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CO_2 availability affects elemental composition (C:N:P) of the marine diatom *Skeletonema costatum*

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ABSTRACT: The effect of variable CO_2 concentrations on the elemental composition (C:N:P) of marine diatoms was investigated in 2 strains of *Skeletonema costatum* (Grev.) Cleve. Five or 6 concentrations of dissolved molecular carbon dioxide [CO_2 (aq)], ranging from 0.5 to 39 µmol l^{-1}, were applied in dilute batch cultures. In both strains, elemental ratios were clearly dependent on [CO_2 (aq)]. With decreasing CO_2 concentrations, a decline in C:P and N:P and an increase in C:N was observed. The close correlation between C:P or N:P and [CO_2 (aq)] corresponded to a ca 45 to 65% decrease in elemental ratios from highest (≥30 µmol l^{-1}) to lowest (ca 1 µmol l^{-1}) CO_2 concentrations. C:N at low [CO_2 (aq)] was up to 24% higher than at high [CO_2 (aq)]. To date, the elemental composition of marine phytoplankton has been considered to be independent of CO_2 availability. If dependency of the C:N:P ratio on [CO_2 (aq)] proves to be a general phenomenon in marine phytoplankton, changes in the elemental composition may be expected in response to the currently observed increase in partial pressure of atmospheric CO_2.

KEY WORDS: Redfield ratio · CO_2 · Phytoplankton · Marine diatoms · Cell stoichiometry

INTRODUCTION

In the 1930s, Alfred C. Redfield developed the concept of a constant elemental composition of planktonic biomass in the oceans (Redfield 1934, Redfield et al. 1963). Since then the Redfield ratio of C:N:P = 106:16:1 (by atoms) in marine plankton has become a cornerstone in biogeochemical and ecophysiological studies, in spite of minor revisions in the proportions of these elements (e.g. Takahashi et al. 1985, Anderson & Sarmiento 1994, Anderson 1995). Its application ranges from the use as biological indicator of *in situ* nutrient limitation of phytoplankton (e.g. Perry 1976, Sakshaug & Holm-Hansen 1977, Hecky et al. 1993) to modelling global carbon flux (e.g. Heinze et al. 1991). Redfield et al. (1963) proposed that since N:P ratio of available nutrients closely resembled planktonic composition, both elements would be potentially limiting to phytoplankton growth. With regard to inorganic carbon supply to microalgae they stated that 'clearly carbon does not become a limiting factor in the growth of marine plants in the sea' due to the high concentrations of inorganic carbon compared to phosphorus and nitrogen.

The high concentration of total dissolved inorganic carbon (DIC) in the oceans constitutes a large reservoir for the build-up of algal biomass. However, utilization of inorganic carbon by phytoplankton depends on the chemical form of DIC (molecular CO_2, HCO_3^-, or CO_3^{2-}) present in seawater. Pathways of inorganic carbon acquisition in marine microalgae still represent a controversial issue owing to the limited and sometimes contradictory information available. Cellular uptake of DIC by passive diffusion of CO_2 versus active transport of CO_2 and/or HCO_3^-, interconversion of CO_2 and HCO_3^-, by extracellular and/or intracellular carbonic anhydrase, and intracellular accumulation of CO_2 by an inorganic carbon concentrating mechanism are discussed as factors controlling carbon availability in marine microalgae (for review see Raven 1991, Raven et al. 1993). Studies of stable carbon isotope fractionation by marine microalgae show that diffusive uptake of dissolved molecular CO_2, which represents only

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0.5 to 1% of the total DIC, can satisfy cellular carbon demand for the observed growth rates over a wide range of [CO_2 (aq)] (Degens et al. 1968, Gleitz et al. 1996, H. Kukert & U. Riebesell unpubl.). A different mode of carbon acquisition might be employed by means of either active CO_2 transport or utilization of the external HCO_3^- pool below a critical concentration of molecular CO_2 (Sharkey & Berry 1985, Gleitz et al. 1996, Laws et al. in press). Phytoplankton relying on diffusive transport of CO_2 as carbon source can be subject to transport limitation (Gavis & Ferguson 1975). CO_2 availability, therefore, can affect carbon uptake and fixation by algal cells (Riebesell et al. 1993) and thereby may also influence the elemental composition of the organic matter produced.

In this study, 2 strains of the marine diatom *Skeletonema costatum* were used in dilute batch culture experiments to evaluate the potential effect of variable [CO_2 (aq)] on cell stoichiometry. To date, the Redfield ratio has been considered to be independent of ambient CO_2 concentrations. Variation of elemental ratios in response to changes in [CO_2 (aq)] would provide first evidence that the chemical composition of marine diatoms can be sensitive to changes in atmospheric partial pressure of CO_2 (pCO_2).

