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Impacts of CO_2 enrichment on growth and photosynthesis in freshwater and marine diatoms*

HU Hanhua (胡晗华) [†,**], GAO Kunshan (高坤山) [†, ††]

[†] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

[††] *Marine Biology Institute, Shantou University, Shantou 515063, China*

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Abstract The physiological responses of *Nitzschia palea* Kützing, a freshwater diatom, to elevated CO_2 were investigated and compared with those of a marine diatom, *Chaetoceros muelleri* Lemmermann previously reported. Elevated CO_2 concentration to 700 μl/L increased the dissolved inorganic carbon (DIC) and lowered the pH in the cultures of *N. palea*, thus enhancing the growth by 4%–20% during the whole growth period. High CO_2-grown *N. palea* cells showed lower levels of dark respiration rates and higher I_k values. Light-saturated photosynthetic rates and photosynthetic efficiencies decreased in *N. palea* with the doubling CO_2 concentration in airflow to the bottom of cultures, although the doubling CO_2 concentration in airflow to the surface cultures had few effects on these two photosynthetic parameters. *N. palea* cells were found to be capable of using HCO_3^- in addition to gaseous CO_2, and the CO_2 enrichment decreased their affinity for HCO_3^- and CO_2. Although doubled CO_2 level would enhance the biomass of *N. palea* and *C. muelleri* to different extents, compared with the marine diatom, it had a significant effect on the specific growth rates of *N. palea*. In addition, the responses of photosynthetic parameters of *N. palea* to doubled CO_2 concentration were almost opposite to those of *C. muelleri*.

Keyword: carbon dioxide; *Chaetoceros muelleri*; elevated CO_2; growth; *Nitzschia palea*; photosynthesis

1 INTRODUCTION

Atmospheric CO_2 concentration has been anticipated to be doubled by Year 2100 with the progressive increase in CO_2 emissions (Houghton et al., 1996). Such an increased CO_2 concentration would raise the seawater and freshwater dissolved inorganic carbon by 6% and 0.6%, and decrease pH by 0.279 and 0.298 units, respectively (Stumm and Morgan, 1996). The growth and photosynthetic CO_2 fixation of marine phytoplankton were found to be enhanced by elevated levels of CO_2 (Riebesell et al., 1993; Hein and Sand-Jensen, 1997). Although large rivers (Raymond et al., 1997) and most lakes (Cole et al., 1994) are supersaturated with CO_2 in contrast to the atmosphere, elevation of atmospheric CO_2 may result in a drastic increase of the productivity in eutrophic freshwater systems (Hein, 1997; Schippers et al., 2004a, b). Laboratory experiments showed that doubling of atmospheric CO_2 significantly affected the growth pattern in *Microcystis aeruginosa* (Qiu and Gao, 2002) and *Chlorella pyrenoidosa* (Xia and Gao, 2003) while influencing their photosynthetic behavior.

Diatoms, ubiquitously distributed in freshwater and marine ecosystems, are eukaryotic microalgae that play important roles in biogeochemical cycling of essential nutrients (Falkowski et al., 1998). Marine diatoms are known to account for approximately 25% of global primary production, while freshwater diatoms are dominant in lakes with intermediate dissolved inorganic carbon or even in softwater acidic lakes (Hein, 1997). Theoretically, photosynthesis of diatoms could be limited by CO_2 supply in both seawater and freshwater as the ribulose-1,5-bisphosphate carboxylase-oxygenase

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** Corresponding author: hanhuahu@ihb.ac.cn

