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Increasing CO_2 Concentration Impact upon Natural Phytoplankton Community at Spermonde Island, Indonesia: Mesocosm Study

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Abstract Ocean acidification is one of an environment problem due to increasing CO2 concentration. Ocean acidification have shown a negatively impact to marine organisms especially calcifying organisms such as microalgae, coral reef and other invertebrate organisms. Decreasing in carbonate saturation state on the calcification rates of individual species and communities in both planktonic and benthic habitats were occurred due to seawater acidification.

The study was conducted at three locations: Barru, Takalar and Barrang Lompo Island using mesocosm technique with two different of incubation periods (48 and 96 hours). Six pCO_2 level based on adding acid base treatments were used, which were 280, 380, 550, 650, 750 and 1000 ppm with 4 replicates

In general, alkalinity decreased with increasing CO_2 concentration. However, length of incubation was showed no significant affected to DIC for all CO_2 concentration at Barru and Barrang Lompo mesocosm experiment. PIC and POC showed a varied response between all locations of mesocosms experiment. There was a trend where increasing of POC with increasing of CO_2 concentration. PIC concentration at Barru and Takalar showed a slightl higher at 96 hours than 48 hours of incubation period and for Barrang Lompo mesocosm experiment PIC was higher at 48 hours than 96 hours of incubation period. Chlorophyll a and cell abundance was decresing with increasing CO_2 concentration. Growth, photosynthesis and calcification rate decreased with increasing CO_2 concentration.
Keywords Ocean acidification; Calcifying microalgae; Calcification rate; Natural phytoplankton community; Mesocosm study; Spermonde Islands

1 Introduction

Nowadays, CO_2 concentration in the atmosphere increase dramatically due to increasing human activities. Sources of CO_2 emission mostly are through fossil fuel utilization, cement production and biomass burning (Gattuso et al., 1998). Approximately half of the released CO_2 remains in the atmosphere, presently increasing p CO_2 at a rate of 0.4% per yr (Houghton et al., 1996). Meanwhile, CO_2 uptake by surface seawater increases the concentration of dissolved inorganic carbon and decrease pH. Furthermore, increased concentration of dissolved CO_2 results in a decreased carbonate concentration and therefore, a decreased saturation state (Gattuso et al, 1998, 1999). Those conditions create ocean acidification.

Ocean acidification and the related changes in seawater chemistry may directly impact marine organisms and ecosystem. It will also lead to a decrease in the saturation state of seawater with respect to calcite and aragonite, two common forms of calcium carbonate secreted by marine calcifying organisms (Orr et al., 2005). Zeebe and Gladrow (2003) have calculated that, if the pCO_2 was doubled in the sea water, the concentration of carbonate should be reduced by 50% approximately, and the pH by 0.35 unit. Calcification of marine calcareous organisms is strongly dependent on the carbonate saturation state of seawater, suggesting that ocean acidification will adversely affect marine calcifying taxa, with potentially severe implication for marine ecosystems such as coral reefs (Kleypas et al., 1999). Seawater acidification and the related decrease in carbonate saturation state negatively impacted on the calcification rates of individual species and communities in both

planktonic and benthic habitats (Bijma et al., 1999; Delille et al., 2005).

Ocean acidification impact to marine organisms have been well investigated at marine temperate and subtropic ecosystem at the monoculture species of laboratory experimental condition. Langer et al (2006) found that foraminifera, coccolithophores and corals each showed a decrease in calcification with increasing CO_2 concentration. Cellular calcification could be depressed consequently to a rise of pCO_2 in seawater. For example, by increasing pCO_2 in phytoplankton culture by the mean of strong acid addition, a significant decrase of the calcification rate and of the calcification photosynthesis ratio was induced in *Gephyrocapsa oceanica* (Riebelsell et al, 2000; Rost and Riebelsell, 2002). Gattuso et al (1998) found that Calcification rate increase exponentially as a function of increasing aragonite saturation state above the 100% saturation level and reaches a plateau at saturation values greater than 300%. They also explained that calcification changes as a function of calcium concentration in several taxonomic groups. For example, the rates of calcification and photosynthesic $^{14}CO_2$ fixation increase as a function of external Ca^{2+} concentration in cells harvested from the exponential growth phase of a high-calcifying strain of the coccolithophoreid *Emiliana huxleyi* (Xu et al., 2011). The rate of photosynthesis and calcification are closely coupled, ad are saturated at 10 mM Ca^{2+} compared with the Ca^{2+} concentrationof 8 mM in seawater. Lea et al. (1995) found that the calcification rate of the foraminifera *Orbulina universa* is proportional to the degree of carbonate saturation. However this process has investigated poorly at natural phytoplankton community of tropical ecosystem through the mesocosm study. On the other hands, there is very important ecosystem that contribute to decrease CO_2 concentration through photosyntetic which is coral reef ecosystem. It is important to understand ocean acidification process and their impact to marine organisms especially in the tropical systems. The aim of this research examined deeply natural phytoplankton community response to increase CO_2 concentration which is part of ocean acidification process.

2 Methods

2.1 Mesocosms experiment

Mesocosms experiment have been conducted at three different locations (Barru, Takalar Regency and Barrang Lompo Island) on June – August 2013 to examine the impact of ocean ocidification upon marine organisms in focus on phytoplankton at tropical system. The treatments in this experiment were six pCO_2 level through manipulating culture media through adding acid-base solution for to get expected CO_2 concentrations. Six treatments of pCO_2 level were 280, 380, 550, 650, 750 and 1000 ppm with 4 replicates for each treatment. There was two kinds of incubation periods such as 48 hours and 96 hours. 24-2L water bottles were incubated at 1 m depth below the surface and randomly placed (Figure 1).

Figure 1 Incubated water bottles at 1 m depth below the surface during mesocosam experiment.

2.2 Growth rates

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

$$\mu = \frac{(\ln c_0 - \ln c_1)}{\square} \Delta t^{-1}$$

where c_0 is the initial count (cells mL^{-1}), c_1 is the final count (cells mL^{-1}), and Δt is the time between the two counts (days).

2.3 Chlorophyll a Analysis

A volume of 2L was filtered through 25mm MF300 glass fibre filters (Fisher Scientific, Massachusetts,

USA) and flash-frozen in liquid nitrogen. Samples were stored at -80℃ 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 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.

