Short- and long-term effects of irradiance and CO_2 availability on carbon fixation by two marine diatoms

Ana Bartual and J. Angel Gálvez

Abstract: Unbuffered and nutrient-replete dilute batch cultures of *Skeletonema costatum* Cleve and *Phaeodactylum tricornutum* Bohlin were grown at high and low CO_2 availability conditions and two incident irradiances, 150 and 30 μmol photons·m^{-2}·s^{-1}. Long-term combined effects of such light and CO_2 availability conditions on carbon fixation rates of both diatoms were compared. At saturating light, *P. tricornutum* showed higher photosynthetic rates than *S. costatum* at both CO_2 conditions. However, under subsaturating light, carbon fixation rates of *P. tricornutum* were higher than observed for *S. costatum* only at low CO_2. *Skeletonema costatum* showed a strong reduction in photosynthetic rates only when both resources, irradiance and CO_2, were low. Short-term alterations of light and CO_2 availability on carbon fixation showed that the response of *S. costatum* differed considerably from long-term trends: the short-term reduction in CO_2 availability at both light levels resulted in a considerable decrease in the maximum photosynthetic rates. This effect was much less noticeable in *P. tricornutum*. The results show that, at saturating light, both diatoms maintain maximum photosynthetic rates under low CO_2 levels, but only *P. tricornutum* is well adapted to rapid changes in this resource. This capacity of adaptation seems to be light dependent, since light limitation altered the responses of both diatoms to low CO_2 availability conditions.

Key words: CO_2, ^{14}C fixation, irradiance, *Phaeodactylum tricornutum*, *Skeletonema costatum*.

Résumé : Dans un milieu dilué non-tamponné et saturé en nutriments, les auteurs ont cultivé le *Skeletonema costatum* Cleve et le *Phaeodactylum tricornutum* Bohlin, en présence de faibles ou hautes teneurs en CO_2, avec deux niveaux d'irradiance incidente, 150 et 30 μmol photons·m^{-2}·s^{-1}. Ils ont comparé les effets combinés à long terme de telles conditions de disponibilité en lumière et en CO_2, sur les taux de fixation du carbone chez les deux diatomées. En lumière saturante, le *P. tricornutum* montre des taux photosynthétiques plus élevés que le *S. costatum*, avec les deux teneurs en CO_2. Cependant, en lumière sub-saturante, les taux de fixation du carbone chez le *P.tricornutum* sont plus élevés que ceux observés chez le *S. costatum,* seulement avec la basse teneur en CO_2. Le *S. costatum* montre une forte réduction des taux photosynthétiques seulement lorsque les deux sources, irradiance et CO_2, sont faibles. Des altérations à court terme de la disponibilité de la lumière et du CO_2 sur la fixation du carbone montrent que, dans ces conditions, la réaction du *S. costatum* diffère grandement des tendances à long terme : la diminution à court terme de la disponibilité du CO_2, aux deux intensités lumineuses, conduit à une diminution considérable des taux maximums de photosynthèse. Cet effet est beaucoup moins évident chez le *P. tricornutum*. Les résultats montrent qu'en lumière saturante, les deux espèces de diatomées maintiennent des taux de photosynthèse maximums sous de faible teneurs en CO_2, mais seul le *P. tricornutum* est bien adapté aux changements rapides de cette ressource. Cette capacité d'adaptation semble dépendre de la lumière, puisqu'une limitation de la lumière altère les réactions des deux diatomées sous des conditions de faible disponibilité en CO_2.

Mots clés : CO_2, fixation du ^{14}C, irradiance, *Phaeodactylum tricornutum*, *Skeletonema costatum*.

[Traduit par la Rédaction]

Introduction

As aquatic photoautotrophs, diatoms are dependent on light and CO_2 for photosynthesis. In natural conditions, light is considerably attenuated with water depth and CO_2 represents less than 1% of total dissolved inorganic carbon (TDIC), corresponding to a concentration between 10 and 20 μM. This range is much lower than the saturation level for most carboxylase enzymes, including the enzyme Rubisco (ribulose biphosphate carboxylase–oxygenase). In addition to CO_2, more than 60% of the photosynthetically generated ATP and approximately 95% of the NADPH generated by the electron transport chain in the light reactions of photosynthesis are required for carbon fixation (Falkowski and Raven 1997). Consequently, under nutrient-replete conditions, the limitation of this first step of photosynthesis depends on the supply of CO_2 at the site of Rubisco, but it is dependent also on energy availability.

Marine autotrophs have developed different strategies for efficient harvesting of light. These strategies have been

Received 22 May 2002. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 21 March 2003.

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 doi: 10.1139/B03-013

widely studied and described for most groups of algae (Richardson et al. 1983; Geider et al. 1996; Falkowski and LaRoche 1991). In addition, most phytoplankton species prevent possible carbon limitation of photosynthesis by activating a so-called carbon concentrating mechanism (CCM) (Raven and Johnston 1991). This mechanism increases the CO_2 concentration at the site of Rubisco using CO_2 or HCO_3^- (>90% of TDIC) as a carbon source and increases the cell affinity for CO_2. The modes of inorganic carbon uptake in such CCM vary among phytoplankton species from diffusive CO_2 uptake to active HCO_3^- or CO_2 uptake and the presence of an external carbonic anhydrase (eCA) enzyme that catalyzes the dehydration of HCO_3^- to CO_2 (see review by Colman et al. 2002). Early in the last decade, different authors demonstrated that growth rates of some diatoms were close to limitation by inorganic CO_2 in the present-day ocean (Riebesell et al. 1993; Chen and Durbin 1994). The implication of these results is that some diatoms, under light saturation, may be capable of increasing their production in response to the present increase in atmospheric CO_2 levels. Since then, there has been little discussion regarding the possibility of CO_2 as a potential limiting substrate for primary production in the ocean. In a recent study, Tortell and Morel (2002) provided evidence that in natural diatom-dominated assemblages, both mechanisms associated with CCM, extracellular carbonic anhydrase activity and direct HCO_3^- uptake, may occur, but HCO_3^- utilization is more prevalent in natural marine phytoplankton communities, thus showing the controversy associated with this aspect. Other physiological studies show that many diatoms have developed direct uptake of HCO_3^- as a response to low CO_2 availability (Colman and Rotatore 1995; Korb et al. 1997; Tortell et al. 1997; Burkhardt et al. 2001). Such direct uptake of HCO_3^- is necessarily associated with an energetic cost. Consequently, part of the available energy in the cell will be necessary for carbon acquisition (Kübler and Raven 1994; Raven 1997). The attenuation of light in the ocean has to be taken into account in this respect, as the optimal use of DIC depends on the optimal harvesting of light, especially if an active uptake of inorganic carbon, in contrast with a diffusive CO_2 uptake, is required. Carbon–light colimitation for diatom photosynthesis seems possible under low light, low CO_2 conditions, but definitive studies remain to be done.

