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Effect of phytoplankton cell geometry on carbon isotopic fractionation

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Abstract—The carbon isotopic compositions of the marine diatom *Porosira glacialis* and the marine cyanobacterium *Synechococcus* sp. were measured over a series of growth rates (μ) in a continuous culture system in which the concentration and carbon isotopic composition of CO_2(aq) were determined. These data were compared with previously published isotopic results of growth rate experiments using the marine diatom *Phaeodactylum tricornutum* and the marine haptophyte *Emiliania huxleyi*. Systematic relationships were found to exist between $\mu/[CO_2(aq)]$ and carbon isotopic fractionation (ϵ_P) for each species. Maximum isotopic fractionation (ϵ_f) for *P. glacialis*, *E. huxleyi*, and *P. tricornutum* was ~25‰, suggesting that this value may be typical for maximum fractionation associated with Rubisco and β-carboxylases for marine eukaryotic algae. By contrast, ϵ_f determined for *Synechococcus* clone CCMP838 was ~7‰ lower. The slopes of the lines describing the relationship between ϵ_P and $\mu/[CO_2(aq)]$ for eukaryotic algal species were different by a factor of more than 20. This result can be accounted for by differences in the surface area and cellular carbon content of the cells. Comparison of chemostat experimental results with calculated results using a diffusion based model imply that the algae in the experiments were actively transporting inorganic carbon across the cell membrane. Our results suggest that accurate estimates of paleo-$[CO_2(aq)]$ from ϵ_P measured in sediments will require knowledge of growth rate as well as cell surface area and either cell carbon quota or cell volume. Given growth rate estimates, our empirical relationship permits reliable calculations of paleo-$[CO_2(aq)]$ using compound-specific isotopic analyses of C_{37} alkadienones (select haptophytes) or fossilized frustules (diatoms). *Copyright © 1998 Elsevier Science Ltd*

1. INTRODUCTION

The fractionation of ^{13}C by marine phytoplankton has been the subject of recent theoretical, laboratory, and field studies (e.g., Arthur et al., 1985; Popp et al., 1989; Rau et al., 1989; Freeman and Hayes, 1992; Goericke et al., 1994; Hinga et al., 1994). If the carbon isotopic composition of phytoplankton is primarily controlled by the concentration of ambient CO_2(aq) (e.g., Degens et al., 1968; Pardue et al., 1976; Mizutani and Wada, 1982; among others), and if the relationship between isotopic fractionation associated with photosynthesis (ϵ_P) and $[CO_2(aq)]$ can be quantified (e.g., Freeman and Hayes, 1992), then the prospect exists for investigating variations in ancient oceanic $[CO_2(aq)]$.

However, carbon isotopic fractionation by marine photoautotrophs potentially depends on many factors. These factors can include cell wall permeability, carbon demand or growth rate, cell size, and the ability of the cell to actively assimilate inorganic carbon (e.g., Raven and Johnson, 1991; Fry and Wainright, 1991; Rau et. al., 1992; Francois et al., 1993; Goericke et al., 1994; Rau et al., 1996). Recently, Laws et al. (1995, 1997) and Bidigare et al. (1997), using results of analyses of marine microalgae grown in chemostat cultures, were able to demonstrate systematic quantitative relationships between cell growth rate, $[CO_2(aq)]$ and carbon isotopic fractionation. In this paper we extend this work and explore the relationship between cell size, carbon quota, and cell geometry on carbon isotopic fractionation. We demonstrate that these factors can significantly influence isotopic fractionation in marine phytoplankton. These results have important implications for the interpretation of the carbon isotopic record of marine sedimentary organic matter.

2. MATERIALS AND METHODS

We combined results of chemostat growth experiments for the marine diatom *Phaeodactylum tricornutum* from Laws et al. (1995, 1997) and for the marine haptophyte *Emiliania huxleyi* from Bidigare et al. (1997) with new results from the marine cyanobacterium *Synechococcus* and the Antarctic marine diatom *Porosira glacialis*. To simulate conditions in the modern ocean, we used only results of experiments with *P. tricornutum* in which $[CO_2(aq)]$ exceeded 10 μmol/kg (see Laws et al., 1997). Results for *E. huxleyi* include calcifying (clone B92/11) and noncalcifying (clone BT6) strains.

