NOTE

Carbon isotope fractionation by a marine diatom: dependence on the growth-rate-limiting resource

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ABSTRACT: The large temporal and spatial variability in carbon isotope fractionation of marine phytoplankton (ε_p) is thought to reflect differences in environmental conditions. Meaningful interpretation of this variability requires an understanding of the processes responsible for phytoplankton isotope fractionation. While numerous factors have been suggested to potentially influence ε_p, recent theoretical and experimental evidence has emphasized the primary role of phytoplankton growth rate (μ) and CO_2 concentration ([CO_2aq]) in controlling ε_p. Experimental examination of the relationship of ε_p with μ and [CO_2aq] in studies using different experimental approaches, however, has yielded inconsistent results. Here we directly compare new and previously published data on ε_p as a function of CO_2 concentration and growth rate for the marine diatom *Phaeodactylum tricornutum*. When grown under nitrogen-deficient conditions (nitrate-limited chemostat), ε_p of *P. tricornutum* decreases with increasing growth rate. In contrast, under N-replete conditions ε_p values are considerably lower at comparable growth rates and CO_2 concentrations and are largely insensitive to a 3-fold increase in growth rate due to increasing photon flux density. In both experimental approaches, ε_p shows a relatively small CO_2 sensitivity in the range of CO_2 concentrations naturally occurring in the ocean (8 to 25 µmol kg^{-1}). Below ca 5 µmol CO_2 kg^{-1}, a strong decline in ε_p with decreasing [CO_2aq] is observed. The apparent difference in ε_p responses between nitrate-limited and light-controlled cultures of *P. tricornutum* suggests a principal difference in carbon acquisition for different growth-rate-limiting resources. A mechanistic explanation is proposed and potential implications for the interpretation of phytoplankton carbon isotope fractionation are discussed.

KEY WORDS: δ^{13}C · Isotope fractionation · CO_2 · *Phaeodactylum tricornutum* · Growth limitation

The carbon isotopic composition (δ^{13}C) of marine organic matter differs considerably from that of the dissolved inorganic carbon (DIC) from which it was formed. This difference is mainly due to the large discrimination against ^{13}C during photosynthetic carbon fixation. Large spatial and temporal variability in suspended and sedimentary organic matter δ^{13}C shows that marine photosynthetic carbon isotope fractionation (ε_p) varies in space and time, both seasonally (Fogel et al. 1992) and over geological time (Arthur et al. 1985, Jasper et al. 1994). This variability is thought to reflect differences in the environmental conditions under which the organic matter was synthesized. Understanding the mechanisms of photosynthetic carbon isotope fractionation could provide a means for reconstructing growth conditions at the time of organic matter production.

One of the main factors considered to be responsible for the observed variability in phytoplankton carbon isotope fractionation is the concentration of dissolved CO_2 ([CO_2aq]; Deuser et al. 1968, Mizutani & Wada 1982, Rau et al. 1989). Experimental evidence for a dependence of ε_p on [CO_2aq] in marine phytoplankton was first provided by Degens et al. (1968). More recently, based on theoretical considerations it has been pointed out that ε_p is ultimately determined by the ratio of cellular carbon fixation to carbon flux into the cell (Francois et al. 1993, Goericke et al. 1994). With carbon fixation corresponding to the product of cellular carbon content and specific growth rate, and carbon flux related to [CO_2aq], experimental results became directly comparable to theoretical predictions (Laws et al. 1995, Rau et al. 1996).

This was substantiated by experimental results of Laws et al. (1995), who found an inverse relationship between ε_p and the ratio of algal growth rate (μ) and [CO_2aq] (μ/[CO_2aq]). Whereas subsequent experiments using the same experimental approach (i.e. chemostat cultures) have yielded compatible ε_p responses (Bidigare et al. 1997, Laws et al. 1997, Popp et al. 1998), no clear relationships were obtained in stud-

ies using batch culture incubations (Hinga et al. 1994, Thompson & Calvert 1994, 1995, Johnston 1996, Burkhardt et al. 1999a). While part of the discrepancy may be due to poorly constrained experimental conditions in batch culture incubations or species-specific differences in isotope fractionation, the principal disagreement in some of these results has remained a matter of concern. For instance, at high $[CO_2aq]$ and μ, N-limited chemostat incubations have consistently yielded ε_p values of about 25‰, i.e. close to isotope fractionation of the carboxylating enzyme RUBISCO (Laws et al. 1995, 1997, Bidigare et al. 1997, Popp et al. 1998). At similar $[CO_2aq]$ and μ in batch culture and continuous culture incubations in which μ was controlled by photon flux density (PFD), maximum isotope fractionation was found to be much lower (Hinga et al. 1994, Johnston 1996, Korb et al. 1996, Burkhardt et al. 1999a).