2.4 PIC and POC Analysis

Two aliquots of 150mL were each filtered down onto ashed (heated in a muffle furnace at 500℃ for 3 hours) 25mm MF300 glass fibre 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.

2.5 Data Analysis

All parameter measured were analyzed descriptively using Excel Program. To determine the effect of CO_2 treatments among location of mesocosms experiment, the data was analyzed using one-way analysis of variance (ANOVA), with an a priori contrast to test for differences between control and treatment mesocosms.

3 Results and Discussion

3.1 The effect of increasing CO_2 to carbonate system

3.1.1 Alkalinity

Alkalinity is one of carbonate system component. It plays a role in a maintain an equilibrium of carbonate concentration in the water. Alkalinity at mesocosm experiment that run at three different locations showed a varied response within CO_2 treatments (Figure 2).

Figure 2a showed that there was trend for decreasing alkalinity with increasing CO_2 concentration for both incubation periods at Barru mesocosm experiment. The lowest alkalinity was found at 96 hours incubation period, which was account for 87.2 mg/L. However, alkalinity at 48 hours incubation period tend to be higher than 96 hours incubation periods for all CO_2 concentration treatments.

Alkalinity at Takalar mesocosm experiment was similar pattern with Barru, which was a trend to decrease of alkalinity with increase of CO_2 concentration for both two periods of incubation (Figure 2b). Howerver, there was no significance different of alkalinity between CO_2 concentrations and length of incubation periods.

Alkalinity was slightly different trends between incubation periods amongs CO_2 concentration at mesocosm experiment for Barrang Lompo Island (Figure 2c). It showed that there was a variation of alkalinity between incubation periods. There was slightly difference of alkalinity amongs CO_2 concentrations for both two incubation periods.

3.1.2 Dissolved inorganic carbon (DIC)

Dissolved inorganic carbon (DIC) showed a similar pattern among CO_2 concentrations between two different lengths of incubation for Barru and Barrang Lompo mesocosms experiment (Figure 3a and 3c). Statistically, there was no significant difference of DIC between CO_2 concentrations for both periods of incubation. For Barru mesocosm experiment, DIC was slightly lower at 1000 ppm CO_2 concentration than others CO_2 treatments for both incubation periods. CO_2 concentration of 750 ppm showed a lower DIC than other CO_2 treatments for two periods of incubation at Barrang Lompo mesocosm experiment. Interestingly, there was a different pattern of DIC between CO_2 treatments and length of incubations for Takalar mesocosm experiment (Figure 3b). DIC tended to be lower at 46 hours of incubation period than 96 hours incubation period for all CO_2 treatments.

The different DIC between locations of mesocosm experiment were due to different of other carbonate chemistry variables, such as pH, pCO_2, and HCO_3^-. Increasing CO_2 concentration also could change carbonate chemistry at the water column. Adding CO_2 to seawater increases aqueous CO2, bicarbonate, and

hydrogen ion concentrations; the latter lowers pH because pH = –log10[H+]. Carbonate ion concentration declined, however, because of the increasing H+ concentrations (Doney et al., 2008).

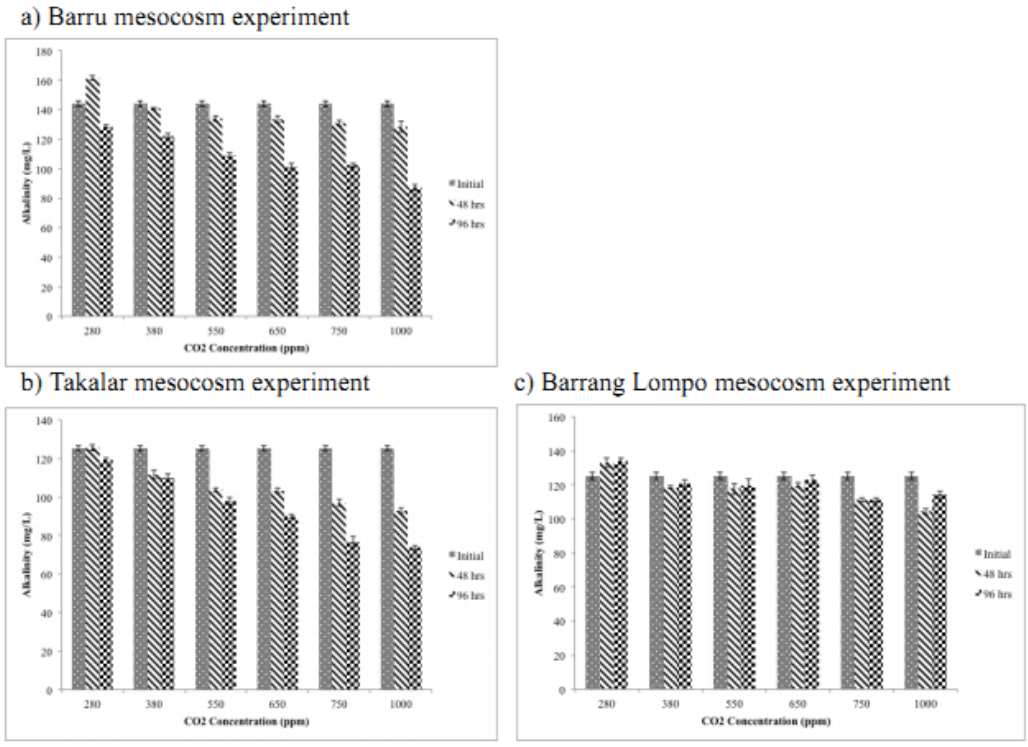

Figure 2 Mean of alkalinity at six CO_2 concentration treatments for two incubation periods (X ± SE). a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