To study this aspect, we used two marine diatom species, *Phaeodactylum tricornutum* Bohlin and *Skeletonema costatum* Cleve, known to differ in their responses to changes in light and CO_2. Provided that neither light nor nutrients are limiting resources, *P. tricornutum* has not shown any effect of CO_2 availability on its growth rate or C:N:P ratio (Burkhardt et al. 1999). Only unnaturally low TDIC concentrations affect inorganic carbon uptake in *P. tricornutum* (Nimer et al. 1997). In contrast, growth rates and the C:N:P ratio of *S. costatum* are CO_2 dependent (Burkhardt and Riebesell 1997; Burkhardt et al. 1999) under nutrient- and light-replete conditions. These trends have been related to the possible mechanism for carbon acquisition, which has been widely studied in *P. tricornutum* (Holdsworth and Colbeck 1976; Patel and Merrett 1986; Dixon and Merrett 1988; Colman and Rotatore 1995; Rotatore et al. 1995; Johnston and Raven 1996; John-McKay and Colman 1997; Laws and Bidigare 1997; Iglesias-Rodríguez and Merrett 1997). All of these authors agree that *P. tricornutum* has the ability to use HCO_3^- as photosynthetic carbon source, but the HCO_3^- transport mechanism seems to vary between different strains involving either eCA-mediated CO_2 uptake or active HCO_3^- uptake through the plasmalemma. The strain used in our work lacks eCA activity (John-McKay and Colman 1997). The absence of eCA implies an increase in the direct HCO_3^- uptake if CO_2 availability in the surrounding medium is low (Colman et al. 2002), which is necessarily energy dependent. In such conditions, a reduction in light availability should affect carbon fixation of *P. tricornutum*. Also, Burkhardt et al. (2001) have shown strong evidence for HCO_3^- use in this strain, with a preferential use of CO_2 when it is highly available, as substrate for inorganic carbon uptake at light saturation. In the case of *S. costatum*, Korb et al. (1997) have shown, by using carbon isotope discrimination methods, that this diatom can use CO_2 or HCO_3^- as an inorganic carbon source, and Nimer et al. (1997) have demonstrated that *S. costatum* grown under light saturation is capable of taking up HCO_3^- actively when CO_2 concentration in the surrounding medium is low. In both diatoms, the process by which inorganic carbon enters the cell as part of the CCM seems to be sensitive to reductions in irradiance, as active HCO_3^- uptake can provide an alternative carbon source when the CO_2 is restricted.

This analysis would not be complete if different time scales for responding to changes in CO_2 concentrations were not considered, since short-term responses to environmental changes usually differ from long-term adjustments (Hein and Sand-Jensen 1997). In this study, we report on the combined effects of changing light and CO_2 availability on the carbon fixation of two marine diatom species, *P. tricornutum* and *S. costatum*, on a short- and long-term scale.

Materials and methods

Carbon equilibria in a closed system: setup of cultures

To study the effects of inorganic carbon availability on growth and production, experiments were performed in a closed system to avoid carbon fluxes between the gas (air) and the liquid phase (culture). In this kind of system, there are six chemical forms (H_2CO_3, HCO_3^-, CO_2(aq), CO_3^{2-}, H^+, and OH^-) that take part in the main reactions of the carbonate system. Their respective concentrations depend on pH, total alkalinity (TA), and TDIC ($HCO_3^- + CO_2 + CO_3^{2-}$). By changing the pH, the proportion of each form changes, resulting in an alteration of TA without altering TDIC. By changing TDIC, TA varies but pH can be maintained unaltered.

On the basis of the chemical characteristics of the carbon system outlined above, the diatoms *P. tricornutum* and *S. costatum* were grown under two different initial CO_2 concentrations, high CO_2 (HCO_2) and low CO_2 conditions (LCO_2), with an equal TDIC. The CO_2 concentration of the experimental cultures was prepared by adjusting the initial pH of the seawater to a value of 8 (HCO_2, [CO_2(aq)] = 20 ± 0.35 μM) and 9 (LCO_2, [CO_2(aq)] = 2 ± 0.92 μM) by using 1 N NaOH and 1 N HCl. These pH settings were controlled daily in the cultures using a WTW 3000 microprocessor pH meter (WTW, Ft. Myers, Fla.) and a combined AgCl–KCl

pH electrode, calibrated with NBS buffers at the experimental temperature.

Algae were obtained from the Alfred Wegener Institute for Polar and Marine Research (Bremerhaven, Germany) culture collection. Originally, *S. costatum* was isolated from the North Sea. The strain of *P. tricornutum* used was CCAP 1052/1A. Prior to the experiments, both species were maintained in the laboratory under constant temperature (15 ± 1°C) and continuous white light (100 µmol photons·m^{-2}·s^{-1}). Experimental cultures were grown in 2-L borosilicate glass bottles (sterilized at 180°C for 4 h) containing 0.2-µm-filtered seawater enriched with f/2 medium ([NO_3^-] = 882 µM and [PO_4^{3-}] = 36 µM) (Guillard and Ryther 1962) and a final concentration of silicate of 211 µM, thus ensuring nutrient-replete conditions throughout the course of the experiments. Bottles were carefully filled with filtered seawater to avoid air bubbles inside.