Confounded by the inconsistency of ε_p versus $\mu/[CO_2aq]$ relationships obtained in different studies, we have attempted to directly compare isotope fractionation data generated by the 2 approaches. Unfortunately, the use of different microalgal species or differences in the μ and $[CO_2aq]$ ranges covered in the various studies in most cases precludes direct comparison between data sets. At least for 1 species, the marine diatom *Phaeodactylum tricornutum*, however, ε_p has been measured at nearly identical μ and $[CO_2aq]$ in both chemostat (Laws et al. 1995, 1997) and batch culture experiments (Johnston 1996, Burkhardt et al. 1999a, this study). As will be shown in the following, direct comparison of these data sets reveals similarities in the general trend of ε_p with $[CO_2aq]$. Considerable differences exist in the responses of ε_p to changes in μ as well as in the absolute values of ε_p under comparable μ and $[CO_2aq]$.

Materials and methods. *Experiments:* Dilute batch cultures of 2 different strains of the marine diatom *Phaeodactylum tricornutum* Bohlin (clone CCAP 1052/1A and CCMP 1327) were grown in 0.2 µm-filtered natural seawater enriched with nitrate, silicate, phosphate, trace metals and vitamins at concentrations of f/2 medium (Guillard & Ryther 1962). Depending on the batch of seawater, salinity varied between 30.5 and 31.5. Prior to the experiments, stock cultures were adapted to experimental conditions of the respective treatments for at least 9 cell divisions. A range of $[CO_2aq]$ (0.2 to 22.4 µmol kg^{-1}, see Table 1) in the growth medium was obtained by manipulating the carbonate system through addition of NaOH or HCl. This treatment changes alkalinity and pH of the medium and leaves DIC approximately constant. Pre-adapted cultures were incubated in 2.4 l borosilicate glass bottles, sealed with PBT-lined screw caps. The bottles were closed without headspace and cells were kept in

suspension by inversion of the bottles 2 to 3 times d^{-1}. The batch cultures were kept in Rumed 1200 light-thermostats at a temperature of 15°C under continuous light and an incident PFD of 15 and 30 µmol m^{-2} s^{-1} for clone CCAP 1052/1A and 150 µmol m^{-2} s^{-1} for clone CCMP 1327. Results from these experiments are combined with those of Burkhardt et al. (1999a), who incubated *P. tricornutum* clone CCAP 1052/1A at a PFD of 150 µmol m^{-2} s^{-1} under continuous light and a light/dark cycle of 16/8 h.

To minimize pH drift and associated shifts in carbon speciation as well as a change in $\delta^{13}C$ of DIC ($\delta^{13}C_{DIC}$) during experimental incubations, cells in all treatments were harvested at particulate organic carbon (POC) concentrations below 50 µmol kg^{-1}. As shown by Burkhardt et al. (1999b), at POC concentrations below this level, pH changes of the unbuffered medium and changes in $\delta^{13}C_{DIC}$ due to photosynthetic ^{13}C discrimination remained below 0.05 units and 0.4‰, respectively. To allow at least 8 cell divisions under experimental conditions, initial POC concentrations in the growth medium were kept below 0.1 µmol kg^{-1}. Bacterial counts on DAPI-stained samples using epifluorescence microscopy (Hobbie et al. 1977) indicated that bacterial biomass never exceeded 1% of algal biomass in the cultures and its contribution to total POC was therefore considered negligible.

Carbonate system: DIC was determined coulometrically in duplicate with a system similar to that described by Johnson et al. (1987). Total alkalinity was titrated in duplicate with an automated, temperature-controlled system at 20°C, and was determined using the Gran-plot approach (Almgren et al. 1988). $[CO_2aq]$ was calculated from DIC concentrations, total alkalinity, temperature, salinity, and concentrations of phosphate and silicate using dissociation constants of Goyet & Poisson (1989).