Chemostat experiments using *Synechococcus* (CCMP838) and *Porosira glacialis* (CCMP980) were performed following the methods originally described in Laws et al. (1995). Both algae were obtained from the Center for Culture of Marine Phytoplankton, Boothbay, Maine, USA. *Synechococcus* was grown in a nitrate-limited chemostat using a polycarbonate medium reservoir and growth chamber at room temperature (22-23°C) and on continuous light. *P. glacialis* was grown in chemostat culture on continuous light and at constant temperature (either −0.1 or 2.0°C). Target temperatures in these experiments were maintained to within ±0.05°C by circulating a water-glycol mixture from a temperature-controlled water bath through the jacket of the glass growth chamber. We measured daily or bi-weekly (depending on the growth rate) cell density, chlorophyll fluorescence, concentration of total dissolved inorganic carbon (DIC), total alkalinity (to calculate $[CO_2(aq)]$), and the $\delta^{13}C_{DIC}$ in the growth chamber. The growth medium consisted of seawater collected from station ALOHA (Karl and Lukas, 1996), enriched with appropriate concentrations of essential

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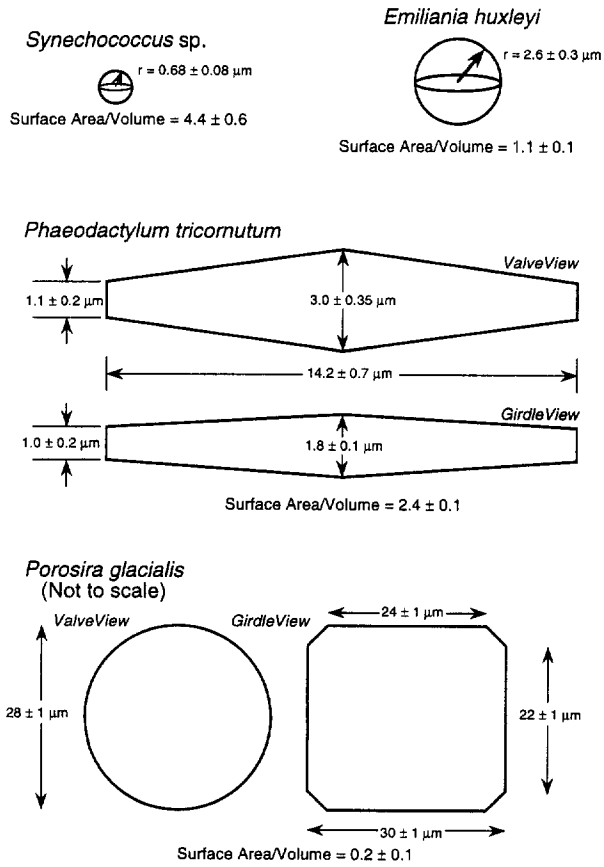

Fig. 1. Schematic diagrams of *Synechococcus* sp., *E. huxleyi*, *P. tricornutum*, and *P. glacialis*. Average cell dimensions and standard deviations are given as well as calculated average surface area to volume ratios. Note that *P. glacialis* is illustrated at 1/5 scale.

nutrients, and sterile filtered (0.22 μm) into a previously autoclaved medium reservoir. Studies at a particular growth rate did not begin until the culture had been at steady-state for at least four doubling times so that more than 93% of the biomass harvested at the end of the experiment had been grown with the concentration of phytoplankton and the concentration and isotopic composition of DIC constant. Collection, processing of algae and water samples, and all calculations were identical to those of Laws et al. (1995, 1997).

Surface area and volume of the eukaryotic phytoplankton species analyzed were calculated using geometric cell dimensions measured with a calibrated micrometer using a Zeiss IM35 inverted microscope. We chose to use optical measurements because electronic methods (e.g., Coulter Counters) can underestimate cell volume, particularly for nonspherical cells (Montagnes et al., 1994). Our strains of *Emiliania huxleyi* had geometrical forms closely resembling a sphere, thus only the average cell diameter was measured and used for surface area and volume calculations (Fig. 1). The form of *P. tricornutum* used in these experiments is fusiform (see Fig. 4 in Borowitzka et al., 1977). For surface area and volume determinations, its shape can be adequately described by the geometric form illustrated in Fig. 1 (Edler, 1979). Cell widths in the middle and at the tips of the cells were measured in girdle view and in valve view as well as cell length for calculation of cell surface area and volume. *Porosira glacialis* is a large centric diatom with the dimensions given in Fig. 1. Surface area and volume were calculated assuming cell geometry could be adequately described by a cylinder with each end capped by the frustum of a right cylinder cone. Because of difficulty resolving optically the geometric dimensions of *Synechococcus* (CCMP838), cell radius was estimated from the cellular carbon conversion factor of Verity et al. (1993; 470 fg-C μm^{-3}) assuming spherical geometry. Cell size estimates agree well with those reported in recent literature (Kana and Gilbert, 1987; Campbell et al., 1994). Carbon biomass for all algae was determined from cell counts and particulate organic carbon analyses. Cell counts were determined by microscopic analyses, flow cytometery (Coulter EPICS 753 w/dual laser bench), and/or by using a Celloscope^R cell counter. Particulate carbon analyses were performed using a Perkin-Elmer model 2400 CHN analyzer. Carbon biomass was found to be proportional to biovolume raised to the 0.88 power (pg C = 0.284 × (biovolume, $\mu m^3)^{0.875}$, r^2 = 0.99), a relationship consistent with that of Verity et al. (1993).