Experimental design

Cells adapted to low (pH 9) and high (pH 8) CO_2 concentrations were grown under two irradiances: 150 µmol photons·m^{-2}·s^{-1} (high irradiance (HE)) and 30 µmol photons·m^{-2}·s^{-1} (low irradiance (LE)). Combining these treatments made up the experimental conditions HE-HCO_2, LE-HCO_2, HE-LCO_2, and LE-LCO_2. Cultures were started using an inoculum of 1500–2000 cells/mL. For each treatment, exponentially growing cell cultures were preadapted to the experimental conditions for 3–4 days prior to the experiment (i.e., at least six cell divisions). When cultures reached the exponential growth phase, coincident with an increase in pH of 0.1 unit, the 2-L culture was divided into three subcultures of 500 mL. Two of them were directly used for ^{14}C uptake estimations (photosynthesis (*P*) versus irradiance (*E*) curves), the first one with the original pH and light conditions and the second one with the pH changed to the other condition (e.g., HCO_2 (pH 8) was changed to LCO_2 (pH 9)) but with no change in light intensity. The third subculture was kept at the original pH but incubated under the opposite light condition (e.g., HE was changed to LE) for 3 h. Afterwards, a third *P* versus *E* curve was recorded. This experimental design allowed us to study the short-term effect of changing CO_2 availability and light intensity on carbon fixation. Also, by comparing the results obtained from cells adapted during 3–4 days to different light and CO_2 conditions, it was possible to distinguish between long- and short-term effects of light and CO_2 on the subsequent rate of CO_2 fixation.

Since the initial CO_2 concentration of the media was fixed by changing the pH, it was necessary to ensure that there was not any direct effect of pH on photosynthesis. This was tested for *S. costatum*. Cells were grown at saturating light conditions and the medium was prepared at pH 8.3, but in this case, the TDIC level was reduced and, consequently, also CO_2 availability. The medium at lower DIC conditions was prepared by bubbling acidified seawater (0.05 N HCl) with air and readjusting the initial pH by adding 0.05 N NaOH. The final DIC obtained was 0.18 mM.

Photosynthetic carbon fixation (*P* versus *E* curves)

Photosynthetic carbon fixation was determined by the addition of $NaH^{14}CO_3$ to exponential cultures. The *P* versus *E* responses based on isotopic carbon fixation were determined using a photosynthetron (Lewis and Smith 1983). An appropriate volume of experimental culture was placed in a test-tube and $NaH^{14}CO_3$ was added to a final activity of 0.5 µCi·mL^{-1} (1 µCi = 37 kBq). After mixing and dark incubation in a water bath at 20°C for some minutes, culture aliquots (5 mL) were placed into 15-mL glass scintillation vials, sealed to avoid invasion of atmospheric CO_2, and placed into compartments with various layers of nickel mesh that produced 18 different incident light intensities ranging from 2 to 2500 µmol photons·m^{-2}·s^{-1}. Samples were illuminated by a 2000-W tungsten halogen lamp through a water-cooled Plexiglas chamber. The temperature was kept at 20°C. Five aliquots of 50 µL of culture were added to 3 mL of medium (0.2-µm-filtered seawater) and 100 µL of 6 N NaOH to measure the total activity (^{14}C disintegrations per minute). Irradiance at each position was measured before and after each *P* versus *E* curve with a 4π sensor (Biospherical Instrument Inc., San Diego, Calif.) placed into an empty scintillation vial. After 1 h of incubation, 10 µL of 6 N HCl was added to each vial. Subsequently, open vials were shaken for 24 h to drive out the CO_2 from the medium. Then, 6 N NaOH and 10 mL of scintillation cocktail (Quickzint 1) were added to each vial and the activity was measured using a Packard Tri-Carb 460C liquid scintillation cocktail. The dark carbon fixation measured was subtracted from light assimilation values. Photosynthetic rates were estimated using Joint Global Ocean Flux Study protocols (UNESCO 1994). Maximum rates of carbon fixation (P_{max}) and the photosynthetic efficiency (α) from the 24 *P* versus *E* curves obtained (12 for each species) were compared. The values of α were obtained from linear regression of light-dependent values of carbon fixation within the 0–100 µmol photons·m^{-2}·s^{-1} range (eight or nine points). The P_{max} values were calculated from the average photosynthetic rates obtained for four or five values at high irradiance.

Cell counts and chlorophyll *a* (Chl *a*) content

Cell number and Chl *a* content were quantified for each sample used to record a *P* versus *E* curve. Subsamples (10 mL) were fixed with Lugol's iodine solution and cell number was determined with an inverted microscope (Utermöhl 1958). For Chl *a* quantification, five samples per culture were filtered through Whatman GF/F filters and kept frozen until analysis. Chl *a* was estimated fluorometrically after homogenization and extraction for 1 h in 90% acetone at 4°C in the dark.

CO_2 measurement

The concentration of CO_2 was calculated from measurements of TDIC, TA, pH, salinity, temperature, and phosphate and silicate concentrations assuming the dissociation constants of Mehrbach et al. (1973). TDIC was measured coulometrically with a UIC-5012 coulometer (UIC, Inc., Joliet, Ill.) according to Johnson et al. (1993). Samples were taken without any air in 100-mL sterilized (4 h at 400°C) borosilicate glass bottles and fixed with $HgCl_2$ (35 g·L^{-1}). The average TDIC in the culture media was 2.04 ± 0.06 mM. TA of the medium was measured with a Gran titration technique using a Methrom 713 pH meter connected to an automatic

burette 665 Dosimat titration cell (Metrohm, Herisau, Switzerland).

Statistics

Photosynthetic efficiencies (α) were compared using a slopes comparison *t*-test (Zar 1984). For the comparison of Chl *a* concentrations, one-way ANOVA and post hoc Tukey test were applied (Fry 1993). The significance levels were set at $p < 0.05$.

Results

Long-term effects

Skeletonema costatum exhibited marked effects of CO_2 availability and light levels on P_{max} and α values (Figs. 1 and 2). The highest values of both parameters were obtained for cells adapted to high irradiance and low CO_2 (Figs. 1*a* and 2*a*) and lowest values when both resources were reduced (Figs. 1*c* and 2*c*). However, under high irradiance, α showed a significantly higher value for cells adapted to low CO_2 level ($p < 0.05$) (Fig. 2*a*), while P_{max} was not affected by CO_2 ($p < 0.05$) (Fig. 1*a*).

For cells adapted to the same pH at different TDIC (Table 1), P_{max} was lower at lower TDIC availability. This result shows that *S. costatum* is especially affected by CO_2 alterations induced by pH changes and that a reduction in TDIC, which alters not only CO_2 but also HCO_3^- concentrations, affected carbon fixation.