Stable carbon isotope fractionation: Samples were filtered using precombusted (500°C, 12 h) Whatman GF-C glass fiber filters. All filters were acidified with 0.1 N HCl prior to measurements and dried for 2 h at 60°C. POC concentration and $\delta^{13}C_{POC}$ were measured in duplicate on the same filters with a continuous flow isotope ratio mass spectrometer (Europa Scientific, ANCA SL 20-20). $\delta^{13}C_{DIC}$ was analyzed on unfiltered subsamples preserved with HgCl$_2$ with a Finnegan MAT Delta-S mass spectrometer after acidification with 100% H$_3$PO$_4$ at 25°C in a vacuum extraction line as described by Mackensen et al. (1996). The carbon isotopic composition is reported in δ-notation relative to Pee-Dee belemnite as a standard:

$$\delta^{13}C\left[\frac{(^{13}C/^{12}C)\,\text{sample}}{(^{13}C/^{12}C)\,\text{standard}} - 1\right]1000 \qquad (1)$$

The isotopic composition of dissolved molecular CO_2 ($\delta^{13}C_{CO_2}$) was calculated from $\delta^{13}C_{DIC}$ following the equation provided by Rau et al. (1996) based on Mook et al. (1974):

$$\delta^{13}C_{CO_2} = \delta^{13}C_{DIC} + 23.644 - \frac{9701.5}{T_K} \qquad (2)$$

where T_K is the absolute temperature in Kelvin.

The isotope fractionation associated with photosynthetic CO_2 fixation, ε_p, was calculated relative to the isotopic composition of CO_2 in the bulk medium according to Freeman & Hayes (1992):

$$\varepsilon_p = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{POC}}{1 + \delta^{13}C_{POC}/1000} \qquad (3)$$

Growth rates: Cell concentrations of *Phaeodactylum tricornutum* were determined with a Coulter 'Multisizer' on duplicate 20 ml subsamples, preserved with Lugol's iodine (Edler 1979). Average 24 h growth rates (μ_{L+D}) were calculated from daily changes in cell concentration according to

$$\mu_{L+D} = \frac{\ln N_1 - \ln N_0}{\Delta t} \qquad (4)$$

where N_0 and N_1 are cell concentrations at the beginning and the end of the time interval Δt. During incubations, cell numbers were determined daily for at least 3 d in control bottles run in parallel to each experimental set-up. No subsamples were taken from the experimental vessels during incubations to avoid disturbance of the closed system, but cell concentrations were determined before and after incubation and μ calculated from these were compared to those in the control bottles. The cellular carbon content was derived from measurements of POC and cell concentration.

Photosynthetic carbon isotope fractionation mainly occurs through enzymatic CO_2 fixation during the photoperiod. Thus, to account for differences in the photoperiodic length (16/8 h light/dark cycle vs continuous light), instantaneous growth rates (μ_i) were calculated according to

$$\mu_i = \frac{(L+D)\mu_{L+D}}{L - D_r} \qquad (5)$$

where L and D are the lengths of the light and dark periods, respectively, and r accounts for respiratory carbon loss during the dark period. The latter was assumed to be 0.15 (Laws & Bannister 1980).

Data comparison: Results obtained in this investigation and in the work of Burkhardt et al. (1999a) are directly comparable with earlier work on ε_p of *Phaeodactylum tricornutum* in relation to μ and [CO_2aq] by Laws et al. (1995, 1997) and Johnston (1996). These investigators used *P. tricornutum* clones CCMP 1327 (Laws et al. 1995, 1997) and CCAP 1052/1A (Johnston 1996), i.e. the same 2 strains grown in this study. Experiments were performed in (1) nitrate-limited, light-saturated chemostat cultures (Laws et al. 1995, 1997), and (2) nutrient-replete batch culture incubations under variable light conditions (Johnston 1996, Burkhardt et al. 1999a, this study). Thus, the main difference between the experimental set-ups is the factor which controls algal growth rate, i.e. the rate of nutrient supply in approach 1 versus PFD in approach 2.

To allow direct comparison of the data sets, it was necessary to convert some of the data to yield identical units. Cell doubling rates reported by Johnston (1996) were transformed to specific μ by multiplying with ln2. Differences between the studies also exist with respect to the light/dark cycle. Whereas incubations by Johnston (1996) and in part by Burkhardt et al. (1999a) were conducted at a light/dark cycle of 16/8 h, all other experiments were performed under continuous light. To account for this difference, μ_i, i.e. μ during the photoperiod (see above), was used for data comparison. [CO_2aq] for the data of Johnston (1996) was calculated assuming temperature, salinity, nutrient concentrations (P, Si), and pH as given in the paper and a pCO_2 value of 360 ppm (medium continuously bubbled with air). This yielded a value of 11.7 µmol CO_2 kg^{-1}. ε_p was calculated from $\delta^{13}C_{POC}$ presented in Johnston (1996) according to Eq. (3) assuming $\delta^{13}C_{CO_2}$ to equal –9.06‰. The latter was calculated from the $\delta^{13}C$ of the air bubbled through the medium, measured to be –7.98‰ (Johnston 1996), and an equilibrium fractionation between gaseous and dissolved CO_2 as given by Mook (1986).

