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INTERACTION OF CO_2 AND LIGHT AVAILABILITY ON PHOTOPHYSIOLOGY OF TROPICAL COCCOLITOPHORIDS (EMILIANIA HUXLEYI, GEPHYROCAPSA OCEANICA, AND OCHOSPHAERA SP.)

Nita Rukminasari*, Muhammad Lukman, & Khusnul Yaqin

Marine Science and Fisheries Faculty, Universitas Hasanuddin, Indonesia

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**Corresponding author*: E-mail : nita.r@unhas.ac.id

Abstract

The study to examine the calcification rate, adaptation, and the biotic response of three tropical coccolithophorids (Emiliania huxleyi, Gephyrocapsa oceanica, and Ochosphaera sp) to changes in CO_2 concentration. Three selected calcifying coccolitophorids were grown at batch culture with CO_2 system at two levels of CO_2 (385 and 1000 ppm) and two light dark periods. The parameters measured and calculation including growth rate, particulate organic carbon content, particulate inorganic carbon content, chlorophyll a, cell size, photosynthetic, organic, inorganic carbon production, photosynthesis, and calcification rate. The results showed that there was a different response to carbonate chemistry changes and dark and light periods in any of the analyzed parameters. The growth rate of three selected calcifying microalgae tested was decreasing significantly at high concentrations of CO_2 (1000 ppm) treatment on 14:10 hour light: dark periods. However, there was no significant difference between the two CO_2 concentrations where they were illuminated by 24 hours light in growth rate. The increasing CO_2 concentration and light-dark periods were species-specific responses to photosynthesis and calcification rate for three selected calcifying microalgae.

Keywords: Calcification rate; Calcifying microalgae; Ocean acidification, Spermonde Islands.

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INTRODUCTION

Human activities cause an increase dramatically of CO_2 concentration in the atmosphere Fuel utilization, cement production, and biomass burning are sources of CO_2 emission (Cornwall et al., 2013). There are an increasing pCO2 rate in the atmosphere led to significant changes to the seawater carbonate system and cause a decrease in pH of 0.3–0.5 units by the end of the century (Cornwall et al., 2013; Pujiastuti et al., 2021). This condition could increase concentration of dissolved CO_2 due to a decreased carbonate concentration and saturation state (Cornwall et al., 2013; Hofmann & Bischof, 2014; Rickaby et al., 2016; Ginting et al., 2021). This phenomenon is called ocean acidification, which may directly impact marine organisms and ecosystems. When ocean acidification occurred, the decreasing pH and carbonate ion concentration also increasing concentration of bicarbonate and hydrogen ions were due to the absorption of CO_2 into the surface ocean changes in seawater chemistry (Singh et al., 2016). Ocean acidification also could lead to the decreased concentration of two command forms of calcium carbonate (calcite and aragonite), which are secreted by marine calcifying organisms (Yong et al., 2016). The carbonate saturation state of seawater strongly affected a calcification of marine calcareous organisms, concequently rine calcifying taxa and ecosystem will get a severe impact from ocean acidification (Celussi et al., 2017).

Coccolithophores play a major role in the global carbon cycle and are known to be sensitive to rising p CO_2 (Raeesossadati et al., 2014; Rokitta & Rost, 2012). The expected changes in the ocean carbonate chemistry will thus likely affect the performance of coccolithophores and may change global biogeochemical cycling in the future (Beardall & Raven, 2013). (Rickaby et al., 2016) found that increasing CO2 concentration could cause decreasing in the calcification of foraminifera, coccolithophores, and corals. As a result of pCO_2 in seawater raised, cellular calcification of calcifying organisms could be depressed. For example, Riebesell et al. (2000); Stoll et al. (2002) found that when a strong acid addition to phytoplankton culture, there was increasing in pCO_2, consequently the calcification rate and of the calcification photosynthesis ratio of Gephyrocapsa oceanica was a significant decreased. Rickaby et al., (2016) also found that the calcification rate increases

exponentially as a function of increasing the aragonite saturation state above the 100% saturation level. They also explained that several taxonomic groups showed the changing of calcification due to the changes in calcium concentration. For example, at a high-calcifying strain of the coccolithophoreid Emiliana huxleyi, the rates of calcification and photosynthetic 14CO2 fixation increase as a function of external Ca2+ concentration in cells (Pantorno et al. 2013). The rate of photosynthesis and calcification are closely twice higher at 10 mM Ca2+ compared with the Ca2+ concentration of 8 mM in seawater.