In contrast with the results obtained for *S. costatum*, *P. tricornutum* did not show significant differences in P_{max} and α in cells adapted to high irradiance at both CO_2 levels (Figs. 1*b* and 2*b*). However, at low irradiance, P_{max} showed lower values at lower CO_2 levels (Fig. 1*d*), while α remained unchanged (Fig. 2*d*).

Species comparison

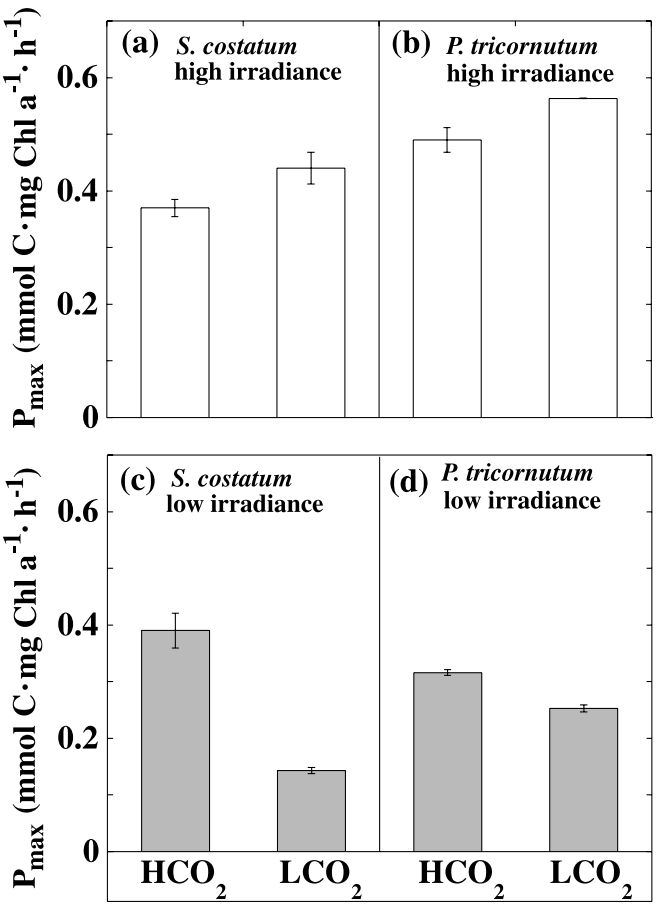
P_{max} and α values

Under high light and high CO_2 conditions, *P. tricornutum* showed higher P_{max} and α values than *S. costatum* (Figs. 1*a*, 1*b*, 2*a*, and 2*b*). When both species were grown under limited light but a high concentration of CO_2, *S. costatum* exhibited a higher P_{max} than *P. tricornutum* (Figs. 1*c* and 1*d*), but the maximum light utilization coefficient (α) was higher for the latter species ($\alpha_{P3} = 0.52$ mmol C·m^2·mg Chl a^{-1}·mol photons^{-1} compared with $\alpha_{S3} = 0.42$ mmol C·m^2·mg Chl a^{-1}·mol photons^{-1}. When grown under low CO_2 conditions, *P. tricornutum* showed a higher P_{max} than *S. costatum* at both irradiances (Fig. 1). While α was equal for both species under high light (Figs. 2*a* and 2*b*), it was considerably higher for *P. tricornutum* under low light (Figs. 2*c* and 2*d*) ($\alpha_{P4} = 0.47$ mmol C·m^2·mg Chl a^{-1}·mol photons^{-1} relative to $\alpha_{S4} = 0.28$ mmol C·m^2·mg Chl a^{-1}·mol photons^{-1}).

Chlorophyll a content

Table 2 shows the cellular Chl *a* content in *S. costatum* and *P. tricornutum* grown under both irradiance conditions. Chlorophyll *a* content in *S. costatum* was independent of irradiance, but it was higher at low CO_2 levels. In contrast, *P. tricornutum* exhibited a higher Chl *a* content in cells grown under low light, and only at this limiting irradiance was a noticeable CO_2 dependence observed. In *P. tricornutum*, the effects of irradiance and CO_2 on Chl *a* content were significant, while for *S. costatum*, only CO_2 had a significant effect (Table 3).

Fig. 1. Maximum photosynthetic rates (P_{max}) for (*a* and *c*) *Skeletonema costatum* and (*b* and *d*) *Phaeodactylum tricornutum* grown under different irradiances and CO_2 availability conditions (long-term effects) for a TDIC of 2.01 ± 0.07 mM. Data are presented as means ± SD of two cultures (eight values for each treatment) for Fig. 1*a* and one culture (four values for each treatment) for Figs. 1*b*, 1*c*, and 1*d*.



Short-term responses of cells adapted to high irradiance

Short-term alterations in CO_2 availability only affected P_{max} values under both irradiances. *Skeletonema costatum* grown under high irradiance showed a strong decrease in P_{max} when CO_2 availability was reduced from 20 to 2.5 µM (pH 8 to pH 9) (Fig. 3*a*). However, for cells adapted to low CO_2 availability, carbon fixation rates did not vary significantly when CO_2 was increased from 2 to 37 µM (pH 9 to pH 8) (Fig. 3*a*). In both cases, the reduced irradiance did not change P_{max} significantly in the short-term response ($p < 0.05$). The P_{max} of *P. tricornutum* cells adapted to high CO_2 availability was less affected than that of *S. costatum* when CO_2 was reduced from 20.5 to 2.7 µM (pH 8 to pH 9) (Fig. 3*b*). The short-term response to reduced CO_2 or irradiance resulted in a slight decrease in P_{max} of *P. tricornutum*. As also found for *S. costatum*, an increase in

Fig. 2. Linear regression of light-dependent portion of *P* versus *E* curves and corresponding values of photosynthetic efficiency (α) for (*a* and *c*) *Skeletonema costatum* and (*b* and *d*) *Phaeodactylum tricornutum* grown at high CO_2 (solid line, solid circles) and low CO_2 (dashed line, open circles) at high and low irradiances (long-term effects) for a TDIC of 2.01 ± 0.07 mM. Results from slope *t* test comparison are also shown (significance level at $p < 0.05$). *S*, *S. costatum*; *P*, *P. tricornutum*; 1, HE-HCO_2; 2, HE-LCO_2; 3, LE-HCO_2; 4, LE-LCO_2.