In order to directly compare ε_p as a function of [CO_2aq] between the different data sets, we grouped the available data into 3 categories according to μ_i: (1) low growth rates, $\mu_i = 0.5$ d$^{-1} \pm 10\%$ (2) intermediate growth rates, $\mu_i = 1.0$ d$^{-1} \pm 10\%$, and (3) high growth rates, $\mu_i = 1.5$ d$^{-1} \pm 10\%$.

All data used in the comparison, including data obtained in this study, are listed in Table 1. Data obtained at μ_i outside these relatively narrow ranges are listed separately (Table 2; open symbols in Fig. 1). This includes data measured at a PFD of 150 µmol m^{-2} s^{-1} and a light/dark cycle of 16/8 h (Burkhardt et al. 1999a). Although 24 h average growth rate were similar to those obtained in parallel experiments under continuous light, μ_i calculated according to Eq. (5) were significantly higher in 16/8 h light/dark cycle incubations than the range given in Category C (see Table 2). As discussed in detail in Burkhardt et al. (1999a), the difference in the light/dark cycle had no effect on the isotope fractionation of *Phaeodactylum tricornutum* (Fig. 1C).

Table 1. Experimental results for *Phaeodactylum tricornutum* from various studies obtained in nitrate-limited chemostats (Laws et al. 1997) and light-controlled batch culture incubations (Johnston 1996, Burkhardt et al. 1999a, this study); * and ** denote the use of strains CCMP 1327 and CCAP 1052/1A, respectively

[CO$_2$aq] (µmol kg^{-1})	pH	DIC (mmol kg^{-1})	μ_i (d^{-1})	$\delta^{13}C_{POC}$ (‰)	ε_p (‰)	C/cell (pg cell^{-1})	Source
(A) Low growth rates							
34.71	7.67	2.27	0.50	−40.47	25.72	6.87	Laws et al. (1997)*
22.23	7.83	2.17	0.50	−47.70	24.35	9.57	
6.49	8.26	1.86	0.50	−32.99	19.85	7.94	
3.56	8.46	1.76	0.50	−29.73	20.63	7.24	
0.98	8.82	1.45	0.50	−17.30	10.65	5.82	
11.70	8.20	~1.89	0.55	−21.10	12.30	−	Johnston (1996)**
14.90	8.20	2.125	0.49	−25.01	14.84	18.02	This study**
7.96	8.45	2.136	0.51	−23.18	12.93	16.91	
3.85	8.72	2.147	0.50	−21.81	11.51	15.86	
1.80	8.98	2.164	0.47	−19.62	9.26	13.76	
0.60	9.32	2.179	0.48	−14.73	4.24	12.49	
(B) Intermediate growth rates							
11.98	8.06	2.05	1.00	−38.10	20.58	7.53	Laws et al. (1997)*
0.92	8.82	1.36	1.04	−20.60	11.08	−	
11.70	8.20	~1.89	1.16	−20.80	11.99	−	Johnston (1996)**
22.42	8.10	2.028	1.06	−25.25	15.09	9.29	This study**
11.58	8.31	2.058	1.03	−24.50	14.31	9.04	
6.91	8.50	2.117	0.90	−23.98	13.76	8.75	
3.85	8.71	2.076	1.00	−23.73	13.51	9.20	
2.79	8.83	2.065	1.02	−20.14	9.79	8.82	
2.27	8.91	2.114	0.98	−18.86	8.69	8.80	
(C) High growth rates							
10.27	8.11	2.01	1.40	−35.14	18.36	11.00	Laws et al. (1997)*
0.64	8.94	1.39	1.38	−16.15	7.42	4.95	
11.70	8.20	~1.89	1.41	−21.10	12.40	−	Johnston (1996)**
37.69	7.82	2.060	1.63	−26.58	16.68	10.03	Burkhardt et al. (1999a)**
14.48	8.24	2.066	1.39	−25.58	15.64	9.90	
7.22	8.59	2.079	1.41	−24.48	14.49	9.24	
4.36	8.76	2.078	1.64	−22.69	12.64	8.40	
3.21	8.89	2.064	1.63	−26.91	17.03	8.68	
2.10	9.02	2.066	1.64	−20.76	10.64	9.01	

Table 2. As in Table 1 except for results obtained at growth rates outside the defined ranges of Categories A to C