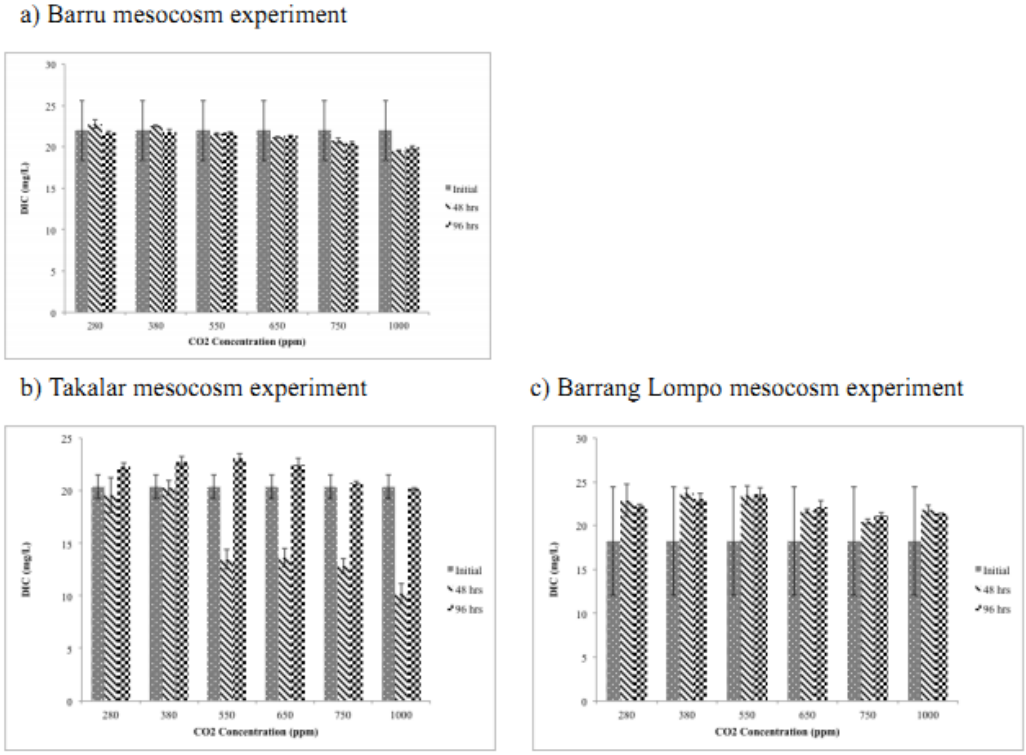

Figure 3 Mean of DIC at six CO_2 concentration treatments for two incubation periods (X ± SE). a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

3.1.3 Particulate Organic Carbon (POC)

Particulate organic carbon (POC) represents one of the main pools of organic carbon observed in the waters. POC for three different locations of mesocosm experiment showed different patterns (Figure 4). However, there was a similar pattern of POC between mesocosm experiment at Takalar and Barrang Lompo, which were POC higher at 96 hours incubation than 48 hours incubation period for all CO_2 treatments (Figure 4b and 4c). Interestingly, at Barru mesocosm experiment, POC was higher at 48 hours incubation period than 96 hours incubation period for all CO_2 treatments (Figure 4a). The highest POC was found at Takalar mesocosm experiment at 96 hours incubation period for 750 ppm CO_2 concentration account for 229 μg/L and the lowest POC was 15.59 μg/L at Barru mesocosm experiment, 96 hours incubation period for 280 ppm CO_2 concentration. Generally, increasing CO_2 concentration did not affect significantly to POC production for both incubation period. However, for Barru mesocosm experiment POC production was higher at 48 hours incubation period than 96 hours incubation periods for all CO_2 treatment. This result showed that length of incubation period affected significantly on POC production. Increasing length of incubation period decreased significantly of POC production.

3.1.4 Particulate Inorganic Carbon (PIC)

Particulate inorganic carbon was varied within location of mesocosm experiments. Figure 5 showed that there was a general trend of PIC which was increasing PIC with increasing CO_2 concentration. For Barru and Takalar mesocosm experiment, PIC concentration was higher at 96 hours incubation period than 48 hours (Figure 5a and 5b). However, there was no significant different of PIC concentration between incubation periods for all CO_2 treatments. On the other hand, PIC concentration at Barrang Lompo mesocosm experiment showed a higher at 48 hours of incubation period than 96 hours for almost all CO_2 treatments (Figure 5c). This trend was contradicted with two mesocosm experiments at two different locations. The different trend of PIC concentration between Barru, Takalar dan Barrang Lompo mesocosm experiments probably due to different of water quality parameters (Salinity, pH, DO, DIC and alkalinity). Based on DIC and alkalinity data (previous subpart of this report), there were a different trend of DIC and alkalinity between Barru, Takalar and Barrang Lompo. This finding indicated that PIC concentration was strong related with DIC and alkalinity was affected significantly by carbonate system in the waters. Engel et al (2005) found that several species of dissolved inorganic carbon (DIC) exist in seawater, namely CO_2, HCO_3^-, and CO_3^-, the proportions of which are largely was determined by the total DIC concentration, pH, and total alkalinity (TA).

1.3.5 Ratio of particulate inorganic carbon (PIC) to particulate organic carbon (POC) at three different locations of mesocosm experiment

Ratio of PIC to POC indicated a physiology process into the cell that correlated with carbonate systems especially concentration of CO_2. The change in [PIC] : [POC] ratios was attributed to a decrease in calcification rates, as well as an increase in organic carbon production (Figure 6). Our results showed that there was decreasing PIC:POC ratio with increasing CO_2 concentration for 96 hours incubation periods for mesocosm experiment at Barru, interestingly, there was no significant difference of PIC:POC ratio for all CO_2 concentration for 48 hours incubation period. (Figure 6a). This result was consistent with previous research, which was showed the ratio of particulate inorganic carbon (PIC) to particulate organic carbon (POC) production decreased with increasing CO_2 concentration (Riebesell et al., 2000; Zondervan et al., 2001, 2002). Interestingly, the PIC:POC ratio tended to increase with increasing CO_2 concentration at two others mesocosm experiment (Takalar and Barrang Lompo Island) for both incubation periods. The PIC:POC value was higher at 48 hours than 96 hours incubation periods for almost all CO_2 concentrations (Figure 6b and 6c). The highest PIC:POC ratio was found at Barrang Lompo mesocosm experiment for 1000 ppm of CO_2, account for 2.11 at 48 hours incubation period. The lowest PIC:POC ratio was 0.37, which was found at Takalar mesocosm experiment for 280 ppm of CO_2 concentration, 48 hours incubation period. This result was contradicted with previous research. It was probably due to different carbonate system, which affected the value of PIC and POC at two locations of mesocosm experiment.