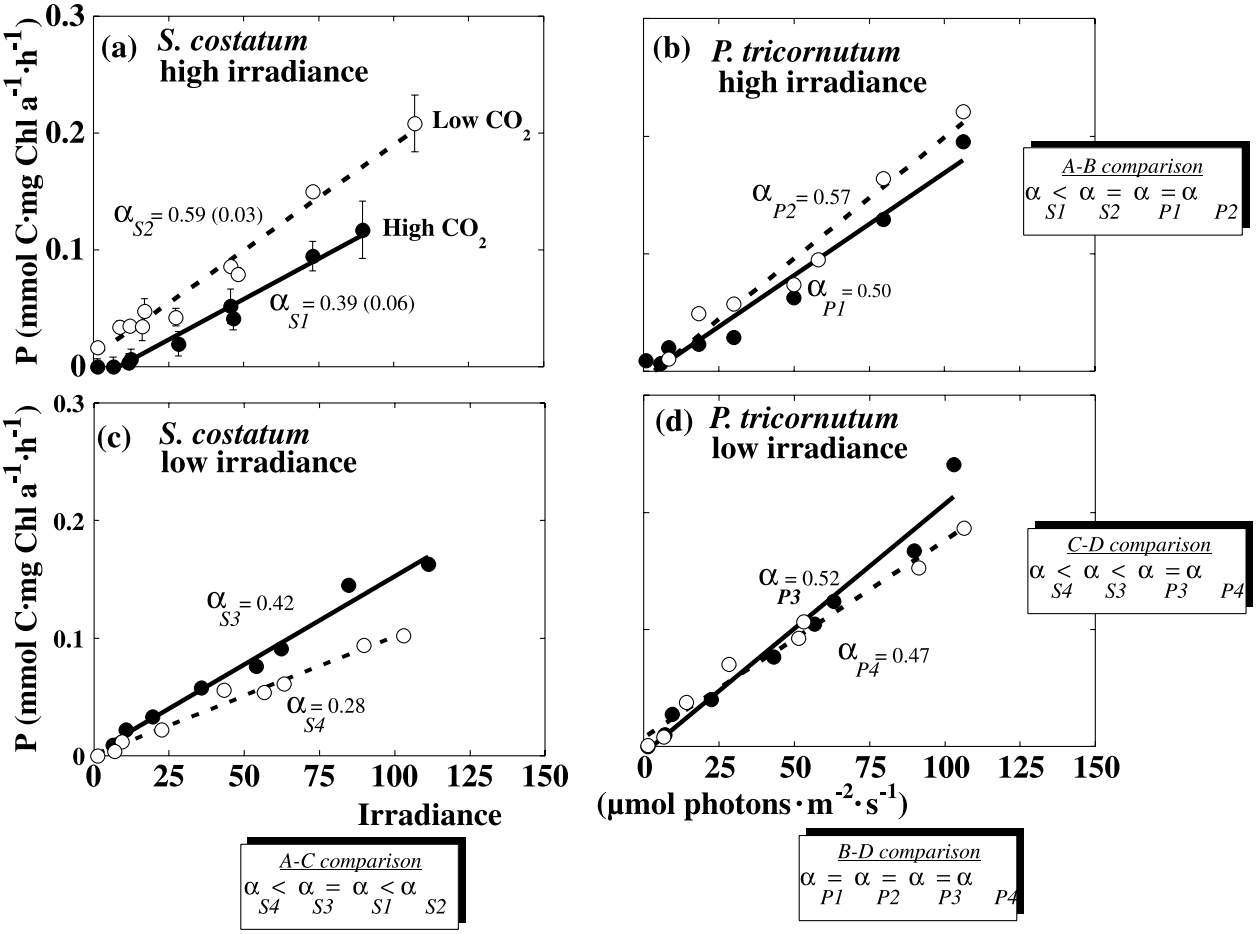


Table 1. Photosynthetic parameters of *Skeletonema costatum* grown under 150 μmol photons·m^{-2}·s^{-1}, natural pH (8.3 at 20°C), and two different DIC concentrations.

	DIC = 2.03 mM, CO_2 = 19 μM	DIC = 0.18 mM, CO_2 = 0.02 μM
α (mmol C·m^2·mg Chl a^{-1}·mol photons^{-1})	0.211	0.214
P_{max} (mmol C·mg Chl a^{-1}·h^{-1})	0.315	0.197

Note: DIC, dissolved inorganic carbon.

CO_2 from 3.2 to 37.4 μM (pH 9 to pH 8) did not enhance the P_{max} value.

Short-term responses of cells adapted to low irradiance

Similar to high irradiance adapted *S. costatum*, cells grown under low irradiance and high CO_2 level exhibited a strong decrease in P_{max} when CO_2 was reduced from 16 to 1.37 μM (pH 8 to pH 9) (Fig. 4*a*). In *P. tricornutum*, the observed decrease in P_{max} values after CO_2 was reduced was substantially less (pH 8 to pH 9) (Fig. 4*b*) compared with *S. costatum*. The short-term responses of both species to changes in irradiance from 30 to 150 μmol photons·m^{-2}·s^{-1} resulted in increasing P_{max} values under low CO_2 conditions. Under high CO_2 conditions, this increase in irradiance led to higher values of P_{max} in *S. costatum* but to lower values in *P. tricornutum* (Fig. 4).

Discussion

Changes in light intensity and inorganic carbon availability in natural well-mixed water columns occur on very different time scales. Phytoplankton can be exposed to rapid changes in light intensity, while CO_2 and HCO_3^- availability shows little variation. The study of combined effects of these major photosynthetic resources on short- and long-term changes in carbon fixation reveals interesting aspects of the physiological adaptation of marine diatoms. It is essential to

Fig. 3. High irradiance. (*a*) *Skeletonema costatum*: short-term changes in P_{max} as a consequence of changes in CO_2 availability (pH 8 to pH 9) or irradiance (HE = 150 µmol photons·m^{-2}·s^{-1} to LE = 30 µmol photons·m^{-2}·s^{-1}) of cells adapted originally to high irradiance and high CO_2 (reference value as solid square) or low CO_2 (reference value as open square). Data are presented as means ± SD of two different cultures (eight P_{max} values for each CO_2–light treatment). (*b*) Same treatments for *Phaeodactylum tricornutum*. Data are presented as means ± SD of one culture (four P_{max} values for each treatment).

