

ORIGINAL ARTICLE

## Effects of Increased CO<sub>2</sub> and Temperature on the Growth of Four Diatom Species (*Chaetoceros debilis*, *Chaetoceros didymus*, *Skeletonema costatum* and *Thalassiosira nordenskioldii*) in Laboratory Experiments

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### Abstract

We examined the combined impacts of future increases of CO<sub>2</sub> and temperature on the growth of four marine diatoms (*Skeletonema costatum*, *Chaetoceros debilis*, *Chaetoceros didymus*, *Thalassiosira nordenskioldii*). The four strains were incubated under four different conditions: present (pCO<sub>2</sub>: 400ppm, temperature: 20°C), acidification (pCO<sub>2</sub>: 1000ppm, temperature: 20°C), global warming (pCO<sub>2</sub>: 400ppm, temperature: 25°C), and greenhouse (pCO<sub>2</sub>: 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. nordenskioldii* showed similar growth rates under current and elevated temperature. None of the four species appeared affected in their cell growth by elevated CO<sub>2</sub> concentrations. *Chaetoceros* spp. showed increase of pH per unit fluorescence under elevated CO<sub>2</sub> concentrations, but no difference in pH from that under current conditions was observed for either *S. costatum* or *T. nordenskioldii*, implying that *Chaetoceros* spp. can take up more CO<sub>2</sub> 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 CO<sub>2</sub> than are *S. costatum* and *T. nordenskioldii*.

**Key words** : Future temperature and CO<sub>2</sub> 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 CO<sub>2</sub> level in 2100 is predicted to be twice the current CO<sub>2</sub> 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 CO<sub>2</sub> emission scenario B1) and possibly up to 6.4°C, the high CO<sub>2</sub> 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

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(Fu et al., 2007).

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 CO<sub>2</sub>-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 CO<sub>2</sub> concentration can stimulate the abundance of chain forming diatom *Skeletonema costatum*, and Tortell et al. (2008) suggested that elevated CO<sub>2</sub> enhanced the growth of *Chaetoceros* spp. in the Southern Ocean. The diatom *Phaeodactylum tricorutum* showed faster growth and greater carbon fixation at higher concentrations of carbon dioxide (Matsuda et al., 2001, Wu et al., 2010). Therefore, atmospheric CO<sub>2</sub> rise and associated increase in seawater pCO<sub>2</sub> may not adversely affect marine diatom production (Wu et al., 2010). However, CCMs can be down-regulated by increasing CO<sub>2</sub> levels, reducing their CO<sub>2</sub>-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 CO<sub>2</sub> availability might affect competition and succession of phytoplankton species (Burkhardt et al., 2001; Rost et al., 2003). For instance, Tortell et al. (2002) showed that increasing CO<sub>2</sub> concentration changed the relative success of phytoplankton groups, with the prymnesiophyte *Phaeocystis* sp. favored at pCO<sub>2</sub> 150 ppm and diatoms at 750 ppm. However, it is likely that CO<sub>2</sub> dependence variations are not necessarily consistent even among diatoms.

Until recently, most studies have focused singly on

the effects of CO<sub>2</sub> 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 nordenskiöldii* under current and three simulated future conditions of water temperature and CO<sub>2</sub> 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. nordenskiöldii*) 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 SiO<sub>4</sub>. 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 CO<sub>2</sub> gas at high precision concentrations of commercially prepared air/CO<sub>2</sub> mixtures (400 ppm and 1000 ppm, air balance). The salinity was maintained at about 31 psu, light intensity at 60 µmol m<sup>-2</sup>s<sup>-1</sup>, and the photoperiod at a cycle of 12h light: 12h dark (Table 1).

