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Effect of CO_2 concentration on C : N : P ratio in marine phytoplankton: A species comparison

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Abstract

The effect of variable concentrations of dissolved molecular carbon dioxide, $[CO_{2,aq}]$, on C : N : P ratios in marine phytoplankton was studied in batch cultures under high light, nutrient-replete conditions at different irradiance cycles. The elemental composition in six out of seven species tested was affected by variation in $[CO_{2,aq}]$. Among these species, the magnitude of change in C : N : P was similar over the experimental CO_2 range. Differences in both cell size and day length-dependent growth rate had little effect on the critical CO_2 concentration below which a further decrease in $[CO_{2,aq}]$ led to large changes in C : N : P ratios. Significant CO_2-related changes in elemental ratios were observed at $[CO_{2,aq}] < 10$ μmol kg^{-1} and correlated with a CO_2-dependent decrease in growth rate. At $[CO_{2,aq}]$ typical for ocean surface waters, variation in C : N : P was relatively small under our experimental conditions. No general pattern for CO_2-related changes in the elemental composition could be found with regard to the direction of trends. Either an increase or a decrease in C : N and C : P with increasing $[CO_{2,aq}]$ was observed, depending on the species tested. Diurnal variation in C : N and C : P, tested in *Skeletonema costatum*, was of a similar magnitude as CO_2-related variation. In this species, the CO_2 effect was superimposed on diurnal variation, indicating that differences in elemental ratios at the end of the photoperiod were not caused by a transient buildup of carbon-rich storage compounds due to a more rapid accumulation of carbohydrates at high CO_2 concentrations. If our results obtained under high light, nutrient-replete conditions are representative for natural phytoplankton populations, CO_2-related changes in plankton stoichiometry are unlikely to have a significant effect on the oceanic carbon cycle.

The elemental composition (C : N : P) of phytoplankton is known to depend on both nutrient concentrations (e.g., Perry 1976; Sakshaug and Holm-Hansen 1977; Hecky et al. 1993) and light regime (Goldman 1980, 1986; Laws and Bannister 1980). Cell stoichiometry may also differ between species, independent of the environmental conditions. In spite of this variability, a constant elemental molar ratio of C : N : P = 106 : 16 : 1 has been reported by Redfield et al. (1963) for marine plankton in the open ocean when large water masses are considered. The elemental composition of plankton is reflected in a remarkably uniform ratio of changes in the concentration of inorganic nutrients with depth in the deep ocean as a result of the remineralization of biogenic sinking particles (Redfield 1934; Redfield et al. 1963; Takahashi et al. 1985; Andersen and Sarmiento 1994). A constant stoichiometry of marine export production, often called the ''Redfield ratio,'' is thus commonly used in the calculation of carbon fluxes from nutrient concentrations (e.g., Broecker et al. 1985; Six and Maier-Reimer 1996).

In a study of the marine diatom *S. costatum*, Burkhardt and Riebesell (1997) observed changes in the elemental composition in response to variable CO_2 concentrations. These results contradict the commonly accepted notion that elemental ratios of marine phytoplankton are unaffected by CO_2 availability. The authors argued that changes in the Redfield ratio may be expected upon the currently observed increase in atmospheric pCO_2 if dependence of the elemental composition proves to be a general phenomenon in marine phytoplankton. However, while C : P and N : P varied by up to 65% over the experimental CO_2 range of 0.5–38 μmol kg^{-1}, the largest changes in the elemental composition occurred at CO_2 concentrations lower than typically encountered in ocean surface waters. CO_2-related changes in C : N were even smaller and pointed in the opposite direction.

Burkhardt and Riebesell (1997) emphasized that the study of more species is a prerequisite to evaluate the biogeochemical relevance of CO_2-related changes in elemental composition of marine phytoplankton. The controversial debate regarding inorganic carbon acquisition by marine microalgae (e.g., Riebesell et al. 1993; Laws et al. 1995, 1997; Korb et al. 1997; Nimer et al. 1997; Raven 1997; Tortell et al. 1997; Burkhardt et al. unpubl. data) indicates that species may differ in their mechanisms of carbon uptake. If HCO_3^- is involved in inorganic carbon acquisition, either through direct uptake by an energy-dependent transport mechanism or through extracellular, catalyzed conversion of HCO_3^- to CO_2 by carbonic anhydrase (CA), we may expect little sensitivity of the elemental composition to variable $[CO_{2,aq}]$ in the bulk medium. By contrast, irrespective of the inorganic carbon source (CO_2 or HCO_3^-) and the mechanism of carbon acquisition (passive diffusion or active transport), cellular carbon demand varies with both growth rate and size of an algal cell. If CO_2 is taken up, we may thus expect greater sensitivity of cell stoichiometry to changes in $[CO_{2,aq}]$ in larger species during growth under high light, nutrient-replete conditions.

