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Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: Theoretical considerations and experimental results

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Abstract—The carbon isotopic composition of the marine diatom *Phaeodactylum tricornutum* ($\delta^{13}C_p$) was measured over a series of growth rates (μ) in a continuous culture system in which both $\delta^{13}C_{CO_2}$ and $[CO_2]_{aq}$ were determined. In accord with theory, a linear relationship was found to exist between $\mu/[CO_2]_{aq}$ and ϵ_p ($\equiv 1000(\delta^{13}C_{CO_2} - \delta^{13}C_p)/(1000 + \delta^{13}C_p)$, the biological fractionation associated with carbon fixation. The range of $[CO_2]_{aq}$ in the continuous culture system was 13–31 μmol kg^{-1}. Measurements of $\delta^{13}C_{CO_2}$ and $[CO_2]_{aq}$ in the mixed layer of the equatorial Pacific and estimates of $\delta^{13}C_p$ obtained from the $\delta^{13}C$ of chlorophyll *a* combined with the regression line fit to the *P. tricornutum* data give phytoplankton growth rates that are in excellent agreement with those estimated via other techniques. Measurement of ϵ_p and $[CO_2]_{aq}$ in the field can provide an estimate of in situ phytoplankton growth rates without the potential artifacts associated with incubation methodologies. These findings also suggest that accurate estimations of ancient CO_2(aq) concentrations will require knowledge of both ϵ_p and phytoplankton growth rate.

INTRODUCTION

Stable isotopic characterization of marine organic matter can potentially provide important insights into the environmental conditions under which carbon fixation occurs. The interpretation of the isotopic data, however, has been confounded by the fact that organic matter in oceanic environments and in underlying sediments is a complex mixture of living organisms and detritus. Within the last few years the use of compound-specific isotopic analyses has allowed for a more precise identification of the origins of organic matter (Hayes et al., 1987, 1989, 1990; Freeman et al., 1990; Jasper and Hayes, 1990; Kenig et al., 1994). In particular, the isolation of pigments uniquely associated with phytoplankton (Bidigare et al., 1991) allows one to focus on living photoautrophic organic matter that forms the basis of the food chain.

In the past, it has been assumed that the major factor controlling the carbon isotopic composition of phytoplankton and individual biomolecules was the availability of aqueous carbon dioxide [CO_2(aq)] (e.g., Arthur et al., 1985; Hayes et al., 1989; Rau et al., 1989; Popp et al., 1989; Jasper and Hayes, 1990). Theoretical considerations, however, indicate that the fractionation of carbon by phytoplankton is a function of both intracellular and extracellular CO_2 concentrations (Farquhar et al., 1982). It is reasonable to assume that the difference between these concentrations will reflect CO_2 demand (Rau et al., 1992; Francois et al., 1993) and hence, the growth rate of the phytoplankton when transport of CO_2 into the cell is controlled by diffusion. In this paper, we explore the theoretical implications of this assumption and show, using both laboratory and field data, that knowledge of both $[CO_2]$aq and the difference between the stable carbon isotopic composition of the CO_2(aq) and phytoplankton biomass may be used to estimate in situ growth rates of natural phytoplankton populations.

MATERIALS AND METHODS

Laboratory Studies

The marine diatom *Phaeodactylum tricornutum* Bohlin (clone CCMP1327, Center for Culture of Marine Phytoplankton, Boothbay Harbor, ME, USA) was grown in a nitrate-limited continuous culture system similar to that described by Laws and Bannister (1980) at a temperature of 22°C. In such a system, the growth rate of the phytoplankton at steady state must equal the growth chamber dilution rate, which equals the overflow rate divided by the volume of the growth chamber (Laws and Bannister, 1980). In our experiments, the growth chamber volume was measured on three occasions, and the standard deviation of the measurements was 0.15% of the mean. The principal uncertainty defining the growth rate of the phytoplankton is the day-to-day variability in the overflow rate, which is determined by the precision of the peristaltic pumping system. The day-to-day reproducibility of our growth chamber dilution rate was ±0.01 d^{-1}, or roughly 1–2% of the dilution rates used in this study.

The light required by the culture for photosynthesis was provided by a bank of daylight fluorescent lamps either continuously or on a 12 h:12 h light:dark (L:D) cycle. The concentration of nitrate in the growth medium was 100 μM. The partial pressure of CO_2 in the gas used to aerate the growth chamber was controlled using mass flow controllers to adjust the flow rates of tank CO_2 (2.06% CO_2 in air, $\delta^{13}C_{CO_2} = -45.1‰$) and CO_2-free air. Sampling for isotopic analysis of the particulate carbon in the growth chamber was not begun until the culture had completed at least four doublings at a given growth rate and the $\delta^{13}C$ of the dissolved inorganic carbon (DIC) in the growth chamber had stabilized to ±0.1‰ from day to day (Fig. 1). The biomass of *P. tricornutum* in the growth chamber was monitored each day from cell counts measured with a Celloscope® cell counter and in vivo fluorescence measured with a Turner model 111 fluorometer.

