Effects of Increased CO₂ and Temperature on the Growth of Four Diatom Species (Chaetoceros debilis, Chaetoceros didymus, Skeletonema costatum and Thalassiosira nordenskioeldii) in Laboratory Experiments

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Abstract

We examined the combined impacts of future increases of CO₂ and temperature on the growth of four marine diatoms (Skeletonema costatum, Chaetoceros debilis, Chaetoceros didymus, Thalassiosira nordenskioeldii). The four strains were incubated under four different conditions: present (pCO₂: 400ppm, temperature: 20°C), acidification (pCO₂: 1000ppm, temperature: 20°C), global warming (pCO₂: 400ppm, temperature: 25°C), and greenhouse (pCO₂: 1000ppm, temperature: 25°C) conditions. Under the condition of higher temperatures, growth of S. costatum was suppressed, while C. debilis showed enhanced growth. Both C. didymus and T. nodenskioeldii showed similar growth rates under current and elevated temperature. None of the four species appeared affected in their cell growth by elevated CO₂ concentrations. Chetoceros spp. showed increase of pH per unit fluorescence under elevated CO₂ concentrations, but no difference in pH from that under current conditions was observed for either S. costatum or T. nodenskioeldii, implying that Chetoceros spp. can take up more CO₂ per cell than the other two diatoms. Our results of cell growth and pH change per unit fluorescence suggest that both C. debilis and C. didymus are better adapted to future oceanic conditions of rising water temperature and CO₂ than are S. costatum and T. nodenskioeldii.

Key words: Future temperature and CO₂ increase, Acidification, Global warming, Greenhouse, Diatom

1. Introduction

Global warming gases (greenhouse gases; mainly carbon dioxide) are continuously being emitted into the atmosphere, with carbon dioxide increasing in the atmosphere by 0.4% each year. Its concentration has increased by about 30% since the Industrial Revolution (Feng et al., 2009), and the CO₂ level in 2100 is predicted to be twice the current CO₂ level (Houghton et al., 2001; Alley et al., 2007). If the current rate of increase atmospheric carbon dioxide continues, it will lower ocean surface pH about 0.35 units (Caldeira and Wickett, 2003), and increase the surface temperature by at least 1.1 °C (low CO₂ emission scenario B1) and possibly up to 6.4 °C, the high CO₂ emission scenario "A1FI " suggested by the year 2100 (Huertas et al., 2011). Such ocean acidification and seawater temperature increases can affect the biogeochemical cycles of the ocean, including the physiology, species composition, and interspecific competition of marine organisms.
Diatoms account for approximately 25% of global primary production in marine ecosystems, playing an important role in the marine carbon cycle (Hu and Gao, 2008). Diatoms typically require larger amounts of carbon dioxide than is in solution in the oceans, as in general their growth in seawater has been proved to be CO₂-limited. To cope with this deficiency, diatoms are known to have carbon-concentrating mechanisms (CCMs) (Rost et al., 2003; Giordano et al., 2005). Operation of the CCMs ensures that internal carbon dioxide concentrations are close to saturating the photosynthetic capacity for its fixation (Engel et al., 2005). Kim et al. (2006) reported that enhanced CO₂ concentration can stimulate the abundance of chain forming diatom *Skeletonema costatum*, and Tortell et al. (2008) suggested that elevated CO₂ concentration can stimulate the abundance of chain forming diatom *Skeletonema costatum*, and Tortell et al. (2002) showed that increasing CO₂ concentrations resulted in a higher growth rate and carbon fixation at higher concentrations of carbon dioxide (Matsuda et al., 2001, Wu et al., 2010). Therefore, atmospheric CO₂ rise and associated increase in seawater pCO₂ may not adversely affect marine diatom production (Wu et al., 2010). However, CCMs can be down-regulated by increasing CO₂ levels, reducing their CO₂-transfer capacity (Giordano et al., 2005). Moreover, recent studies have demonstrated that phytoplankton species and functional groups differ regarding the efficiency and regulation of their CCMs, indicating that changes in CO₂ availability might affect competition and succession of phytoplankton species (Burkhart et al., 2001; Rost et al., 2003). For instance, Tortell et al. (2002) showed that increasing CO₂ concentration changed the relative success of phytoplankton groups, with the prymnesiophyte *Phaeocystis* sp. favored at pCO₂ 150 ppm and diatoms at 750 ppm. However, it is likely that CO₂ dependence variations are not necessarily consistent even among diatoms.