(RubisCO), with the $K_{1/2}$ (CO_2) values of 30–60 μmol/L, was much undersaturated at the CO_2 concentrations of waters (approx. 10 μmol/L at atmospheric equilibrium at 25°C) (Badger et al., 1998). However, diatoms along with other microalgal species have been found to be capable of concentrating CO_2 (CO_2 concentrating mechanisms, CCMs) within the cell (Tortell et al., 1997; Badger et al., 1998; Raven and Falkowski, 1999; Reinfelder et al., 2000; Giordano et al., 2005), which usually involves an extracellular carbonic anhydrase (CA) that facilitates the conversion of bicarbonate (HCO_3^-) to CO_2 (Nimer et al., 1999). Elevated CO_2 levels have been found to down-regulate the capacity of CCMs (Giordano et al., 2005) or activity of extracellular CA (Burkhardt et al., 2001; Chen and Gao, 2003; Rost et al., 2003). Responses of diatoms to CO_2 enrichment may differ according to their different environments or evolutionary histories. In particular, owing to the difference of buffering capability in freshwater and marine systems, freshwater and marine diatoms may respond differently to elevated CO_2. Although the responses of marine diatoms to elevated CO_2 have been studied (Riebesell et al., 1993; Tortell et al., 1997; Tortell et al., 2000; Burkhardt et al., 2001; Hu and Gao, 2001; Chen and Gao, 2003; Rost et al., 2003), few reports are found to be concerned with those of freshwater diatoms. Study of freshwater diatoms and their physiological responses to elevated CO_2 is conducive to the evaluation of the effect of elevated atmospheric CO_2 in freshwater ecosystem. Furthermore, the comparison of the responses to doubled CO_2 by freshwater and marine diatoms is important in understanding their different adaptations to the elevating CO_2.

The present study intended to investigate the physiological responses of a freshwater diatom, *Nitzschia palea* Kützing, to doubled CO_2, and then its responses were compared with those of a marine diatom, *Chaetoceros muelleri* Lemmermann reported in our previous studies (Hu, 2001; Hu and Gao, 2001).

2 MATERIALS AND METHODS

2.1 Samples and growth conditions

Nitzschia palea Kützing were collected from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Culture experiments were performed according to Hu and Gao (2001). The alga was grown in 500 mL Erlenmeyer flasks with 450 mL DM medium (Beakes et al., 1988) and aerated with sterile filtered ambient (350 μl/L) or elevated CO_2 (700 μl/L) air at 200 mL/min in plant growth chamber (E7 Conviron, Winnipeg, Canada) at 50 μmol photons/(m^2s) and a 14 h light:10 h dark photoperiodic cycle. Temperature was fluctuation according to the photoperiodic cycle with the highest 26°C at noon, and the lowest 22°C at night. Aeration was used either in or above the culture medium.

2.2 General analyses

Cell density was estimated by measuring the optical density (OD) at 665 nm with a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). A linear regression between OD_{665} and dry weight (DW) was made and OD_{665} was employed to determine the dry biomass in cultures. All cultures were initiated at OD_{665} of about 0.02. OD_{665} was monitored every 24 h until a stationary phase was reached. Specific growth rates (μ) were calculated as the slope of logarithmic dry biomass against the time (days). Chlorophyll *a* contents were determined according to Jeffrey and Humphrey (1975) with 90% acetone extracts. The pH value was estimated with a pH meter (420A, Orion, Allometrics, Baton Rouge, Louisiana, USA). Samples (5 mL each) were processed with an ultrasonic cleaner (8892, Cole-Parmer Inc., Vernon Hills, Illinois, USA) at ambient temperature for 2–3 min without disrupting the cells then precipitated by centrifugation (680 g, 10 min). Dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) in the cell-free supernatant medium were measured with a total organic carbon analyzer (TOC-5000A, Shimadzu, Kyoto, Japan). DIC speciation and concentration were determined according to Stumm and Morgan (1996).