Samples were taken daily from the continuous-light chemostat and every 3 h over a 24-h cycle from the 12 h:12 h cyclostat for determination of the concentration and isotopic composition of the DIC. DIC and $\delta^{13}C_{DIC}$ were determined using a system modified after Kroopnick (1985). Briefly, 9.67 mL of unfiltered seawater (preserved with 5 μL saturated $HgCl_2$) and 1.20 mL of 25% H_3PO_4 were sparged with N_2 in a 13 mL column fitted at the base with a fritted glass disk. The sparged CO_2 from acidification of the DIC was

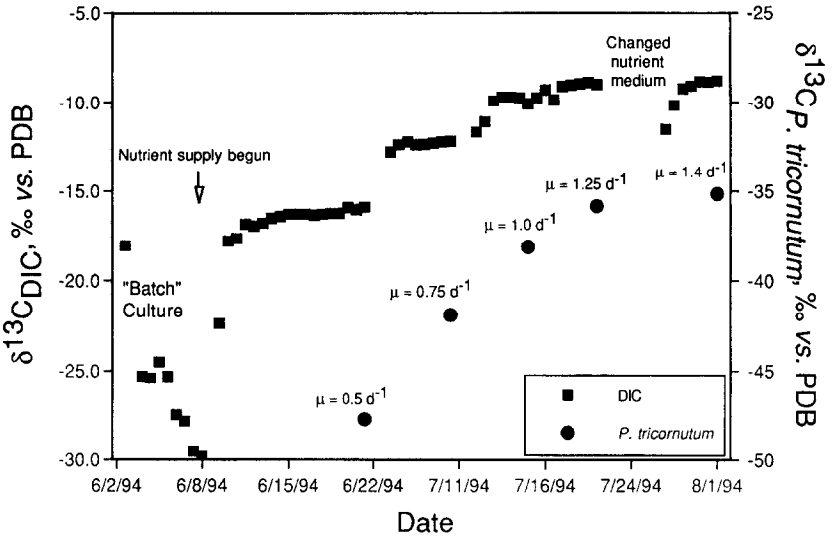

FIG. 1. Isotopic compositions of DIC and *P. tricornutum* plotted as a function of time. From 6/2/94 to 6/8/94 the system was operated as a batch culture. Variations in the isotopic composition of DIC during the week of 7/24/94 reflect changes in growth rate as the *P. tricornutum* culture adjusted to a new nutrient reservoir.

trapped using liquid nitrogen (LN_2) on a multiloop trap and transfered to a vacuum distillation line where the quantity of CO_2 was determined manometrically (MKS Baratron model 122). The concentration of DIC based on the *P-V* calculation yielded an accuracy and precision of less than 10 μM.

The abundance of CO_2(aq) was determined from concentrations of DIC, phosphate, and silicate, as well as total alkalinity (Roy et al., 1994). Apparent dissociation constants used in this calculation were from Dickson (1990a,b) and Roy et al. (1993). The apparent constants were corrected for the effects of pressure (Millero, 1979). Phosphate and silicate analyses were performed using the colorimetric techniques described in Strickland and Parsons (1972) on a Technicon Autoanalyzer II continuous flow system. Total alkalinity was determined by the Gran method using a computer-controlled titration. Precision and accuracy as determined by analyses of an alkalinity reference standard was less than 10 μeq kg^{-1}. Overall, the day-to-day precision of our calculation of [CO_2]aq was within 10% of the mean at steady state. The isotopic composition of CO_2(aq) was determined from the relative abundances of bicarbonate, carbonate and CO_2 (see above) and the temperature-fractionation relationships of Deines et al. (1974) and Mook et al. (1974).

