high CO2 concentrations, the growth of *Acropora hyacinthus* and *Isopora palifera* corals is impeded. As the concentration of carbon dioxide dissolved in seawater increases both pH and alkalinity decrease, and changes in the seawater carbonate balance reduce the level of aragonite, the mineral used by corals to build their limestone skeletons; these changes can disrupt the growth process [2,19].

Coral growth depends on environmental conditions, most of which are not fixed and tend to change due to disturbances originating from natural phenomena and human activities [20]. The water quality data (Table 1) show an increase in the partial carbon dioxide concentration (pCO2) directly proportional to the concentration of carbon dioxide dissolved in water ($CO2_{(aq)}$). This is in accordance with Henry's law which states that the amount of gas dissolved in a solution will be directly proportional to the partial pressure of the gas in solution at equilibrium [21]. Research has shown [22,23,24] that at 400 ppm pCO2, $CO2_{(aq)}$ was 10.9 ppm, equivalent to pH 8.08; 597 ppm pCO2 (pH = 7.89; $CO2_{(aq)}$ = 16 ppm); 1004 ppm pCO2 (pH = 7.7; $CO2_{(aq)}$ = 27.4 ppm). Alkalinity reflects the buffering capacity of carbonate ions, with higher the alkalinity lowering fluctuations in pH [25]. Increased pCO2 also causes reduced oxygen solubility in water, as seen in Table 1. Furthermore, the solubility of oxygen in water is influenced by temperature and salinity [26]; the higher the temperature and salinity, the lower the solubility of oxygen in water, and vice versa. Thus elevated temperatures and higher pCO2 will act in synergy to reduce oxygen levels, which can negatively affect growth. Imbalances in the acid-base equilibrium can cause damage to exoskeleton components such as calcareous shells; the dissolving of calcium carbonate (CaCO3) and accumulation of metabolic waste products can weaken an organism, disrupt oxygen transport processes, and if prolonged will cause death [27].

4. Conclusion

An increase in carbon dioxide (CO2) concentration had a negative effect on the growth of *Acropora hyacinthus* and *Isopora palifera* in terms of weight. All corals originating from the inner zone (Karanrang Island), the middle zone (Badi Island) and the outer zone (Kapoposang Island) of the Spermonde Archipelago experienced an increase in weight at ambient partial concentration of carbon dioxide (390 ppm pCO2). However the impact of predicted increases in pCO2 under near future conditions is likely to inhibit coral weight gain and may result in high mortality or even extirpation of widespread and ecologically important corals.

Acknowledgments

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