2.3 Photosynthetic activity

Exponentially growing cells were harvested and re-suspended in fresh medium. Their photosynthetic activity was assayed by measuring the rate of O_2 evolution under different irradiances using a Clark-type O_2 electrode (Hansatech Instruments Ltd, King's Lynn, Norfolk, UK). The temperature was kept at 25°C by a circulating water bath. Data were treated by non-linear fitting technique using model $P=P_m \times \tanh(\alpha \times I/P_m) + R_d$ (Henley, 1993), where P is the photosynthetic rate, and I is the light level. I_c, the light intensity at which net photosynthetic rate is zero, was calculated as R_d/α. I_k, the light intensity at which photosynthesis is initially

saturated, was calculated as P_m/α. The ascending slope at limiting irradiances, α, was calculated to assess the photosynthetic efficiency.

2.4 Inorganic carbon-dependent photosynthetic oxygen evolution

Cells were harvested by centrifugation at 1 500 g for 10 min at 25°C, washed twice with CO_2-free fresh medium and resuspended in CO_2-free fresh medium buffered with 30 mmol/L Bis-Tris Propane (pH 8.0). Inorganic carbon-dependent, photosynthetic oxygen evolution was measured using a Clark-type oxygen electrode (Hansatech Instruments Ltd, King's Lynn, Norfolk, UK) at 25°C. Cell suspension was placed in the O_2 electrode chamber, illuminated at a photon flux density of 300 μmol photons/(m^2s) and the cells allowed to reach CO_2 compensation concentration (as shown by the cessation of oxygen evolution). Then aliquots of $NaHCO_3$ were added sequentially to the cell suspension to create increasing DIC concentrations. Parameters for the photosynthetic response to DIC were obtained by fitting net photosynthetic rates at various levels of DIC with the Michaelis-Menten formula: $V=V_m\times[S]/(K_{1/2}(DIC)+[S])$, where $K_{1/2}(DIC)$ is the DIC concentration required to give a half maximal photosynthetic rate and V_m is the inorganic carbon-saturated photosynthesis rate. The CO_2 supply rate from spontaneous dehydration of HCO_3^- was estimated according to Miller and Colman (1980).

2.5 Statistics

The data were expressed as the mean values ± standard deviation (SD). Statistical significance of the data was tested with one-way analysis of variance (ANOVA) or *t*-test, with the significant level set at 0.05.

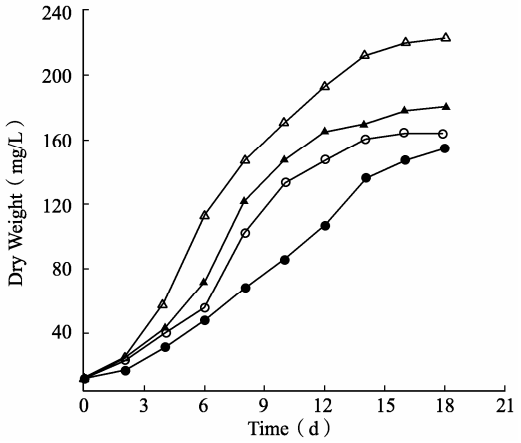

Fig.1 Time course of dry weight (mg/L) **of *Nitzschia palea* cultured under ambient (●, ▲) with elevated CO_2 (○, Δ) aerated to the surface of cultures (●, ○) or to the bottom of flasks (▲, Δ)**

3 RESULTS

3.1 Growth and changes in the medium

As shown in Fig.1, elevated CO_2 to 700 μl/L enhanced the growth of *Nitzschia palea* during the whole growth period. With enriched CO_2 in the airflow to the surface of cultures or to the bottom of flasks, the maximum dry biomass were 4% and 20% respectively higher than the controls ($P < 0.05$, *t*-test). Specific growth rates of *N. palea* cultured in enriched CO_2 were 0.40–0.45/day, which were significantly higher in comparison with 0.29–0.37/day in the controls ($P < 0.05$, *t*-test).