Samples of *P. tricornutum* for carbon isotopic analysis were filtered (Whatman GF/C precombusted at 500°C for at least 4 h) from 50 mL of water. The filters were wrapped in precombusted aluminum foil and placed immediately in LN_2 and stored under LN_2 until analysis. Frozen samples were placed in precombusted quartz tubes, vacuum dried, cupric oxide added, the tubes sealed, and the samples combusted at 680°C for at least 8 h (Wedeking et al., 1983). Isotopic abundances were measured on cryogenically purified CO_2, using either a Finnigan MAT 252 or Delta-S mass spectrometer (Santrock et al., 1985). Analytical uncertainty for all carbon isotopic analyses was less than 0.1‰. Carbon isotopic compositions are reported in δ-notation relative to PeeDee belemnite (PDB). Particulate carbon concentrations were determined by two methods: manometrically from the amount of CO_2 produced when the samples for particulate carbon isotopic analysis were combusted and from 50 mL aliquots from the growth chamber collected on precombusted GF/C filters and analyzed on a Perkin-Elmer model 2400 CHN elemental analyzer. Agreement between the two methods was quite good (r^2 = 0.96), and the average of the two measurements was used in subsequent calculations.

Field Studies

The field work was undertaken during the spring of 1992, as a part of the U.S. JGOFS-NOAA Equatorial Pacific (EqPac) Study to investigate the effects of the 1991–1993 El Niño on physical, chemical, and biological processes in the upper ocean (Murray et al., 1992). Samples were collected during cruises on the R/V *Thomas G. Thompson* (TT007, February–March, 1992) and R/V *Baldridge* (Spring Leg 3, April–May, 1992). Stations were occupied in the vicinity of the equator (12°N–12°S) along 135–140°W. Despite temporal differences, mixed layer hydrographic properties (density and temperature) were similar for these two cruises. During the initial cruise, suspended particulate samples (5–7 m^3) were collected from the upper mixed layer (40–80 m), using a Multiple Unit Large Volume in situ Filtration System (MULVFS; J. Bishop, University of Victoria). The filtration system sampled particles in the 1–53 μm size range. Subsamples, each representing 0.6–0.9 m^3 of seawater, were analyzed for stable carbon isotopic compositions of lipid ($\delta^{13}C_{lipid}$) and chlorophyll *a* ($\delta^{13}C_{chl}$) following the methods described in Bidigare et al. (1991) and of bulk organic matter ($\delta^{13}C_{bulk}$) after acid fuming. Shipboard measurements of salinity, temperature, and pressure (University of Washington), nutrients (C. Garside, Bigelow Laboratory for Ocean Sciences), and total CO_2 and alkalinity (D. Archer, University of Chicago) were used to calculate $[CO_2]_{aq}$ as described above. Unfortunately, measurements of $\delta^{13}C_{DIC}$ are not available from the initial cruise. During the second cruise, however, measurements of $\delta^{13}C_{DIC}$ were performed along the 140°W transect (J. Zhang and P. Quay, University of Washington). Values of $\delta^{13}C_{DIC}$ measured in surface waters (10°N–10°S) were relatively invariant and averaged 1.55 ± 0.08‰ (n = 25). Carbon dioxide system parameters and $\delta^{13}C_{DIC}$ values determined during the first and second cruises, respectively, were used to calculate $\delta^{13}C_{CO_2}$ using the procedures described in the previous section.

THEORY

It is straightforward to show from the theoretical analysis of Farquhar et al. (1982) that if there is no intrinsic discrimination by dark respiration and photorespiration, the effects of diffusion and carboxylation on the carbon isotopic composition of plants can be rather accurately described by the equation:

$$\epsilon_p = A + (B - A)C_i/C_e, \quad (1)$$

where C_i and C_e are the total ($^{12}CO_2$ plus $^{13}CO_2$) internal and external CO_2 concentrations, respectively, A and B are the