In this study, we tested the effect of CO_2 availability on C : N : P ratios in seven species of marine phytoplankton, which covered a wide range of cell sizes. Our main goal was the quantification of changes in elemental ratios in response to variable CO_2 concentrations rather than the identification of the underlying mechanisms. In addition, we investigated two other aspects that may be critical to the interpretation of CO_2-related effects on the elemental ratios.

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First, even under light-saturated and nutrient-replete conditions, the rate of photosynthetic carbon assimilation may vary by a factor of >2 within a species in response to variable day length (Burkhardt et al. unpubl. data). As a consequence of enhanced carbon flux into the cell, we expect an increase in the critical concentration below which passive diffusion of CO_2 becomes insufficient to satisfy cellular carbon demand (Riebesell et al. 1993; Wolf-Gladrow and Riebesell 1997). Differences in growth rate may thus affect the C : N : P vs. $[CO_{2,aq}]$ relationship. To test this, we incubated each of the species under continuous light and in a 16 : 8 or 18 : 6 light : dark (LD) cycle at six CO_2 concentrations.

Second, C : N : P ratios in phytoplankton exhibit diurnal variation when grown in an LD cycle as the result of an uncoupling of photosynthetic carbon fixation from nutrient assimilation (Cosper 1982; Cuhel et al. 1984). In experiments of Burkhardt and Riebesell (1997), samples were taken at the end of an 18-h photoperiod. Differences in elemental ratios over the range of $[CO_{2,aq}]$ could thus reflect differences in the ability of a cell to accumulate carbohydrates during the day for subsequent use as an energy reserve in dark respiration. In this case, we might expect no differences in C : N : P at the end of the night between cells growing at variable $[CO_{2,aq}]$. To test this, we monitored diurnal variation in C : N : P ratios of the diatom *S. costatum* at two CO_2 concentrations during growth at a 16 : 8 irradiance cycle and compared the results to growth under continuous light conditions.

Materials and methods

The diatoms *Phaeodactylum tricornutum, S. costatum, Asterionella glacialis, Thalassiosira weissflogii, Thalassiosira punctigera,* and *Coscinodiscus wailesii* and the dinoflagellate *Scrippsiella trochoidea* were obtained from the culture collection at the Alfred Wegener Institute. Prior to the experiments, all species were grown under conditions identical to the respective treatments for at least nine cell divisions to ensure adaptation of the cells to CO_2 and light supply. Experimental setup, sampling protocol, and analytical methods were the same as in previous studies and are described in detail by Burkhardt and Riebesell (1997). In the following, we will briefly summarize experimental design and measurements.

All experiments were performed in 2.4-liter dilute batch cultures at 15°C and an incident photon flux density of 150 μmol photons m^{-2} s^{-1}. In addition to experiments under continuous light, cells were grown in an 18 : 6 (*T. weissflogii*) or a 16 : 8 (all other species) LD cycle. Aged, 0.2-μm–filtered seawater from the North Sea was enriched with a trace metal mix and vitamins at concentrations of f/2 medium (Guillard and Ryther 1962). Nitrate, silicate, and phosphate were added to final concentrations of ca. 100, 100, and 6.25 μmol kg^{-1}, respectively. Salinity varied between 30.5 and 31.5 psu, depending on the batch of seawater. CO_2 concentrations were adjusted by the addition of HCl or NaOH, which resulted in changes in pH and alkalinity at a constant concentration of dissolved inorganic carbon (DIC). To cover an experimental $[CO_{2,aq}]$ range from 1.5 to 37.7 μmol kg^{-1}, pH varied from 9.1 to 7.8. It is important to note that the degree of pH variation in our experiments was identical with pH changes that would accompany corresponding changes in $[CO_{2,aq}]$ in the ocean. In ocean surface waters, $[CO_{2,aq}]$ is typically in the range of 10–20 μmol kg^{-1}. The incubation bottles were inoculated at low cell densities to permit nine or more cell divisions prior to sampling. During the experiments, <2% of DIC was taken up by the cells, so pH and carbon speciation was largely unaffected by algal growth. pH was measured with a microprocessor pH meter (WTW, pH 3000) using a combined AgCl/KCl electrode, calibrated with National Bureau of Standards (NBS) buffer solutions. Total alkalinity was determined from linear Gran plots (Gran 1952) after potentiometric titration in duplicate in a temperature-controlled automated system (Metrohm pH-713, coupled with Metrohm Dosimat 665). DIC was measured in duplicate by coulometric titration (UIC, model 5012) in an automated gas extraction system (Johnson et al. 1993). $[CO_{2,aq}]$ was calculated from DIC, total alkalinity, temperature, salinity, and concentrations of phosphate and silicate, assuming dissociation constants according to Mehrbach et al. (1973).