3. RESULTS AND DISCUSSION

Several models describing carbon isotopic fractionation in plants exist based on the work of Farquhar et al. (1982). Early studies assumed variations in [CO_2(aq)] were paramount in controlling carbon isotopic fractionation (ϵ_P, cf. Freeman and Hayes, 1992) in marine algae (e.g., Arthur et al., 1985; Popp et al., 1986; Rau et al., 1989; among others). Recently, physiological effects have been investigated (e.g., Rau et al., 1992; Francois et al., 1993; Goericke et al., 1994; Rau et al., 1996). Building upon this work, Laws et al. (1995, 1997) and Bidigare et al. (1997), using results of laboratory experiments and field observations, demonstrated that, over the range of growth rate and [CO_2(aq)] combinations found in contemporary seawater, a linear relationship existed between growth rate (μ), [CO_2(aq)] and ϵ_P in a marine diatom and a marine haptophyte such that

$$\epsilon_P = \epsilon_t + (\epsilon_f - \epsilon_t)\left(\frac{K_1}{K_2} - \frac{\mu}{K_2 CO_2}\right) \quad (1)$$

where CO_2 is the [CO_2(aq)] in the growth medium, ϵ_t and ϵ_f are the discrimination factors associated with diffusion of CO_2(aq) into the plant and fixation of CO_2(aq) within the plant, respectively, and K_1 and K_2 are rate constants for the flux of CO_2(aq) into and out of the plant, respectively. Implicit in Eqn. 1 is the assumption that CO_2(aq) enters the cell via passive diffusion. Several studies, however, suggest that one or more inorganic carbon species is actively transported across cell membranes (see reviews by Aizawa and Miyachi, 1986; Raven, 1991a,b). When *P. tricornutum* was grown in chemostat culture with [CO_2(aq)] < 7 μmol kg^{-1}, ϵ_P was found to be a nonlinear function of μ/CO_2, implying that CO_2(aq) entered the cell by mechanisms in addition to diffusion from the bulk medium (Laws et al., 1997). Nonetheless, the $\epsilon_P - \mu/CO_2$ relationship was well approximated by a linear fit for chemostat cultures of *P. tricornutum* with [CO_2(aq)] exceeding 10 μmol kg^{-1}. We further examine below the implications of passive diffusion vs. active (facilitated) transport of CO_2(aq). With the exception of *Synechococcus* (CCMP838), results of chemostat experiments are analogous to those of *P. tricornutum* grown at [CO_2(aq)] exceeding 10 μmol kg^{-1} and are well approximated by linear relationships between ϵ_P and μ/[CO_2(aq)], but those relationships are species-specific (Fig. 2a; Table 1).

One factor potentially affecting ϵ_P and our interpretation of the mechanism by which CO_2(aq) enters the cell is ϵ_f, the flux-weighted average of isotope effects associated with all carbon-fixation reactions active in the cell. Laws et al. (1995) suggested that if $K_1 \cong K_2$, then ϵ_P should approach ϵ_f in the limit as $\mu/CO_2 \rightarrow 0$. The values of ϵ_P predicted by the linear regression for the eukaryotic species at $\mu/CO_2 = 0$ converge on a value of ~25‰ (Fig. 2a). Maximum fractionations of 25-