A control group (present condition- pCO<sub>2</sub>: 400ppm, water temperature: 20°C) and three experimental

groups (acidification condition - pCO<sub>2</sub>: 1000ppm, water temperature: 20°C; global warming condition- pCO<sub>2</sub>: 400ppm, water temperature: 25°C; greenhouse condition - pCO<sub>2</sub>: 1000ppm, water temperature: 25°C) were set up to simulate three possible future conditions of ocean water based on the IPCC A2 Scenarios (IPCC, 2007) (Table 1). Each level of CO<sub>2</sub> gas was injected into the incubation dishes until their pH values stabilized, and then phytoplankton were inoculated at 50 to 100 cells mL<sup>-1</sup> for each dish. The culture dishes (treatments replicated in triplicate), with their tops open to allow the air-CO<sub>2</sub> mixtures to exchange with their interiors, were arranged in a air-tight sealed transparent acrylic container (1400mm X 250mm X 400mm) for each treatment inside the incubators set at 20°C (i.e., containers for present and acidification condition) and 25°C (i.e., containers for global warming and greenhouse condition), respectively. The concentration of carbon dioxide in each dish was maintained by continuously injecting the air-CO<sub>2</sub> mixture into each container at 400 ppm and 1,000 ppm.

Growth of the phytoplankton cultures was checked by measuring *in vivo* fluorescence (FSU) (Turner Designs 10-AU) daily after the third day of incubation, along with the pH changes in the medium with a pH meter (Orion Inc.). The experiments were terminated

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<sub>0</sub>: initial value of FSU, N<sub>t</sub> : FSU value at the end of the experiment.

With uptake of CO<sub>2</sub> 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<sub>2</sub> (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<sub>2</sub> concentration (i.e., pH increase as a proxy for CO<sub>2</sub> uptake).

One-way analysis of variance (n=2 or 3) and

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

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

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<sup>-1</sup> (*C. debilis*) to 0.96 doublings d<sup>-1</sup> (*T. nordenskioldii*). 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<sup>-1</sup> and 0.68 doublings d<sup>-1</sup>, respectively) compared with present and acidification conditions (0.87 doublings d<sup>-1</sup> and 0.85 doublings d<sup>-1</sup>, 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<sub>2</sub> 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

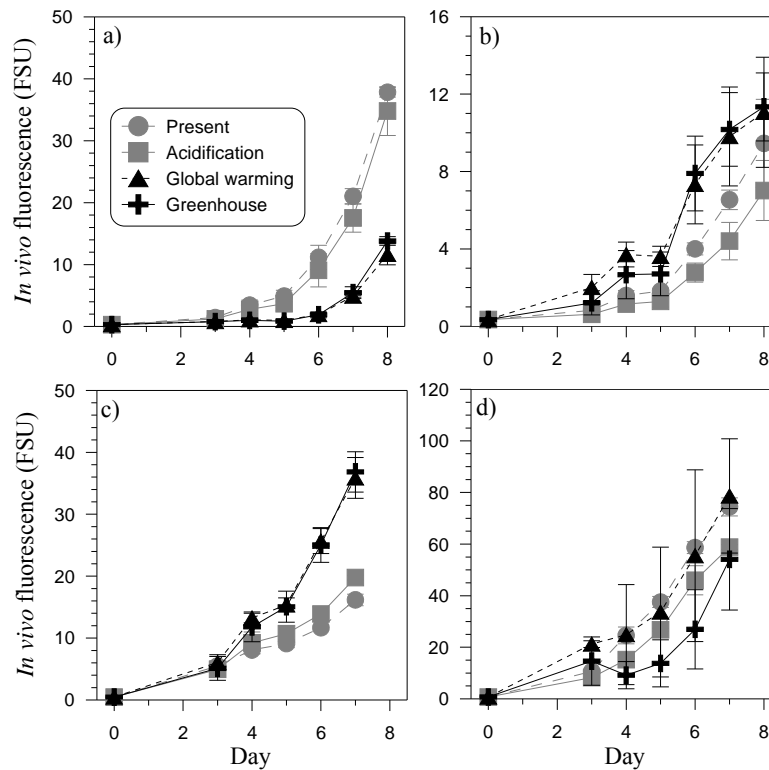
opposite from the results for *S. costatum*. *Chaetoceros didymus* also showed slightly better growth in the acidification condition (0.77 doublings d<sup>-1</sup>) than under the present condition (0.73 doublings d<sup>-1</sup>). Its growth was not different between global warming and greenhouse condition, which suggests that water temperature increase had a greater effect on the growth than did the elevated CO<sub>2</sub> level (Table 2).