Fig.2 shows that the pH values of cultures aerated to surface increased by about 2.0 pH units during the whole growth period, while they only changed a little in those cultures aerated to bottom. However, the average pH values of the medium aerated with enriched CO_2 were significantly lower

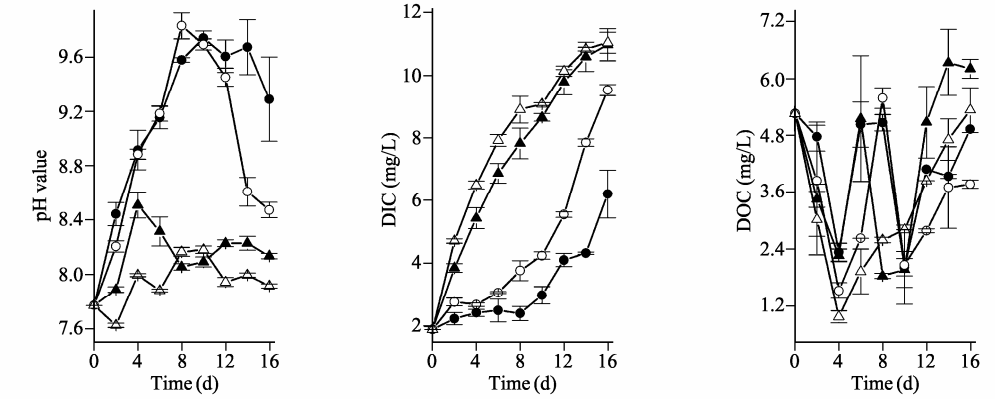

Fig.2 Changes of pH value, dissolved inorganic carbon (DIC)**, and dissolved organic carbon** (DOC) **in *Nitzschia palea* cultures aerated with ambient (●, ▲) and elevated CO_2 (○, Δ) to the surface of cultures (●, ○) or to the bottom of flasks (▲, Δ)**

than the controls by an average of 0.32–0.42 pH units ($P < 0.05$, ANOVA), and the surface aeration reduced pH values more than subsurface aeration at the end of the stationary phase. The DIC concentrations in the medium increased with time and were elevated to a higher level by doubled CO_2, in particular, with aeration to surface the increased extent of DIC concentrations was much greater. The dissolved organic carbon contents in the medium changed slightly but were reduced to a lower level with enriched CO_2.

3.2 Photosynthetic characteristics

Fig.3 shows the relationship between photosynthesis and irradiance in *N. palea* cultured under ambient with elevated CO_2. A photoinhibition at irradiances up to about 500 μmol photons/(m^2s) was observed. Saturating irradiance for photosynthesis was low and the I_k values were 55.9–90.9 μmol photons/(m^2s). Light compensation points were 21.3–50.1 μmol photons/(m^2s). Chlorophyll *a*-specific light-saturated photosynthetic rates ($P_m^{chl\ a}$) and dark respiration rates ($R_d^{chl\ a}$) changed within 131.0–207.0 and 48.3–105.6 μmol O_2/(mg chl *a* h) respectively, and chlorophyll *a*-specific apparent photosynthetic efficiency ($\alpha^{chl\ a}$) ranged from 1.5–2.7 [μmol O_2/(mg chl *a* h)]/[μmol photons/(m^2s)] (Table 1).