MATERIAL AND METHODS

Diatom cultures. Two strains of *Skeletonema costatum*, SK A and SK B, were used in laboratory experiments. SK A was collected in the North Sea by vertical net tows and maintained in stock culture for several years. SK B was provided by the Biologische Anstalt Helgoland, where cells were isolated from North Sea phytoplankton net samples in February 1996. Mean cell diameter of SK B cells (12.9 µm) exceeded that of SK A (4.7 µm) by a factor of 3. Dilute batch cultures were pre-adapted to experimental conditions for at least 5 d. Experiments were designed to permit at least 9 cell divisions under the specific conditions of the respective treatment. To avoid large pH drift and corresponding changes in DIC speciation, cultures were inoculated at low cell concentrations (SK A: ca 4×10^4 cells l^{-1}; SK B: ca 1×10^4 cells l^{-1}) and harvested at ca 2.5×10^7 cells l^{-1} (SK A) or 6×10^6 cells l^{-1} (SK B), which corresponds to ca 30 µmol l^{-1} inorganic carbon, 5 µmol l^{-1} nitrate and 0.5 µmol l^{-1} phosphate assimilated during the experiment. Bacterial biomass never exceeded 1% of algal biomass in the cultures and its contribution to elemental ratios was therefore considered negligible.

Experiments were performed in 2.4 l borosilicate glass bottles, tightly closed with PBT-lined screw caps to avoid air bubbles in the culture vessel. SK A was grown in f/2-enriched (Guillard & Ryther 1962) aged 0.2 µm filtered natural seawater. Nutrient enrichment in SK B cultures was modified to nitrate, silicate, and phosphate concentrations of 100, 100, and 6.25 µmol l^{-1}, respectively. The diatoms were incubated in a Rumed 1200 light-thermostat at 15°C with a light-dark cycle of 18 h:6 h and an incident photon flux density of 150 µmol photons m^{-2} s^{-1}.

Experimental carbonate system. An intrinsic property of the carbonate system is the interdependency of its components. In order to vary [CO_2 (aq)], at least 1 of the parameters DIC, alkalinity, and pH needs to be changed. Concentration of molecular CO_2 in ocean surface water depends on the physical parameters atmospheric pCO_2, air-sea gas exchange, mixing events, temperature, salinity, and the biological processes photosynthesis, respiration and calcite production. Thus, either DIC concentration or, in the case of calcite production, alkalinity are affected. Whenever DIC concentration has been altered, changes in [CO_2 (aq)] are accompanied by changes in pH.

In routine experiments, concentrations of DIC within treatments were approximately the same (DIC const) (Table 1). Five concentrations of dissolved molecular carbon dioxide [CO_2 (aq)], ranging from 1.5 to 32 µmol l^{-1}, were adjusted by keeping pH fixed at values between 7.88 and 9.03. The respective pH was achieved by addition of 1 N HCl or 1 N NaOH to the culture medium. Further gas exchange was prevented by closing the incubation vessels tightly without headspace immediately after pH adjustment to ensure constancy of pH and [CO_2 (aq)]. In experiments with SK A (DIC const), triplicate bottles were incubated at each of the 5 CO_2 (aq) levels. Because of reasonable agreement in elemental ratios between replicates, incubation of only 1 bottle at each [CO_2 (aq)] was considered sufficient in further experiments.

To evaluate potential pH effects on cell physiology, which might be reflected in the chemical composition of the cells, control experiments with SK A were performed at identical pH of 8.21 to 8.23 (pH const) in 6 parallel treatments of different [CO_2 (aq)]. Unlike variation in [CO_2 (aq)] under natural conditions, concentration of molecular CO_2 in this treatment was manipulated by alteration of both DIC concentration and alkalinity (Table 1). To achieve lower CO_2 (aq) concentrations at constant pH, 1 N HCl was first added to the culture medium, which resulted in a decrease in alkalinity and supersaturation of CO_2 (aq) relative to atmospheric pCO_2. [CO_2 (aq)] was then decreased by bubbling with air. Because of the open system, DIC decreases until [CO_2 (aq)] approaches equilibrium with the atmosphere, accompanied by an increase in pH. In the following step, pH was readjusted to initial values by addition of 1 N NaOH. The incubation vessels were

Table 1 *Skeletonema costatum*. Experimental carbonate system. Two strains (SK A, SK B) were incubated at either constant concentrations of dissolved inorganic carbon (DIC const) or at constant pH (pH const). pH and concentrations of dissolved molecular carbon dioxide [CO_2 (aq)] were calculated from DIC, total alkalinity (tAlk), temperature (15°C), salinity (32 psu) and concentrations of phosphate (6.25 µmol l^{-1}) and silicate (100 µmol l^{-1})