In treatments with an alternating LD cycle, cells were harvested during the last hour of the photoperiod to permit a direct comparison of elemental ratios between species. In continuous light experiments, cells were harvested at the same time of day as in LD cycle experiments. Samples for the C : N : P analysis were filtered on precombusted (500°C, 12 h) GF/C filters (Whatman), which were stored at −25°C until analysis. With the exception of the time-series experiment, C : N ratios of *S. costatum* and *T. weissflogii* were measured in triplicate on a carbon–hydrogen–nitrogen analyzer (Carlo Erba, model 1500). All other C : N samples were measured in duplicate on a mass spectrometer (ANCA-SL 20-20, Europa Scientific). To remove any inorganic carbon, filters were acidified with 0.1 N HCl prior to analysis. Total particulate phosphorus was measured spectrophotometrically (Parsons et al. 1984) after digestion with a 1% potassium persulfate solution for 1 h at 121°C in an autoclave (Grasshoff et al. 1983; Andersen and Hessen 1991).

In the time-series experiment, 12 bottles in each treatment were inoculated with the appropriate amount of preadapted *S. costatum* to achieve final particulate organic carbon (POC) concentrations of ca. 50 μmol kg^{-1}. After 3 d of incubation, the first bottle of each treatment was harvested completely. Each of the remaining bottles was then sampled accordingly in consecutive 2-h intervals to obtain a 24-h record for the parameters of interest. In treatments including a dark phase, the first sample was taken at the beginning of the light period.

Cell concentrations of *P. tricornutum* were measured with a Coulter Multisizer. Cell concentrations of the other species were determined microscopically. Daily cell counts in control bottles indicated that no prolonged lag phase occurred during the experiments. Growth rates were calculated according to the equation $\mu = \ln(POC_{i+1}/POC_i)/(t_{i+1} - t_i)$, where POC_{i+1} and POC_i are concentrations of POC at time t_{i+1} and t_i, respectively.