The effect of increasing pCO2, temperature, and nutrient depletion on growth, cococcophere thickness, sinking and calcification rate of calcifying microalgae and coccolithoporids were well documented. (Xu & Gao, 2012a) found that E. huxleyi seemed able to adapt to tremendous changes in light and temperature. Studies showed that E. huxleyi cells are resistant to high light levels with negligible photoinhibition (Olson et al., 2017) and it has been hypothesized that its coccoliths around the cells can dissipate the high solar radiation (Rickaby et al., 2016). Other studies showed that the photoinhibition tolerance of this organism was independent of coccoliths (Beardall & Raven, 2013; Rickaby et al., 2016), and calcification was supposed not to provide protective functions to dissipate energy under high irradiances (Benthien et al., 2007). Pantorno et al (2013) found that the N-limited cells were significantly smaller than the N-replete cells at the exponential growth phase of E. huxleyi. Gephyrocapsa oceanica showed low capability in adapt to to high CO2 conditions, consequently, there will be a significant change in photosynthetic production and elemental stoichiometry of this species for a scenario of ocean acidification projected for the end of this century. Jin, Gao, and Beardall (2013) found that there are several parameters that could be used as indicators for specific adaptation to the elevated CO2 level, including. chl a content cell volume, POC, PON content, and POC, PON production rates

Two variables, in particular, are known to influence the coccosphere thickness; specifically, day length (Guan & Gao, 2010; Xu et al., 2011; Xu & Gao, 2012b) and pCO2 availability (De Bodt et al., 2010; Engel et al., 2005; Iglesias-Rodriguez et al., 2008; Langer et al., 2006; Muller et al., 2009; Zondervan, 2007)

whereby coccosphere thickness decreased with increasing of pCO2 and longer daylength period. Recent studies have suggested that such changes in the coccosphere thickness and arrangement ultimately modify the internal cell light environment and hence the underlying photobiological signatures and photo-acclimation status (Guan & Gao, 2010; Zhou et al., 2012); therefore, we specifically examined how photobiological properties of three tropical coccolithophore species were influenced by daylength and pCO_2 availability (via a fully multifactorial experiment) and whether taxonomic and treatment patterns were consistent with associated changes in coccosphere thickness.

RESEARCH METHODS

Culturing and experiment conditions

Monospecific cultures of three selected calcifying algae (Emiliania huxleyi; RCC 962, Gephyrocapsa oceanica; RCC 1804 and Onchosphaera sp; RCC 1366)) were grown in sterile filtered (0.2 mm) seawater enriched with K and K2 media [Guillard and Ryther, 1962]. The incident photon flux density was 350 mmol/m2*s and a 14/10 hours of light/dark cycles were applied. Experiments were carried out at optimum growth temperature. Cells were acclimated to experimental conditions for approximately 15 generations and grown in semi continous cultures [Zondervan et al., 2002]. Low cell density was maintained during the experiment with the initial cell density for Emiliania huxleyi, Gephyrocapsa oceanica and Onchosphaera sp were 150,000 cell/ml, 35,000 cells/ml, and 30,000 cell/ml, respectively.

Controlling and monitoring the carbonate system

In this experiment a CO_2 treatment was using CO_2 system. The carbonate system was adjusted by bubbling CO_2 in the air into the culture media. This was achieved with 2 air cylinders; one containing air and the other 10% CO_2. The air was passed through a sodalime CO_2 scrubber and mixed with the 10% CO2. A mass flow controller regulated the rate which these two combined based on the target pCO_2 to give the desired concentration, and is able to control this to within 2ppm. The CO_2 in air was bubbled through the cultures at a rate of 150mL min-1.

Several parameters of the carbonate chemistry were measured every day. Alkalinity was measured by taking 20mL aliquots from each culture and running a

titration against HCl acid of a known normality. The pH was measured using the probe on the titrator as it is calibrated every day.

To measure the DIC in the media, 15mL aliquots were filtered through 25mm MF300 glass fibre filters (Fisher Scientific, Massachusetts, USA). The filtrate was transferred to glass vials that had been heated in a muffle furnace at 500°C for 3 hours to remove any carbon on them and was then analyzed using a total carbon analyzer (Shimadzu TOC-VCSH Total Organic Carbon Analyzer with ASV-I autosampler).