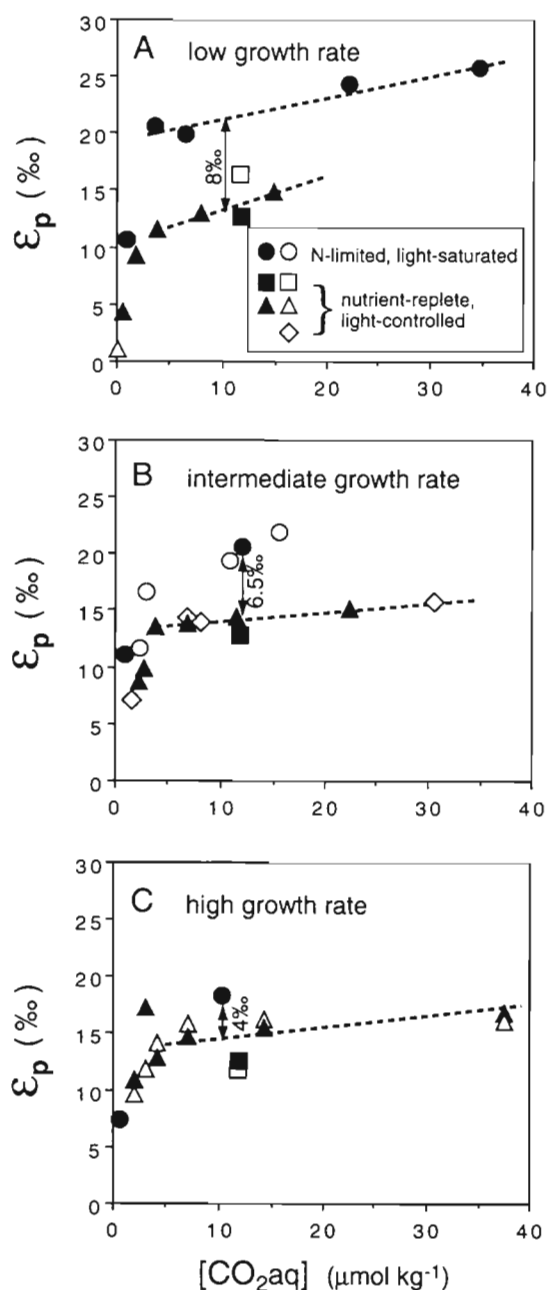| [CO$_2$aq] (µmol kg^{-1})	pH	DIC (mmol kg^{-1})	μ_i (d^{-1})	$\delta^{13}C_{POC}$ (‰)	ε_p (‰)	C/cell (pg cell^{-1})	Source
(A) Low growth rates							
11.70	8.20	~1.89	0.29	−24.80	16.14	−	Johnston (1996)**
0.23	9.58	2.199	0.34	−11.52	0.98	14.17	This study**
(B) Intermediate growth rates							
15.61	7.97	2.10	0.75	−41.88	22.04	8.20	Laws et al. (1997)*
10.72	8.10	2 02	1.25	−35.82	18.94	9.15	
2.93	8.51	1.68	0.75	−26.79	16.76	6.21	
2.36	8.58	1.67	0.75	−21.09	11.83	6.99	
30.21	7.90	2.084	1.19	−26.31	16.22	15.86	This study*
7.59	8.47	2 081	1.18	−24.83	14.67	15.07	
6.69	8.51	2.060	1.26	−24.95	14.80	12.41	
1.16	9.12	2.066	1.33	−17.64	7.24	12.97	
(C) High growth rates							
11.70	8.20	~1.89	1.97	−21.20	12.30	−	Johnston (1996)**
37.69	7.82	2.060	2.27	−25.92	15.99	9.70	Burkhardt et al. (1999a)**
14.48	8.24	2.066	2.30	−25.65	15.70	9.57	
7.22	8.59	2.079	2.21	−25.51	15.57	8.91	
4.36	8.76	2.078	2.21	−23.96	13.95	8.07	
3.21	8.89	2.064	2.23	−21.71	11.62	8.35	
2.10	9.02	2.066	2.21	−19.58	9.42	8.68	

Fig. 1. Carbon isotope fractionation, ε_p, in relation to CO_2 concentration, $[CO_2aq]$, for *Phaeodactylum tricornutum* in 3 growth rate categories: (A) low growth rate, $\mu_i = 0.5$ $d^{-1} \pm 10\%$ (B) intermediate growth rate, $\mu_i = 1.0$ $d^{-1} \pm 10\%$; (C) high growth rate, $\mu_i = 1.5$ $d^{-1} \pm 10\%$ (compare Table 1). Data were obtained in N-limited, light-saturated chemostat cultures by Laws et al. (1997) (circles), and in nutrient-replete, light-controlled batch culture incubations by Johnston (1996) (squares), Burkhardt et al. (1999a) (triangles in C), and this study (triangles in A and B, diamonds). Culture experiments used 2 different strains of *P. tricornutum*, clone CCMP 1327 (circles and diamonds) and CCAP 1052/1A (squares and triangles). Dashed lines represent linear regressions for data at $[CO_2aq] > 3.5$ μmol kg^{-1}. Open symbols represent data outside the range of growth rates in Categories A to C (compare Table 2) and are not included in linear regressions