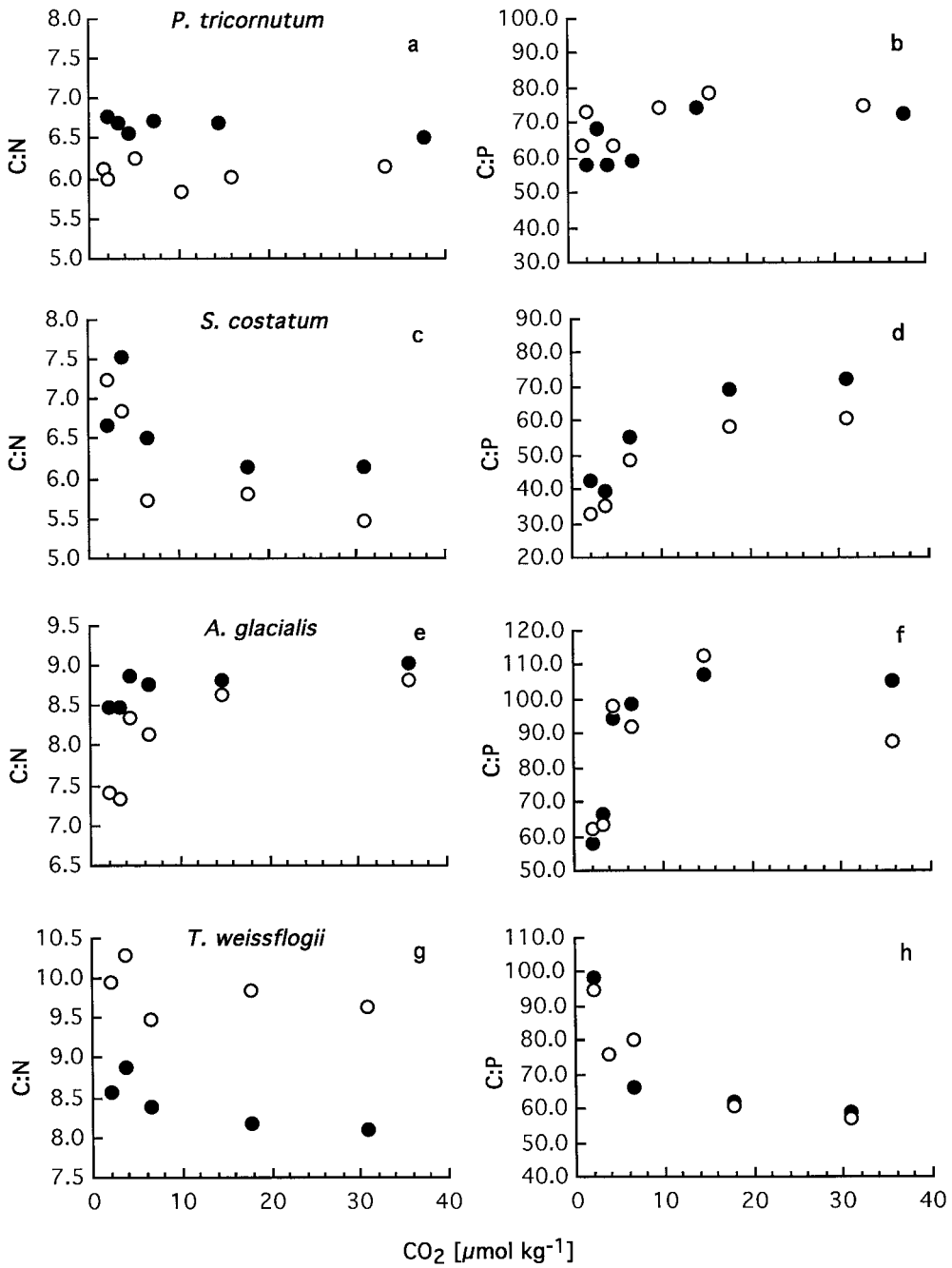

Fig. 1. Variation in C : N and C : P as a function of $[CO_{2,aq}]$ during growth at an LD cycle (closed symbols) or under continuous light (open symbols) in seven species of marine phytoplankton. Data are shown for the diatoms *P. tricornutum* (a, b), *S. costatum* (c, d), *A. glacialis* (e, f), *T. weissflogii* (g, h), *T. punctigera* (i, j), and *C. wailesii* (k, l) in the order of increasing cell size and for the dinoflagellate *S. trochoidea* (m, n).

Results

C : N and C : P molar ratios as a function of $[CO_{2,aq}]$ are shown for the diatoms in Fig. 1a–l in the order of increasing cell size and are shown for the dinoflagellate *S. trochoidea* in Fig. 1m,n. CO_2-related trends in N : P (data not shown) closely followed trends in C : P. In *S. costatum* grown in a 16 : 8 LD cycle (Fig. 1c,d), a decrease in C : N and an increase in C : P in response to increasing $[CO_{2,aq}]$ were consistent with the results that Burkhardt and Riebesell (1997) obtained in an 18 : 6 LD cycle. Under continuous light, both C : N and C : P were slightly lower but showed the same response to variable $[CO_{2,aq}]$. *C. wailesii* (Fig. 1k,l) and *S. trochoidea* (Fig. 1m,n) exhibited a similar increase in C : N and a decrease in C : P with decreasing $[CO_{2,aq}]$. In contrast to *S. costatum*, however, C : N ratios were higher in these species

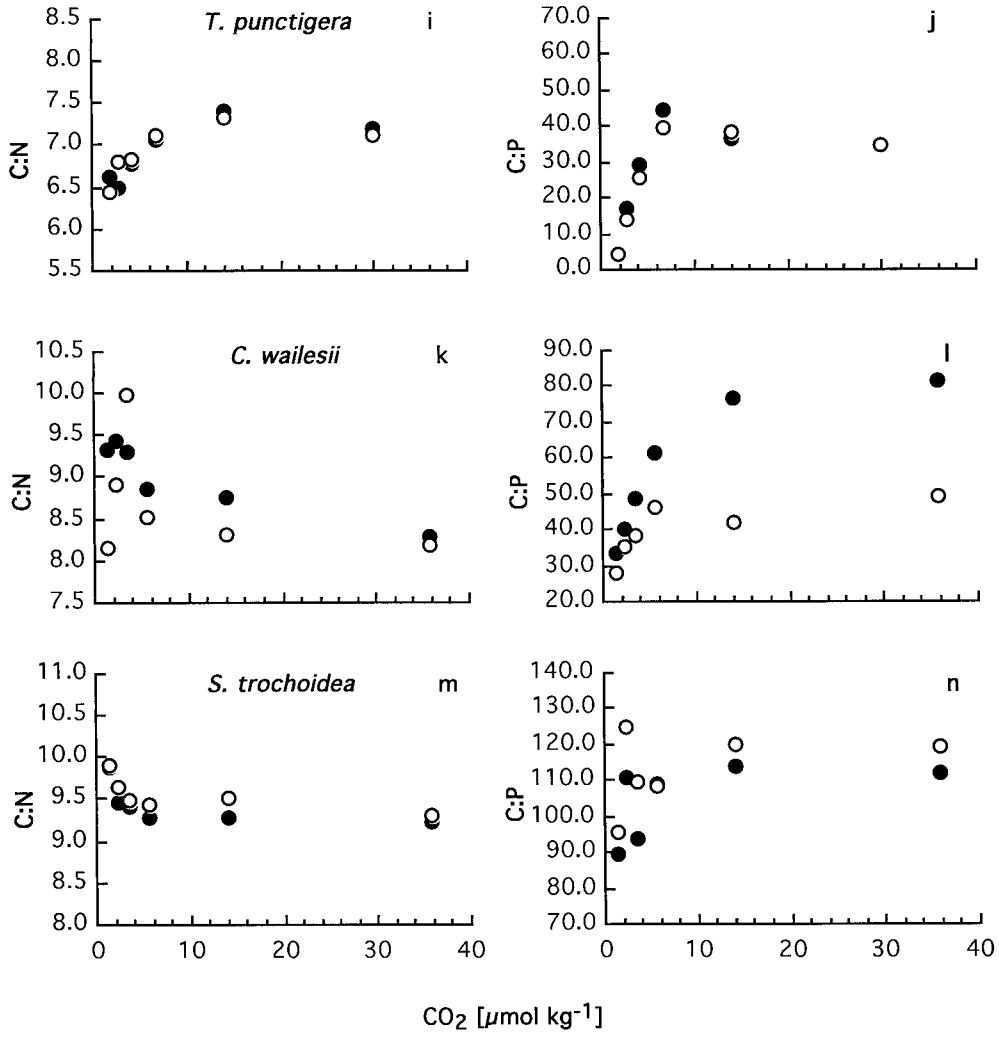

Fig. 1 Continued.