*Thalassiosira nordenskioldii* grew the fastest among the four diatom species (0.88-0.96 doublings d<sup>-1</sup>). However, fluorescence for *T. nordenskioldii* 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).

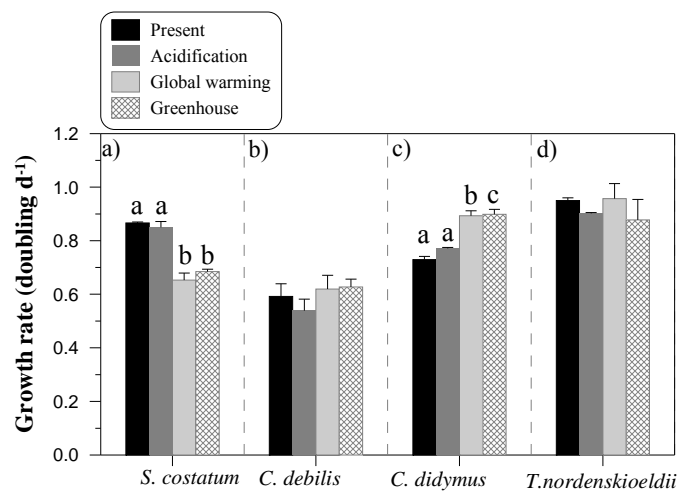
The pH in the culture media varied quite differently among species over the incubation period (Fig. 3), with the least variation observed in *C. debilis* cultures that grew the slowest and the most in *T. nordenskioldii* cultures that grew the fastest. The pH quickly rose for all treatments in the *T. nordenskioldii* cultures (Fig. 3d) such that addition of concentrated CO<sub>2</sub> 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. nordenskioldii* and other phytoplankton cultures were likely due to cellular uptake of CO<sub>2</sub> from the media, uptake which took place mostly in the later days (e.g., Day 4 or 5 for *T. nordenskioldii*) 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<sub>2</sub> and temperature increase

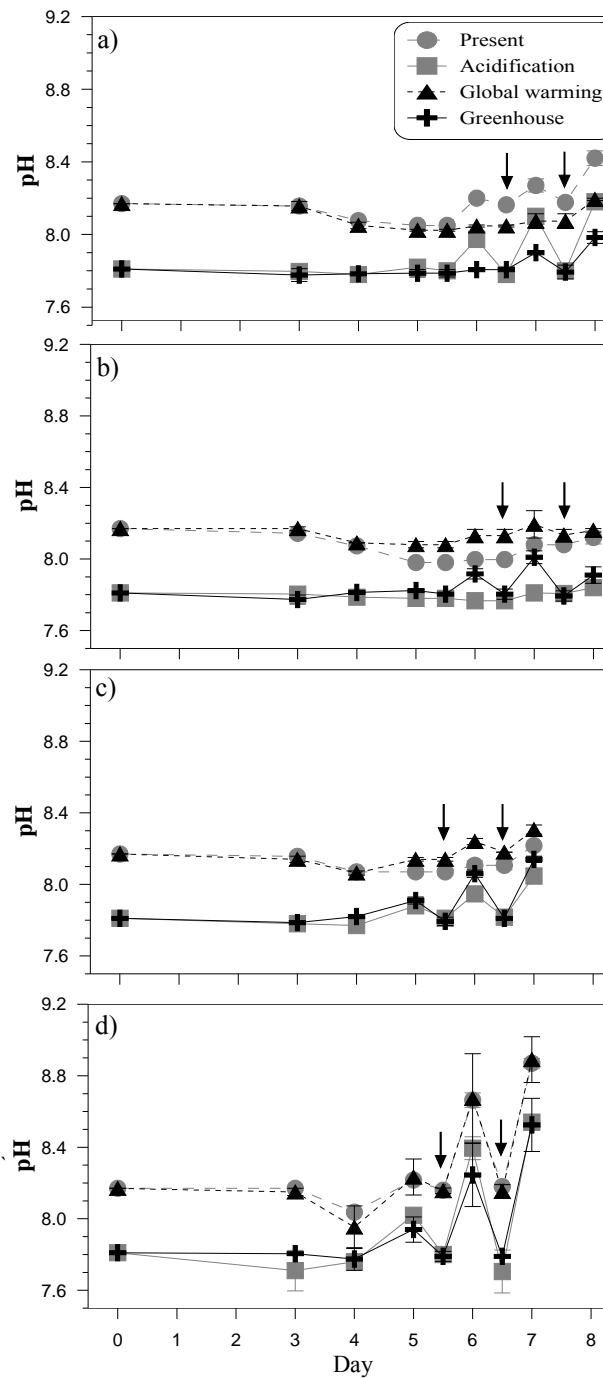
Condition	<i>Skeletonema costatum</i>	<i>Chaetoceros debilis</i>	<i>Chaetoceros didymus</i>	<i>Thalassiosira nordenskioldii</i>
CO <sub>2</sub>	Neutral	Neutral	Neutral	Neutral
Temperature	Negative	Neutral	Positive	Neutral
Future ocean	Negative	Positive	Positive	Neutral