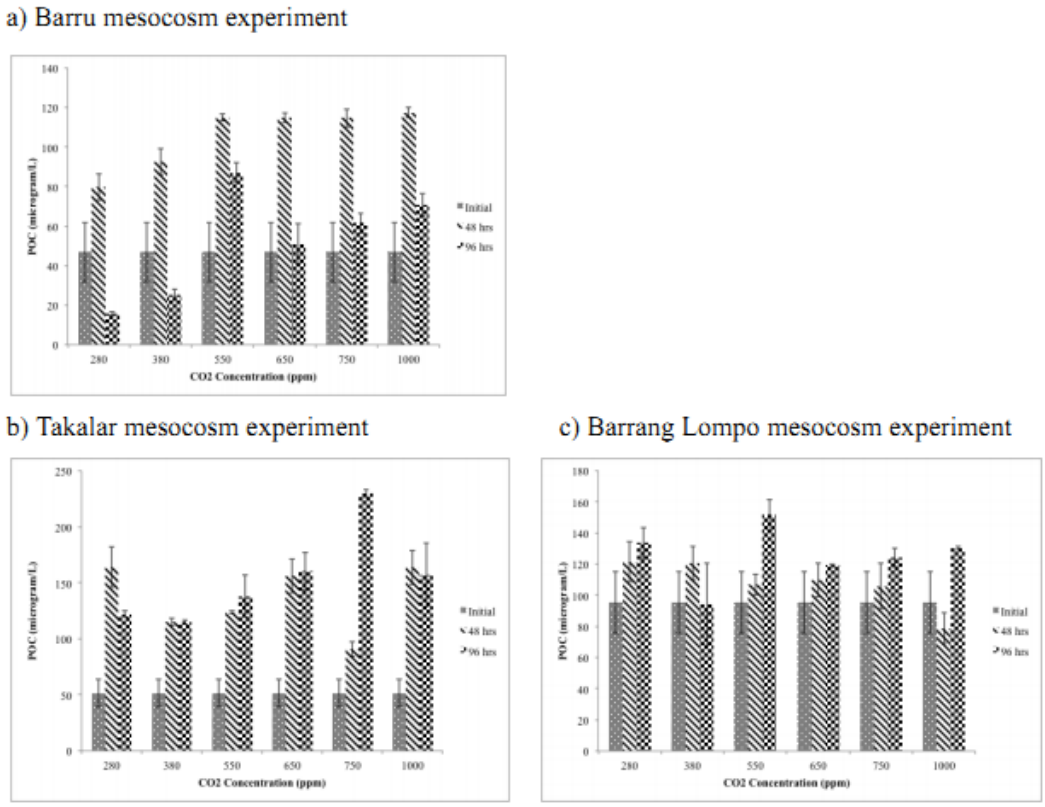

Figure 4 Mean of POC (X ± SE) at six CO_2 concentration treatments for two incubation periods. a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

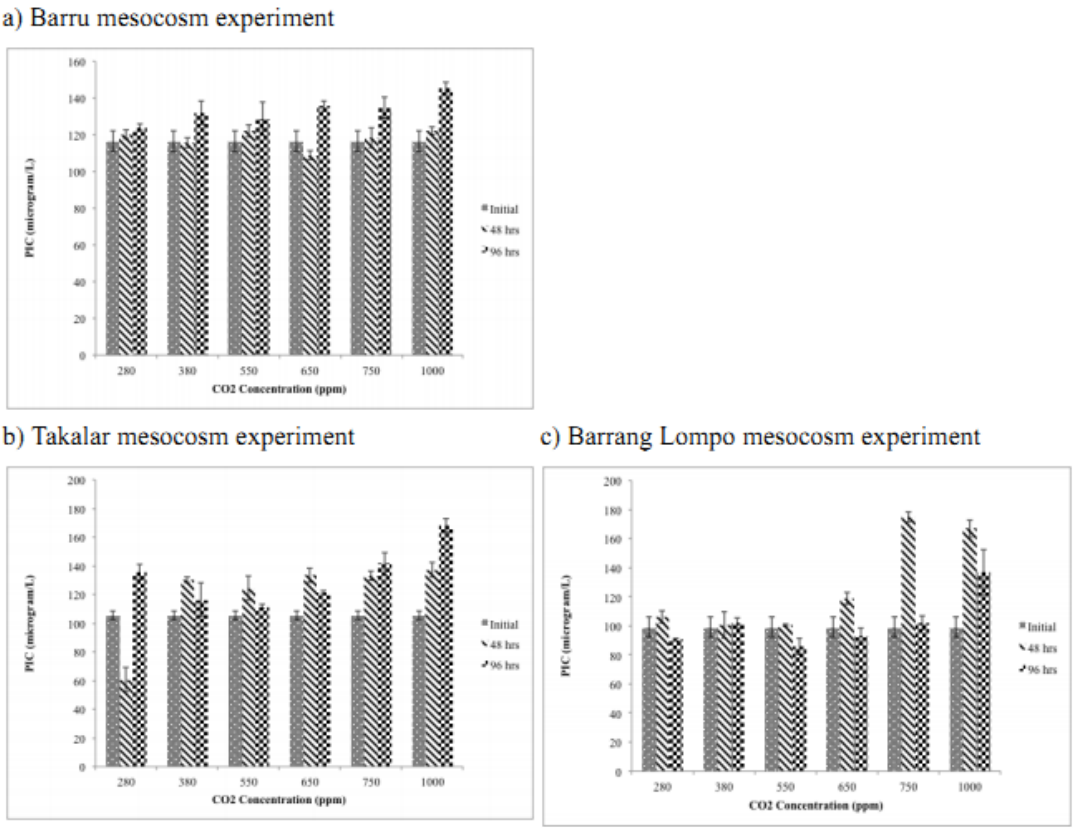

Figure 5 Mean of PIC (X ± SE) at six CO_2 concentration treatments for two incubation periods. a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