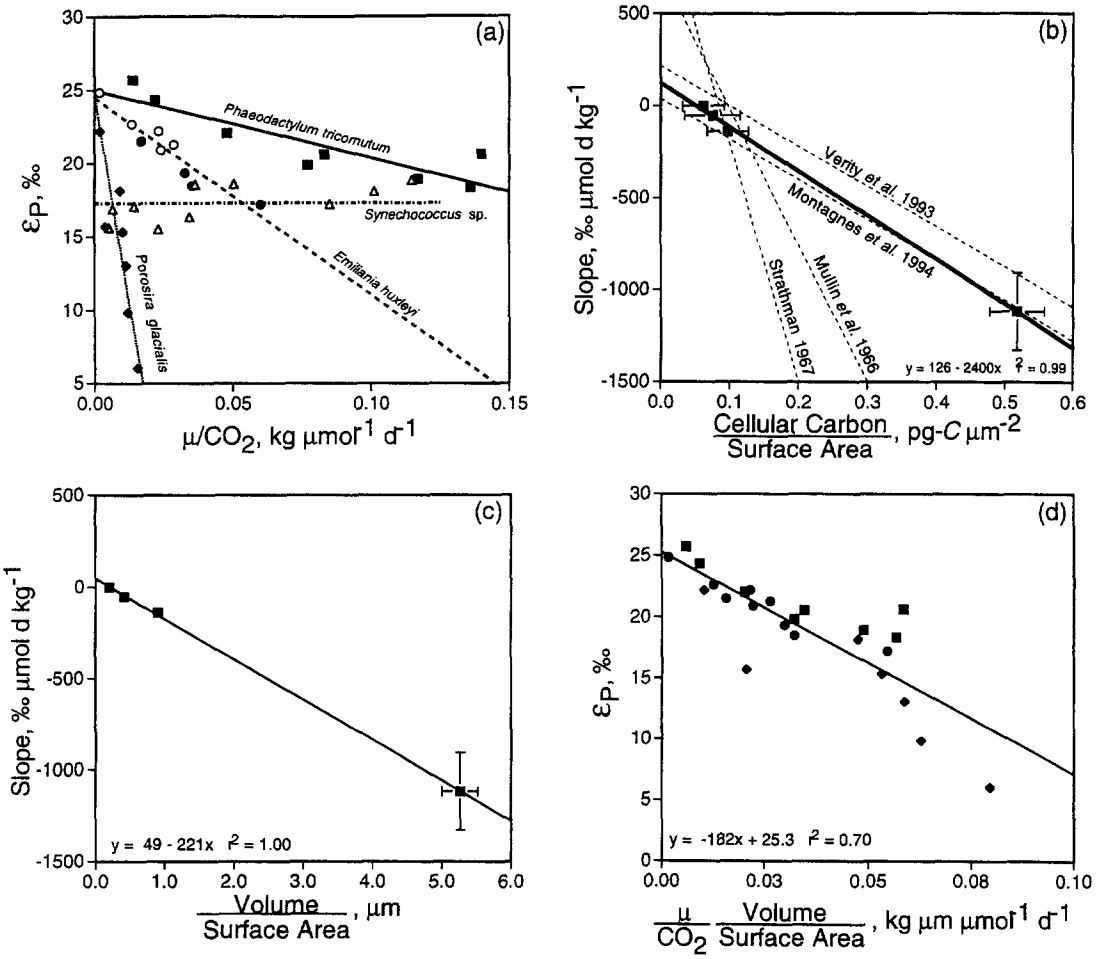

Fig. 2. Results of chemostat experiments. (a) Plot of ϵ_P *vs.* $\mu/[CO_2(aq)]$ for *P. tricornutum* (filled squares), *P. glacialis* (filled diamonds), *E. huxleyi* (noncalcifying clone open circles and calcifying clone filled circles), and *Synechococcus* sp. (open triangles). The lines represent geometric mean regression analysis (reduced major axis) and are described by: *P. tricornutum* ($\epsilon_P = 25.5 - 52.6\mu/CO_2$, $r^2 = 0.78$, n = 8); *E. huxleyi* ($\epsilon_P = 24.6 - 137.9\mu/CO_2$, $r^2 = 0.87$, n = 9); *Synechococcus* sp. (mean $\epsilon_P = 17.3$, n = 10) *P. glacialis* ($\epsilon_P = 25.5 - 1118.2\mu/CO_2$, $r^2 = 0.75$, n = 7). See also Laws et al. (1995, 1997) and Bidigare et al. (1997). (b) Plot of the slope of the ϵ_P vs. $\mu/[CO_2(aq)]$ relationship shown in 2a vs. the cellular-carbon-to-surface-area ratio. Also shown are relationships (dashed lines) based on cellular carbon estimated using cellular carbon-biovolume equations from the literature citations noted. Surface area was determined from the geometry of the cell (Fig. 1). The error bars in the y-axis represent the 95% confidence limit in the slope of the regression, error along the x-axis are standard deviations of the analyses. (c) Plot of the slope of the ϵ_P vs. $\mu/[CO_2(aq)]$ relationship shown in 2a vs. the volume-to-surface-area ratio. Surface area and volume were determined from the geometry of the cell (Fig. 1). The error bars in the y-axis represent the 95% confidence limit in the slope of the regression, error along the x-axis are standard deviations of the analyses. (d) Plot of ϵ_p vs. the product $\mu/[CO_2(aq)]$ and volume-to-surface-area ratio for the eukaryotic species shown in Fig. 2a.

28‰ have been suggested by Raven and Johnson (1991) and by Goericke et al. (1994). If a linear fit to these results is valid, then ~25‰ would appear to be a typical value for maximum fractionation associated with Rubisco and β-carboxylases for marine eukaryotic algae. Results of experiments with *Synechococcus* (CCMP838) show no systematic variation in ϵ_P with change in either $[CO_2(aq)]$ or growth rate, indicating that carbon isotopic fractionation by this organism was independent of the supply of $CO_2(aq)$ to the cell and the $CO_2(aq)$ demand within the cell. ϵ_P for these experiments average 17.3 ± 1.2‰ (n = 10). For comparison, isotopic fractionation of 21.5‰ was determined for Rubisco isolated from the freshwater cyanobacterium, *Anacystis nidulans* (Guy et al., 1987). This latter measurement did not account for effects of β-carboxylations during carbon fixation. Isotopic analyses of Rubisco in C_3 plants yield ε-values of 27-28‰ (see review by Goericke et al., 1994), also larger than the 25‰ we determined using our chemostat experiments. These results suggest that ~17‰ may be a value typical for maximum fractionation associated with Rubisco and β-carboxylases in *Synechococcus* clone CCMP838. Most importantly, the source of Rubisco appears to affect the maximum fractionation and must be considered when interpreting results of isotopic analyses of marine organic materials.