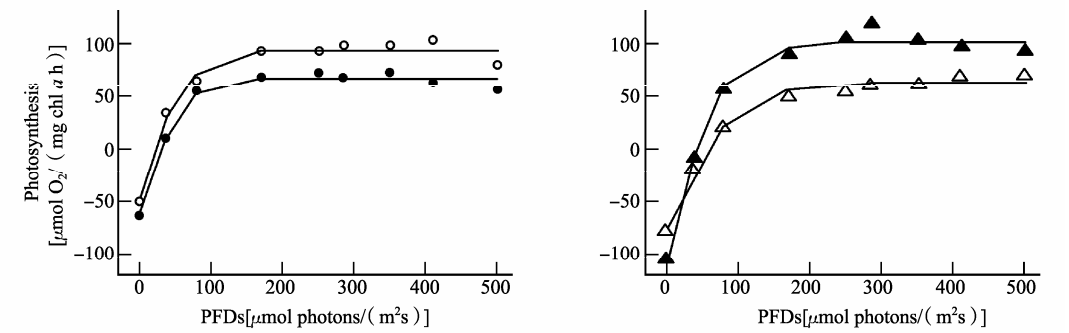

Fig.3 Representative curves of chlorophyll *a*-specific photosynthesis versus irradiance for *Nitzschia palea* cultures aerated with ambient (●, ▲) and elevated CO_2 (○, Δ) to the surface of cultures (●, ○) or to the bottom of flasks (▲, Δ)

Table 1 Photosynthetic parameters of *Chaetoceros muelleri* and *Nitzschia palea* cultured under ambient with elevated CO_2*

Photosynthetic parameter	Species	Surface (μl/L CO_2)		Bottom (μl/L CO_2)	
		350	700	350	700
$P_m^{chl\ a}$ μmol O_2/(mg chl *a* h)	*C. muelleri*	280.1±11.8	286.5±12.1	175.0±6.8	232.4±7.0
	N. palea	131.0±7.7	141.3±7.6	207.0±7.6	138.6±7.4
$\alpha^{chl\ a}$ [μmol O_2/(mg chl *a* h)]/[μmol photons/(m^2s)]	*C. muelleri*	2.5±0.3	3.9±0.5	2.7±0.3	4.7±0.4
	N. palea	2.3±0.3	2.3±0.3	2.7±0.3	1.5±0.2
$R_d^{chl\ a}$ μmol O_2/(mg chl *a* h)	*C. muelleri*	-52.7±10.1	-82.3±11.2	-22.1±6.3	-62.2±6.6
	N. palea	-64.1±7.2	-48.3±7.1	-105.6±7.1	-76.3±6.9
I_k μmol photons/(m^2s)	*C. muelleri*	112.8±4.7	74.2±3.1	66.0±2.5	49.6±1.5
	N. palea	55.9±3.3	62.4±3.4	75.7±2.8	90.9±4.9
I_c μmol photons/(m^2s)	*C. muelleri*	21.2±4.1	21.3±2.9	8.3±2.4	13.3±1.4
	N. palea	27.4±3.1	21.3±3.2	38.6±2.6	50.1±4.5

* Data of *Chaetoceros muelleri* from Hu, 2001; Hu and Gao, 2001. Data are represented in means ±SD (n=3). Chl: chlorophyll

The doubling CO_2 concentration in the airflow to the culture surface (lower DIC in medium) had few effects on light-saturated photosynthetic rates and photosynthetic efficiencies (P>0.05, t-test); however, bottom aeration with doubled CO_2 (higher DIC in medium) caused them to decrease significantly ($P < 0.05$, t-test). Dark respiratory rates decreased with the doubling of CO_2, while elevated CO_2 gave rise to the increase of I_k values. Light compensation points increased with the doubling CO_2 concentration in the airflow to the bottom of cultures, though the doubling CO_2 concentration in the airflow to the surface of the cultures had few effects on it.

The observed rates of photosynthetic O_2 evolution in *N. palea* cultured under ambient with elevated CO_2 were compared with the theoretical rates of CO_2 supply from the uncatalyzed dehydration of HCO_3^- at 25°C, pH 8.0 (Fig. 4b). The rates of inorganic carbon-dependent photosynthetic

oxygen evolution exceeded the supply of CO_2 from the uncatalyzed dehydration of HCO_3^-, indicating an ability to use HCO_3^- in *N. palea* at DIC concentrations below approximately 0.7 mmol/L. The observed rates of O_2 evolution were greater than the theoretical rates of CO_2 supply in *N. palea* at DIC concentrations below 0.24 mmol/L under all culture conditions. At saturating DIC concentrations, small additions of inorganic carbon would have no effect on the rate of photosynthetic oxygen evolution.