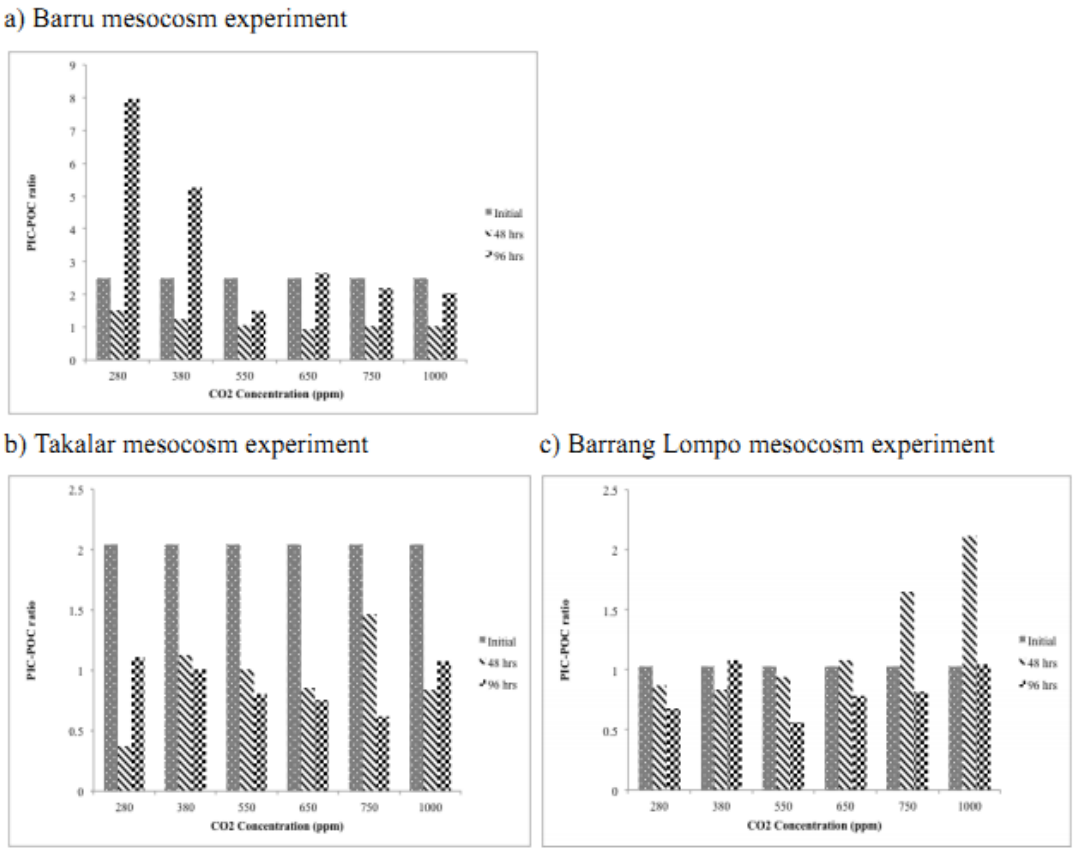

Figure 6 Mean of PIC/POC ratio (X ± SE) at six CO_2 concentration treatments for two incubation periods. a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

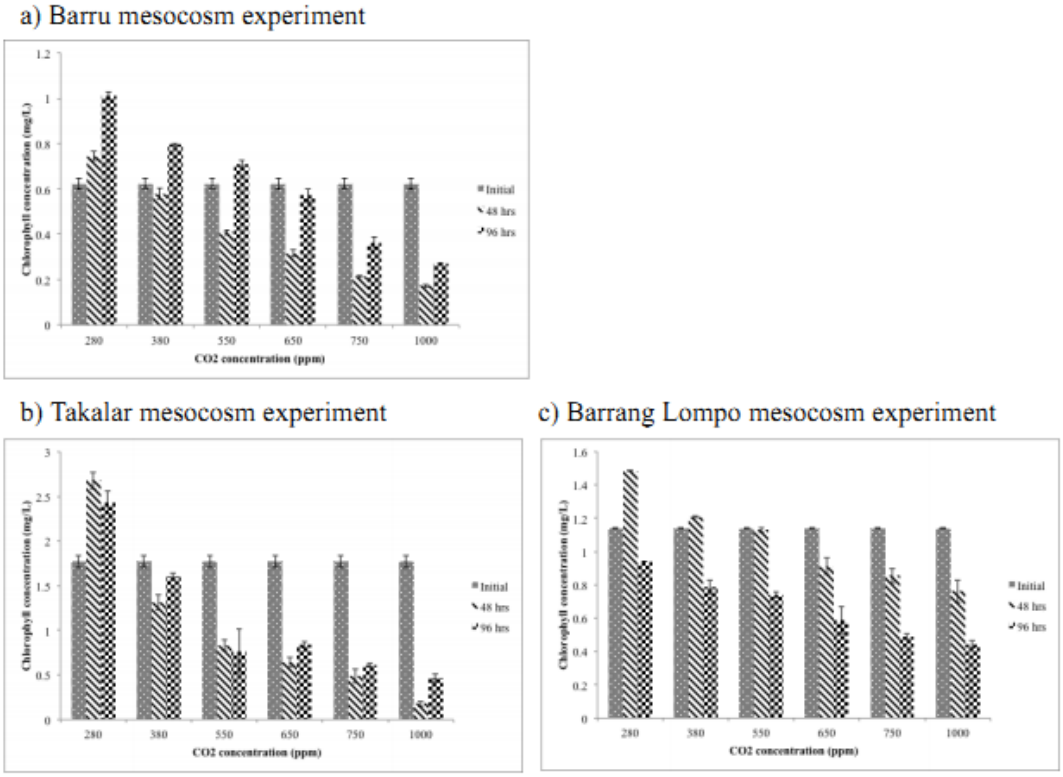

Figure 7 Mean of chlorophyll a concentration at six CO_2 concentration treatments for two incubation periods (X ± SE). a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

3.2 The effect of increasing CO_2 to chl a, cell abundance, growth rate and photosynthesis rate

3.2.1 Chlorophyll a concentration

Chlorophyll a concentration showed a varies trends between CO_2 concentration treatment for each location during two periods of incubation (Figure 7). Figure 7 showed that Chl a concentration for 96 hours incubation periods of mesocosm experiment at Barru was higher than 48 hours of incubation period at all CO_2 concentration treatments (Figure 7a). However, there was a similar trends of chl a concentration between 48 and 96 hours of incubation periods among CO_2 concentration treatments. Chl a concentration tend to decrease with increasing of CO_2 concentration. The lowest Chl a was found at 1000 ppm of CO_2 concentration for both 48 and 96 hours of incubation period. This result showed that there was a significant effect of increasing CO_2 concentration in phytoplankton Chl a concentration production.

Chlorophyll a concentration tends to be lower at 48 hours than 96 hours incubation period with increasing of CO_2 concentration, except at 280 and 550 ppm CO_2 for mesocosm experiment at Takalar (Figure 7b). The lowest Chl a concentration was found at 1000 ppm CO_2 for 48 hours incubation period account for 0.1782 mg/L and the highest Chl a concentration was 2.678 mg/L at 280 ppm CO_2 treatment in 48 hours incubation period.