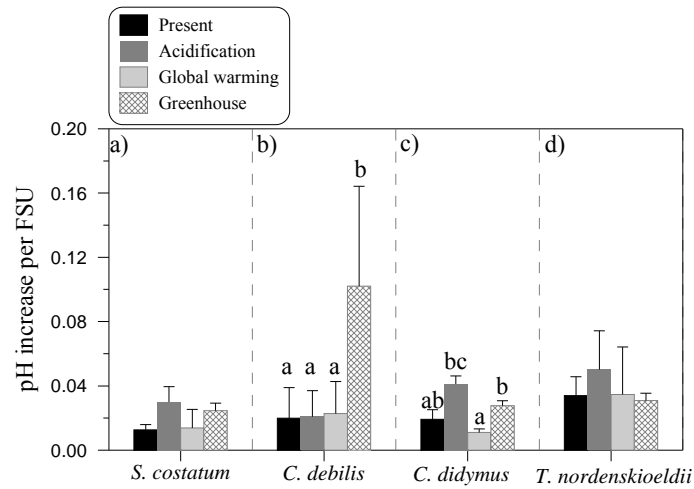
**Fig. 1.** Changes of fluorescence during experimental periods for four diatom species under present, acidification, global warming and greenhouse conditions. (a) *Skeletonema costatum*, (b) *Chaetoceros debilis*, (c) *Chaetoceros didymus*, (d) *Thalassiosira nordenskiöldii*.



**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 nordenskiöldii* 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 nordenskiöldii*. The arrows indicate the time points when concentration CO<sub>2</sub> was added to phytoplankton culture.



**Fig. 4.** Changes in the pH value per fluorescence units (FSU) during last two days for four diatom species under present, acidification, global warming and greenhouse conditions. Characters indicate significant difference at  $\alpha=0.05$  level for Tukey's honestly significant difference tests. The vertical bars represent 95% confidence interval ( $n=2$  for *Thalassiosira nordenskiöldii* culture in acidification and greenhouse condition, other all species culture conditions in  $n=3$ ).

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 CO<sub>2</sub>, and thus those species with higher values might be better suited to future conditions. Cultures of *S. costatum* and *T. nordenskiöldii* 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 CO<sub>2</sub> 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 CO<sub>2</sub> level. Growth of *S. costatum* is generally unaffected by elevation of CO<sub>2</sub> concentration (Chen and Gao 2003). No changes in its growth were observed even at very low CO<sub>2</sub> concentration ( $<4 \mu\text{mol L}^{-1}$ ) (like results of Goldman, 1999). Cultures of the diatom *Chaetoceros muelleri* showed lower growth and/or no response in elevated CO<sub>2</sub> concentrations (Gao et al., 2012). In contrast, in a mesocosm study, both *S. costatum* and *Chaetoceros* spp. showed increased growth rates with increased  $p\text{CO}_2$  (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 CO<sub>2</sub> 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 nordenskiöldii* 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 CO<sub>2</sub> 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 CO<sub>2</sub> may not only increase their growth but also their uptake of CO<sub>2</sub>, leading to potentially faster growth rates and greater carbon fixation by this genus. Thus, in the likely warmer, higher-CO<sub>2</sub> 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 CO<sub>2</sub> and temperature showed no negative effects of more CO<sub>2</sub> 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 CO<sub>2</sub> and temperature.

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