As indicated in Table 1, the carbon contents per cell differ noticeably within and between data sets and are considerably higher under low μ in this study. If μ_i were expressed on a carbon content per cell basis, values in the low μ_i category of our study would be somewhat higher than those calculated from changes in cell concentration. However, since ε_p was insensitive to changes in μ_i in light-controlled batch cultures (see below), a correction for the observed differences in cellular carbon contents was not critical to the interpretation of the results. In this context it is worth noting that differences in cellular carbon contents between the 2 strains of *Phaeodactylum tricornutum*, CCAP 1052/1A and CCMP 1327, had no detectable effect on ε_p. Despite a factor of almost 2 difference in C content between the 2 clones, ε_p values are practically identical when grown under the same conditions in batch culture incubations (Fig. 1B, compare triangles and diamonds).

Results and discussion. *Isotope fractionation in relation to growth rate and CO_2 concentration:* A close agreement is obtained between all data sets with respect to the general trend of ε_p versus $[CO_2aq]$ (Fig. 1). In the range of $[CO_2aq]$ naturally occurring in the ocean (ca 8 to 25 μmol kg^{-1}), ε_p of *Phaeodactylum tricornutum* shows a relatively small CO_2 dependence, increasing by 2 to 3‰ with $[CO_2aq]$ increasing from 5 to 25 μmol kg^{-1}. Below ca 5 μmol CO_2 kg^{-1}, a strong decline in ε_p with decreasing $[CO_2aq]$ is observed. This trend is consistently found in each of the 3 μ_i categories in light-controlled batch cultures and is clearly indicated also for nitrate-limited chemostats at low μ_i (Fig. 1A). Although the limited number of data points from chemostat experiments precludes determining the relationship of ε_p versus $[CO_2aq]$ at intermediate and high μ_i (Fig. 1B,C), the available data indicate that the trend observed at low μ_i (Fig. 1A) may also hold true at higher growth rates. This interpretation is further supported when data outside the narrow range of growth rates selected in the 3 categories are included (open symbols in Fig. 1).

With respect to the absolute values of ε_p, reasonable agreement is further obtained between the results of Johnston (1996), Burkhardt et al. (1999a), and the present study (Fig. 1). As the data of Johnston (1996) correspond to only a single $[CO_2aq]$ in each μ_i category, they do not provide information on the CO_2 dependence of ε_p. However, they support the finding that changes in μ_i in response to changing PFD have little or no effect on isotope fractionation in *Phaeodactylum tricornutum* (with the exception of 1 high ε_p value at PFD < 10 μmol m^{-2} s^{-1}, see Fig. 3A in Johnston 1996). Close agreement in ε_p responses is also obtained between the 2 strains of *P. tricornutum*, clone CCAP 1052/1A and CCMP 1327, used in our study (Fig. 1B,

triangles and diamonds, respectively). This shows that possible strain-specific differences in isotope fractionation between these 2 clones — if present — are much smaller than differences obtained in response to different experimental conditions.

Strong differences in the absolute values of ε_p evidently exist between results obtained under nitrate-controlled (Laws et al. 1995, 1997) and light-controlled growth (Johnston 1996, Burkhardt et al. 1999a, this study). ε_p values are considerably higher in N-limited cultures at all μ_i under similar [CO_2aq] (Fig. 1). In addition, whereas a 3-fold increase in μ_i due to increasing PFD has virtually no effect on ε_p, a similar increase in μ_i due to enhanced nitrate supply results in significantly lower ε_p values. This causes the offset in ε_p between nitrate- and light-controlled cultures to drop from 8‰ at low μ_i to 4‰ at high μ_i (Fig. 1). How can apparent differences in ε_p between N- and light-controlled incubations be reconciled with our present understanding of the basic mechanisms regulating isotope fractionation?

Mechanisms of isotope fractionation: The key variables determining carbon isotope fractionation in photosynthetic carbon fixation are: (1) the inorganic carbon source entering the cell, i.e. CO_2 or HCO_3^-, which differ in their isotopic composition by ca 8 to 10‰, and (2) the ratio of CO_2 leakage out of the cell (efflux) to carbon flux into the cell (influx).