Until recently, most studies have focused singly on the effects of CO₂ or water temperature on phytoplankton growth, and studies of the combined effects have been limited to dinoflagellates (Fu et al., 2008) and certain blue-green algae (Fu et al., 2007). Few studies have been performed for dominant diatoms. Our study was performed to examine growth of the marine diatoms *Skeletonema costatum*, *Chaetoceros debilis*, *Chaetoceros didymus*, and *Thalassiosira nordenskioldii* under current and three simulated future conditions of water temperature and CO₂ level: acidification, global warming, and intense greenhouse conditions. The consumption of carbon dioxide under these conditions was indirectly estimated by determining pH in the culture media.

2. Materials and Methods

From diatom cultures maintained at the Korea Institute of Ocean Science and Technology (KIOST), four species (*S. costatum*, *C. debilis*, *C. didymus*, and *T. nordenskioldii*) were used in this study. Seawater for culturing diatoms was collected from the Korea Strait (salinity range, 29.9-34.8 psu from 2006 to 2008) 18.5 km from the southern coast of Korea. The seawater was passed through a membrane filter (pore size 0.2μm, Advantec), and was enriched with f/2 medium with SiO₄. The experimental culture media were autoclaved (15 min, 121°C). Crystal rising dishes (diameter 115mm x height 65mm, DURAN™ borosilicate glass) were used, and the incubator (Lab-tech co.) was modified to facilitate the inflow of carbon dioxide gas required for the experiment. We used CO₂ gas at high precision concentrations of commercially prepared air/CO₂ mixtures (400 ppm and 1000 ppm, air balance). The salinity was maintained at about 31 psu, light intensity at 60 μmol m⁻²s⁻¹, and the photoperiod at a cycle of 12h light: 12h dark (Table 1).

A control group (present condition- pCO₂: 400ppm, water temperature: 20°C) and three experimental
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Environmental parameters

<table>
<thead>
<tr>
<th>Environmental parameters</th>
<th>Present</th>
<th>Acidification</th>
<th>Global warming</th>
<th>Greenhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$CO$_2$ (ppm)</td>
<td>400</td>
<td>1000</td>
<td>400</td>
<td>1000</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20±1.0</td>
<td>20±1.0</td>
<td>25±1.0</td>
<td>25±1.0</td>
</tr>
<tr>
<td>pH</td>
<td>8.17±0.1</td>
<td>7.81±0.1</td>
<td>8.17±0.1</td>
<td>7.81±0.1</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>31.4±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photoperiod (hr)</td>
<td>12 light : 12 dark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>Filtered (0.2µm) and sterilized seawater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient</td>
<td>Adding the F/2 media 132µL and silicate (10mM) 1mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chamber</td>
<td>0.5 liter volume Duran Borosilicate Glass, ø115mm×h65mm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Initial experimental conditions for four diatom species grown under present, acidification, global warming and greenhouse conditions

when the populations were in late exponential phase or early stationary phase. A sample of 15 mL was taken from each bottle for each measurement of FSU and pH, and the growth rates, as doubling per day ($\mu$), were calculated by applying

$$\mu = (\log_2 N_t - \log_2 N_0) / t$$

where $t$ is length of incubation (days), $N_0$: initial value of FSU, $N_t$: FSU value at the end of the experiment.

With uptake of CO$_2$ by phytoplankton, pH in the culture medium can increase substantially. To maintain the pH values more or less at the initial levels of the experiments, small amounts of (1-5 mL) freshly sterilized culture medium hypersaturated with CO$_2$ (10,000 ppm) were added to the culture dishes. The volume of culture medium in the culture dishes was maintained > 70% of the initial volume until the end of experiment.

The FSU normalized pH values for the last two samplings (generally between Day 6 and 7 or Day 7 and 8) of the incubation were calculated to examine species-specific capability of chlorophyll synthesis (i.e., growth) per unit uptake of CO$_2$ concentration (i.e., pH increase as a proxy for CO$_2$ uptake).

One-way analysis of variance (n=2 or 3) and
Tukey's honestly significant difference tests (Tukey's HSD) were performed to compare the mean growth rates among the four conditions. Normality of the variations was examined from the distribution of values around the means.

