Photophysiology response of non-calcifying microalgae Chaetoceros sp. on increasing anthropogenic carbon dioxide and temperature

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Abstract. Increased use of fossil fuels, land use change, and deforestation, causing an increase in carbon emissions in the atmosphere, is estimated to 4.1± 0.1 GtC/year [1-3], partial pressure (pCO2) in the atmosphere has increased from pre-industrial 280 ppm to 379 ppm in 2005; or an increase of about 280 ppm to about 395 μatm and is estimated to reach a value of about 900 μatm by the end of the 21st century [2-4] 1500 μatm pCO2 between 2100-2200 [5]. The increasing CO2 it causing decrease pH of the ocean surface layer account for 0.3-0.5 units by 2100 [2-3,6-7] and changed chemical equilibrium, so increasing atmospheric CO2 causing climate change and ocean acidification [8,9]. Seawater carbonate chemistry is governed by a series of chemical reactions:

\[
\text{CO}_2(\text{atmos}) \leftrightarrow \text{CO}_2(\text{aq}) + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \leftrightarrow 3\text{H}^+ + \text{CO}_3^{2-} \]

The next stage of the dissociation of bicarbonate into carbonate (CO$_3^{2-}$) is followed by releasing a hydrogen ion. The process experienced by CO$_2$ gas after diffusion into the ocean waters becomes dissolved CO$_2$ (Figure 1).
The ability of the ocean to absorb CO$_2$ in the atmosphere depends on the rate of dissolution of CaCO$_3$ in the water column or sediment CaCO$_3$ $\Leftrightarrow$ Ca$^2+$ + CO$_3^{2-}$. The CaCO$_3$ minerals derived from the shells and skeletons of marine organisms, including calcifying plankton, corals and coralline algae, and other invertebrates. In the pelagic environment, carbonate falls through the water column and behind released or kept in shallow marine sediments or in the sea [10, 11].

The partial pressure of CO$_2$ in the oceans leads to increasing concentrations of CO$_2$ and bicarbonate (HCO$_3^-$), while the concentration of carbonate ions (CO$_3^{2-}$) decreased. These changes in the speciation of dissolved inorganic carbon (DIC) in which the pH value dropped, the phenomenon ocean acidification [7]. In many ways, ocean acidification related to carbonate chemistry changes has been shown to have an impact on marine organisms [12]. Especially for phytoplankton, which became the basis of marine food webs and the driver of biological carbon pump, these changes have consequences for the future [13, 14].

Increasing concentrations of carbon dioxide as a climate change impacts such as rising sea temperatures and increasing ocean acidity that result in dissolution of calcium carbonate (CaCO$_3$), this is a major threat to life-threatening extinction of algae including phytoplankton, and even affect the rate of population growth, chlorophyll-a on calcifying and non-calcifying phytoplankton productivity [15, 16]. The study was conducted to analyze the photophysiology response of calcifying microalgae (Emiliania huxleyi) and non-calcifying microalgae (Chaetoceros sp.) on increasing anthropogenic carbon dioxide and temperature.

2. Materials and methods

2.1. Materials
Monoclonal cultures of Emiliania huxleyi and Chaetoceros sp. were conducted at Plankton and Water Quality Laboratory of the Research Institution for Coastal Aquaculture (RICA) Maros. Containers used for monitoring the growth of cells were glass containers with 2 liters media equipped with aeration devices and fluorescent lamps. The cells were incubated at 23-25°C room temperature. Media used seawater with salinity 30 ppt screened using a 0.2 micron membrane filter, then it was autoclaved at 115°C for 30 minutes.

2.2. Experimental setup
The container was conditioned with 3 levels including 385 ppm CO$_2$ concentration (ambient); 0.075% balance N$_2$ mix (equal to 750 ppmv CO$_2$ scenario in 2050) and 0.1% CO$_2$, balance N$_2$ mix (equal to 1000 ppmv CO$_2$, the scenario in 2100) [7, 17, 18]. Research was carried out at the 3 x 3 factorial in a completely randomized design. The tested treatments were concentration factor of carbon dioxide (CO$_2$) including 385 ppm, 750 ppm, 1000 ppm. Temperature factor consists of 30°C, 32°C and 34°C with 3 replications per unit. Initial density of Emiliania huxleyi was 10$^4$ cells mL$^{-1}$ and Chaetoceros sp. was 1.5 x 10$^5$ cells mL$^{-1}$. For growing phytoplankton was using f2 media. Cell density was calculated daily using the Neubauer hemocytometer Improved.
Total population by 4 field observations (A, B, C, and D) was calculated with the equation (1):

\[ \sum_{sel} = \frac{A+B+C+D}{4} \times 10^4 \text{ sel mL}^{-1} \]  