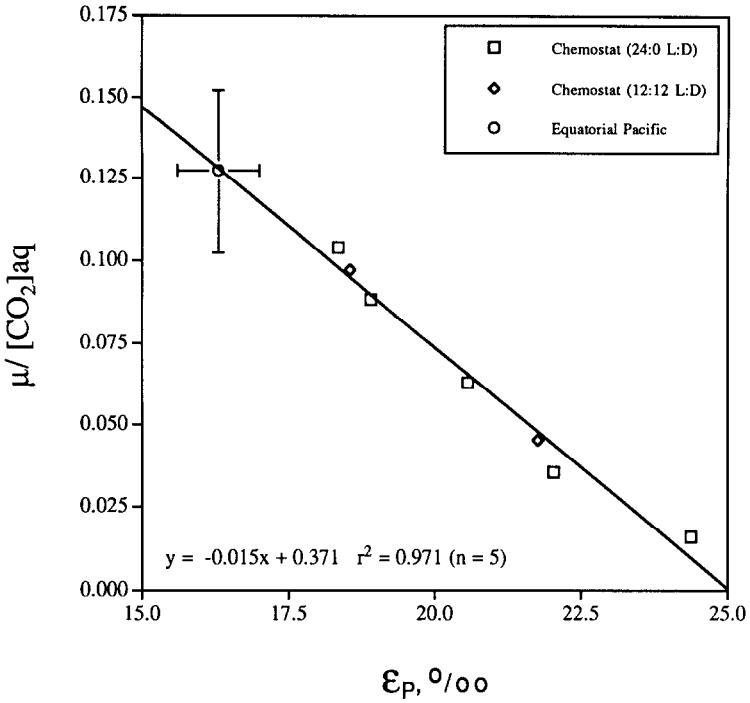

FIG. 2. Relationship between $\mu/[CO_2]aq$ and ϵ_p for *P. tricornutum* grown in a chemo(cyclo)stat system under light:dark (L:D) cycles of 24h:0h and 12h:12h. The geometric mean model II regression equation corresponds to the 24:0 L:D cycle, where $[CO_2]aq$ ranged from 13 to 31 μmol kg^{-1}. The open circle is the mean of the range of reported growth rates (0.585 d^{-1}) in the equatorial Pacific multiplied by 2.35 to correct for L:D cycle and respiration effects (see text) and divided by the $[CO_2]aq$ of 10.8 μmol kg^{-1}. The corresponding ϵ_p is 16.3‰. The error bars indicate the range of reported growth rates (0.47 − 0.70 d^{-1}; Greene et al., 1994) and the variability associated with ϵ_p (±1 standard deviation, Table 1). For the laboratory data, the standard error of the ϵ_p measurements is ±0.05‰, and the standard error of the $\mu/[CO_2]aq$ measurements is about ±5% of the mean value at the given growth rate.

discrimination factors associated with diffusion of CO_2 into the plant and fixation of CO_2 within the plant, respectively, and ϵ_p is the isotope discrimination factor of the plant defined by

$$\epsilon_p = 1000(\delta_e - \delta_p)/(1000 + \delta_p), \quad (2)$$

where δ_e and δ_p are the $\delta^{13}C$ of the external CO_2 and plant carbon, respectively. Similar equations have been derived by O'Leary and Osmond (1980) and Vogel (1980). We now assume that the growth rate μ of the cell is related to C_i and C_e by an equation of the form:

$$\mu = K_1 C_e - K_2 C_i; \quad (3)$$

i.e., the gross transport of CO_2 into the cell is proportional to C_e, the gross transport of CO_2 out of the cell is proportional to C_i, and growth rate is proportional to the net transport of CO_2 into the cell. Substituting $(K_1C_e - \mu)/K_2$ for C_i in Eqn. 2 gives:

$$\epsilon_p = A + (B - A)(K_1 - \mu/C_e)/K_2. \quad (4)$$

In other words, if Eqn. 3 accurately describes the relationship between μ, C_e, and C_i and if isotope discrimination effects due to respiration are negligible, then ϵ_p is expected to be approximately a linear function of μ/C_e. A similar conclusion has been reached by Goericke et al. (1994). If $K_1 \cong K_2$, then ϵ_p should approach B in the limit as $\mu/C_e \to 0$. B is the combined fractionation due to Rubisco and β-carboxylase carboxylations, and its likely value is 25–28‰ (see review by Goericke et al., 1994).

RESULTS

The $\delta^{13}C$ results from the continuous light chemostat experiment are shown in Fig. 1. The difficulty of relating the $\delta^{13}C$ of the particulate organic matter (POM) to the $\delta^{13}C$ of the DIC during batch culture growth is apparent in this figure. During approximately one week of batch culture growth, the $\delta^{13}C_{DIC}$ dropped from −18 to −30‰. This problem could be circumvented by operating a large batch culture (i.e., CO_2 supply is large relative to utilization) but would require filtering a large volume of water to obtain a sufficient amount of particulate carbon for the $\delta^{13}C_{POM}$ measurement. A more practical solution to the problem is to operate a chemostat. Our results indicate that the $\delta^{13}C_{DIC}$ is stable to ±0.1‰ from day to day in a chemostat system (Fig. 1).