Interestingly, the pattern of Chl a concentration for mesocosm experiment at Barrang Lompo Island was slightly different from the other locations of mesocosm experiment (Figure 7c). Figure 4 showed that Chl a conentration at all CO_2 treatments were higher at 48 hours than 96 hours of incubation period. However, the trend of Chl a concentration was similar between 48 hours and 98 hours of incubation period, which was decreasing of Chl a concentration with increasing of CO_2 conentration.

3.2.2 Cell abundance

Cell abundance was varied between CO_2 concentrations and length of incubation periods for all mesocosms experiment (Figure 8). For Barru mesocosm experiment, increasing CO_2 concentration affected significantly in decreasing cell abundance for both incubation periods (Figure 8a) and cell abundance was higher at 96 hours than 46 hours incubation period for all CO_2 treatments. Cell abundance at Takalar and Barrang Lompo mesocosm experiment showed a similar trend with increasing CO_2 concentration for both incubation periods (Figure 8b and 8c), which were a slightly different in cell abundance between 48 and 96 hours incubation period for all CO_2 treatments. The highest number of cell was found at Barrang Lompo mesocosm experiment account for 3250 cell and the lowest number of cell was found at Barru mesocosm experiment which was accounted for 100 cells.

3.2.3 Growth rate of phytoplankton

Results showed increasing CO_2 concentration affected negatively on growth rate of phytoplankton. All locations of mesocosms experiment showed a negative growth rate of phytoplankton with increasing CO_2 concentration for both incubation periods (Figure 9). However, the longer incubation period was the lower negative value of phytoplankton growth for all CO_2 treatments. The highest negative value of growth rate was found at Barru mesocoms experiment on 1000 ppm CO_2 concentration for 48 hours incubation periods (Figure 9a). On the other hand, the positive value of growth rate was found at the same location of experiment on 280 ppm CO_2 concentration for 96 hours incubation periods. This result was consistent with previous research, which showed that specific growth rate of *E. huxleyi*, which de- creased with pCO_2 and might have influenced differences in net PIC accumulation. Because net specific growth rates were derived from changes in cell concentration, we have to speculate whether cell division rate was directly affected by the CO_2 treatment or whether different loss rates (i.e., by grazing, sinking, or autolysis) occurred (Engel et al., 2005).

3.2.4 Photosynthesis rate

Photosynthesis rate is strong correlated to growth rate and POC production of phytoplankton. This study found that photosynthesis rate for all mesocosm experiments was negative due to the growth rate of those experiment were negative. However, photosynthesis rate was varied between CO_2 treatments and length of incubation period for all mesocosm experiments (Figure 10).

Figure 10 showed that increasing CO_2 concentration affected on decreasing photosynthesis rate at all location

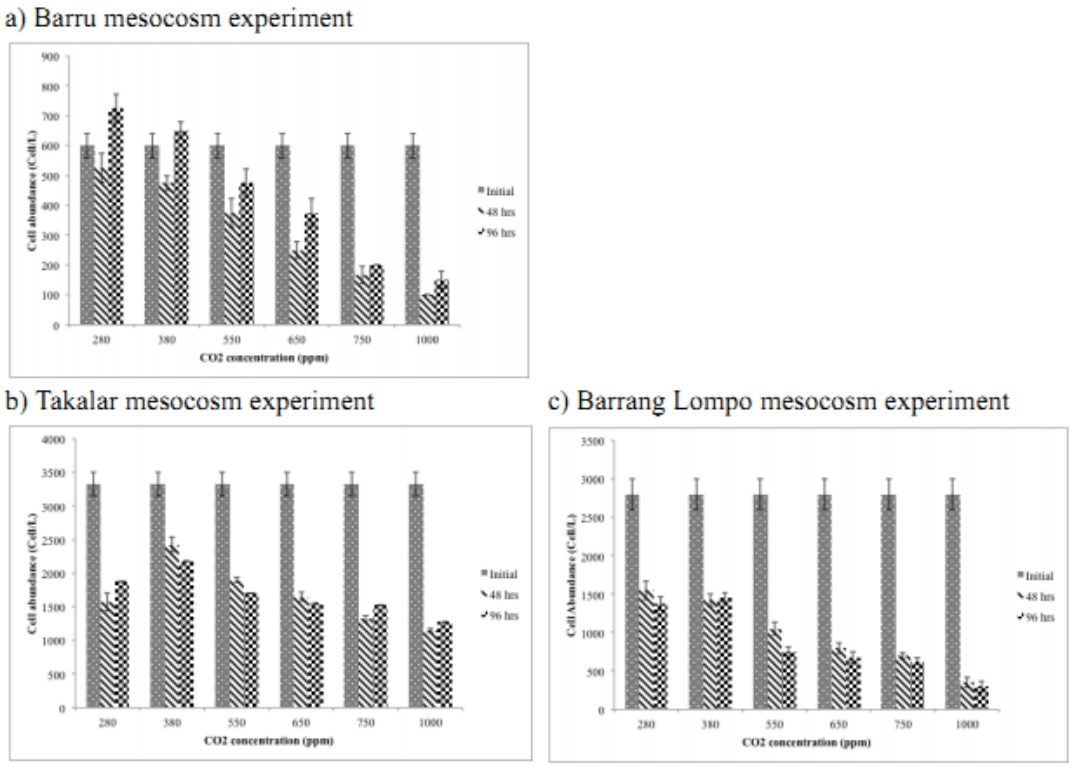

Figure 8 Mean of cell abundances at six CO_2 concentration treatments for two incubation periods (X ± SE). a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