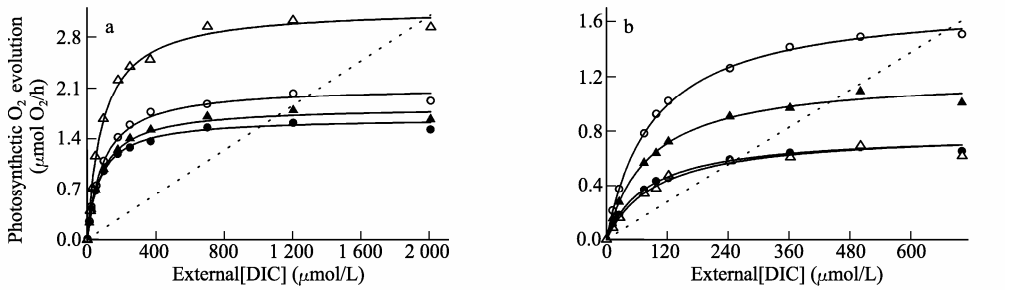

Fig.4 The observed rate (solid line) of O_2 evolution in *Chaetoceros muelleri* (a: from Hu 2001; Hu and Gao 2001) **and *Nitzschia palea*** (b) **cultured under ambient (●, ▲) with elevated CO_2 (○, Δ) aerated to the surface of cultures (●, ○) or to the bottom of flasks (▲, Δ) and the theoretical rates** (dot line) **of CO_2 supply from the uncatalyzed dehydration of HCO_3^- at 25°C, pH 8.0, in 4 mL of fresh F2AW or DM medium buffered with 30 mmol/L Bis-tris propane**

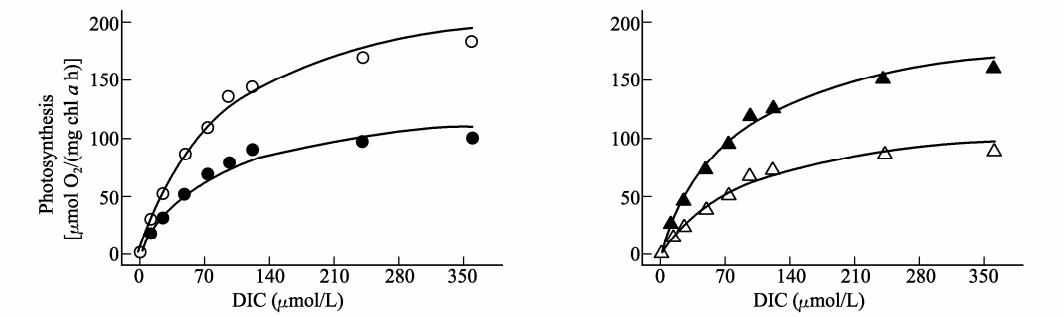

Fig.5 Photosynthesis CO_2 assimilation as a function of DIC concentration for *Nitzschia palea* cultures aerated with ambient (●, ▲) and elevated CO_2 (○, Δ) to the surface of cultures (●, ○) or to the bottom of flasks (▲, Δ)

The measurements were conducted at 300 μmol photons/(m^2s) and 25°C

The cells of *N. palea* required 81.9–101.0 μmol/L DIC or 1.45–1.79 μmol/L CO_2 to give half maximal photosynthetic rate at pH 8.0 (Fig.5, Table 2). The DIC–saturated photosynthetic rates were 125.1–244.8 μmol O_2/(mg chl *a* h), and aeration to surface with doubled CO_2 resulted in higher levels of V_m values, while bottom aeration with doubled CO_2 showed lower V_m values. Under doubling CO_2 concentration, the affinity for CO_2 decreases as indicated by increasing $K_{1/2}(CO_2)$ of photosynthesis.