Growth Rates and Cell size

Cells were counted daily by taking a 2mL sample from each culture and measured microscopically using a Neubauer hemocytometer slide (Fisher Scientific, Loughborough, UK). Growth rates (μ) were calculated using the following equation:

$$\mu = (\ln c_1 - \ln c_o) / \Delta t^{-1} \quad (1)$$

where c0 is the initial count (cells mL-1), c1 is the final count (cells mL-1), and Δt is the time between the two counts (days). A volume of 50 mL was filtered onto 47mm 0.2μm Cyclopore polycarbonate membranes (Whatman, Maidstone, UK), washed with a weak alkaline solute, ion, and stored in Petri dishes. The dishes were placed in a desiccator to dry for 24 hours for subsequent analysis by scanning electron microscopy (SEM) via collaborators at the Marine Biological Association, Plymouth, UK (Dr. Fred Verret). The resulting SEM images provided a determination of (i) the identification of any malformations of the lithes between treatments and (ii) an estimate of cell size. Dimensions of the cells were calculated by the imaging software ImageJ to determine the cell diameter, from which the cell volume could then be calculated (assuming spherical cells) using the standard equation for the volume of a sphere:

$$V = \frac{4}{3}\pi r^3 \quad (2)$$

A total of 7 intact cells were identified and measured from the SEMs each.

Chlorophyll an Analysis

A volume of 150mL was filtered through 25mm MF300 glass fiber filters (Fisher Scientific, Massachusetts, USA) and flash-frozen in liquid nitrogen. Samples were stored at -80°C for later analysis. Pigments were extracted from the cells by grinding the filters each in 5mL of 90% acetone and then refrigerating in the dark for 2 hours. Samples were then centrifuged at 4500rpm for 5 minutes, and the

supernatant was pipetted into cuvettes. Absorbance was measured at 630nm, 644nm, and 750nm using a spectrophotometer (U-3000, Hitachi High Technologies, Wokingham, UK) relative to acetone blanks to correct the readings. Chlorophyll concentrations were finally determined using the equations of Ritchie (2006, and normalized to cell concentration and volume.

PIC and POC Analysis

Two aliquots of 150mL were each filtered down onto ashes (heated in a muffle furnace at 500°C for 3 hours) 25mm MF300 glass fiber filters (Fisher Scientific, Massachusetts, USA) and then placed in a desiccator to dry for 24 hours. Samples were stored in cryotubes to be analyzed at a later date. One of each pair of filters was acidified with ~2M HCl to drive off inorganic carbon, and further dried for 24 hours. The total carbon on each filter was measured using a carbon analyzer (Shimadzu TOC-VCSH Total Organic Carbon Analyzer with ASV-I autosampler) calibrated using a glucose standard. Particulate organic carbon (POC) was measured on the acidified filters, and particulate inorganic carbon (PIC) was calculated by subtracting PIC from the total carbon measured on the non-acidified filters. Both PIC and POC were then normalized to cell concentration and volume.

Data Analysis

Growth rate, POC/cell, and PIC/cell data were analyzed using Two Way ANOVA for comparing the data between species, light-dark periods, and CO_2 concentrations. Descriptive analysis was also used as a supplement data analysis. All graph and figure were made by an excel program. Statistical analyses were conducted using the SPSS version 15 software program. Assumptions of normally distributed residuals and equal variance among ANOVA cells were tested. The Kruskal–Wall is test produces an F-statistic produced by performing a parametric ANOVA on rank-transformed data. For colonies grown, a one-way ANOVA was applied (the assumptions were met), and the treatment effect was tested using an a priori contrast of control versus treatment tanks.

RESULTS AND DISCUSSION

Result

Carbonate chemistry

Variables of carbon chemistry were measured including pCO_2, total alkalinity, pH, DIC, HCO_3, $CO_{,3,}$ and CO_2. The mean and SE of carbon chemistry variables was shown at Tabel 1.

Tabel 1 showed that three calcifying microalgae responded differently to carbon chemistry variables at different CO_2 concentrations and L:D periods. In general, a pH value between CO_2 concentration treatment and L:D periods showed a similar pattern for three selected calcifiying microalgae which was lower at high CO_2 concentration (1000 ppm CO_2) treatment (Fig. 1). A higher pH value was found at Ochosphaera sp for not only CO_2 concentration treatments but also for L:D period treatments.