(2)

Growth rate \((\mu)\) was determined as equation 2:

\[ \mu = \frac{\ln c_i - \ln c_0}{\Delta t} \]  

(3)

with \(c_i\) and \(c_0\) being the cell concentration at the beginning and the end of the experiment, respectively, and \(\Delta t\) the duration of the incubation in days.

2.3. Measurements chlorophyll-a

To determine chlorophyll-a, 100–200 ml culture suspension was filtered in duplicate on cellulose-nitrate Millipore filter paper with pores of 0.2 m (diameter 0.47 mm) using a vacuum pump a pressure of 50 mm Hg rapidly frozen in liquid nitrogen and subsequently stored at \(-80^\circ\text{C}\). Filter paper containing phytoplankton were wrapped with aluminum foil and paper desiccator was dried and put in the fridge to do the extraction.

The chlorophyll-a of phytoplankton was extracted with 90% acetone in a centrifuge tube, scalable, crushed and left for 24 hours in a light-tight box 5 ml volume of the extract was made, centrifuge rotation 4500 rpm for 15 min, the clear solution was measured using a spectrophotometer absorbance with wavelengths 664 nm, 647 nm, and 630 nm according to the method of Strickland & Parsons (1972).

The equation is based on the APHA [19]:

\[
\text{Chlorophyll-a (mg/m}^3\text{)} = \frac{[1.185 \times \text{OD}_{664} - (1.54 \times \text{OD}_{647}) - (0.08 \times \text{OD}_{630})]}{\text{Vs} \times \text{d}} \times \text{V1} 
\]

(4)

where:

\[
\begin{align*}
\text{OD}_{664} &= \text{Abs} \lambda_{664} - \text{Abs} \lambda_{750} \\
\text{OD}_{647} &= \text{Abs} \lambda_{647} - \text{Abs} \lambda_{750} \\
\text{OD}_{630} &= \text{Abs} \lambda_{630} - \text{Abs} \lambda_{750} \\
\text{V1} &= \text{acetone extract volume (ml)} \\
\text{Vs} &= \text{volume of water filtered (L)} \\
\text{d} &= \text{width diameter cuvette (1 cm)}
\end{align*}
\]

2.4. Statistics

The data were analyzed with Excel program, SPSS 20, and curve expert. Normality of data was confirmed using the Shapiro-Wilk test. Variables were log-transformed if this improved the homogeneity of variances, as tested by Levene’s test. Significant differences between treatments were tested using ANOVA, followed by post hoc of the means Bonferroni \((\alpha = 0.05)\), relationships between peak population, chlorophyll content and \(\text{CO}_2\) concentrations were tested by means of linear correlation.

3. Results and discussion

3.1. Performance and growth population of Chaetoceros sp.

Performance of phytoplankton cell Chaetoceros sp. was observed with a microscope magnification 400 times in Figure 2.
Figure 2. Cells performance of *Chaetoceros* sp. were observed with a microscope magnification of 400 times.

One of the essential factors for the growth of *Chaetoceros* sp. biomass was light, which played a role in the process of photosynthesis. This laboratory-scale study simulated sunlight that normally was used by phytoplankton with a capacity of 1500-2000 lux daylight lamp. As it was converted into energy units (mol photon.m-2.s-1), the daylight lamp irradiation capacity for culture was taking place from 20.76 to 27.68 mol photon.m-2.s-1.

This value was smaller as compared with optimal light in open waters, where the optimum light for photosynthesis of phytoplankton ranged from 204.64 to 241.20 mol photon.m-2.s-1. The life cycle of the development of the vegetative *Chaetoceros*, was sexual and "resting" spores. *Chaetoceros* commonly grown through vegetative cell division, during cell division and part epiteka hypoteka, each would form a new cell with a smaller size [20].

The growth of *Chaetoceros* sp. includes several phases of growth was the lag phase in which there was a slight increase in the number of cells in a relatively long time due to adaptation to changes in the culture medium. Later in the exponential phase occurred rapidly increasing the number of cells, then the stationary growth phase cells where cell division occurred slowly due to a decrease in the limiting factors such as nutrients, light, pH, CO₂ and other chemical, physical factors [21].