4 DISCUSSION

The DIC concentration in seawater is high and relatively constant (2 mmol/L), whereas the DIC concentration in freshwater ranges from very high (1–10 mmol/L) in alkaline, hardwater habitats to less than 10 μmol/L in acidic, softwater habitats (Talling, 1985). The supply of inorganic carbon may limit photosynthesis and growth of phytoplankton under certain circumstances (Hein, 1997; Chen and Gao, 2003). During intense blooms, in particular, the growth of freshwater and marine phytoplankton can be limited by the CO_2 supply (Riebesell et al., 1993; Hein and Sand-Jensen, 1997; Ibelings and Maberly, 1998; Qiu and Gao, 2002; Schippers et al., 2004a). Thus, atmospheric CO_2 increase could potentially promote phytoplankton productivity (Schippers et al., 2004b). The growth of *Nitzschia palea* at varied concentration levels of CO_2 indicated that inorganic carbon might limit its growth when the phytoplankton population was dense.

Table 2 Parameters for the responses of photosynthesis to dissolved inorganic carbon of *Chaetoceros muelleri* and *Nitzschia palea* cultured under ambient with elevated CO_2*

Photosynthetic parameter	Species	Surface (μl/L CO_2)		Bottom (μl/L CO_2)	
		350	700	350	700
V_m μmol O_2/(mg chl *a* h)	*C. muelleri*	298.9±4.1	326.8±4.8	399.9±5.6	577.8±5.5
	N. palea	135.6±3.8	244.8±3.9	209.7±4.9	125.1±5.6
$K_{1/2}$(DIC) μmol/L	*C. muelleri*	74.9±1.2	92.7±2.3	85.4±1.6	88.4±7.8
	N. palea	81.9±4.7	92.4±1.9	84.9±1.6	101.0±1.9
$K_{1/2}$(CO_2) μmol/L	*C. muelleri*	0.69±0.01	0.85±0.02	0.78±0.01	0.81±0.07
	N. palea	1.45±0.05	1.64±0.03	1.51±0.03	1.79±0.03

* Data of *Chaetoceros muelleri* from Hu, 2001; Hu and Gao, 2001. Data are represented as means ±SD (n=3). Chl: chlorophyll

Schippers et al. (2004b) suggested that increased phytoplankton productivity because of atmospheric CO_2 elevation was proportional to the increased atmospheric CO_2, though it was reported that unlike terrestrial plants, phytoplankton would not show a significant response to the atmospheric CO_2 increase (Raven, 1997; Raven and Falkowski, 1999; Tortell et al., 2000). Under eutrophic conditions, the CO_2 concentration in the water would decrease and the pH increase when carbon is assimilated by the species. Accordingly the air-water flux of CO_2 would be enhanced, and make the phytoplankton more responsive to increased atmospheric CO_2 (Schippers et al., 2004b). Our results show that the biomass of the freshwater diatom increased by about 4%–20% in response to the CO_2 rise. The increase in high air-water exchange rate (airflow to the bottom of flasks, 20%) was more significant than that in low air-water exchange rate (airflow to surface of cultures, 4%), which was in agreement with our previous finding in the marine diatom, *Chaetoceros muelleri* (31% and 11% respectively) (Hu, 2001; Hu and Gao, 2001). Schippers et al. (2004b) predicted doubling of atmospheric CO_2 could result in an increase of the productivity of 10%–40%, and Hein and Sand-Jensen (1997) found 15%–19% stimulation of primary production in response to elevated CO_2 concentration.