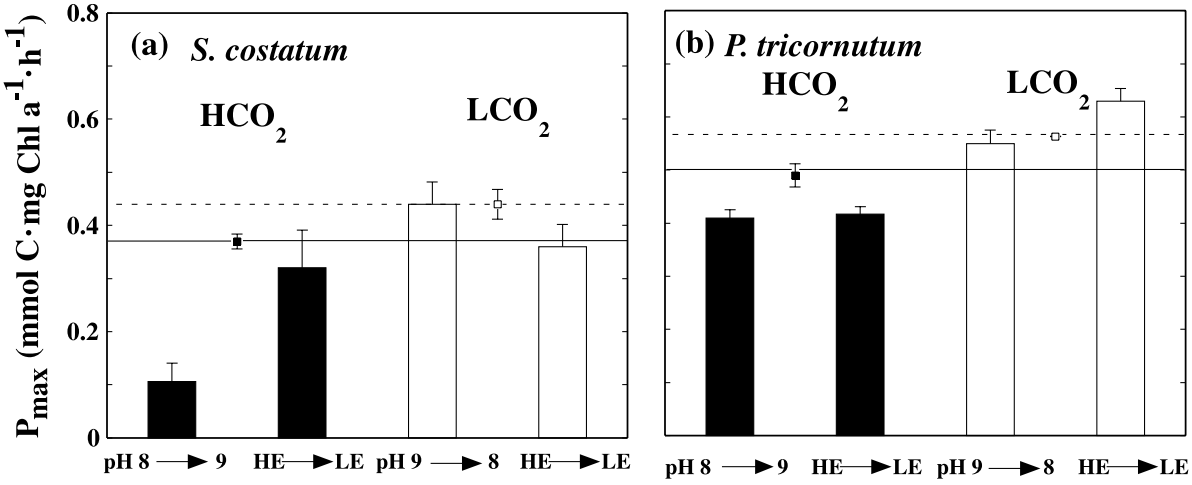


Table 2. Chlorophyll *a* content (pg/cell) of *Skeletonema costatum* and *Phaeodactylum tricornutum* grown at high (150 µmol photons·m^{-2}·s^{-1}) and low (30 µmol photons·m^{-2}·s^{-1}) irradiance and high (HCO_2) and low CO_2 (LCO_2) concentrations.

	S. costatum	*P. tricornutum*
High irradiance		
HCO_2	0.164±0.033*a*	0.372±0.04*c*
LCO_2	0.262±0.020*b*	0.355±0.05*c*
Low irradiance		
HCO_2	0.198±0.010*a*	0.854±0.08*c*
LCO_2	0.298±0.006*b*	0.513±0.13*e*

Note: Data are presented as means ± SD. Values followed by a different letter are significantly different at the 5% confidence level (one-way ANOVA and Tukey's test).

Table 3. Two-way ANOVA *F* values of Chl *a* cell content of *Skeletonema costatum* and *Phaeodactylum tricornutum* grown under two irradiances (30 and 150 µmol photons·m^{-2}·s^{-1}) and two CO_2 levels (pH 8 and pH 9).

	CO_2	Irradiance	Interaction
P. tricornutum	12.1*	41.10**	13.13*
S. costatum	50.27**	n.s.	n.s.

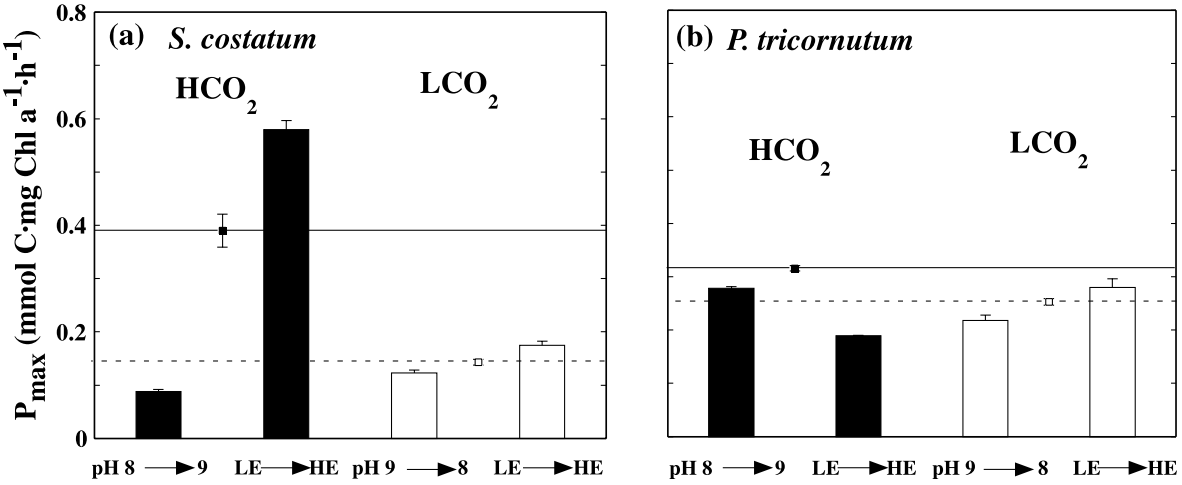
Note: Significance levels: *$p < 0.05$; **$p < 0.01$; n.s., no significant difference ($p > 0.05$).

consider that, on a long-term scale, the cellular carbon demand varies depending on growth rate and the net rate of synthesis of organic compounds, irrespective of the carbon source (HCO_3^- or CO_2) mainly used by the cell or the uptake mechanisms (passive diffusion or active transport). In contrast, on the short-term scale, the fast responses of photosynthetic parameters to external alterations in light or inorganic carbon may be due to changes in the reactivity or activity of photosynthetic components rather than to their cellular concentration. As an example, the time constant for light-dependent activation of the enzyme Rubisco ranges between 2.6 and 5.7 min for diatoms (MacIntyre and Geider 1996). Consequently, the physiological adaptations required for growth at a given inorganic carbon and light intensity will influence how the cells respond to rapid changes in these factors. In this context, the comparison of *P* versus *E* parameters of cells adapted to different light and CO_2 conditions under changes in both resources can reveal the underlying adaptation mechanisms, since P_{max} is primarily limited by the rate of carbon procurement, while α is a function of light harvesting and energy conversion efficiencies (Geider and Osborne 1992). The observed changes in P_{max} values under different levels of ambient CO_2 should be directly related to carbon uptake mechanims for the studied species.