Population growth was characterized by the occurrence of cell division that started on day 2 after passing through a phase of adaptation. Thus, exponential phase occurred from day 2 to day 9 were seen at the peak of the population, then on days 9 through a phase in which the stationary cell division has begun to stagnate (no cells tend to grow even experiencing death) until the 10th day of the death phase was characterized by the declining number of cell populations, occurred at all levels of CO₂ concentration and temperature. Population growth on the highest daily concentration level was 385 ppm CO₂ at 30°C and 32°C (Figure 3).

**Figure 3.** Daily population growth (cells mL-1) *Chaetoceros* sp. on increasing CO₂ concentration (385 ppm, 750 ppm and 1000 ppm) and temperature (30°C, 32°C 34°C)
3.2. The peak population density and growth rate of Chaetoceros sp.
The highest Chaetoceros sp. cell abundance of \(2.2 \times 10^6\)±54037 cells mL\(^{-1}\) at a concentration of 385 ppm CO\(_2\) at 30°C, then decreased at 750 ppm CO\(_2\) concentration : \(1.4 \times 10^6\)±76675 sel mL\(^{-1}\); 1,2\( \times 10^5\)±69755 cell mL\(^{-1}\); and \(1.1 \times 10^5\)±41633 cells mL\(^{-1}\), respectively at 30°C, 32°C, 34°C and the lowest abundance at 9.7\( \times 10^5\)±7486 cell mL\(^{-1}\) on 1000 ppm CO\(_2\) concentration and 34°C (Table 1).

| CO\(_2\) conc. (ppm) | Temperature 30°C | Temperature 32°C | Temperature 34°C |
|---------------------|------------------|------------------|------------------|
| 385                 | 2.2\( \times 10^6\) ± 54037 | 2.1\( \times 10^6\) ± 90947 | 1.8\( \times 10^6\) ± 48883 |
| 750                 | 1.4\( \times 10^6\) ± 76677 | 1.2\( \times 10^6\) ± 69755 | 1.1\( \times 10^6\) ± 41633 |
| 1000                | 1.1\( \times 10^6\) ± 3550 | 1.08\( \times 10^6\) ± 5707 | 9.7\( \times 10^5\) ± 7486 |

Growth rate of Chaetoceros sp. was the highest at a concentration of 385 ppm CO\(_2\) namely: \(0.3743\)±0.00105 at 30°C, decreased at 750 ppm CO\(_2\) concentration, namely \(0.282\)±0.0077, \(0.272\)±0.0114, and \(0.2687\)±0.00417 at 30, 32, and 34°C, respectively. Meanwhile, the lowest was found in the concentration of 1000 ppm CO\(_2\) and 34°C, namely \(0.253\)±0.00086. The data showed that the trend of growth rate decreased with the increasing of CO\(_2\) concentration and temperature (Figure 4). Analysis of variance (ANOVA) shows that interaction affected by increasing CO\(_2\) concentration and temperature significantly influences on the growth rate of Chaetoceros sp. (\(P<0.005\)). Chaetoceros sp. trend of population decline was occurred with the increasing of CO\(_2\) concentration and temperature.

3.3. Chlorophyll content
Chlorophyll content described of phytoplankton productivity value, the observations obtained at the highest concentration of 385 ppm of CO\(_2\), decreased with increasing CO\(_2\) concentration. Chlorophyll content of Chaetoceros sp. listed in Table 2. The chlorophyll contented highest at concentration of 385 ppm CO\(_2\) at 35.94±1.62 mg.cm\(^{-3}\) decrease on the CO\(_2\) concentration of 750 ppm; 19.22±1.12; 15.47±1.23; 8.01±1.28 mg.cm\(^{-3}\); and the lowest at CO\(_2\) concentration of 1000 ppm account for 3.63±0.24 mg.cm\(^{-3}\) and 34°C, the trend of the data showed that the chlorophyll content dropped with the increase of CO\(_2\) concentration and the correlation between cell density with chlorophyll content showed at Figure 5a.b.
Table 2. Mean±SE chlorophyll content (mg.cm\(^{-3}\)) in the CO\(_2\) concentration (ppm) and temperature (°C). The Standard error are calculated from triplicate sample.

| CO\(_2\) conc. (ppm) | 30     | 32     | 34     |
|---------------------|--------|--------|--------|
| 385                 | 35.94 ± 1.62 | 33.06± 0.59 | 27.99± 1.26 |
| 750                 | 19.22 ± 1.12  | 15.47± 1.23 | 8.01 ± 1.28  |
| 1000                | 7.35 ± 0.43   | 4.01 ± 0.18  | 3.63 ± 0.24  |

Figure 5. (a. left). Chlorophyll content of Chaetoceros sp. on increasing CO\(_2\) concentration and temperature, (b. right). Correlation of between cell abundance with chlorophyll content of Chaetoceros sp.