Treatment	pH	DIC (mmol l^{-1})	tAlk (meq l^{-1})	[CO_2 (aq)] (µmol l^{-1})
SK A	7.89	2.11	2.24	30.6
DIC const	8.28	2.13	2.45	12.0
	8.59	2.14	2.71	5.4
	8.85	2.14	3.01	2.7
	9.03	2.14	3.26	1.5
SK B	7.88	2.14	2.25	31.9
DIC const	8.12	2.16	2.37	18.3
	8.51	2.16	2.63	6.8
	8.72	2.17	2.85	3.9
	8.93	2.17	3.12	2.1
SK A	8.22	5.94	6.51	38.9
pH const	8.23	2.17	2.47	14.0
	8.21	0.76	0.95	5.1
	8.22	0.48	0.65	3.1
	8.22	0.17	0.32	1.1
	8.21	0.07	0.21	0.5

then sealed with screw caps immediately to prevent further exchange with atmospheric CO_2. To achieve higher [CO_2 (aq)] at constant pH, Na_2CO_3 was added and pH then readjusted to initial values with 1 N HCl.

[CO_2 (aq)] before and after the experiments was calculated from DIC, total alkalinity (tAlk), temperature, salinity and concentrations of phosphate and silicate, assuming dissociation constants according to Mehrbach et al. (1973). pH was measured with a microprocessor pH meter (WTW pH 3000) using a combined AgCl/KCl electrode, calibrated with NBS buffer solutions. In addition, pH was calculated from DIC and tAlk. The difference between measured and calculated pH was, on average, less than 0.05 pH units. tAlk was calculated from linear Gran-plots (Gran 1952) after potentiometric titration of duplicate 100 ml samples with 0.05 N HCl (Bradshaw et al. 1981, Brewer et al. 1986). DIC samples were stored at 4°C in 300 ml borosilicate glass bottles without headspace after addition of 1 ml of 50% saturated $HgCl_2$ solution. DIC was measured in triplicate by coulometric titration (UIC coulometer) in an automated gas extraction system (Johnson et al. 1993).

C:N:P measurements. Samples for determination of C:N:P were filtered on precombusted (500°C, 12 h) GF/C glass fiber filters (Whatman), rinsed with 3 × 5 ml 0.17 M Na_2SO_4 solution, and stored at −25°C until analysis. Analysis of particulate organic carbon (POC) and nitrogen (PON) was performed on triplicate filters on a Carlo Erba 1500 CHN analyzer after removal of inorganic C with 0.1 N HCl. Triplicate filters for determination of total particulate phosphorus (TPP) were placed directly in 10 ml borosilicate tubes with teflon-lined screw caps and stored frozen at −25°C. Upon analysis, TPP filters were digested in a 1% potassium persulfate solution in an autoclave at 121°C for 1 h. Subsequently, TPP was measured spectrophotometrically by the ammonium-molybdate method (Strickland & Parsons 1972) as orthophosphate in 1 cm cells at 885 nm. C:N:P was calculated from molar concentrations of POC:PON:TPP.

Cell size, cell concentration and growth rates. Cells were enumerated on 20 ml samples preserved in Lugol's solution under an inverted microscope. At least 400 cells were counted in duplicate for calculation of cell concentrations. For determination of cell size, at least 25 individual cells were chosen at random in the microscopic sample. Cell counts before and after the experiment served as an estimate of growth rates at the various CO_2 (aq) concentrations. To avoid disturbance of the closed system, no further subsamples were taken from the incubation vessels during the experiment. Growth rates, however, were compared to rates obtained from subsequent cell counts twice a day over a 3 d period in pre-cultures. These values were also used to define the duration of the experiment (72 h at [CO_2 (aq)] ≥ 2 µmol l^{-1}, 96 h at [CO_2 (aq)] < 2 µmol l^{-1}) which permitted 9 cell divisions prior to sampling. Growth rates (µ) were calculated according to the equation $\mu = \ln(N_1/N_0)/t$, where N_0 and N_1 are cell concentrations at the beginning and the end of the experiment and t equals duration of incubation in days.

RESULTS

Elemental ratios

Both strains of *Skeletonema costatum* (SK A and SK B) showed decreases in C:P and N:P, but increases in C:N, towards lowest [CO_2 (aq)] (Figs. 1 & 2). Minimum C:P and N:P ratios were respectively only 41 to 55% and 35 to 49% of maximum values over the range of CO_2 (aq) concentrations (>30 to <2 µmol l^{-1}) tested (Figs. 1b, c & 2b, c). The effect of [CO_2 (aq)] on C:N was also significant, but less pronounced, with C:N increasing by up to 24% from highest to lowest CO_2 (aq) concentrations (Figs. 1a & 2a). Results from 3 independent incubations of SK A at each of 5 experimental CO_2 concentrations are shown in Fig. 1. Elemental ratios of the other strain, SK B, were similar with C:P and N:P ratios being slightly lower than in SK A. In spite of differences in cell diameter by a factor of 3, both strains showed similar variation in C:N:P ratios with changing [CO_2 (aq)] (Fig. 1).