Although elevated CO_2 enhanced the growth of *N. palea* and *C. muelleri*, they made different physiological responses to the doubling of CO_2 concentration. In contrast to the DM medium (freshwater, *N. palea*), the aeration with enriched CO_2 did not decrease the pH values of the f/2AW medium (seawater, *C. muelleri*) significantly but resulted in an even greater increased extent of the DIC concentrations after the exponential phase (Hu, 2001; Hu and Gao, 2001). Accordingly, enhancement of growth due to the enriched CO_2 occurred in different growth phases: for *N. palea* during the whole growth period, for *C. muelleri* after the exponential phase. Therefore, the growth rates of *C. muelleri* were not significantly different between the cultures with different CO_2 treatments (Hu, 2001; Hu and Gao, 2001), consistent with the experimental findings of Tortell et al. (1997) in another marine diatom; while elevated CO_2 concentrations gave rise to higher specific growth rates in *N. palea*. It was indicated that the growth of *C. muelleri* was not limited by the availability of DIC before the late exponential phase, while the growth of *N. palea* was limited by the CO_2 supply even with low cell density. In addition, the enhanced extent of biomass in *C. muelleri* by doubled CO_2 was greater than that of *N. palea*, which might be attributed to the higher photosynthetic efficiency (2.5–4.7, [μmol O_2/(mg chl *a* h)] / [μmol photons $m^{-2}s^{-1}$)]) in the former (Table 1).

The DIC concentration was high (>2.0 mmol/L) in the media of *C. muelleri*, and it had the ability to take up HCO_3^- (Hu and Gao, 2001), so its growth was not likely to be limited by the DIC concentration before the late exponential phase. When the cell density was rather high, doubled CO_2 increased the availability of DIC, thus the growth of the marine diatom would be stimulated in the stationary phase. The enhanced growth of the marine diatom should be related to the increase of photosynthesis (P_m and α) at elevated CO_2. However, the DIC concentration was relatively low (< 0.2 mmol/L) in the media of *N. palea*, and the change of HCO_3^- concentration with doubled CO_2 was not as much as that in the media of *C. muelleri*, while the CO_2 concentration was increased by 100% in the media. Therefore, an increase of the growth of *N. palea* might be mainly contributed to the doubled free (dissolved) CO_2. In the present study the photosynthesis of *N. palea* was saturated with

external CO_2 when the DIC concentration was as high as 0.7 mmol/L. It was indicated that the growth of *N. palea* was limited by the supply of CO_2 before the late exponential phase especially with low air-water exchange rate. On the other hand, the DIC concentration in the culture of *N. palea* increased fast with time at high air-water exchange rate in particular, suggesting that *N. palea* was counted firstly on free CO_2 to drive photosynthesis. It was evident that *N. palea* had a limited capacity for HCO_3^- utilization. The decrease of P_m and α for *N. palea* grown in doubled CO_2 concentration with high air-water exchange rate might be related to the negative feedback from CO_2 concentration, which was also covered in other studies (Xia and Gao, 2003).

In conclusion, the different physiological responses to elevated CO_2 concentrations between *N. palea* in the present study and *C. muelleri* in our previous studies (Hu, 2001; Hu and Gao, 2001) might be related to their capacity for dissolved inorganic carbon utilization. *N. palea* ($K_{1/2}$(DIC) = 81.9–101.0 μmol/L) had a relatively lower affinity for DIC than *C. muelleri* ($K_{1/2}$(DIC) = 74.9–92.7 μmol/L). The photosynthesis of *N. palea* and *C. muelleri* were saturated with external CO_2 when the DIC concentration was above 0.7 and 2.0 mmol/L (Fig.4), respectively, which also suggested that the latter had a greater capacity of HCO_3^- usage than the former. The growth of the marine diatom would not be limited by the supply of inorganic carbon due to its stronger HCO_3^- utilization ability when the cell density was relatively low, and thus elevated CO_2 had little effects on its growth. In contrast, the HCO_3^- utilization capacity was limited in the freshwater diatom, and the doubling of CO_2 in the airflow would enhance the dissolution of CO_2 and lower the pH values of cultures to a greater extent owing to the lower buffering capacity in freshwater media. Therefore, even if the cell density was low, the growth of *N. palea* would show a significant increase at the elevated CO_2 level. It is anticipated that the influences of doubling of atmospheric CO_2 on the freshwater diatom would be more significant compared with the marine diatom at the ecosystem level.

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