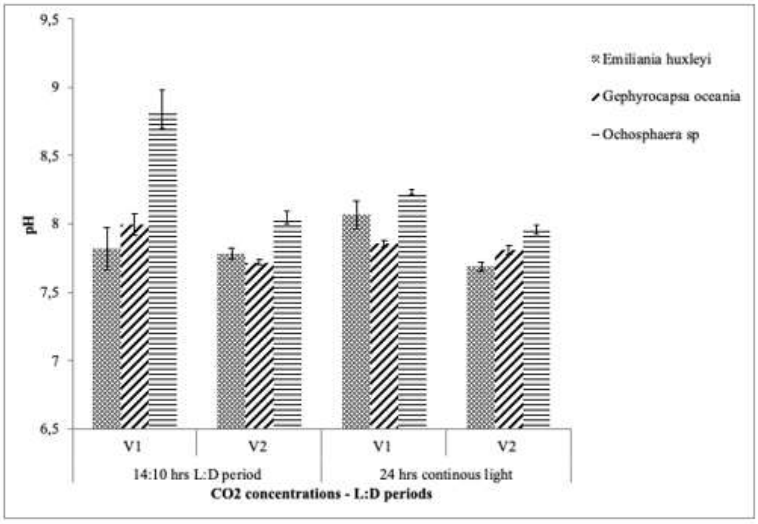

Figure 1. pH value (Mean±SE, N=3) of three calcifying microalgae on two different concentrations of CO2 and L:D periods. Note: V1 385 ppm CO_2 treatment, V2 1000 ppm CO_2 treatment

Emiliania huxleyi showed a positive response on L:D periods in terms of alkalinity (Fig. 2). Alkalinity at *E. huxleyi* was higher at 24 hrs continuous light than others calcifying microalgae at 14:10 hrs L:D periods. Interestingly, Ochosphaera sp showed a similar response on alkalinity value for CO_2 concentrations and L:D periods. Alkalinity for *Ochosphaera* sp was lower for both treatments than the other two calcifying microalgae. However, the difference of alkalinity was not significant between both treatments.

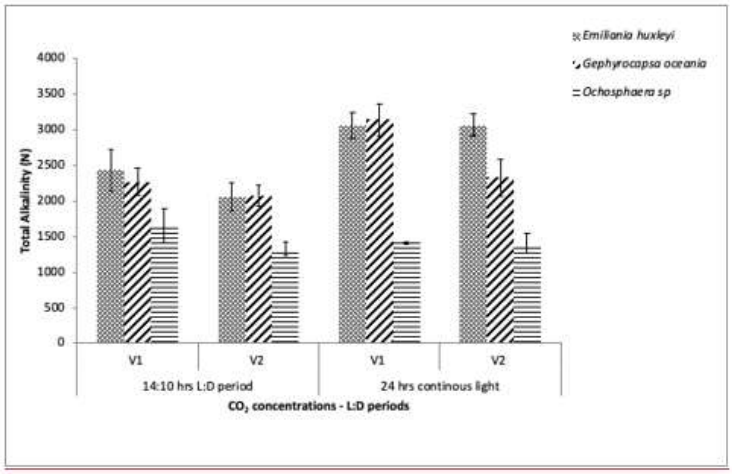

Figure 2. Total Alkalinity (Mean±SE, N=3) of three calcifying microalgae on two different concentrations of CO_2 and L:D periods. Note: V1 385 ppm CO_2 treatment, V2 1000 ppm CO2 treatment

Selected calcifying microalgae (*Emiliania huxleyi, Gephyrocapsa oceania* and *Ochosphaera* sp) showed a various responses in relation to dissolved inorganic carbon (DIC) on CO_2 concentrations and L:D periods (Fig. 3). The lowest DIC was found at Emiliania huxleyi sp for both treatments (CO_2 concentrations and L:D periods). On the other hand, Ochosphaera sp responded positively in terms of DIC on CO_2 concentrations and L:D periods. There was no significant difference of DIC between CO_2 concentrations and L:D periods.

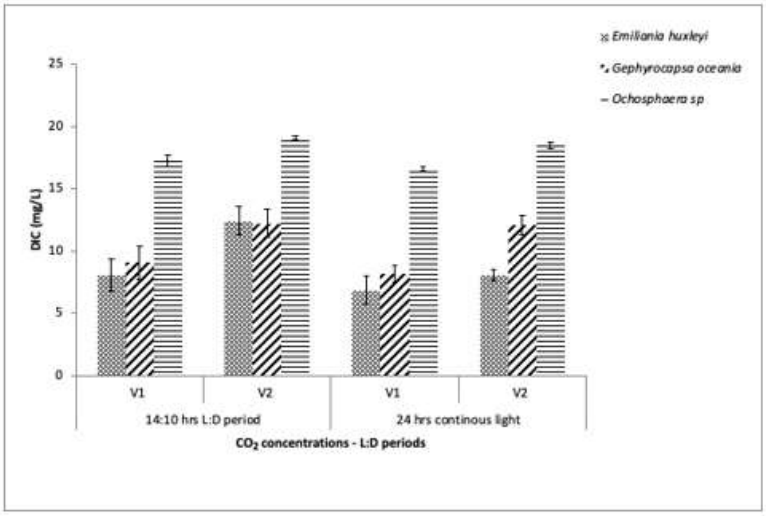

Figure 3. Dissolved Inorganic Carbon/DIC (Mean±SE, N=3) of three calcifying microalgae on two different concentrations of CO_2 and L:D periods. Note: V1 385 ppm CO_2 treatment, V2 1000 ppm CO_2 treatment