The slopes of the $\epsilon_P - \mu/[CO_2(aq)]$ lines describing the eukaryotic algae grown in chemostat culture (Fig. 2a) differ by a factor of more than 20, indicating that this relationship varies

Table 1. Results of *Synechococcus* sp. and *Porosira glacialis* chemostat studies.

Sample	DIC mmol/kg	CO_2 μmol/kg	μ d^{-1}	μ/CO_2 kg d^{-1} μmol^{-1}	$\delta^{13}C_{CO_2}$ ‰	$\delta^{13}Cp$ ‰	εp ‰
Synechococcus sp.							
SN1	2.061	12.90	0.30	0.023	−14.85	−29.91	15.53
SN2	2.033	11.60	0.40	0.035	−12.09	−27.98	16.35
SN3	2.038	13.70	0.50	0.037	−12.24	−30.25	18.58
SN4	2.000	11.91	0.60	0.050	−10.46	−28.52	18.59
SN5	1.963	8.24	0.70	0.085	−9.86	−26.61	17.21
SN6	2.071	13.76	0.20	0.015	−14.71	−31.26	17.08
SN7	2.182	18.40	0.10	0.005	−16.89	−32.03	15.64
SN8	2.142	14.96	0.10	0.008	−16.72	−33.05	16.89
SN9	1.877	6.10	0.70	0.115	−7.76	−26.12	18.85
SN10	1.887	9.80	0.90	0.101	−9.65	−27.25	18.10
Porosira glacialis							
PG1	2.167	23.00	0.09	0.004	−36.59	−51.47	15.69
PG2	2.076	18.70	0.22	0.012	−31.83	−44.29	13.04
PG3	2.106	23.40	0.27	0.016	−28.35	−34.16	6.02
PG4	2.361	26.00	0.27	0.010	−30.59	−45.23	15.34
PG5	2.347	25.79	0.32	0.012	−28.31	−37.77	9.83
PG6	2.362	23.08	0.21	0.009	−30.53	−47.81	18.15
PG7	2.643	79.90	0.17	0.002	−37.89	−58.81	22.22

greatly among species. For example, if *P. tricornutum* and *P. glacialis* were growing on the same inorganic carbon source in the same water mass at $\mu = 0.35\ d^{-1}$ with a $[CO_2(aq)] = 25$ μmol/kg, our experimental results predict that these organisms would have δ-values differing by 15‰. The mechanisms underlying these differences must be understood before the carbon isotopic record of marine organic matter can be interpreted with confidence.

Because the flux of $CO_2(aq)$ into and out of an algal cell is expected to be roughly proportional to the surface area of the cell, and specific growth rate equals the net $CO_2(aq)$ flux per cell divided by carbon per cell, Laws et al. (1995) suggested that the rate constants K_1 and K_2 should vary as a function of surface area, carbon content, and cell wall permeability (see also Francois et al., 1993; Goericke et al., 1994; Rau et al., 1996). The fact that ϵ_P predicted by the linear regression equations at $\mu/CO_2 = 0$ for eukaryotic algae equals ~25‰ suggests that for each alga, K_1 is proportional to or equal to K_2 since variation in these terms among different species would result in variability in the intercept (see Eqn. 1). Equality or proportionality in these terms makes ecological sense. $CO_2(aq)$ would be expected to cross the cell membrane under conditions of no or low growth and high $[CO_2(aq)]$ only by diffusional processes as little would be gained by expending energy to transport $CO_2(aq)$ into the cell. If we assume $K_1 \cong K_2$, Eqn. 1 may be simplified

$$\epsilon_P = \epsilon_t + (\epsilon_f - \epsilon_t)\left(1 - \frac{\mu}{KCO_2}\right) \qquad (2)$$

where the slope should reflect the dependence of K on the carbon-to-surface-area ratio of the cell, and the intercept is independent of cell size and shape.

To determine if differences in the slopes of the lines describing the ϵ_P *vs.* $\mu/[CO_2(aq)]$ relationships for the algae shown in Fig. 2a can be accounted for by variations in surface area and cellular carbon, we plotted the carbon-to-surface-area ratio determined from cell counts, CHN analyses and the geometry of the cells (Fig. 1) vs. slope (see caption Fig. 2; we assume the slope for *Synechococcus* = 0). These results (Fig. 2b) indicate that differences in surface area and cellular carbon accounts for 99% of the variation between the slopes in Fig. 2a.