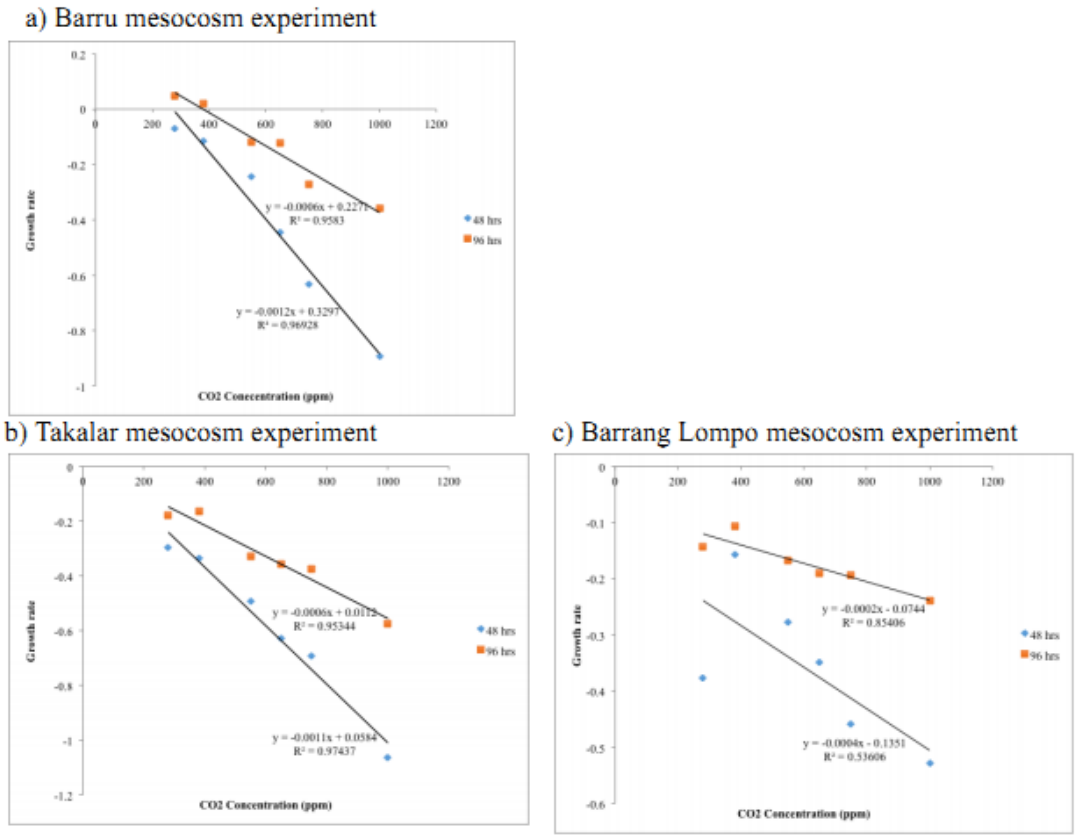

Figure 9 Mean of growth rate at six CO_2 concentration treatments for two incubation periods. a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

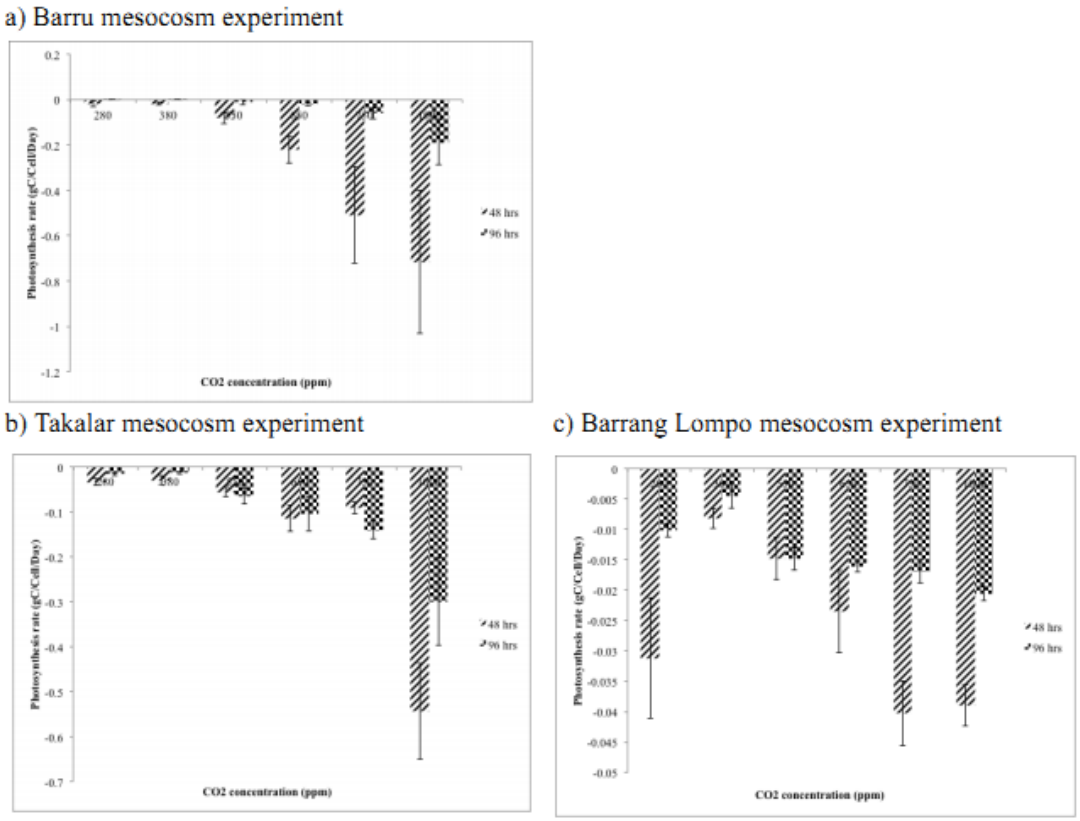

Figure 10 Mean of photosynthesis rate (X ± SE) at six CO_2 concentration treatments for two incubation periods. a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