A lower proportion of HCO_3^- relative to CO_2 uptake could account for the high ε_p values in N-limited compared to light-controlled cultures (Fig. 1). Reduced HCO_3^- utilisation may be caused, for example, by reduced synthesis of transport proteins for HCO_3^- uptake in N-limited cells. Explaining up to 8‰ higher ε_p values by reduced HCO_3^- utilisation (Fig. 1A) would imply that the ratio of carbon efflux to influx remains approximately constant. With a reduction in HCO_3^- uptake this would require that CO_2 uptake increases proportionally. Support for this mechanism could be gained if a stronger reliance on CO_2 in N-limited cultures would be reflected in a stronger CO_2 dependence of ε_p. This is not substantiated by the data comparison. An equally low dependence of ε_p on CO_2 at concentrations above 5 μmol kg^{-1} is indicated in both N-limited and light-controlled *Phaeodactylum tricornutum*.

Alternatively, the observed differences in ε_p could be explained by differences in the efflux to influx ratio without invoking a difference in the source of inorganic carbon. The higher the ratio of efflux to influx, the stronger the discrimination against ^{13}C (Sharkey & Berry 1985), in the extreme case up to the kinetic fractionation of the carboxylating enzymes (25 to 27‰ for the dominating carboxylase RUBISCO). Carbon influx is the sum of CO_2 diffusive transport into the cell and active uptake of CO_2 and/or HCO_3^-. For a given cellular carbon content, the rate of carbon fixation is a direct function of μ_i. Thus, an increase in μ_i at constant influx reduces carbon efflux and lowers discrimination against ^{13}C, i.e. decreases ε_p. The observed lack of a response of ε_p to a 3-fold increase in μ_i in light-controlled *Phaeodactylum tricornutum* (Fig. 1) implies that carbon influx increased with increasing μ_i. In fact, constant ε_p at increasing μ_i can only be explained if carbon influx increased sufficiently to keep the ratio of influx to fixation constant. While enhanced carbon influx could be due to an increase in the permeability of the cell membrane for CO_2 and/or to active carbon uptake, the presence of a highly efficient carbon concentrating mechanism in *Phaeodactylum tricornutum* (Patel & Merret 1986, Burns & Beardall 1987, Rotatore et al. 1995) stresses the importance of the latter mechanism in the regulation of carbon influx. In contrast, rising μ_i in response to increasing N supply led to a decrease in ε_p, suggesting that carbon uptake in N-limited cultures was not as tightly coupled to carbon fixation as in light-controlled incubations.

These observations indicate a principal difference in the regulation of carbon acquisition for different growth-rate-limiting resources. Evidently, changes in N supply and PFD causing identical changes in μ can have very different effects on active carbon uptake. This may be understood when considering the relevant cellular processes affected by different limiting resources. In the following we will therefore briefly outline some of the photochemical constraints which may be of relevance to the regulation of active carbon uptake.

Photochemical constraints: During photosynthetic growth, CO_2 and other nutrients are reduced and transformed into new cell biomass. The entire process consumes electrons (NADPH) and ATP, which are ultimately generated by the photochemical reactions. For any given composition of biomass at steady-state growth, ATP and NADPH must be delivered in a ratio (ATP/e$^-$) which matches the requirement to synthesize biomass. Any other process, such as active carbon uptake, will enhance the ATP/e$^-$ ratio that must be met by the photochemical reactions. Vice versa, if the photochemical reactions yield higher ATP/e$^-$ ratios, the ATP in excess of what is minimally required to synthesize the biomass might be used for active uptake.

The ATP/e$^-$ ratio is determined by both linear (non-cyclic) and non-linear (cyclic) electron flow during photosynthesis. Linear electron flow refers to the direct transfer of electrons from water to ferredoxin and NADPH as terminal electron acceptors in a reaction sequence mediated by photosystems PSII and PSI. While the ATP/e$^-$ ratio might vary during linear electron flow due to the involvement of the Q-cycle (re-oxidation of reduced plastochinone through the

cytochrome b6f complex), it increases significantly during non-linear electron flow. Non-linear flow involves cyclic PSI transport (electron transfer from ferredoxin to the plastochinone pool) and the Mehler reaction (electron flow through both photosystems with subsequent reduction of as much O_2 as is generated by the oxidation of water). An increase in the proportion of non-linear to linear electron transport increases the ATP/e^- ratio and thus the amount of chemical energy available for processes such as active carbon transport.

The relative contribution of non-linear electron flow to steady-state photosynthesis and its regulation under variable environmental conditions is poorly quantified. Clearly, one major site for regulation is the electron transfer from ferredoxin to competing pathways. There, cyclic PSI transport and the Mehler reaction compete directly with other electron transfer pathways, most prominently the reduction of NADP (required in the photosynthetic carbon reduction cycle and in the reduction of nitrate to nitrite) and the reduction of nitrite reductase (catalyzing the reduction of nitrite to ammonia). The critical question is: How would the proportion of non-linear to linear electron transport be affected by differences in the growth-rate-limiting resource?