The results of applying Eqn. 4 to both the laboratory and field data are shown in Fig. 2. The regression line was fit to the continuous light chemostat data. However, note that the data from the 12:12 L:D cycle study and the EqPac field study work lie very close to the regression line. In the continuous light chemostat work $[CO_2]aq$ was negatively correlated with growth rate and varied from 13 μmol kg^{-1} at the highest growth rate (1.4 d^{-1}) to 31 μmol kg^{-1} at the lowest growth rate (0.5 d^{-1}). In the 12:12 L:D cycle experiment, $[CO_2]aq$ declined from an average of 125 μmol kg^{-1} during the first

three hours of the photoperiod to 10 μmol kg^{-1} during the last three hours of the photoperiod. The two L:D cycle data points in Fig. 2 are taken from the second and third three-hour intervals in the photoperiod, during which time the growth rate of *P. tricornutum* averaged 1.8 d^{-1}. The $\delta^{13}C$ of *P. tricornutum* showed no temporal trend during the 12 h dark period in the cyclostat and averaged -35.9 ± 0.1‰. This result is consistent with the assumption that there is little fractionation of carbon associated with respiration. Because of this fact, the ϵ_p of *P. tricornutum* will reflects the growth rate of the phytoplankton carbon biomass during the photoperiod and not the 24 h average growth rate. Since respiration removes particulate carbon in the dark, the growth rate of particulate carbon during the photoperiod must be at least twice the 24 h average growth rate when cells are growing on a 12:12 L:D cycle.

The growth rates of equatorial Pacific phytoplankton estimated by various techniques are summarized in Table 1. The results are quite consistent and imply a growth rate of 0.6 $\pm$ 0.1 d^{-1}. We multiplied these 24 h growth rates by 2.35 to estimate the growth rate of phytoplankton carbon during the photoperiod (see below). The stable carbon isotopic compositions determined for bulk organic matter, lipid, chlorophyll *a*, and CO_2(aq) exhibited only minor variations along the survey transect, and averaged -21.1, -25.5, -23.2, and -6.60‰, respectively (Table 2). The lipid fraction was depleted in ^{13}C by 4.4‰ relative to bulk organic matter. The determination of $\delta^{13}C$ for phytoplankton carbon ($\delta^{13}C_p$) in natural samples is confounded by the presence of heterotrophic and detrital organic carbon in the POM. In order to circumvent this problem, we estimated $\delta^{13}C_p$ from the $\delta^{13}C$ of the chlorophyll *a* ($\delta^{13}C_{chl}$) and the $\delta^{13}C_{plant} - \delta^{13}C_{chl}$ relationship shown in Fig. 3 (after Kennicutt et al., 1992). The calculated $\delta^{13}C_p$ averaged -22.5 ± 0.7‰, a value which is 1.4‰ lighter than that measured for the bulk POM. The ϵ_p values determined for equatorial Pacific phytoplankton averaged 16.3 $\pm$ 0.7‰ (Table 2).

DISCUSSION

The value of ϵ_p predicted by the regression line in Fig. 2 at $\mu/CO_2 = 0$ is 25.0‰. This value is consistent with the expected fractionation of 25–28‰ due to Rubisco and β-carboxylase carboxylations. This fact suggests that either $K_1 \cong K_2$ in Eqn. 4 or else $B \cong A$. In fact, the magnitude of A is believed to be much smaller than B (Farquhar et al., 1982; O'Leary, 1984; Goericke et al., 1994). Therefore, the implication is that $K_1 \cong K_2$.

The agreement between theoretical calculations and experimental results suggests that the measurement of [CO_2]aq and ϵ_p may be used to estimate in situ growth rates of natural phytoplankton populations. The appeal of the method is that it requires no incubations and gives a time-integrated estimate of growth rate. There are, however, several cautionary notes to consider when using the proposed method.

First, as already noted, it is impossible to directly measure $\delta^{13}C_p$ in the field. The solution to this problem is to isolate the chlorophyll *a*, measure $\delta^{13}C_{chl}$ and estimate $\delta^{13}C_p$ from the regression line in Fig. 3. The $\delta^{13}C_{plant} - \delta^{13}C_{chl}$ relationship is sufficiently constrained that the use of this regression line to estimate $\delta^{13}C_p$ would seem to be a reasonable and accurate solution to the problem.

Second, it is reasonable to assume that the rate constants K_1 and K_2 will be a function of cell size. The fluxes of CO_2 into and out of the cell should be roughly proportional to the surface area of the cell, and the growth rate will equal the net flux divided by the carbon per cell. Assuming spherical geometry, the surface area of the cell would be expected to vary as cell volume raised to the 0.67 power. Carbon per cell varies approximately as cell volume raised to the 0.75–0.99 power for marine phytoplankton (Mullin et al., 1966; Moal et al., 1987; Verity et al., 1992; Montagnes et al., 1994). Therefore K_1 and K_2 would be expected to vary as cell volume raised to approximately the $0.67 - 0.75 = -0.08$ power to $0.67 - 0.99 = -0.32$ power. Assuming that most phytoplankton in the open ocean have cell volumes in the range 1–1000 μm^3, the range of associated K_1 and K_2 values would be within a factor of 2–9. *P. tricornutum* has a cell volume of about 100 μm^3 (Strathmann, 1967). Obviously, if K_1 and K_2 have the same dependence on cell size, the ratio K_1/K_2 will be independent of cell size, and the intercept of the ϵ_p vs. μ/[CO_2]aq regression should, therefore, be independent of cell size. The slope of the regression line, however, would reflect the dependence of K_2 on cell size. Since most natural communities of phytoplankton include many species with a variety of cell sizes, it is unlikely that the range of K_2 values between natural com-

Table 1. Comparison of phytoplankton growth rate (μ) estimates for equatorial Pacific phytoplankton.