Ecophysiology responses of three calcifying microalgae to CO_2 concentration and L:D periods

Physiology variables were measured including growth rate, Coccosphere thickness, chl/cell, PIC/cell and POC/cell. (Table 2.) Study found that CO_2 concentration affected significantly on growth rate for all calcifying microalgae tested on 14:10 hrs L:D periods (Fig. 4). Interestingly, 24 hours continuous light and high CO_2 concentrations negatively affected only for *Gephyrocapsa oceania*, while ~~for~~ *Emiliania huxleyi* and *Ochosphaera* sp showed no significant difference of growth rate for both treatments (CO_2 concentrations and L:D periods).

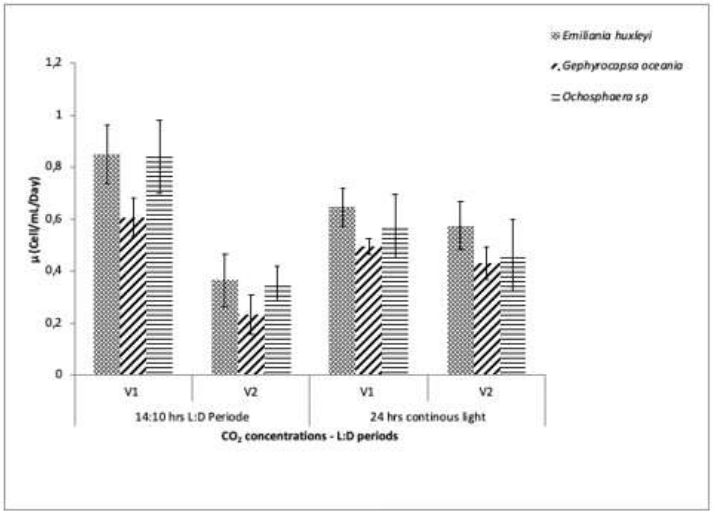

Figure 4. Growth rate (Mean±SE, N=3) of three calcifying microalgae at two different concentration of CO_2 and L:D periods. Note: V1 385 ppm CO_2 treatment, V2 1000 ppm CO2 treatment

Table 1. Carbon chemistry variables of three calcifying microalgae (*Emiliania huxleyi*, *Gephyrocapsa oceania*, and *Ochosphaera sp*) at two different L:D periods.

L:D Period - Species	Measured pH	Calculated TA (μmol/Kg)	DIC (μmol/Kg)	HCO_3(μmol/Kg)	CO_3(μmol/Kg)	pCO_2 (μ atm)
14:10 hrs – L:D periods						
Emiliania huxleyi V1 (385 ppm CO_2)	7.82±0.15	2435.89±293.24	4384±3.29	1922.52±228.58	229.20±36.78	441.56±74.41
Emiliania huxleyi V2 (1000 ppm CO_2)	7.78±0.04	2059.00±192.68	8531±3.63	1890.95±184.29	96.38±8.22	1079.67±143.76
Gephyrocapsa oceania V1(385 ppm CO_2)	7.99±0.08	2274.00±293.24	6618±1.46	1824.16±142.63	217.87±24.83	409.67±36.93
Gephyrocapsa oceania V2(1000 ppm CO_2)	7.72±0.02	2083.00±145.38	10210±0.58	1878.09±140.93	98.13±2.97	1134.44±138.74
Ochosphaera sp V1(385 ppm CO_2)	8.84±0.14	1648.22±241.75	15653±1.13	1360.21±218.35	163.01±19.83	261.78±73.53
Ochosphaera sp V2(1000 ppm CO_2)	8.05±0.05	1338.89±93.60	19133±0.23	1220.76±123.40	75.10±5.92	465.44±50.38
24 hrs continuous light						
Emiliania huxleyi V1 (385 ppm CO_2)	8.07±0.10	3067.17±183.02	5613±1.48	2335.92±121.72	315.08±30.01	422.50±0.42
Emiliania huxleyi V2 (1000 ppm CO_2)	7.69±0.03	3068.00±157.83	7717±0.17	2712.98±155.93	164.97±6.00	1237.08±4.10
Gephyrocapsa oceania V1(385 ppm CO_2)	7.86±0.02	3148.00±224.81	7705±0.55	2383.63±150.89	330.89±36.38	422.00±0.56
Gephyrocapsa oceania V2(1000 ppm CO_2)	7.81±0.03	2335.17±258.09	12563±0.72	1609.32±286.52	93.16±15.62	1023.50±4.34
Ochosphaera sp V1(385 ppm CO_2)	8.24±0.02	1413.00±17.01	16673±0.14	889.40±157.08	119.96±22.28	210.17±1.00
Ochosphaera sp V2(1000 ppm CO_2)	7.96±0.03	1410.17±132.86	18557±0.67	934.75±154.95	66.61±12.74	477.50±1.87