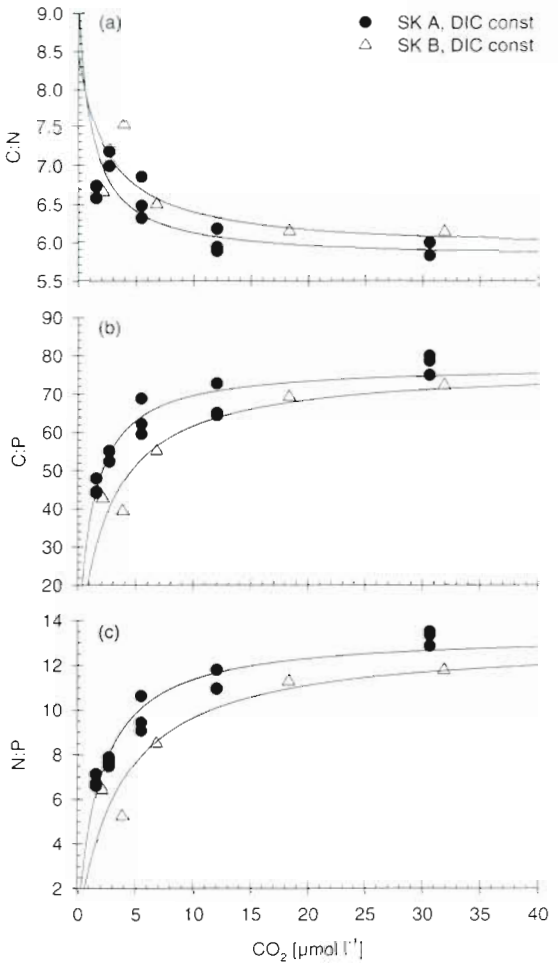

Fig. 1 *Skeletonema costatum*. Variation in (a) C:N, (b) C:P, and (c) N:P ratios of SK A (●) and SK B (Δ) at constant DIC in relation to [CO_2 (aq)]. Each data point represents the mean value of triplicate measurements of C, N, and P. C:P and N:P curves are derived from a least-squares fit using the Michaelis-Menten equation (see Table 2). The C:N curve was calculated as C:N = C:P/N:P

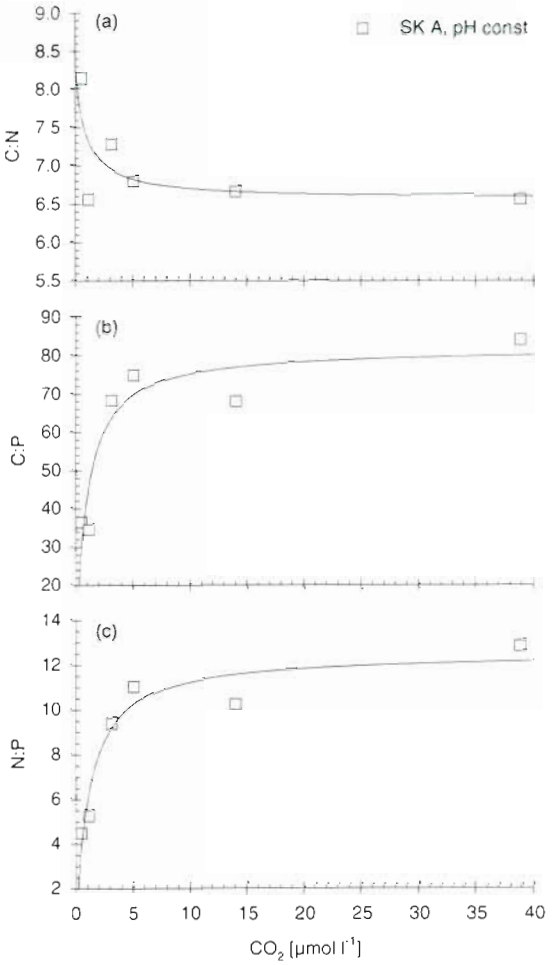

Fig. 2. *Skeletonema costatum*. Variation in (a) C:N, (b) C:P, and (c) N:P ratios of SK A at constant pH in relation to [CO_2 (aq)]

If [CO_2 (aq)] of ocean surface water varies in response to biological activity or, over longer time scales, by air-equilibration in response to increasing pCO_2, alkalinity remains unaffected whereas DIC and pH change. As mentioned above, only 1 of the parameters DIC, alkalinity, and pH can be kept constant when manipulating seawater. In the experimental carbonate system, DIC was kept constant when changing [CO_2 (aq)]. Corresponding changes in pH in this system were of the same magnitude as under natural conditions. If the observed variation in C:N:P were caused by changes in pH and not CO_2 concentration, one would expect no variation in elemental ratios of *Skeletonema costatum* at variable [CO_2 (aq)] but constant pH. Fig 2 shows data from the control experiment with SK A, in which pH was kept constant at all CO_2 levels. C:N:P ratios varied by the same order of magnitude as in experiments with constant DIC and variable pH. Although absolute values in Fig. 2 differ somewhat from Fig 1, identical trends in elemental ratios were taken as indication that variability in elemental ratios was caused by changes in supply of CO_2 rather than pH effects on cell physiology.