at both irradiance cycles, approaching maximum values between 9.5 and 10 at low [$CO_{2,aq}$]. While C : P in *S. trochoidea* was close to the Redfield ratio of 106, C : P in *C. wailesii* was low similar to that of *S. costatum* (ca. 80 at high [$CO_{2,aq}$]) when grown in a 16 : 8 LD cycle and even lower under continuous light (ca. 50 at high [$CO_{2,aq}$]). *A. glacialis* (Fig. 1e,f) and *T. punctigera* (Fig. 1i,j) also exhibited a systematic increase in C : P with increasing [$CO_{2,aq}$], yielding values close to the Redfield ratio at high [$CO_{2,aq}$] in *A. glacialis* but very low C : P ratios in *T. punctigera.* In these species, however, C : N showed an opposite trend, with minimum values observed at the lowest CO_2 concentrations. Unlike any other species tested, C : N and C : P in *T. weissflogii* both decreased as [$CO_{2,aq}$] increased. In this diatom, we observed high C : N ratios (ca. 9.5–10.5) under continuous light, being ca. 1.5 units higher than in an 18 : 6 LD cycle. Elemental ratios of the smallest species *P. tricornutum* were not affected by changes in [$CO_{2,aq}$] in either of the irradiance cycles (Fig. 1a,b).

S. costatum exhibited diurnal variation in C : N and C : P when grown in an LD cycle but not under continuous light (Fig. 2). After the onset of the dark period, both C : N and C : P decreased with time and continued to decrease for 6–8 h during the photoperiod. During the second half of the photoperiod, C : N and C : P ratios increased rapidly, reaching a maximum when the light was turned off. The offset in elemental ratios between high and low CO_2 concentrations persisted over the entire 24-h period. Average values of the relatively invariable C : N and C : P ratios under continuous light were similarly low to elemental ratios at the end of the dark period.

P. tricornutum was the only species exhibiting no dependence of growth rate on [$CO_{2,aq}$] over the entire experimental CO_2 range (Fig. 3). In all other species, growth rate showed no significant variation at CO_2 concentrations above 10 μmol kg^{-1} but clearly declined at [$CO_{2,aq}$] $<$ 4–6 μmol kg^{-1}. In general, the correlation of growth rate with [$CO_{2,aq}$] showed the same trend in experiments with an LD cycle (Fig. 3a,b) as under continuous light (Fig. 3c,d).

Discussion

Our species comparison of CO_2-related effects on elemental composition in marine phytoplankton is a continuation of

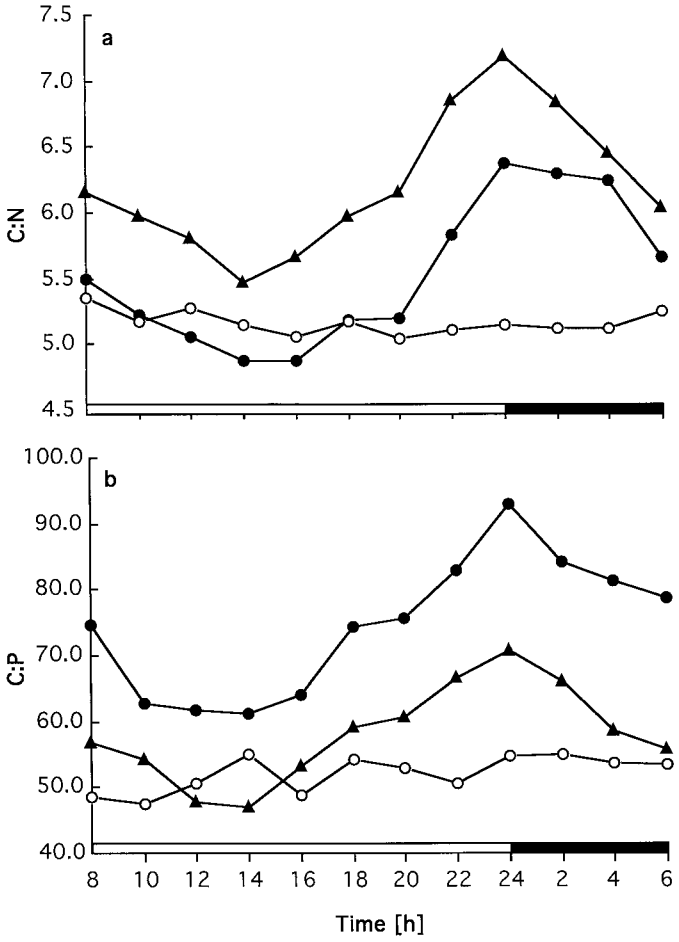

Fig. 2. *S. costatum.* Diurnal variation in (a) C : N, and (b) C : P at high [$CO_{2,aq}$] (27 μmol kg^{-1}; circles) and low [$CO_{2,aq}$] (3 μmol kg^{-1}; triangles) during growth in a 16 : 8 LD cycle (closed symbols) or under continuous light (open symbols).