Method (reference)	μ (d^{-1})
Phytoplankton Community	
Dilution technique (Feb-Mar 1992 EqPac Study; Landry et al., 1994)	0.52 ± 0.40[a]
	0.58 ± 0.37[b]
Calculated (^{14}C assimilation and C:chl ratios; Greene et al., 1994)	0.47 - 0.70
Stable isotopes ($\delta^{13}C_{CO_2}$, $\delta^{13}C_{chl}$, and [CO_2(aq)]; this study)	0.58 ± 0.05
Prochlorococcus spp.	
Cell cycle analysis (1992 EqPac study; Vaulot et al., 1994)	0.51 - 0.63

[a]without added nutrients (n = 16)

[b]with added nutrients (n = 16)

Table 2. Stable carbon isotopic compositions and aqueous CO_2 concentrations (μmol kg^{-1}) determined during the 1992 EqPac spring survey cruises. The stable isotopic compositions for phytoplankton were estimated from the $\delta^{13}C_{chl}$ values and the regression equation given in Figure 3.

			$\delta^{13}C$ (‰) vs. PDB						
Latitude	Stn	Depth	Bulk	Lipid	Chl	Phyto	$CO_2(aq)$	$[CO_2(aq)]$	ε_p(‰)
3°N	5	77 m	-21.1	-26.7	-23.6	-23.0	-6.61	10.76	16.8
2°N	6	43 m	-20.8	-24.8	-22.3	-21.6	-6.61	11.04	15.3
1°N	7	42 m	-21.5	-26.1	-23.8	-23.2	-6.61	11.17	17.0
1°S	9	48 m	-20.9	-24.5	-22.4	-21.7	-6.61	11.32	15.4
2°S	10	47 m	-21.0	-24.3	-23.0	-22.3	-6.60	11.14	16.1
5°S	12	43 m	-21.3	-26.1	-23.2	-22.5	-6.58	10.57	16.3
12°S	15	38 m	-21.4	-25.8	-23.9	-23.3	-6.59	9.39	17.1
		Mean	-21.1	-25.5	-23.2	-22.5	-6.60	10.77	16.3
		Std Dev	0.3	0.9	0.6	0.7	0.01	0.66	0.7

munities in the ocean would be as large as 2 to 9. In a study in the North Atlantic, for example, Murphy and Haugen (1985) found from an analysis of 74 oceanic, 17 slope water, and 39 neritic samples, that 73–84% of eucaryotic cells passed a 3-μm filter, and of these, 35–75% passed a 0.8-μm filter but were retained on a 0.2-μm filter. Of the procaryotic cells, 95–99% passed a 3-μm filter. The percentage of the procaryotes that passed a 0.8 μm-filter was seldom greater than 50%. Thus, assuming that the average diameter of phytoplankton cells is 1 μm in a natural marine community is unlikely to be in error by more than a factor of 2 in most cases. It is possible that at some times and places cell size effects may change the slope of the ϵ_p vs. $\mu/[CO_2]aq$ relationship sufficiently to invalidate the use of a single equation to predict phytoplankton growth rates from ϵ_p and $[CO_2]aq$ data in all parts of the ocean, but these cases are likely to be the exception rather than the rule.

Third, if transport of CO_2 across the cell membrane is driven by diffusion, then K_1 and K_2 will reflect the permeability of the cell membrane. Significant differences in membrane permeability may exist between species. Obviously, this question has not been addressed by studies on a single species.

A fourth concern is the fact that ϵ_p reflects the growth rate of the phytoplankton during the photoperiod, not the 24 h average growth rate. Thus, a correction to the growth rate calculated from the regression line in Fig. 2 must be made to account for L:D cycle effects. Both cyclostat studies (Laws and Bannister, 1980) and field work conducted in subtropical latitudes (Laws et al., 1990) suggest that when phytoplankton are growing on a 12:12 L:D cycle, the respiratory loss of carbon during the dark period is about 10–20% of that fixed during the preceding photoperiod. The implication is that growth rates during the photoperiod are 2.2–2.5 times the 24 h average growth rates. The correction factor becomes larger as the photoperiod becomes shorter. Choosing a mean correction factor of 2.35 in the case of a 12:12 L:D cycle should produce an error of less than 7% in the estimated growth rate due to uncertainty in the dark respiration rate. The growth rate of equatorial Pacific phytoplankton estimated from $[CO_2]aq$ and ϵ_p is 0.58 d^{-1} (Table 1 and Fig. 2), a value which is virtually identical to the mean (0.585 d^{-1}) of reported values (0.47–0.70 d^{-1}; Greene et al., 1994).