To describe CO_2-dependent variation in C:P and N:P, it was assumed that elemental ratios (E) approach maximum values (E') at saturating CO_2 levels. Therefore, the Michaelis-Menten equation $E = E' [CO_2 (aq)]/(K_E + [CO_2 (aq)])$, where K_E is the half-saturation constant, was used for an iterative, non-linear least-squares fit to C:P and N:P measurements. C:N curves were calculated as C:N = C:P/N:P (Figs. 1 & 2). Calculated constants and their standard deviations for all treatments are listed in Table 2.

Table 2. *Skeletonema costatum*. Results from a least-squares fit to CO_2-dependent variation in C:P and N:P. Maximum elemental ratios E' and half-saturation constants K_E were calculated according to the Michaelis-Menten equation for C:P and N:P molar ratios of 2 clones (SK A, SK B). Standard deviations are given in parentheses (for further explanations see text)

Clone	Treatment	E'	K_E (µmol l^{-1})
SK A	DIC const	(C:P)' = 77.47 (0.51)	$K_{C:P}$ = 1.14 (0.04)
		(N:P)' = 13.40 (0.57)	$K_{N:P}$ = 1.80 (0.31)
SK B	DIC const	(C:P)' = 76.86 (0.02)	$K_{C:P}$ = 2.46 (0.01)
		(N:P)' = 13.06 (1.16)	$K_{N:P}$ = 3.55 (1.10)
SK A	pH const	(C:P)' = 82.01 (0.74)	$K_{C:P}$ = 0.90 (0.04)
		(N:P)' = 12.50 (0.78)	$K_{N:P}$ = 1.13 (0.31)

Cell quota and growth rates

Table 3 summarizes cellular carbon, phosphorus, and nitrogen contents (cell quota) for SK A at constant DIC. Most of the variation in C:P ratios was accounted for by decreasing amounts of organic carbon per cell, whereas P/cell was largely unaffected by changes in CO_2 concentrations. N/cell was also variable, but to a greater degree than C/cell, which resulted in high C:N and low N:P at low [CO_2 (aq)]. Growth rates determined for the different treatments (Table 3) equalled those obtained from repeated cell counts in pre-cultures (data not shown), indicating that the cells grew in the experimental growth phase throughout the experiments. Growth rate decreased at CO_2 concentrations below ca 2 µmol l^{-1} to a minimum value of 1.6 d^{-1}. At higher CO_2 levels, growth rates were largely unaffected by variation in [CO_2 (aq)] and SK A grew at maximum rates of 2.1 d^{-1}.

DISCUSSION

Response to variable CO_2 concentration

This study provides the first evidence that the C:N:P elemental ratios of a common marine diatom can be affected by the availability of CO_2 (aq). As indicated by identical trends at constant pH in the control experiment, pH effects on algal physiology did not seem to have accounted for the observed changes in elemental ratios. Under controlled conditions with sufficient light and excess nutrients, variation in CO_2 concentrations appeared to be the cause of changes in chemical composition of the cells in this study. Differences in cell size between the 2 strains were not reflected in the CO_2 dependency of C:N:P ratios.

CO_2 availability not only affected elemental ratios of *Skeletonema costatum* but also limited its rate of growth as indicated by a decrease in growth rate of SK A from 2.1 d^{-1} at high [CO_2 (aq)] to 1.6 d^{-1} at the lowest [CO_2 (aq)] (Table 3). These results concur with other studies in which growth limitation of marine diatoms was observed at low CO_2 concentrations (Riebesell et al. 1993, Chen & Durbin 1994).

Table 3. *Skeletonema costatum*. Cell quota for C, N, P and average growth rates (µ) of SK A, grown at different CO_2 concentrations [CO_2 (aq)] at constant DIC

[CO_2 (aq)] (µmol l^{-1})	C/cell (pg C)	N/cell (pg N)	P/cell (pg P)	µ (d^{-1})
30.6	17.70	3.54	0.58	2.1
12.0	14.49	2.84	0.58	2.1
5.4	14.98	2.77	0.65	2.0
2.7	12.29	2.05	0.61	1.9
1.5	12.03	2.09	0.65	1.6