the work by Burkhardt and Riebesell (1997). Their study was motivated by the observation of CO_2-dependent growth rates and primary production in monospecific cultures of marine diatoms (Riebesell et al. 1993; Chen and Durbin 1994) and in mixed phytoplankton assemblages in marine mesocosms (Chen and Durbin 1994), as well as in the open ocean (Hein and Sand-Jensen 1997). An increase in growth rate toward higher CO_2 concentrations implies that cellular uptake of CO_2 is a significant component of inorganic carbon acquisition during algal photosynthesis. In addition to passive diffusion of CO_2, active transport of CO_2 and/or HCO_3^- may function as a compensatory transport mechanism at low [$CO_{2,aq}$] (*see* Raven 1997 for a recent review) to maintain constant growth rates. Algal growth rates are expected to be insensitive to changes in [$CO_{2,aq}$] if HCO_3^- is the only carbon source or if active uptake of HCO_3^- is induced in response to a decrease in [$CO_{2,aq}$].

The critical concentration below which algal growth becomes limited by diffusive CO_2 supply depends on cell size, growth rate, and presence of extracellular CA (Riebesell et al. 1993; Wolf-Gladrow and Riebesell 1997). If inorganic carbon transport across the plasmalemma occurs mainly by CO_2 uptake, we may thus expect that (1) large cells are more sensitive to decreasing [$CO_{2,aq}$] than small cells if they grow at a similar rate, and (2) cells of similar size but different growth rates differ in their sensitivity to changes in [$CO_{2,aq}$] regarding both elemental ratios and growth rate. The reason is that higher CO_2 concentrations are required to satisfy carbon demand in large or rapidly growing cells.

In our experiments, the seven species covered a wide size range of surface-equivalent spherical radius (r) from 1.3 to 91.8 μm. Burkhardt et al. (unpubl. data) determined that there are large differences in the minimum CO_2 concentration ($CO_{2,min}$) at which cellular carbon demand for growth can, theoretically, be satisfied by CO_2 uptake. While $CO_{2,min}$ = 1–2 μmol kg^{-1} in the smallest species ($r \leq 6$ μm), values were higher in *T. weissflogii* (r = 8.9 μm) and *T. punctigera* (r = 21.5 μm), ranging from 4 to 9 μmol kg^{-1}. The largest diatom, *C. wailesii* (r = 91.8 μm), represents a special case, because $CO_{2,min}$ was always >50 μmol kg^{-1}, thus exceeding even the highest experimental CO_2 concentrations employed in our study. This species depends on an additional carbon source, such as CA-catalyzed conversion of HCO_3^- to CO_2 near the outer cell surface or direct uptake of HCO_3^-. Nevertheless, inorganic carbon supply becomes insufficient to maintain high growth rates at CO_2 concentrations below 10 μmol kg^{-1} (Fig. 3b,d) in this species, indicating that inorganic carbon acquisition of *C. wailesii* is not independent of [$CO_{2,aq}$]. Although growth rate of *C. wailesii* becomes sensitive to [$CO_{2,aq}$] at higher concentrations compared to the smaller species tested, the large size-related differences in $CO_{2,min}$ between species are not reflected in a similarly large range of CO_2 concentrations, below which growth rate depends on [$CO_{2,aq}$].

A comparison of Figs. 1, 3 indicates that large variability in elemental ratios coincides with a CO_2-related decrease in algal growth rates. We interpret these results as evidence for a direct effect of CO_2 availability on the elemental composition of marine phytoplankton, although they do not exclude the possibility that the algae make use of the large HCO_3^- pool in seawater in addition to CO_2 uptake. Regardless of the carbon uptake mechanism, differences in cell size cause little variation in the CO_2 concentration below which large changes in the elemental composition of the algae are observed.