Finally, there is a possibility that under conditions of low $[CO_2]aq$, cells may obtain inorganic carbon via active transport. Many marine phytoplankton, including *P. tricornutum*, are capable of utilizing bicarbonate and concentrating inorganic carbon (Burns and Beardall, 1987; Raven and Johnston, 1991, 1994). Since CO_2 is the inorganic species utilized by photosynthesizing *P. tricornutum* (Dixon and Merrett, 1988; Johnston and Raven, 1991), use of bicarbonate ultimately involves carbonic anhydrase (CA) for converting bicarbonate into CO_2. The impact of active transport on the relationship shown in Fig. 2 could occur in several ways. First, active transport would lead to more rapid growth than the regression line in Fig. 2 would predict at a given concentration of $CO_2(aq)$. This effect, taken by itself, would make data lie above the regression line. Secondly, the use of bicarbonate could lead to isotope shifts unassociated with changes in growth rate due to the fact that at isotopic equilibrium bicarbonate is enriched in ^{13}C relative to $CO_2(aq)$, the fractionation factor ranging from 8.4‰ at 30°C to 12.0‰ at 0°C (Deines et al., 1974; Mook et al., 1974). If the conversion of bicarbonate to CO_2 occurred intracellularly, then the CO_2 available for photosynthesis would be isotopically heavier and ϵ_p would be smaller than in the case of purely diffusional transport. This second effect therefore, taken by itself, would make data lie to the left of (i.e., below) the regression line. Insofar as causing deviations from the regression line is concerned, the two effects would therefore tend to counterbalance one another. For example, if in seawater containing 5 μmol kg^{-1} $[CO_2]aq$ *P. tricornutum* were able to grow 0.75 d^{-1} faster during the photoperiod by obtaining all its carbon via active transport of bicarbonate rather than by diffusion of

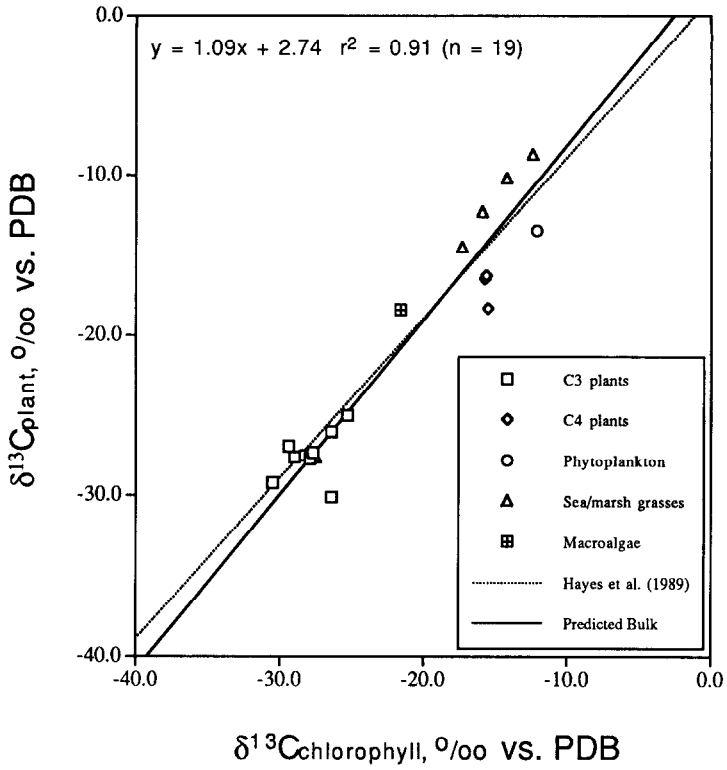

FIG. 3. Relationship between the stable carbon isotopic composition of bulk plant organic matter and chlorophyll *a* determined for a variety of plant types. Data for C_3 plants, C_4 plants, phytoplankton (*Isochrysis* sp. and *Anabaena* sp.) and macroalgae (*Laminaria* sp.) are taken from Galimov and Shirinskiy (1975), Bidigare et al. (1991), and Kennicutt et al. (1992). Isotope values for sea (*Zostera* sp.) and marsh (*Salicornia* sp. and *Spartina* sp.) grasses were determined using the methods described in Bidigare et al. (1991). The predicted line represents the major axis model II regression equation. The Hayes et al. (1989) line was calculated by mass balance, assuming that the chlorin ring and phytol chain of chlorophyll *a* are enriched and depleted in ^{13}C by 0.5 and 4.0‰ relative to plant biomass, respectively (Hayes et al., 1989, 1990). The differences between measured and predicted plant carbon isotopic compositions are likely caused by variations in biochemical composition among various plant types.