Table 2. Ecophysiology responses of three selected calcifying microalgae to changing CO_2 concentrations and L:D periods

L:D Period - Species	Growth rate (μ)	Chl/Cell (pg/cell)	PIC (pmol/cell)	POC (pmol/cell)	Cell size(μm)
14:10 hrs – L:D periods					
Emiliania huxleyi V1 (385 ppm CO_2)	0.8507±0.1126	405.35±44.13	3.24±1.41	1.81±0.57	2.99±0.27
Emiliania huxleyi V2 (1000 ppm CO_2)	0.3655±0.1008	293.11±106.73	0.98±0.23	2.36±0.75	-0.22±2.17
Gephyrocapsa oceania V1(385 ppm CO_2)	0.6069±0.0736	181.75±45.05	4.73±1.01	2.72±0.48	6.59±0.80
Gephyrocapsa oceania V2(1000 ppm CO_2)	0.2363±0.0756	190.70±56.74	1.75±0.49	3.36±0.58	1.91±1.78
Ochosphaera sp V1(385 ppm CO_2)	0.8418±0.1398	1600.25±366.13	1.23±0.63	3.86±0.68	0.47±2.57
Ochosphaera sp V2(1000 ppm CO_2)	0.3528±0.0672	644.26±181.18	0.57±0.25	4.68±1.33	1.35±2.18
24 hrs continuous light					
Emiliania huxleyi V1 (385 ppm CO_2)	0.6465±0.6465	415.50±100.09	1.67±1.42	2.24±0.46	6.11±0.61
Emiliania huxleyi V2 (1000 ppm CO_2)	0.5769±0.5769	742.95±118.94	1.88±1.18	4.05±0.23	-0.89±2.05
Gephyrocapsa oceania V1(385 ppm CO_2)	0.4965±0.4965	149.42±54.36	2.62±0.79	3.22±0.59	5.27±1.28
Gephyrocapsa oceania V2(1000 ppm CO_2)	0.4326±0.4326	209.39±71.47	2.04±0.69	5.13±0.36	-3.49±4.01
Ochosphaera sp V1(385 ppm CO_2)	0.5743±0.5743	247.11±70.53	0.58±0.61	4.28±1.27	-0.08±2.71
Ochosphaera sp V2(1000 ppm CO_2)	0.4629±0.4629	312.89±57.08	0.71±0.26	4.30±0.98	0.43±2.58

This finding indicated that selected calcifying microalgae responded differently to CO_2 concentrations and L:D periods in terms of growth rate. This finding also showed high CO_2 concentrations could inhibit the growth of selected calcifying microalgae tested. There was a significant difference in growth rate at two CO_2 concentrations for three calcifying microalgae tested. The inhibition growth of calcifying microalgae at high CO_2 concentration possibly due to the disturbance of carbonate chemistry in the cell. Concequently, microalgae required more energy to maintain the balancing of the pH on the cell.

Gephyrocapsa oceania and *Ochosphaera* sp showed a higher chlorophyll/cell at high CO_2 concentration on 24 hours continuous light. The highest chlorophyll/cell was found at 24 hours continuous light and high CO_2 concentration for Ochosphaera sp, accounting for 0.01 pg/L. On the other hand, Emiliania huxleyi had a low chlorophyll/cell for both CO_2 concentrations and L:D periods.

High CO_2 concentration reduced significantly ~~of~~ coccosphere thickness for selected calcifying microalgae algae. This finding indicated that high CO_2 concentration decreased the calcification rate of selected calcifying microalgae. Three calcifying microalgae responded differently on CO_2 concentrations and L:D periods in terms of PIC/Cell and POC/Cell L:D period of 14;10 hours showed a high PIC/Cell for *Emiliania huxleyi* and Gephyrocapsa oceania at low and high CO_2 concentration, respectively. On the

other hand, PIC/Cell at 24 hours continous light and high CO_2 concentration were the highest for Emiliania huxleyi accounting for 0.008 µg/L. In general, the POC/Cell of three calcifying microalgae was higher at high CO_2 concentration than low CO_2 concentration at both L:D periods, statistically, there was a difference significantly of POC/cell for the three microalgae tested. This finding indicated that CO_2 concentration is possibly affected in accumulating organic carbon in the cell.