Actually, on the basis of the experimental design, the results obtained could be discussed in terms of either a direct pH effect or a CO_2 effect on carbon fixation. In the case of *P. tricornutum*, changes in carbon fixation rates were much more dependent on irradiance than CO_2 availability, but this was not the case for *S. costatum*. However, the carbon fixation rates obtained for this diatom grown at different DIC levels at a constant pH of 8.3 (Table 1) revealed equivalent trends compared with cells adapted to pH 9. Burkhardt and Riebesell (1997) also obtained similar trends in growth and elemental composition of *S. costatum* adapted to different pHs at a constant DIC compared with cells adapted to different CO_2 levels at a constant pH. We took this as indication that the variability in carbon fixation rates was mainly caused by changes in CO_2 rather than direct pH effects, and results will be discussed in such terms.

The minor variation in the P_{max} of *P. tricornutum*, observed at each irradiance in response to rapid CO_2 reduction, suggests that this species responds faster than *S. costatum* to decreasing CO_2 availability. This indicates that *P. tricornutum* is capable of activating an inorganic carbon uptake mechanism on a short time scale to maintain P_{max}

Fig. 4. Low irradiance. (*a*) *Skeletonema costatum*: short-term changes in P_{max} as a consequence of changes in CO_2 availability (pH 8 to pH 9) or irradiance (LE = 30 μmol photons·m^{-2}·s^{-1} to HE = 150 μmol photons·m^{-2}·s^{-1}) of cells adapted originally to low irradiance and high CO_2 (reference value as solid square) or low CO_2 (reference value as open square). Data are presented as means ± SD of one culture (four P_{max} values for each treatment). (*b*) Same treatments for *Phaeodactylum tricornutum*. Data are presented as means ± SD of one culture (four P_{max} values for each treatment).



when CO_2 is suddenly restricted. Such a mechanism may be permanently operative and regulated by CO_2 availability. Neither a short-term increase in CO_2 availability nor a long-term adaptation to high CO_2 levels increased the P_{max} values of *P. tricornutum* at saturating irradiance. Consequently, the P_{max} of this diatom seems to be saturated at the CO_2 levels assayed, between 2.5 and 20 μM (Fig. 1*b*). The lowest concentration of CO_2 used (pH 9, [CO_2] ≈ 2.5 μM) is much lower than the half-saturation constant for the Rubisco carboxylase reaction determined for this diatom (Read and Tabita 1994). Consequently, photosynthetic rates obtained under such conditions are consistent with the existence of a CCM and the rapid activation of a HCO_3^- transport system under low CO_2 conditions. This has been proposed by John-McKay and Colman (1997) for this particular strain, which lacks eCA and would demand energy for direct HCO_3^- uptake. It is also consistent with the results obtained by Burkhardt et al. (2001) for this strain who showed that a decline in CO_2 supply is accompanied by the induction of HCO_3^- uptake, with preferential use of CO_2 as the primary photosynthetic carbon source if present at high concentrations. The energetic requirement associated with active uptake of HCO_3^- under low CO_2 availability conditions would be responsible for lower P_{max} values obtained for cells adapted to low light, low CO_2 conditions compared with high light. Such a mechanism would be further optimized on a longer term scale if the Chl *a* pool for light reactions were also modified (Table 2), as will be discussed below.

Under saturating irradiance, *S. costatum* showed a greater reduction in carbon fixation rates than *P. tricornutum* when CO_2 availability was restricted on a short-term scale (65% P_{max} reduction compared with 12%, respectively) (Fig. 3). This trend was not obtained when long-term P_{max} values were compared (Fig. 1*a*). Such results are consistent with an active uptake of HCO_3^- as proposed for this species (Korb et al. 1997). This is also consistent with the strong reduction in P_{max} (77.3%) in cells grown at low light, low CO_2 conditions, since a reduction in energy availability reduces the supply of ATP for any active step in carbon acquisition. Based on these results, it seems likely that the mechanism of inorganic carbon uptake in *S. costatum* under low CO_2 availability conditions, in contrast with what has been hypothesized for *P. tricornutum*, is related to long-term changes in the cellular composition (synthesis of proteins or enzymes) rather than to a short-term activation of a pre-existing mechanism. The trends in P_{max} observed for *S. costatum* are also in agreement with the described CO_2 dependence of growth rates of this diatom cultured under high light conditions (Burkhardt and Riebesell 1997). Obviously, from the *P* versus *E* curves obtained for *S. costatum*, it is not possible to deduce the mechanism of inorganic carbon uptake, which in any case is not an objective of this work. However, we can affirm that *S. costatum* is able to maintain high photosynthetic rates under low CO_2 on a long-term scale, only when light is not a limiting resource. Consequently, the reduction in photosynthetic rates seems to also be related to the light requirement for carbon acquisition, since at low irradiance, short- and long-term decreases in CO_2 resulted in a reduction in P_{max}. This energetic reduction could also affect Rubisco activity. In fact, P_{max} increased considerably for *S. costatum* grown under low light and high CO_2 when transfered to high light conditions (Fig. 4*a*). This could be related to a light-dependent activation–deactivation of Rubisco, altering the saturating carbon level for Rubisco, as previously observed for this species under natural conditions (MacIntyre et al. 1996). These authors showed a correlation between maximum Rubisco activity and light-saturated photosynthesis. Consequently, at 30 μmol photons·m^{-2}·s^{-1} (light-limited photosynthesis), the Rubisco activity is also subsaturated. This could explain the increase in P_{max} values observed when cultures adapted to low light and high CO_2 conditions were transfered to high light conditions (Fig. 4*a*). In such conditions, Rubisco would be fully activated without any CO_2 limitation.