Nitrate- versus light-controlled growth: Nitrate-limiting conditions at high PFD can be expected to affect the photochemical electron transport in 2 ways: (1) The limited rate of nitrate supply keeps the electron demand for the reduction of nitrate to ammonium low compared to nitrate-replete conditions at similar PFD. This would raise the relative strength of competing electron acceptors favoring the rate of non-linear electron flow. (2) Reduced protein synthesis under N-limiting conditions is likely to be reflected in the cellular activity of the carboxylating enzyme RUBISCO, but may also affect the ability of cells to harvest light efficiently or to synthesize transport proteins involved in carbon uptake. In analogy to nitrate reduction, a decrease in electron demand for photosynthetic CO_2 reduction due to repressed synthesis of RUBISCO would favor competing reactions such as cyclic PSI transport or the Mehler reaction. In this scenario, ferredoxin can be regarded as a controller for the transfer of electrons at branching pathways.

In contrast to N-limited growth, the primary effect of light limitation on photosynthesis is the low rate of photon flux to the reaction center of PSII. At this acceptor site for electrons, however, no competing reactions exist which could provide a similar regulatory mechanism as described above. A decline in PFD under nutrient-replete conditions would therefore lower the total rate of electron flow through both PSII and PSI without having a significant effect on the ATP/e^- ratio. At a given μ_i, higher values of ε_p in N-limited chemostats

compared to light-controlled cultures may then simply reflect higher rates of active carbon uptake, fueled by elevated ATP/e^- ratios.

Other environmental variables: If indeed the ATP/e^- ratio is a key variable in determining active carbon uptake and, hence, ε_p, then any environmental variable influencing this ratio would potentially affect ε_p. A change in the ATP/e^- ratio, for instance, may occur in response to a shift in the nitrogen source (i.e. between NO_3^- and NH_4). Whereas ammonium can be directly utilized by the cell since it is the form of nitrogen used in amino acid synthesis, the reduction of 1 molecule of nitrate to ammonium requires 8 electrons. Shifting from nitrate to ammonium as the external nitrogen source would eliminate this electron sink and favor competing reactions such as non-linear electron transport and/or CO_2 reduction in the Calvin cycle. In case of increased non-linear electron transport, the resulting higher ATP/e^- ratio could support enhanced active carbon uptake, thus leading to increased ε_p. If the 'extra' electrons are primarily channeled into CO_2 reduction in the Calvin cycle, the resulting higher carbon demand would lower ^{13}C discrimination. Reduced ^{13}C discrimination following ammonium spiking of diatom-dominated natural phytoplankton was in fact reported by Dehairs et al. (1997). While these authors suggest that a shift from Calvin cycle carboxylation to β-carboxylation may have led to the observed response, reduced ^{13}C discrimination would also be consistent with enhanced CO_2 reduction.

Differences in the ATP/e^- ratio may also arise depending on the growth-limiting nutrients (e.g. N vs P or Fe). Phosphorus limitation can affect photosynthetic energy conversion by reducing the rate of protein synthesis in the photosynthetic apparatus. In addition, P limitation lowers the rate of synthesis and regeneration of substrates in the Calvin cycle, thereby reducing the rate of light utilization for carbon fixation (Falkowski & Raven 1997). Iron limitation, on the other hand, leads to a loss of PSI and PSII reaction centers, without a corresponding loss of the antenna pigments. Reduced coupling of light harvesting to electron transport in Fe-limited cells drastically lowers the quantum yield of photochemistry (Geider & LaRoche 1994). Although it is not clear what effects P and Fe limitation would have on the ATP/e^- ratio, one might speculate that they may differ from each other as well as from the effect of N limitation.

Conclusions. Whether or not the above scenario adequately describes the origin of the observed differences in carbon isotope fractionation of nitrate- and light-controlled cultures of *Phaeodactylum tricornutum*, the results of this comparison reveal additional uncertainty in the interpretation of carbon isotope data. In addition to growth rate and CO_2 concentration,

carbon isotope fractionation may be affected by the growth-limiting resource. Thus, even if species-specific factors determining isotope fractionation, such as cell size, carbon acquisition pathways, composition and ^{13}C discrimination of the carboxylating enzymes can be accounted for, attempts to reconstruct growth rate or CO_2 concentration from organic matter carbon isotope composition may critically depend on knowledge of the growth-limiting resource at the time of organic matter production. Clearly, a better understanding of the factors determining active carbon uptake, both under controlled laboratory conditions and in the field, would improve our ability to interpret carbon isotope fractionation in marine phytoplankton.

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