Inorganic carbon acquisition

The interpretation of CO_2 effects on algal physiology is complicated by limited knowledge of mechanisms and pathways of inorganic carbon acquisition by marine phytoplankton. RUBISCO (ribulose 1,5-bisphosphate carboxylase-oxygenase) is the primary enzyme of photosynthetic carbon fixation in microalgae (Raven 1996) and uses molecular CO_2 as substrate. Several mechanisms which involve active transport of inorganic carbon through the plasma membrane or the chloroplast envelope have been proposed to actively increase CO_2 concentration at the site of carboxylation (Badger et al. 1980, Badger & Andrews 1982, Badger & Price 1992) Such an inorganic carbon concentrating mechanism might be coordinated with the enzymatic activity of extracellular carbonic anhydrase which would increase external CO_2 concentration by catalyzing dehydration of HCO_3^- (Aizawa & Miyachi 1986, Badger & Price 1994). If changes in external [CO_2 (aq)] affected CO_2 availability at the carboxylation site of RUBISCO or the energy budget of an algal cell, corresponding shifts in metabolic pathways might be reflected in variable elemental composition. The extent to which marine microalgae can make use of the large HCO_3^- pool in seawater and whether they have adopted a common strategy to accommodate variations in [CO_2 (aq)] is not known yet.

In this study, C:N:P ratios of *Skeletonema costatum* showed consistent increases or decreases with decreasing [CO_2 (aq)] (Figs. 1 & 2) over the range of CO_2 concentrations (ca 5 to 25 µmol l^{-1}) typically encountered in the ocean's surface waters (Goericke & Fry 1994). At the lowest experimental [CO_2 (aq)] of ap-

proximately 1 to 2 µmol l^{-1}, however, trends in C:N in all treatments and in C:P and N:P of SK B were reversed. It has been suggested that a different mode of inorganic carbon acquisition might be induced below a critical level of CO_2 supply in marine phytoplankton (Raven 1991, Hinga et al. 1994, Laws et al. in press). Below a CO_2 threshold concentration of approximately 2 µmol l^{-1}, *S. costatum* might have responded by changing its inorganic carbon acquisition pathway to compensate for limited CO_2 supply. Such physiological adaptation should be accompanied by changes in the biochemical composition and might have accounted for reversal of observed trends in changes in elemental ratios. Another possible response to insufficient CO_2 supply could be enhancement of carbon fixation by β-carboxylases. While RUBISCO is considered 'the sole carboxylase involved in converting exogenous inorganic C into at least 95% of cellular organic C' (Raven 1996), Descolas-Gros & Fontugne (1985) found β-carboxylation to increase in batch cultures of *S. costatum* at the end of the exponential growth phase. Whether such shifts in enzymatic pathways also apply to growth at low [CO_2 (aq)] has not been investigated yet.

Factors affecting C:N:P

Compared to variability of C:P or C:N ratios measured under severe P or N limitation, the magnitude of change caused by CO_2 availability can be considered moderate. Elemental ratios found at high [CO_2 (aq)] in this study were close to values reported by Sakshaug & Holm-Hansen (1977) for *Skeletonema costatum* under nutrient-replete conditions during exponential growth (C:N 7.4, C:P 90, N:P 12; continuous culture, N:P of medium 20). In their study, however, C:P covered a wide range from lowest C:P = 39 during exponential growth (N:P of medium = 1.2) to C:P > 900 during starvation in P-deficient cells (N:P of medium = 310 and 830). Large increases in C:N and C:P under N or P limitation appear, to a great extent, to be the result of accumulation of β-1,3 glucan, which is considered a common reserve polysaccharide in marine diatoms (Myklestad 1974, 1977). It can be speculated that, in contrast to an overflow production of carbon under N- or P-limiting conditions, variation in elemental ratios of *S. costatum* in response to changes in [CO_2 (aq)] might result from differences in the allocation of assimilated carbon to intracellular carbon pools. More detailed analysis of carbohydrate, protein, and lipid composition, however, is required to test this hypothesis.

As shown in Table 3, P content per cell remained relatively constant, whereas amounts of intracellular organic carbon decreased at low [CO_2 (aq)]. Regardless of the nutrient status, *Skeletonema costatum* appears to lack storage polyphosphates (Sakshaug & Holm-Hansen 1977). C:P ratios of approximately 40 at [CO_2 (aq)] ≤ 2 µmol l^{-1} were similar to lowest C:P ratios of *S. costatum* in experiments by Sakshaug & Holm-Hansen (1977) and might thus represent minimum values which can be maintained in *S. costatum* irrespective of the kind of environmental factor affecting the chemical composition of the cells.

Whereas a decrease in C:P ratios with decreasing [CO_2 (aq)] fits the classical perception of intracellular shortage of a limiting nutrient, a concomitant increase in C:N appears to violate this concept. In a comparison of P-limited and N-limited chemostat cultures of the marine diatom *Thalassiosira pseudonana*, Perry (1976) observed increases in C:N ratios in response to nutrient exhaustion irrespective of the kind of limiting nutrient. Similar observations of high C:N during N or P starvation were made in other studies (e.g. Sakshaug & Holm-Hansen 1977, Goldman et al. 1979, Laws & Bannister 1980). Harrison et al. (1977) found highly elevated ratios of C:N = 13.2 in silicate-starved and C:N = 14.8 in ammonium-starved *Skeletonema costatum*. Thus, in contrast to C:P ratios, elevated C:N might serve as a general indicator of unfavorable environmental conditions irrespective of the nutrient (CO_2 in this study) in shortest supply.