Table 3. Results of analysis of variance (ANOVA) interaction effects of CO\(_2\) and temperature on the peak population of Chaetoceros sp. and chlorophyll content.

| Source             | DF | F   | P    |
|--------------------|----|-----|------|
| Peak Population    |    |     |      |
| Intercept          | 1  | 7889.611 | .000 |
| CO\(_2\)           | 2  | 354.612  | .002 |
| Temperature        | 2  | 23.115   | .003 |
| CO\(_2\)*Temperature | 4 | 1.758   | .001 |
| Error              | 18 |       |      |

| Source             | df | P | CO\(_2\) (ppm) | P    |
|--------------------|----|---|----------------|------|
| Peak population    |    |   | 385            | 750  | .000 |
| Intercept          | 1  | .000 | 385           | 750  | .000 |
| CO\(_2\) concentration | 2 | .000 | 1000          | .000 |
| Temperature        | 2  | .087 | 385           | .000 |
| CO\(_2\)*temperature | 3 | .003 | 1000          | .002 |
| Error              | 16 | 1000| 385           | .000 |

| Source             | df | P | CO\(_2\) (ppm) | P    |
|--------------------|----|---|----------------|------|
| Peak population    |    |   | 750            | 385  | .002 |
Carbon dioxide (CO$_2$) in the atmosphere and dissolved CO$_2$ in the oceans is an equilibrium state. Consequently, increasing CO$_2$ concentration in the atmosphere affected carbon dioxide concentration in the aquatic environment. The decline in ecosystem CO$_2$ will increase the pH of the water. In contrast, the process of respiration by all components of the ecosystem will increase the amount of CO$_2$, thus decreasing the pH water. The partial pressure of CO$_2$ in the oceans leads to increased concentrations of CO$_2$ and bicarbonate (HCO$_3^-$). The decrease in pH causes the concentration of carbonate ions (CO$_3^{2-}$) decrease. These changes in the speciation of dissolved inorganic carbon (DIC) in which the pH dropped [7].

Results showed that cell growth of *Chaetoceros* sp. was found the highest value at 385 ppm CO$_2$ concentration and temperature of 30°C amount for 0.3743±0.00105 at 30°C, decreased at 750 ppm CO$_2$ concentration, namely 0.282±0.0077, 0.272±0.0114, and 0.2687±0.00417 at 30, 32, and 34°C, respectively. Meanwhile, the lowest value was found at the concentration of 1000 ppm CO$_2$ and 34°C, namely 0.253±0.00086. This condition indicated that the increase in CO$_2$ gave effect on the ability of phytoplankton to perform disrupted cell division due to changes in the carbonate equilibrium in the medium. It also affects the synthesis process on phytoplankton cells, as the first effect of high pCO$_2$ on the thermodynamic equilibrium carbonate from seawater.

Decrease in seawater pH will have an effect on intracellular pH species changes in the pH of seawater will then affect the properties of proteins, cell membrane permeability and other biological functions, including calcium carbonate precipitation rate. Furthermore, a decrease in the pH of seawater will affect aragonite saturation levels. The consequences of high CO$_2$ are decreasing enzyme activity, inefficiency of cellular functions (e.g., proteins synthesis), and metabolic depression. Then, temperature has been considered one of the most important variables affecting algal growth [22-24] and the chemical composition of the marine microalga [25-27].