To test the effect of variable growth rate on CO_2-related variation in C : N : P independent of [$CO_{2,aq}$] and cell size, we compared results from experiments at different LD cycles. In spite of a 6- to 8-h difference in day length, we observed only small differences in 24-h average growth rates between irradiance cycles. To maintain a constant growth rate on a daily basis, rates of photosynthetic carbon fixation during the light period thus needed to be significantly higher in a 16 : 8 or 18 : 6 LD cycle than under continuous light, because no net growth occurs in darkness. These instantaneous growth rates, rather than 24-h average rates, ultimately determine the actual rates of carbon uptake and thus the sensitivity of elemental ratios to CO_2 supply. Instantaneous growth rates were calculated according to

$$\mu_i = \frac{(L + D)\mu_{L+D}}{L - Dr} \quad (1)$$

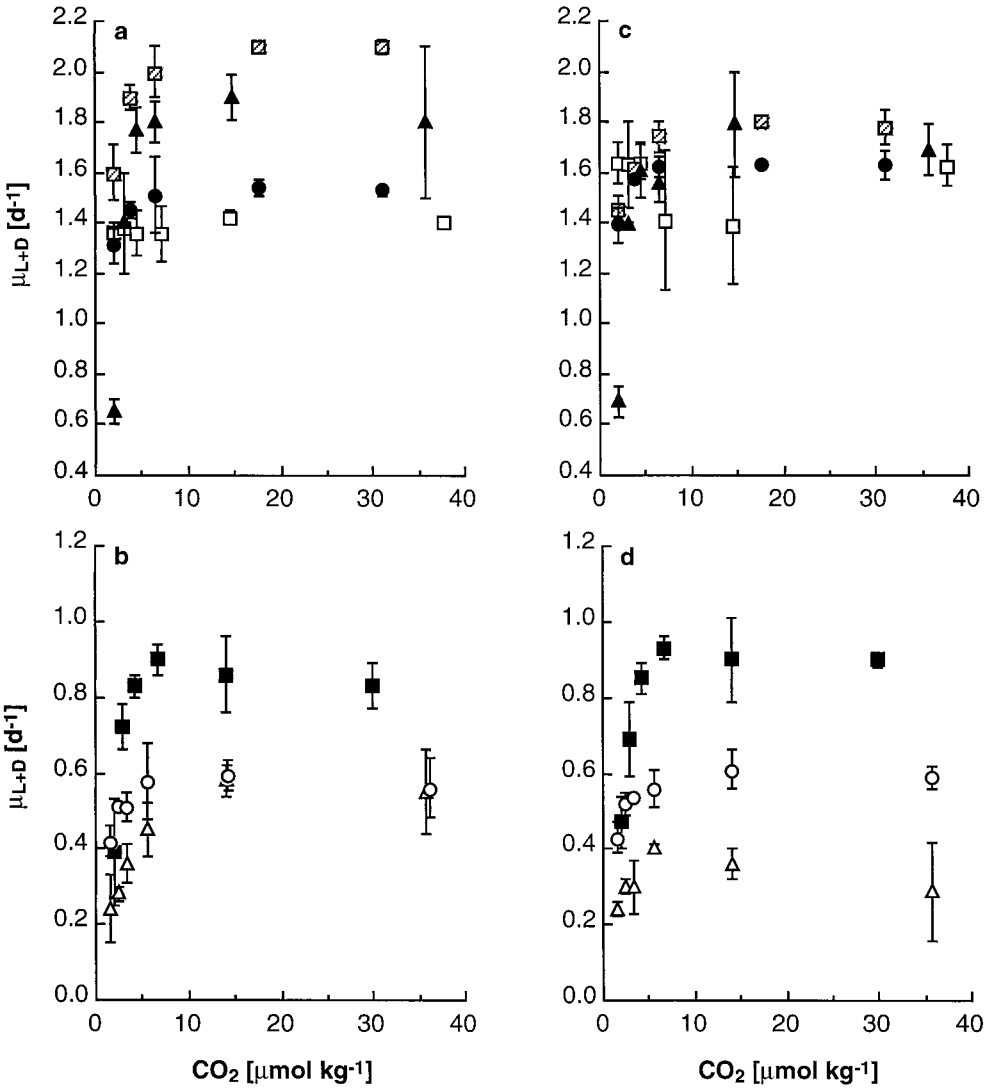

Fig. 3. The 24-h average growth rate (μ_{L+D}) as a function of [$CO_{2,aq}$] in species of small cell size and $\mu_{L+D} > 1$ d^{-1} (a, c) and species of large cell size and $\mu_{L+D} < 1$ d^{-1} (b, d) in an LD cycle (a, b) and under continuous light (c, d). (□) *P. tricornutum,* (▨) *S. costatum,* (▲) *A. glacialis,* (●) *T. weissflogii,* (■) *T. punctigera,* (△) *C. wailesii,* and (○) *S. trochoidea.* Error bars indicate ±1 SD.

where *L* and *D* are duration of the light and dark period, respectively. The rate of dark carbon loss was assumed to equal 15% of the rate of C assimilation during the preceding light period, in which case r = 0.15 (Laws and Bannister 1980).