It has been shown that the carbon biomass of marine microalgae varies as a function of cell volume (e.g., Strathman, 1967; Verity et al., 1993; Montagnes et al., 1994; this study). However, there is currently no consensus on the exact relationship between carbon biomass and cell biovolume (Montagnes et al., 1994), and much of the variability can be traced to artifacts associated with volume changes during fixation of samples and with electronic particle counting (Montagnes et al., 1994). Montagnes et al. (1994) found good agreement between their data and that of Verity et al. (1993) where these artifacts were minimized. To determine if a consistent relationship could be found between cell volume and carbon isotopic fractionation, we calculated cellular carbon from the biovolume of the algae grown in chemostat cultures using four published cellular carbon-biovolume relationships. The resulting cellular carbon-to-surface area ratios calculated using cellular biovolumes determined from cell geometry (Fig. 1) are compared with the experimentally derived slope using the measured $[CO_2(aq)]$ (Fig 2b). Our experimental results are in good agreement with the cellular carbon-biovolume relationships of Verity et al. (1993) and Montagnes et al. (1994) but not with Strathman (1967) and Mullin et al. (1966) perhaps because of artifacts included in these latter relationships. If we accept the relationships of Verity et al. (1993) and Montagnes et al. (1994), it suggests that differences between the slopes of the ϵ_P-$\mu/[CO_2(aq)]$ lines shown in Fig. 2a can also be accounted for by the volume-to-surface-area ratio. In fact, the relationship between slope and the volume-to-surface-area ratio is highly correlated (Fig. 2c). The generality of the relationships shown in Fig. 2b and 2c suggest that when cellular carbon cannot be easily constrained (e.g., modern and ancient field samples), the relationships of Montagnes et al. (1994) or Verity et al. (1993)

can be used to estimate cellular carbon when biovolume is known.

Several authors have suggested that, assuming spherical cell geometry, carbon isotopic fractionation by marine phytoplankton is related to cell size (Francois et al., 1993; Goericke et al., 1994; Laws et al., 1995; Rau et al., 1996). Our results indicate that ϵ_P depends not only on cell size, but also on the shape of the cell. Phytoplankton with geometry that maximize surface-area-to-volume ratios (e.g., penate diatoms, dinoflagellates) should have higher ϵ_P values under the same conditions of μ and $[CO_2(aq)]$ compared with cells with a low surface area to volume ratio, at least in natural settings which mimic our experimental conditions. A similar conclusion was also reached by Francois et al. (1993) and Goericke et al. (1994). The ϵ_P of phytoplankton with low surface area to volume ratios are more sensitive to changes in growth rate and $[CO_2(aq)]$ than that of phytoplankton with high surface area to volume ratios (see Fig. 2a). Maximum differences in δ-values among phytoplankton having different surface area to volume ratios should be observed in environments where growth rates are high and $[CO_2(aq)]$ is low.

The correlation between slope and both the cellular-carbon-to-surface-area ratio (Fig. 2b) and the volume-to-surface-area ratio (Fig. 2c) implies that there is a functional relationship between carbon isotopic fractionation and cell size and cell geometry. This relationship is consistent with the assumption that $CO_2(aq)$ enters the cell via passive diffusion through a surface boundary layer (e.g., Pasciak and Gavis, 1975; Riebesell et al., 1993) and the cell membrane. Our experiments allow this assumption to be tested. The models of Pasciak and Gavis (1975) and Riebesell et al. (1993) allow us to examine $CO_2(aq)$ diffusion through the surface boundary layer. We used Eqn. 14 in Laws et al. (1997), which is based on these models, to compare $[CO_2(aq)]$ in the bulk medium to that at the cell surface. Our calculations indicate that under conditions of highest μ/CO_2, where the effects of a surface boundary layer are expected to be most important, $[CO_2(aq)]$ at the cell membrane was more than 92% of ambient $[CO_2(aq)]$, implying that diffusion of $CO_2(aq)$ from the bulk medium through the boundary layer around the cell was probably not limiting for any of the algae or conditions examined in this study.

If passive diffusion of $CO_2(aq)$ is the mechanism for DIC uptake, then there must be sufficient $CO_2(aq)$ in the bulk medium to satisfy cellular carbon demand. We calculated the uncatalyzed rate of formation of $CO_2(aq)$ from dehydration and dissociation of bicarbonate in the growth chamber medium (using Eqn. 6 in Johnson, 1982; see also Laws et al., 1997) and compared this rate to that of net photosynthetic uptake (μC, where C = carbon cell^{-1}) under conditions of maximum μ/CO_2 to determine if, in the absence of extracellular carbonic anhydrase, conversion of $CO_2(aq)$ from bicarbonate is fast enough to support photosynthesis. Results of this calculation indicate that the rate of photosynthetic uptake was at most 3% of the uncatalyzed rate of formation of $CO_2(aq)$ from bicarbonate. This amount of available $CO_2(aq)$ must be considered a minimum, since the presence of any carbonic anhydrase in our cultures could have dramatically increased the rate of dehydration and dissociation of bicarbonate. Hence, $[CO_2(aq)]$ in the bulk medium cannot be considered a limiting factor in any of our experiments.