CO_2, then ϵ_p would be almost exactly the value predicted by the regression line in Fig. 2, since 0.75/5 = 0.15, which is the shift in $\mu/[CO_2]aq$ predicted when ϵ_p decreases by 10‰.

However, if the conversion of bicarbonate to CO_2 occurred at the cell surface and hence, effectively increased the concentration of CO_2 available for diffusion into the cell, the CO_2 which entered the cell would have nearly the same isotopic composition as the rest of the external CO_2 (U. Riebesell, pers. commun.). This is because the fractionation associated with the CA-mediated conversion of bicarbonate to CO_2 is 10.1‰ (Paneth and O'Leary, 1985). Under these conditions, data would be expected to lie above the regression line in Fig. 2.

CO_2(aq) concentrations in the ocean typically fall in the range 10–20 μmol kg^{-1} (Rau et al., 1992; Francois et al., 1993). Whether marine phytoplankton are actively transporting inorganic carbon at these CO_2(aq) concentrations appears at this time to be largely a matter of speculation (e.g., Morel et al., 1994). Since active transport requires energy, it is reasonable to assume that phytoplankton do not actively transport CO_2 if diffusional transport is adequate. Our experimental data provide a basis for estimating when active transport may become necessary for *P. tricornutum*. Assuming that $K_1 = K_2$ and that A is small compared to B, we conclude that K_1 equals the intercept (0.37) of the regression line in Fig. 2. From Eqn. 3, we can see that the maximum growth rate that can be supported by diffusion is K_1C_e, and this occurs only in the limit as $C_i \rightarrow 0$. Thus, for example, at a $[CO_2]aq$ of 10 μmol kg^{-1}, the maximum growth rate that *P. tricornutum* could possibly achieve without actively transporting inorganic carbon would be 3.7 d^{-1}. This is, however, well above the maximum growth rate of about 1.7 d^{-1} which *P. tricornutum* was capable of achieving under batch culture, continuous light conditions in our experiments. The implication is that *P. tricornutum* would not need to actively transport inorganic carbon at $[CO_2]aq$ in excess of 10 μmol kg^{-1}.

In natural marine systems, phytoplankton growth rates are rarely reported in excess of two doublings per day, i.e., μ = 1.4 d^{-1} (Laws et al., 1987). If the photoperiod were 12 h, then phytoplankton growing at an average rate of 1.4 d^{-1} would be growing at about (2.35) (1.4) = 3.3 d^{-1} during the photoperiod (see above). This is approaching the maximum rate achievable at a $[CO_2]aq$ of 10 μmol kg^{-1} by *P. tricornutum* without active transport of inorganic carbon. We conclude that if the regression line in Fig. 2 is not misleading, marine phytoplankton under most conditions are not now actively transporting inorganic carbon, nor in the geologic past when atmospheric CO_2 levels were apparently higher (Berner,

1994). Active transport may, however, become a factor when growth rates are rapid (e.g., 1.4 d^{-1} with a 12 h photoperiod) and $[CO_2]aq$ is 10 μmol kg^{-1} or less.

Obviously, further studies are needed to clearly define the limitations of using ϵ_p and $[CO_2]aq$ data to estimate the growth rates of marine phytoplankton. If the method proves to be robust, however, its use could greatly facilitate the estimation of phytoplankton growth rates in the ocean. In addition, application of the approach described here to source-specific algal biomarkers (e.g., carotenoids, sterols, fatty acids, alkenones, etc.) could potentially yield information regarding class-specific phytoplankton growth rates. The findings of this study also have important implications regarding the estimation of ancient, upper ocean $CO_2(aq)$ concentrations. Our laboratory measurements document that ϵ_p varies as a function of both $CO_2(aq)$ concentration and phytoplankton growth rate. Therefore, estimates of paleo-$CO_2(aq)$ concentrations based on the stable isotopic composition of organic matter and biomass indicators (e.g., porphyrins and alkenones) need to carefully consider the paleoenvironmental conditions at the time of phytoplankton biomass production and deposition.

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