The highest cellular contents of nitrogen, carbon, and chlorophyll have been found at the extremes of the temperature growth range [28-31], in continuous cultures. Ecological and biogeochemical interaction among calcifying plankton, non-calcifying, seawater carbonate chemistry, and the resultant reciprocal of the concentration of atmospheric CO$_2$ are complex [32, 33].

| Growth rate | Intercept | CO$_2$ concentration | Temperature | CO$_2$*temperature | Error | Source | df | CO$_2$ (ppm) | P | Error | df | CO$_2$ (ppm) | P |
|-------------|-----------|----------------------|-------------|--------------------|-------|--------|----|-------------|---|-------|----|-------------|---|
| Intercept   | 1         |          385          | 750         |                   |       |        |    |             |   |       |    |             |   |
| CO$_2$ concentration | 2       | .001                 | 1000        |                   |       |        |    |             |   |       |    |             |   |
| Temperature | 2         | .003                 | 750         | 385               | .000  |        |    |             |   |       |    |             |   |
| CO$_2$*temperature | 2       | .002                 | 1000        | 385               | .000  |        |    |             |   |       |    |             |   |
| Error       | 16        | 1000                 | 385         | 750               | .000  |        |    |             |   |       |    |             |   |

**Chlorophyll content**

| Chlorophyll content | Intercept | CO$_2$ concentration | Temperature | CO$_2$*temperature | Error | Source | df | CO$_2$ (ppm) | P | Error | df | CO$_2$ (ppm) | P |
|---------------------|-----------|----------------------|-------------|--------------------|-------|--------|----|-------------|---|-------|----|-------------|---|
| Intercept           | 1         | .000                 | 385         | 750                | .000  |        |    |             |   |       |    |             |   |
| CO$_2$ concentration | 2       | .000                 | 1000        |                   |       |        |    |             |   |       |    |             |   |
| Temperature         | 2         | .001                 | 750         | 385               | .000  |        |    |             |   |       |    |             |   |
| CO$_2$*temperature  | 2         | .002                 | 1000        | 385               | .001  |        |    |             |   |       |    |             |   |
| Error               | 16        | 1000                 | 385         | 750               | .003  |        |    |             |   |       |    |             |   |

Consequently, increasing CO$_2$ concentration in the atmosphere affected carbon dioxide concentration in the aquatic environment. The decline in ecosystem CO$_2$ will increase the pH of the water. In contrast, the process of respiration by all components of the ecosystem will increase the amount of CO$_2$, thus decreasing the pH water. The partial pressure of CO$_2$ in the oceans leads to increased concentrations of CO$_2$ and bicarbonate (HCO$_3^-$). The decrease in pH causes the concentration of carbonate ions (CO$_3^{2-}$) decrease. These changes in the speciation of dissolved inorganic carbon (DIC) in which the pH dropped [7].

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The highest cellular contents of nitrogen, carbon, and chlorophyll have been found at the extremes of the temperature growth range [28-31], in continuous cultures. Ecological and biogeochemical interaction among calcifying plankton, non-calcifying, seawater carbonate chemistry, and the resultant reciprocal of the concentration of atmospheric CO$_2$ are complex [32, 33].
Phytoplankton have different sensitivity to CO$_2$ concentration and variation mechanisms against the use of carbon [34]. Most phytoplankton primary production was resulted from the activity of two functional groups: coccolithophorid and diatom [35]. Phytoplankton have different sensitivity to CO$_2$ concentration and variation mechanisms against the use of carbon [34]. Most phytoplankton primary production resulting from the activity of two functional groups: coccolithophorid and diatom [35].

The increase of CO$_2$ concentrations significantly affect the productivity of Chaetoceros sp. as the ANOVA indicating the analysis of diversity effects on increasing CO$_2$ and temperature. The peak population of Chaetoceros sp. occurred as CO$_2$ and temperature significantly affected the plankton population (p <0.005) as similarly the effect of the interaction effect (p <0.005), indicating that the growth Chaetoceros sp. degraded by the increase in CO$_2$ and temperature (Table 3).

4. Conclusion
The increasing CO$_2$ concentration affected the growth of daily population, peak population, the rate of cell growth, and chlorophyll content of Chaetoceros sp.. The highest population growth and chlorophyll content was occurred at 385 ppm CO$_2$ with temperature of 32°C, population decreased with increasing CO$_2$ concentration and temperature. Increasing carbon dioxide and temperature significantly affected on degradation of Chaetoceros sp. productivity.

Acknowledgements
The authors wish to thank for the support of Research Collaboration Essex University United Kingdom with Research Centre Hasanuddin University and parties involved in this study, including technician of water quality and plankton in water quality/planktonology laboratory of RICAFE Maros for their active engagement and providing the data.

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