Although the relationship between ϵ_P and μ/CO_2 of the form described by Eqn. 2 is consistent with a passive diffusion uptake mechanism, we suspected that other mechanisms might yield a similar relationship. In order to explore this further, Eqn. 2 was rewritten in terms of nondimensional variables X and e, where $X = CO_2\ K/\mu$ and $\epsilon = (\epsilon_P - \epsilon_t)/(\epsilon_f - \epsilon_t)$. In terms of these variables Eqn. 2 becomes

$$\epsilon = 1 - \frac{1}{X} \tag{3}$$

Laws et al. (1997) have shown that this relationship can be derived from a more general model of inorganic carbon uptake formulated by Francois et al. (1993). The more general model is basically an algebraic manipulation of the mass balance equations for ^{12}C and ^{13}C, assuming that $CO_2(aq)$ diffuses through the plasmalemma but making no assumptions about what other mechanisms may be associated with CO_2 uptake. If we define C_i to be the $CO_2(aq)$ concentration immediately inside the plasmalemma and $Y = C_i\ K/\mu$, the general model takes the form

$$\epsilon = \frac{Y}{1 + Y} \tag{4}$$

If uptake occurs entirely by diffusion, then $X - 1 = Y$, i.e., gross diffusion of $CO_2(aq)$ into the cell minus carbon fixation equals gross diffusion of $CO_2(aq)$ out of the cell. In that case $\epsilon = \frac{Y}{1+Y} = \frac{X-1}{X} = 1 - \frac{1}{X}$, which is identical to Eqn. 3. If $CO_2(aq)$ enters the cell by some mechanism in addition to passive diffusion, then it is reasonable to assume that Y will exceed $X - 1$. If $Y = \beta X - 1$, where $\beta > 1$, then

$$\epsilon = \frac{Y}{1 + Y} = \frac{\beta X - 1}{\beta X} = 1 - \frac{1}{\beta X} \tag{5}$$

Equation 5 has the same form as Eqn. 3, with βX replacing X, or in terms of dimensional variables, with βK replacing K. The implication of this analysis is that as long as the relationship between the concentration of carbon dioxide in the medium and C_i obey the relationship

$$C_i = \beta CO_2 - \frac{\mu}{K} \tag{6}$$

then there will exist a linear relationship between ϵ_P and μ/CO_2 of the form

$$\epsilon_p = \epsilon_t + (\epsilon_f - \epsilon_t)\left(1 - \frac{\mu}{\beta K CO_2}\right) \tag{7}$$

The linearity of the relationship between ϵ_P and μ/CO_2 under these conditions would be consistent with uptake via passive diffusion, and the apparent permeability of the plasmalemma would be βK. Thus linearity of the relationship between ϵ_P and μ/CO_2, while consistent with uptake via passive diffusion, by no means rules out uptake via other mechanisms. The only criterion for linearity is Eqn. 7, and Eqn. 7 is consistent with passive diffusion only when $\beta = 1$. Active transport of $CO_2(aq)$ requires that $K_1 > K_2$ in Eqn. 1.

If uptake of $CO_2(aq)$ is facilitated by an active transport mechanism, then the value for ϵ_t may be in error. This value is presumed to be 0.7‰, the isotope effect determined for diffu-

Table 2. Pertinent cellular data and comparison of calculated and measured slope of the ϵ_p-μ/CO_2 relationship (see text).

Sample	Surface area μm^2	Volume μm^3	C cell^{-1} pg cell^{-1}	μ d^{-1}	b/μ[1] ‰ μmol d kg^{-1}	Slope[2] ‰ μmol d kg^{-1}
P. tricornutum	100.6	42.5	7.6	1.4	−17	−53
E. huxleyi	87.6	77.1	8.3	0.6	−22	−138
Synechococcus sp.	5.8	1.0	0.36	1.0	−4	0
P. glacialis	3886	20410	2015	0.3	−118	−1118

[1] b calculated using Eqn. 15 in Rau et al. (1996) assuming ϵ_f = 25‰, $P = 1 \times 10^{-4}$ m s^{-1}.
[2] See caption Figure 2a.

sion of CO_2(aq) in fresh water at 25°C (O'Leary, 1984). Although it is not known if isotopic discrimination occurs during active trtransport of CO_2(aq) across the plasmalemma, it is generally assumed that the effect is small (Kerby and Raven, 1985; Berry, 1989; Raven and Johnson, 1991; Hayes, 1993; Goericke et al., 1994). We assume here that the kinetic isotope effect associated with active transport and passive diffusion of CO_2(aq) across the plasmalemma are similar and equal to ~0.7‰. This does not imply that active transport does not affect the magnitude of ϵ_p. This will be addressed below.