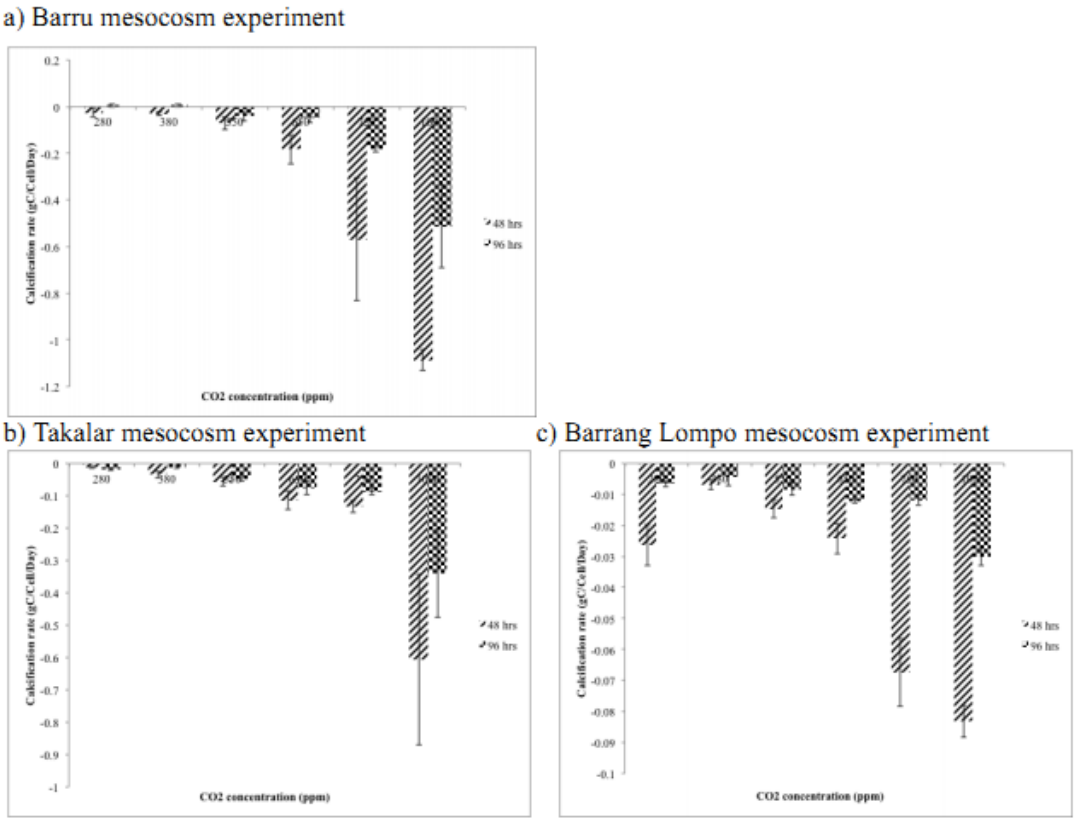

Figure 11 Mean of calcification rate (X ± SE) at six CO_2 concentration treatments for two incubation periods. a) Barru mesocosm experiment, b) Takalar mesocosm experiment and c) Barrang Lompo Island mesocosm experiment.

of mesocosm experiment, even the value of photosynthesis rate were varied within location. The result also showed that the length of incubation period impacted significantly on photosynthesis rate for all CO_2 treatments. There was a similar trend of photosynthesis rate for all mesocosm experiment, which were the longer incubation period was the higher photosynthesis rate for all mesocosm experiments. The lowest negative value of photosynthesis rate was found at Barrang Lompo mesocosm experiment account for -0.0019 gC/Cell/Day (Figure 10c) and the highest negative value of photosynthesis rate was -0.714 gC/Cell/Day at Barru mesocosm experiment for 1000 ppm CO_2 concentration at 48 hours incubation period (Figure 10a). The study finding that photosynthesis rate was negative due to the growth rate for all mesocosm experiments were negative. It showed that there was not organic material was produced during the incubation periods due to the effect of increasing CO_2 concentration. However, this finding was contracdicted with previous research which showed that increasing CO_2 concentration elevated photosynthesis rate at monoculture species. The contradicting of this finding possibly due to natural comunity phytoplanktoan was subjected in this study. Carbon-concentrating mechanisms enable most marine phytoplankton species to accumulate in-tracellular inorganic carbon either as CO_2 or HCO_3^- or both (Giordano et al., 2005). Largely because of these mechanisms, most marine phytoplankton tested in single-species laboratory studies and field population experiments show little or no change in photosynthetic rates when grown under high pCO_2 conditions equivalent to ～760 μatm (Tortell et al., 1997; Hein and Sand-Jensen, 1997; Burkhardt et al., 2001; Tortell and Morell, 2002; Rost et al., 2003; Beardall and Raven, 2004; Giordano et al., 2005; Martin and Tortell,2006).

3.2.5 Calcification rate

Calcification involves the precipitation of $CaCO_3$ from Ca^{2+} and CO_3^{2-} ions in solution. In most cases this involves the generation of microenvironments that allow supersaturation of $CaCO_3$ (Brownlee and Taylor, 2002). Our result found that there was a negative value of calcification rate for all CO_2 treatments for both incubation periods (Figure 11). In general, there was a similar trend of calcification rate or all location of mesocosm experiment. Calcification rate was higher at 48 hours than 96 hours incubation period for all CO_2 concentration. Our finding showed that increasing CO_2 concentration decreased calcification rate for both incubation periods. This finding also showed that length period of incubation affected significantly to calcification rate. The highest and the lowest of calcification rate was 0.006 and -1.088 gC/Cell/Day, respectively (11a). Decrease in the rate of calcification with increasing CO_2 concentration, as derived from changes in the seawater alkalinity by Delille et al. (unpubl.). Although differences in [PIC]:[POC] ratios could also result from an effect of CO_2 on cell growth and organic carbon production by *E. huxleyi*, the differences in [PIC] :[Cell] ratios and in the size and weight of coccoliths observed between the treatments is direct evidence that calcification itself was affected by the changes of the carbonate chemistry. Reduced calcification rates and direct effects of low pH on cell physiology nevertheless might have affected cell division rates. It has been previously demonstrated that many coastal marine phytoplankton species are directly sensitive to the pH of seawater and that the growth rate of some phytoplankton species has an optimum pH, below and above which the rate of cell division decreases (Hinga, 2002).

4 Conclusions

Increasing CO_2 concentration affected significantly to alkalinity, DIC, POC, PIC, physiological aspect of phytoplankton. Alkalinity and DIC were varied between location of mesocosm experiment and the treatments of CO_2 concentration and length period of incubation. In general, alkalinity decreased with increasing CO_2 concentration. However, length of incubation was showed no significant affected to DIC for all CO_2 concentration at Barru and Barrang Lompo mesocosm experiment.

PIC and POC showed a varied response between all locations of mesocosms experiment. POC at Barru showed a higher at 48 hours than 96 hours of incubation period. For Takalar and Barrang Lompo mesoscosm experiment was an apposite trend, which was higher of POC at 96 hours than 48 hours of incubation period. There was a trend where increasing

of POC with increasing of CO_2 concentration. PIC concentration at Barru and Takalar showed a slightl higher at 96 hours than 48 hours of incubation period and for Barrang Lompo mesocosm experiment PIC was higher at 48 hours than 96 hours of incubation period.

Chlorophyll a and cell abundance was decresing with increasing CO_2 concentration for all location of mesocosm experiment. Increasing CO_2 concentration affected negatively to growth, photosynthesis and calcification rate. Growth, photosynthesis and calcification rate decreased with increasing CO_2 concentration.

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