3. Results and Discussion

All of the four diatom species showed exponential growth in the control (i.e., present condition) dishes, but with growth rates ranging from 0.54 doublings d\(^{-1}\) (C. debilis) to 0.96 doublings d\(^{-1}\) (T. nordenskioeldii). A lag phase was observed in the control dishes for all species; growth was generally slower in the first 3 days of incubation.

The four diatom species exhibited different growth responses to various simulated conditions. Skeletonema costatum showed repressed growth under global warming and greenhouse conditions (0.65 doublings d\(^{-1}\) and 0.68 doublings d\(^{-1}\), respectively) compared with present and acidification conditions (0.87 doublings d\(^{-1}\) and 0.85 doublings d\(^{-1}\), respectively) (Fig. 1a and 2a, Tukey’s HSD, p<0.05). Its growth was not different between present and acidification conditions or between global warming and greenhouse conditions (Tukey’s HSD, p>0.05). Increased temperature, but not elevated CO\(_2\) level in the culture, affected the cell growth of this diatom (Table 2).

Chaetoceros debilis grew more or less similarly in all simulated conditions (Fig. 1b and 2b), whereas C. didymus grew much faster under global warming and greenhouse condition (Fig. 1c and 2c), quite the opposite from the results for S. costatum. Chaetoceros didymus also showed slightly better growth in the acidification condition (0.77 doublings d\(^{-1}\)) than under the present condition (0.73 doublings d\(^{-1}\)). Its growth was not different between global warming and greenhouse condition, which suggests that water temperature increase had a greater effect on the growth than did the elevated CO\(_2\) level (Table 2).

Thalassiosira nordenskioldii grew the fastest among the four diatom species (0.88-0.96 doublings d\(^{-1}\)). However, fluorescence for T. nordenskioeldii increased at similar rates for all simulated conditions over the incubation period, resulting in no significant difference in the growth rate (Fig. 1d and 2d).

The pH in the culture media varied quite differently among species over the incubation period (Fig. 3), with the least variation observed in C. debilis cultures that grew the slowest and the most in T. nordenskioeldii cultures that grew the fastest. The pH quickly rose for all treatments in the T. nordenskioeldii cultures (Fig. 3d) such that addition of concentrated CO\(_2\) solution was necessary to bring down the pH in the cultures to the level at the beginning. Such rises in pH in the cultures of T. nordenskioeldii and other phytoplankton cultures were likely due to cellular uptake of CO\(_2\) from the media, uptake which took place mostly in the later days (e.g., Day 4 or 5 for T. nordenskioeldii) of the incubation. The variation differed among the simulated conditions for each species, but generally was positively correlated with cell growth under each condition (Fig. 1 vs. Fig. 3).

**Table 2.** Predicted outcome in future ocean for four diatom species in response to combined impacts of CO\(_2\) and temperature increase

<table>
<thead>
<tr>
<th>Condition</th>
<th>Skeletonema costatum</th>
<th>Chaetoceros debilis</th>
<th>Chaetoceros didymus</th>
<th>Thalassiosira nordenskioldii</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2)</td>
<td>Neutral</td>
<td>Neutral</td>
<td>Neutral</td>
<td>Neutral</td>
</tr>
<tr>
<td>Temperature</td>
<td>Negative</td>
<td>Neutral</td>
<td>Positive</td>
<td>Neutral</td>
</tr>
<tr>
<td>Future ocean</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Neutral</td>
</tr>
</tbody>
</table>
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Fig. 1. Changes of fluorescence during experimental periods for four diatom species under present, acidification, global warming and greenhouse conditions. (a) Skeletonema costatum, (b) Chaetoceros debilis, (c) Chaetoceros didymus, (d) Thalassiosira nordenskioeldii.

Fig. 2. Comparison of growth rate of four diatom species for four different growth conditions. The vertical bars represent 95% confidence interval (n=2 for Thalassiosira nordenskioeldii culture in acidification and greenhouse condition, other all species culture conditions in n=3).
Fig. 3. Changes of pH concentration of four diatom species under present, acidification, global warming and greenhouse conditions during the experimental periods. (a) Skeletonema costatum, (b) Chaetoceros debilis, (c) Chaetoceros didymus, (d) Thalassiosira nordenskioeldii. The arrows indicate the time points when concentration CO₂ was added to phytoplankton culture.
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The FSU normalized pH changes for the last two sampling times (generally between Days 6 and 8 or Days 7 and 8, Fig. 4), during which the cells grew exponentially, followed the same patterns of pH change over the incubation period (Fig. 4). The ratio, along with the results of pH change (Fig. 3), could indicate higher cell growth under future marine conditions of higher concentration of CO₂, and thus those species with higher values might be better suited to future conditions. Cultures of S. costatum and T. nordenskioeldii were not different in pH increase per FSU (pH/FSU) among the four conditions (Fig. 4a and 4d). For C. debilis, increased pH/FSU was observed only in the greenhouse treatment (Fig. 4b). Chaetoceros didymus showed more a complex pattern, the greenhouse condition having a significantly greater pH/FSU increase than the global warming condition, and the pH/FSU of the acidification condition was greater than for both the greenhouse and the present conditions (Fig. 4c).