As occurred for *P. tricornutum* (Figs. 1*b* and 3*b*), neither the short-term increase in CO_2 nor the long-term adaptation

to high CO_2 levels enhanced photosynthesis for *S. costatum* (Figs. 1*a* and 3*a*). Consequently, P_{max} for both diatoms grown under high irradiance seems to be saturated at natural TDIC, and on a long-term scale, energy supply could be the limiting step for maximal carbon uptake under natural conditions where changes in CO_2 may be less extreme but persist much longer than alterations in light availability.

For the analysis of long-term adaptations of both diatoms, Chl *a* contents of cells adapted to different light and CO_2 levels were compared. Chlorophyll *a* content was not light dependent in *S. costatum*, as has been previously reported for this diatom (Gilstad et al. 1993; MacIntyre et al. 1996). However, at both irradiances, there was a carbon dependence (Tables 2 and 3). Alterations in pigment content as a consequence of changing CO_2 availability have been reported in higher plants and macroalgae (García Sánchez et al. 1996). The higher Chl *a* content of *S. costatum* adapted to lower CO_2 levels under both irradiances suggests an effect of the external inorganic carbon concentration on the light harvesting apparatus of this diatom. Sheen (1994) studied the feedback regulation of photosynthesis by carbon metabolites in this diatom based on the initial increase, but long-term decrease, in photosynthesis during chlorosis in higher plants (Stitt 1991). The results presented in our work suggest that low light availability under high CO_2 concentration could promote the synthesis of sugar phosphates by the Calvin cycle in *S. costatum*. This accumulation of carbon skeletons would promote a reduction in electron transport, and as a consequence, the lower Chl *a* content at higher CO_2 could be a long-term response of the photosynthetic apparatus to such electron transport reduction. On the other hand, the higher Chl *a* content of cells adapted to lower CO_2 might function to increase harvesting to increase electron flow and the energy available for active HCO_3^- uptake.

In contrast with the tendency observed in *S. costatum*, CO_2, light, and the interaction of both affected Chl *a* content in *P. tricornutum* (Tables 2 and 3). This diatom adapts to low irradiances by increasing Chl *a* content (Beardall and Morris 1976). In this way, cells increase light capturing to meet energy demands. What is novel from our results is that this increase in Chl *a* content is lower under low CO_2 availability conditions (Table 2). The activation of an active HCO_3^- mechanism under such low CO_2 levels should increase Chl *a* content to a greater extent than under high CO_2 levels, where cells would use CO_2 as a carbon source (Burkhardt et al. 2001) without any energy requirement in carbon uptake. Different processes may explain the lower increase in Chl *a* under low CO_2 conditions. First, it might be a consequence of a reduction in Chl *a* synthesis caused by low carbon availability. If such were the case, *P. tricornutum* grown under high light and low CO_2 conditions should also show a reduction in Chl *a*, but this was not observed. Consequently, the reduction in Chl *a* content seems rather to be related to the combination of low light and low CO_2 availability conditions. Second, under low light conditions, the synthesis of ATP is considerably reduced compared with high light conditions. The available ATP in the cell should be allocated to different metabolic pathways, such as the Calvin–Benson cycle, but also to the acquisition of inorganic carbon and other nutrients (e.g., nitrogen) requiring active uptake and (or) energy for assimilation. It is well known that *P. tricornutum* acquires nitrate by Na^+-dependent active transport (Rees et al. 1980). The use of inorganic carbon obtained by active transport in cells under low CO_2 levels may result in competition for ATP and NADPH between carbon acquisition and nitrate incorporation. The lower Chl *a* content in *P. tricornutum* grown under low light, low CO_2 conditions compared with low light, high CO_2 conditions may be a consequence of the preferential use of ATP for carbon acquisition (similar P_{max} values at pH 8 and pH 9) to the detriment of nitrogen uptake and assimilation. This hypothesis is in agreement with alterations in the biochemical composition of this diatom when grown at both irradiances over a range of pH conditions from 7.9 to 9.5 (Bartual and Gálvez 2002). This could also be tested by studying the kinetics of nutrient incorporation under different CO_2 conditions in a wider range of light and carbon conditions.

In summary, in the case of *P. tricornutum*, photosynthesis is strongly light regulated, being less dependent on inorganic carbon availability even when it is present mainly as HCO_3^-. In contrast, *S. costatum* is more sensitive to changes in carbon availability under limiting or nonlimiting light conditions. Consequently, restrictions on carbon fixation rates in *P. tricornutum* are probably occurring at the level of the light reactions in photosynthesis, which provide energy to the Calvin cycle and other metabolic routes. In *S. costatum*, the limitation seems to be at the level of Rubisco activation–inactivation under changing irradiances or at the level of inorganic carbon flux to the site of Rubisco under low CO_2. Another important conclusion obtained from our work is the higher versatility of *P. tricornutum* compared with *S. costatum* under rapid changes in CO_2 availability. Furthermore, the time necessary to activate any mechanism to use HCO_3^- as an alternative CO_2 source is shorter than in *S. costatum*, as demonstrated by the differential results obtained for both time scales. *Skeletonema costatum*, which is better adapted to low light than *P. tricornutum*, appears to be able to use HCO_3^-, but only after long-term acclimation. Finally, the differential effect of CO_2 availability on Chl *a* content under low and high light conditions in *P. tricornutum* shows the importance of taking into account the combination of both sources (light and CO_2) and the time scale in the study of carbon uptake mechanisms and production. The capacity of diatoms to utilize HCO_3^- efficiently can be considerably reduced under very low light, a much more common natural condition than saturating light.

Acknowledgements

This study was undertaken at the Alfred Wegener Institute for Polar and Marine Research (AWI) in Bremerhaven (Germany), thanks to a short stay grant to A. Bartual from the Consejería de Educación y Ciencia de la Junta de Andalucía from Spain and also with financial support from the AWI. We appreciate Anke Dauelsberg for her laboratory assistance and Rüdiger Roettgers for technical support. We especially thank Dr. Ulf Riebesell for his input and his thorough review of previous versions of the manuscript. We also acknowledge the constructive comments from Dr. Bearn Kroon and the revision of the English by Dr. Kai Bischof. We thank two anonymous reviewers for their critical comments that considerably enhanced the original manuscript. We also appreci-

ate editorial detail and helpful comments regarding both the content and the language of the manuscript.

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