The C:N ratio can also be affected by light availability. Over a range in photon flux density of 4.4 to 209 µmol photons m^{-2} s^{-1}, C:N of the marine diatom *Thalassiosira fluviatilis* varied to an extent (molar C:N = 5.1 to 7.1) similar to CO_2-related changes in C:N in this study (Laws & Bannister 1980). Light-limited growth, however, resulted in minimum C:N values at the lowest growth rates, contrary to maximum C:N during nutrient-limited growth.

Likewise, iron starvation might affect the biochemical composition. Compared to exponentially growing cells, Sakshaug & Holm-Hansen (1977) observed a decrease in C:P and N:P in Fe-deficient cultures of *Skeletonema costatum* similar in magnitude to variation of these ratios in the present study. For C:N they found no systematic trend due to the large scatter of values. On the other hand, C:N:P ratios of the chrysophyte *Pavlova* (*Monochrysis*) *lutheri* (Sakshaug & Holm-Hansen 1977), of the diatom *Phaeodactylum tricornutum* (Greene et al. 1991) and of natural phytoplankton communities from the Southern Ocean (van Leeuwe et al. 1997) appeared to be largely unaffected by Fe starvation.

Ecological significance of CO_2-related C:N:P variation

While variation in the elemental composition of marine diatoms in response to changes in [CO_2 (aq)] is

interesting from a physiological point of view, its ecological significance depends on the degree to which such changes in C:N:P develop and persist under natural conditions. Drastic and rapid changes in C:N and C:P ratios are known to occur in microalgae during nutrient exhaustion (Banse 1974, Sakshaug & Holm-Hansen 1977), which occurs upon termination of algal blooms. Such large deviations from Redfield stoichiometry easily override the comparatively small differences in elemental ratios related to variable CO_2 or light supply.

Above a certain threshold concentration, however, elemental ratios of marine phytoplankton seem to be largely unaffected by concentrations of potentially limiting nutrients. C:N ratios of surface layer particulate organic matter are often similar to or less than the Redfield ratio even at nitrate concentrations below 2 µmol l^{-1} (see Sambrotto et al. 1993 for reference). Further examples of little variation in C:N at low nitrate concentrations are given by Sakshaug & Olsen (1986) for blooms dominated by *Skeletonema costatum* or the coccolithophorid *Emiliania huxleyi* and by Dortch et al. (1985) for mixed algal assemblages. Highly efficient uptake mechanisms for both nitrate (Eppley et al. 1969, Harrison et al. 1996) and phosphate (Perry 1976) may provide the means to compensate for deficiency in nutrient supply.

Particulate organic matter close to the Redfield ratio below the euphotic layer (Copin-Montegut & Copin-Montegut 1983) provides further evidence that the major portion of biogenic sinking flux is not characterized by the extremely high C:N ratios observed upon nutrient exhaustion. Phytoplankton spring blooms, as they occur in high latitude ecosystems, are characterized by high rates of export production because autotrophic production and heterotrophic consumption are largely uncoupled (Bienfang & Ziemann 1992). As long as nutrient supply does not drop below a critical level, CO_2-related changes in the Redfield ratio are of similar magnitude as variability caused by other factors contributing to stoichiometry of sinking particles.

In the subarctic North Pacific, the equatorial Pacific or the Southern Ocean, where high nitrate levels persist throughout the year but phytoplankton stocks are low, iron limitation of phytoplankton growth and biomass represents an important factor controlling primary productivity (Martin & Fitzwater 1988, de Baar et al. 1995, Coale et al. 1996). Since the effect of iron starvation on elemental composition of phytoplankton is moderate or negligible (Sakshaug & Holm-Hansen 1977, Greene et al. 1991, van Leeuwe et al. 1997) and nitrate and phosphate are in ample supply, CO_2-related effects on plankton stoichiometry should be noticable.

Biogeochemical relevance

Over geological time scales, atmospheric pCO_2 increased from a glacial minimum of 180 ppmv to pre-industrial levels of 280 ppmv (e.g. Delmas et al. 1980, Neftel et al. 1982). At a constant temperature, [CO_2 (aq)] in air-equilibrated seawater would show a corresponding increase (Broecker et al. 1979). It needs to be considered, however, that over geological time scales not only [CO_2 (aq)] but also other factors such as nitrate, phosphate, iron and light availability are likely to have varied significantly. Furthermore, shifts in species composition among the major taxonomic groups of marine microalgae might have occurred over such time scales. All these factors are known to influence the average elemental composition of marine phytoplankton, which complicates evaluation of potential changes in the Redfield ratio due to increases in [CO_2 (aq)].