Our results show that diatoms respond in various ways to increased water temperature and CO₂ level, ranging from suppression in growth, to no response, to enhanced growth. More specifically, the diatoms studied appeared to be more affected by water temperature than by CO₂ level. Growth of S. costatum is generally unaffected by elevation of CO₂ concentration (Chen and Gao 2003). No changes in its growth were observed even at very low CO₂ concentration (<4 μmol L⁻¹) (like results of Goldman, 1999). Cultures of the diatom Chaetoceros muelleri showed lower growth and/or no response in elevated CO₂ concentrations (Gao et al., 2012). In contrast, in a mesocosm study, both S. costatum and Chaetoceros spp. showed increased growth rates with increased pCO₂ (Kim et al., 2010). The reduced growth of S. costatum at 25°C (Fig. 1a) is consistent with the observation of Montagnes and Franklin (2001). Their S. costatum cultures grew faster with temperature increase up to 20°C, but reduced growth at 25°C. However, S. costatum is a eurythermal species consisting of many sub-strains (Sarno et al., 2007), growing at water temperatures from 2.0°C to 30°C (Hitchcock, 1980), strains appearing through the year.
off the coasts of Korea and Japan (Oh et al., 2008; Shikata et al., 2008; Park et al., 2009). Maximal growth rates of different strains are found between 20 and 30 °C (Yoder, 1979), and thus more studies for various strains of this species are necessary fully to characterize their responses to temperature and CO2 increases.

The *Chaetoceros* species annually occur around in coastal waters around the world (Guiry and Guiry, 2012), and they mainly occur in spring and autumn in korean seawaters, frequently being the dominant phytoplankton species (Park et al., 2009). However, recent studies show that they usually occur massively in summer (Baek and Kim, 2010) and grow better in the laboratory when grown at temperatures higher than the in situ temperature (Karentz and Smayda, 1984). Our results of higher growth rates for both *C. debilis* and *C. didymus* at high temperature are consistent with previous studies.

*Thalassiosira nordenskioeldii* is reported to occur at high density in cold seasons (winter and spring) in Long Island Sound and Narragansett Bay (Popovich and Gayoso, 1999). It is also known as a cold-water species in Korean waters, present at high density in seasons of low temperature (Choi et al., 1997). Durbin (1974) reported that the species grew slower as the culture temperature rose from 10 °C to 15 °C, but no such growth repression at higher temperature was detected in our study (Fig. 1d).

Phytoplankton groups and species greatly vary in their requirements for the carbon dioxide that is essential for their growth (Riebesell, 2004). Previous studies indicate that diatoms may respond in various ways to increased CO2 as ocean acidification progresses, from better growth (positive response) (Schippers et al., 2004; Kim et al., 2006; Egge et al., 2009), to no effects (neutral response) (Gao et al., 2012), to reduced growth (negative response) (Wu et al., 2010). In this study, enhanced carbon dioxide concentration seemed to have no adverse effects on the growth for the four species examined. For *Chaetoceros* spp., future rise in CO2 may not only increase their growth but also their uptake of CO2, leading to potentially faster growth rates and greater carbon fixation by this genus. Thus, in the likely warmer, higher-CO2 oceans of the future, *Chaetoceros* spp. are apparently better adapted for survival and growth than are the other two species.

4. Conclusions

Growth rate determinations in elevated CO2 and temperature showed no negative effects of more CO2 in any of four diatom species studied; in fact it stimulated their growth. All of the adverse effects were from temperature increase. With their higher carbonate consumption capacity, *Chaetoceros* spp. seem to be better adapted than other two species studied to coastal waters of increased CO2 and temperature.

Acknowledgments

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Reference


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