Calculated μ_i in 16 : 8 or 18 : 6 LD cycles was 50–70% higher than under continuous light in all species. In spite of the large differences in μ_i between irradiance cycles, however, both C : N : P ratios and growth rates did not show greater sensitivity to changes in [$CO_{2,aq}$] at higher μ_i. A higher resolution in the experimental [$CO_{2,aq}$] gradient may be necessary to detect possible differences in CO_2-related effects on C : N : P between the irradiance cycles. Nevertheless, we demonstrated that large changes in the rate of instantaneous growth of a cell in response to variable day length have a relatively small effect on the C : N : P vs. [$CO_{2,aq}$] relationship.

The difference in absolute values of C : N and C : P ratios between irradiance cycles, which we observed in some species, is likely to result from an uncoupling of CO_2 fixation from nutrient assimilation rather than from differences in the rate of carbon supply. This is indicated by the increase in C : N and C : P in *S. costatum* during the second half of the photoperiod (Fig. 2), which probably reflects the rapid accumulation of carbohydrates to provide the metabolic energy and carbon skeletons for biosynthesis during the subsequent dark period. During growth under continuous light, CO_2 fixation and nutrient assimilation in *S. costatum* appear to be closely coupled, so that the primary products of photosynthesis are directly used in biosynthesis rather than for carbon storage. The main results from the time-series experiment are that (1) diurnal variation in C : N and C : P is of a similar magnitude as CO_2-related differences in elemental ratios over a range of [$CO_{2,aq}$] = 3–27 μmol kg^{-1}, and (2) the effect

of CO_2 on the elemental composition in *S. costatum* persists over a 24-h period and is not merely the result of different rates of carbohydrate accumulation during the day under the respective CO_2 concentrations.

Our experiments were aimed at the quantification of CO_2-related changes in C : N : P, and we cannot identify the physiological reasons for the observed variability in elemental ratios. One might speculate that CO_2-limited cells differ from CO_2-replete cells, for example, in their cellular content of pigment-associated proteins or RUBISCO, in their ability to synthesize storage carbohydrates or polyphosphates, or in loss rates of dissolved organic carbon compounds. In our analysis of carbon, nitrogen, or phosphorus cell quota (data not shown), we observed changes in cellular content of each of the elements at variable [$CO_{2,aq}$], but no generalization was possible by which differences in cell quota could be related to variation in elemental ratios.

Under high light, nutrient-replete conditions, CO_2 limitation is likely to affect the ratio of available energy (ATP) and reducing power ($NADPH_2$) in an algal cell (Burkhardt et al. unpubl. data; Riebesell et al. unpubl. data). Consequently, the acquisition of inorganic carbon relative to nitrogen and phosphorus may vary at low [$CO_{2,aq}$] because of the different requirements for ATP and reducing power during cellular uptake and assimilation into organic compounds. As an example, nitrate reduction to the redox state of organic N compounds requires eight electrons, whereas phosphate can be directly incorporated without electron transfer reactions. Similarly, nitrate assimilation into protein differs in its ATP/electron requirement from photosynthetic CO_2 fixation (Turpin 1991). A shift in nutrient uptake ratios thus depends on physiological control mechanisms for expenditure of ATP and $NADPH_2$, which ultimately determines whether the C : N and C : P ratio of a cell increases or decreases under CO_2-limiting conditions. If such a scenario adequately describes carbon acquisition in phytoplankton, we may conclude that the balance between C, N, and P uptake and assimilation is not affected as long as the cells are able to maintain a constant growth rate. In contrast, limited availability of ATP and reduction equivalents under growth-limiting conditions may affect the ratio of their allocation to C, N, and P acquisition. In case that an energy-dependent, active transport of inorganic carbon is required to maintain high growth rate, we may further conclude that algal cells are more sensitive to changes in CO_2 availability under light-limiting conditions. If so, growth at low light intensities may also lead to a considerably greater response of C : N : P ratios to [$CO_{2,aq}$] variation in the range typically encountered in ocean surface waters than under the experimental conditions in our study.

In summary, our study clearly demonstrates the dependence of phytoplankton elemental composition on [$CO_{2,aq}$] that coincides with a simultaneous change in growth rate. While these results are interesting from a physiological point of view, CO_2-related variation in plankton stoichiometry appears to play a minor role in the ocean carbon cycle because large changes in C : N : P ratios are only expected below the CO_2 concentrations of ca. 10–20 μmol kg^{-1} typically observed in ocean surface waters. Although an increase in C : P with increasing [$CO_{2,aq}$] was observed in most species, no generalization can be made with respect to the direction of trends in C : P or C : N ratios as a function of CO_2 concentration. Neither cell size nor day length-dependent growth rates had much effect on the critical CO_2 concentration below which the elemental composition showed large variability.

Unless marine phytoplankton stoichiometry proves to be more sensitive to changes in [$CO_{2,aq}$] under light-limiting conditions than under light saturation as obtained in our experiments, we do not expect the presently observed increase in atmospheric pCO_2 to significantly affect global ocean Redfield ratios. Based on the results of this study, CO_2-related variability in phytoplankton elemental composition is unlikely to act as a feedback mechanism capable of effectively modifying the strength of the biological carbon pump.

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