Unlike the above-mentioned long-term variability, burning of fossil fuel has led to an increase in atmospheric pCO_2 on a much shorter time scale. Within about 200 yr, atmospheric pCO_2 increased from 280 ppmv to present levels of 360 ppmv and is predicted to exceed 600 ppmv by the year 2100 in 'business as usual' emission scenarios (Wigley et al. 1996). Assuming total alkalinity = 2.3 meq l^{-1}, salinity = 35 psu and an increase in surface seawater temperature from 10 to 11°C, an increase in atmospheric pCO_2 to 600 ppmv would raise [CO_2 (aq)] in air-equilibrated seawater from pre-industrial 12.3 µmol l^{-1} to 25.5 µmol l^{-1}. As a result of this increase in [CO_2 (aq)], C:P of *Skeletonema costatum* would be expected to increase by 4.6% (from 70.9 to 74.2), C:N to decrease by 2.3% (from 6.07 to 5.93) according to Table 2 (SK A, DIC const).

The rapid increase in today's pCO_2 and the associated increase in [CO_2 (aq)] of surface seawater represents a uni-directional trend of a single environmental factor which is not accompanied by other factors and has a potential influence on elemental composition of marine phytoplankton. Systematic changes in the elemental composition of marine phytoplankton in reponse to the rapid increase in [CO_2 (aq)] would thus be superimposed on a background scatter due to stochastic variability of other factors affecting the Redfield ratio. Changes in C:P are of particular importance because phosphate is the nutrient which ultimately controls marine phytoplankton biomass (Redfield 1958, Broecker 1982, van Cappellen & Ingall 1996) and therefore C:P is commonly used in global biogeochemical models to quantify inorganic carbon fixation (e.g. Broecker et al. 1985, Six & Maier-Reimer 1996).

Several recent studies recognize deviations from Redfield stoichiometry and caution against calculation

of organic carbon export based on a constant Redfield ratio (e.g Fanning 1992, Sambrotto et al. 1993, Banse 1994). According to Maier-Reimer (1996), 'the assumption of a constant Redfield ratio (for global modelling studies) represents an efficient mechanism to reduce the number of free parameters and, thus, to increase the efficiency of any model prediction'. Deviations from Redfield ratio due to intra- and interspecific variability of cell stoichiometry 'seem to compensate each other in the large-scale chemical distribution in the deep sea' (Maier-Reimer 1996). If changes in elemental composition, however, are caused by factors which exhibit systematic trends, deviations from the Redfield ratio will no longer be compensated by random variability. Knowledge of the magnitude by which relevant factors affect the elemental composition of planktonic organisms, therefore, provides the framework for improved models of large-scale processes.

CO_2-dependent stoichiometry of marine microalgae has not yet been considered a factor contributing to variability of the Redfield ratio. If the observed correlation of elemental ratios with [CO_2 (aq)] proves to be a general trend in marine phytoplankton, changes in the Redfield ratio may be expected in response to the currently observed increase in atmospheric pCO_2. Dependency of elemental ratios on CO_2 concentration would affect the efficiency of the 'biological carbon pump' (Volk & Hoffert 1985) due to variation in the amount of carbon fixed relative to other inorganic nutrients.

Species comparison

As an important next step, it needs to be investigated whether CO_2-dependent variation in phytoplankton stoichiometry is a common phenomenon among marine phytoplankton. *Skeletonema costatum* is a cosmopolitan species with its main distribution in coastal waters, where it frequently dominates phytoplankton blooms (Hasle 1973). It can dominate phytoplankton composition in the Peruvian upwelling system (Sukhanova et al. 1978) and has also been observed in the spring bloom of the Sargasso Sea off Bermuda (Hulburt et al. 1960). In order to assess the potential significance of a CO_2-dependent Redfield ratio in carbon flux on a larger scale, other microalgal species of similar importance to marine primary productivity need to be tested for their response to increasing [CO_2 (aq)].

Caraco et al. (1996) observed a similar dependency of C:P ratios on [CO_2 (aq)] in freshwater species of chrysophytes, chlorophytes and cyanobacteria. C:P in the freshwater chrysophyte *Ochromonas*, grown at 35, 350, and 3500 ppmv CO_2, had variable C:P ratios of 120, 250, and 490, respectively. These results suggest that, in spite of the much higher CO_2 (aq) and lower HCO_3^- concentrations in freshwater, limnetic microalgae might show a similar stoichiometric response to variation in [CO_2 (aq)] as marine phytoplankton. This observation is of particular interest because freshwater and marine phytoplankton might employ different modes of inorganic carbon acquisition in adaptation to their different environments.

In conclusion, results from this study demonstrate clear correlation of C:N:P elemental ratios with concentrations of CO_2 (aq) in 2 strains of the marine diatom *Skeletonema costatum*. The physiological mechanisms responsible for the observed trends and the question whether this variation in Redfield ratios represents a common feature in marine phytoplankton need to be elucidated.

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