The effect of CO_2 concentration and L:D periods on photophysiology variables

Photophysiology variables were measured using FRRF fluorescence photospectrometer, variables measured including Fo/F', Fv/Fm, σ, and Tau. This variable was also examined the photosynthesis activities and efficiency.

CO_2 concentration and light and dark periods affected the photosynthesis of selected calcifying microalgae. They also responded differently on L:D period. Four photophysiology variables values were varied among three calcifying microalgae in response to CO_2 concentration treatments and L:D periods.

Ochosphaera sp showed a higher Fo/F' value at 24 hours continuous light for low and high CO_2 concentrations than the other two species with the value were 21,426.33 and 19105.67, respectively. This finding indicated that *Ochosphaera* sp harvest more fluorescence after the removal of actinic light than the others two calcifying microalgae. The finding also indicated that the L:D period affected significantly ~~to~~ Fo/F' value.

Three selected calcifying microalgae showed a similar pattern of Fv/Fm value. Fig. 8b also showed that the 14:10 hours L:D period had a higher Fv/Fm value for all selected calcifying microalgae than at 24 hours continuous light for both low and high CO_2 concentrations. This finding indicated that L:D period affected in absorption energy for photochemistry.

The variables to show the efficiency of the photosystem in photosynthesis are noted as sigma (σ) and Tau (τ). Similar to Fv/Fm value, σ value was higher at 14;10 hours L:D period than 24 hours continuous light for all selected calcifying microalgae. The study also found that CO_2 concentration was not affected significantly to the sigma value. Interestingly, Ochosphaera sp showed a higher Tau value at 24 hours continuous light for both low and high CO_2 concentration than the other two calcifying microalgae. This finding indicated that ~~that~~

Ochosphaera sp was more efficiency in using light energy for photosynthesis.

The effect of CO_2 concentration and L:D periods on calcification and photosynthesis rate

Calcification involves the precipitation of $CaCO_3$ from Ca^{2+} and CO_3^{2-} ions in solution, which is mostly involving the generation of microenvironments that allow the supersaturation of $CaCO_3$ (Brownlee & Taylor, 2002). Our study found that three calcifying microalgae responded differently to light dark periods and CO_2 concentrations in terms of calcification rate. For Emiliania huxleyi, 24 hours light illumination was not shown a significant response to calcification rate. However, high CO_2 concentration (1000 ppm) at 14:10 hours light-dark period showed a very low calcification rate compare to normal CO_2 concentration on E.huxleyi accounting for 0.226 and 2.181 gC/cell/day, respectively. *Gephyrocapsa oceanica* showed a negative calcification rate for high CO_2 concentration and 24 hours light:dark period. The calcification rate was higher at 24 hrs light: dark period than the 14:10 hrs light-dark period for normal CO_2 concentration (385 ppm). For Ochosphaera sp, CO_2 concentration showed no effect on calcification rate at 24 hrs light: dark period, however at 14:10 hrs light:dark period, high CO_2 concentration affected significantly to calcification rate.

The opposite trend was shown for the photosynthesis rate. *Ochosphaera* sp showed a higher photosynthesis rate at 24 hours light-dark period than the others two microalgae for both CO_2 concentrations accounting for 2.896 and 3.706 gC/Cell/Day, respectively. Photosynthesis rate for *Gephyrocapsa oceanica* showed a similar trend with calcification rate in terms of the effect of the light dark period and CO_2 concentration, which was a higher rate of photosynthesis at 24 hours light; dark periods for both CO_2 concentrations. On the other hand, high CO_2 concentration affected negatively on photosynthesis rate at 14:10 hour light: dark period. Interestingly, the photosynthesis rate for *Emiliania huxleyi* showed a similar trend with calcification rate for the effect of CO_2 concentrations and light: dark periods, which was a higher photosynthesis rate at 24 hours light: dark periods than 14:10 hours light dark period for both CO_2 concentrations.

Three calcifying microalgae showed a different ratio of calcification and photosynthesis on the effect of increasing CO_2 concentrations and light: dark periods.

E.huxleyi and *G. oceanica* at 385 ppm CO_2 concentration and 14:10 hours light-dark period have calcification and photosynthesis ratio > 1. On the other hand, calcification and photosynthesis ratio for all calcifying microalgae at 1000 ppm CO_2 and 24 hours continuous light were < 1.

DISCUSSIONS

Impact of increasing CO_2 and light: dark period on the growth rate

In our experiment growth rate did not show a significant difference with increasing light: dark period for three calcifying microalgae tested. Growth rates were significantly different for three calcifying microalgae with increasing CO_2 concentrations, the range of growth rate for *E. huxleyi, Gephyrocapsa oceanica,* and *Ochosphaera* sp were between 0.3655 and 0.8507/day, 0.2363 and 0.3655/day and 0.8418/day, respectively. All of the calcifying microalgae tested showed a slightly decreased in ~~the~~ growth rate in response to increase CO_2 concentration. Our finding was supported by a previous studies (Rickaby et al., 2016) who found that *E. huxleyi* (RCC1256) displayed a marked decrease in growth rate with increasing CO_2 concentration. This finding indicated that the specific growth rate of calcifying microalgae, decreased with pCO_2 was influenced by the differences in net PIC accumulation. Engel et al., (2005) found that the lower net specific growth rate in the high-CO_2 treatment mirrored cell division rates and was not caused by an enhancement of loss processes. The growth rates of a few microalgae have been shown to increase when grown under CO_2 concentrations that are double or triple the present atmospheric values (Fu et al., 2007).

Impact of increasing CO_2 and light: dark period on the POC and PIC production

An increase in cellular POC production rate was found in the future CO_2 concentration (1000 ppm) compared to the present CO_2 concentration (385 ppm) for all calcifying microalgae tested. ~~In~~ our experiment, it showed that a clear effect of the pCO_2 and light-dark period on the cellular POC production rate was observed for all microalgae tested. Our result is consistent with several previous study (Antorno et al., 2013; Langer et al., 2013; Mongin & Baird, 2014; Olson et al., 2017) that found an increase in the POC production is observed with increasing CO_2 levels.

Our study found that PIC production decreased with increasing CO_2 concentration at 14:10 hours light: dark

period for three calcifying microalgae tested, however, there was no significant difference in PIC production at 24 hours continuous light for all calcifying microalgae tested.

Impact of increasing CO_2 and light: dark period on photosynthesis and calcification rate

Our study found that three calcifying microalgae showed a different response to increase CO_2 concentration and light: dark period on photosynthesis and calcification rate. *Emiliania huxleyi* showed the highest calcification rate for both CO_2 concentrations at 24 hours of continuous light than two other calcifying microalgae. This result indicated that Emiliania huxleyi can utilize HCO_3^- more efficiently than the other two species. It also demonstrated that calcification is a light-dependent and energy-requiring process, with chloroplast as the main regulator (Olson et al., 2017). (Fukuda et al., 2011) found that *E. huxleyi* utilizes HCO_3^- for calcification and produces calcium carbonate crystals by combining HCO_3 with Ca^{2+} together with the coccolith polysaccharides. The calcification reaction promotes oceanic acidification by producing CO_2 according to the following reaction: $Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2$ (Murata, 2006; Shin et al., 2002).

Calcification and photosynthesis ratio could show us the balance between carbon dioxide production (by calcification) and consumption (by photosynthesis) influences the magnitude of oceanic carbon drawn down (Celussi et al., 2017; Patidar, 2017; Raeesossadati et al., 2014; Wang et al., 2016). Our study found that calcification and photosynthesis ratio for all calcifying microalgae at 1000 ppm CO_2 and 24 hours of continuous light were < 1. It was indicated that the carbon dioxide (CO_2) generated by calcification is less than that required for photosynthesis, and an external source of CO_2 is required. On the other hand, *E.huxleyi* and *G.oceanica* generated CO_2 from calcification is greater than the cellular requirement for photosynthesis. The excess of CO_2 is produced due to calcification, and the photosynthesis ration was > 1. This finding supported by previous research who stated that at the cellular scale, a calcification to photosynthesis ratio < 1 indicates that the carbon dioxide (CO_2) generated by calcification is less than that required for photosynthesis, and an external source of CO_2 is required, while a calcification to photosynthesis ratio of >1 implies that the CO_2 generated from calcification is greater than the cellular requirement for photosynthesis, and

excess CO_2 is produced (Beardall & Raven, 2013; Coad et al., 2016; Rickaby et al., 2016; Singh et al., 2016; Djaingsastro et al., 2021).

CONCLUSION

Based on the discussion above, it can be concluded that increasing CO_2 concentration could cause decreasing in growth rate of all calcifying microalgae tested. Furthermore, there was an effect of the pCO_2 and light-dark period on the cellular POC and PIC production rate of three calcifying microalgae tested. Finally, Three calcifying microalgae tested showed a different response to increase CO_2 concentration and light: dark period on photosynthesis and calcification rate.

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