The above discussion indicates that we cannot determine the mechanism by which CO_2(aq) enters the cell based only on linear relationships between ϵ_p and μ/CO_2. We can, however, test the assumption of passive diffusion by comparing our experimental results with results predicted using the fractionation model based on diffusive molecular uptake by Rau et al. (1996). Equation 15 of Rau et al. (1996) provides an explicit solution for b, where b is the slope of the relationship $\epsilon_p = \epsilon_f - b/CO_2$. We can calculate b from our measured growth rate, carbon biomass, and surface area (Table 2). However, direct comparison of the slope of the ϵ_p *vs.* $\mu/[CO_2(aq)]$ relationship determined from the chemostat experiments to that calculated using Eqn. 15 of Rau et al. (1996) requires that we factor out μ. These results (Table 2) indicate that the diffusion-based fractionation model overestimates the slopes obtained from chemostat observations. One factor in Eqn. 15 of Rau et al. (1996) that has a high degree of uncertainty is the apparent cell wall permeability, which is equivalent to βK in Eqn. 7. Values of βK calculated from chemostat experimental results for the eukaryotic species (i.e., those data with high correlation coefficients, see caption Fig. 2) suggest apparent permeability ranging from 1-4 $\times 10^{-5}$ m s^{-1}, values which are consistent with permeability estimated using an energy minimization model for *P. tricornutum* (Laws et al., 1997). These values are a factor of four to ten times lower than the permeability postulated by Rau et al. (1996).

It is reasonable to ask if our calculated apparent permeability is consistent with diffusion of CO_2(aq) across the plasmalemma. Estimates of permeability range from 3.5 x 10^{-3} m s^{-1} (Gutknecht et al., 1977) to 1×10^{-7} m s^{-1} (Raven, 1988). The highest value was determined using an artificial phospholecthin/cholesterol membrane and was not considered by the authors to be representative of plasma membranes in plants (Gutknecht et al., 1977; see also Gimmler et al., 1990). Gimmler et al. (1990) recently found values of ~10^{-6} m s^{-1} typical of plasma membranes in aquatic plants but noted that conductivities of CO_2(aq) were lower for experiments performed in the dark. These results implied that in experiments performed in the light, conductivity of CO_2(aq) across the plasma membrane was augmented by facilitated diffusion or active transport (Gimmler et al., 1990). In one of the algal species studies by Gimmler et al. (1990), Rotatore et al. (1992) subsequently found rapid uptake of CO_2(aq) upon the illumination of darkened cells which could not be attributed to CO_2-fixation. Rotatore et al. (1992) concluded that the facilitated transport of CO_2(aq) resulted in higher permeability and was probably driven by ATP as the energy source via a CO_2-ATPase probably located in the plasmalemma. The energy driving this inorganic-carbon-concentrating mechanism may be derived from pseudocyclic photophosphorylation (Sültemeyer et al., 1993). If this effect occurs in marine phytoplankton, it implies that light level may have an affect on fractionation through the production of ATP which could be independent of growth rate. Wayne et al. (1994) found independent evidence for facilitated CO_2(aq) transport across the plasmalemma in a study of water and CO_2(aq) permeability in single internodal cells of *Chara corallina*. These authors suggested "when cells are grown under low CO_2(aq) conditions, the plasma membrane permeability to CO_2(aq) may increase as a result of changes in the lipid bilayer and/or the addition of CO_2(aq) transport proteins." Taken together, results of these studies argue that measurements of plasmalemma permeabilities to CO_2(aq) greater than 10^{-5} m s^{-1} may include effects of active transport and may not be indicative of permeabilities associated only with passive diffusion.

As noted above, overwhelming evidence exists for active transport of inorganic carbon in aquatic microalgae. Recently, Laws et al. (1997) concluded that at growth rates >0.5 d^{-1}, most of the CO_2(aq) entered the cell of *P. tricornutum* grown in chemostat cultures via active transport. This interpretation was based on the observation that ϵ_p was found to be a nonlinear function of μ/CO_2 at high values of μ/CO_2 although the results were well approximated by a linear relationship for $0 < \mu/CO_2 < 0.3$ kg d^{-1} μmoles^{-1} (Laws et al., 1997). These authors found that the nonlinearity resulted in positive curvature; a result inconsistent with active transport of bicarbonate. Facilitated transport of CO_2(aq) across the plasmalemma could be achieved by either active uptake of CO_2(aq) (perhaps via an CO_2-ATPase) or conversion of bicarbonate to CO_2(aq) by an external carbonic anhydrase followed by diffusion (or active transport) of CO_2(aq) across the cell membrane (Laws et al., 1997). Since several studies have documented that intracellular concentrations of CO_2(aq) exceed concentrations in the growth medium (Zenvirth and Kaplan, 1981; Badger and Andrews, 1982; Burns and Beardall, 1987), and since authors have concluded that CO_2(aq) permeability through the plasma mem-

brane is $\leq 10^{-5}$ m s^{-1} (Raven, 1988; Gimmler et al., 1990), the most parsimonious conclusion is that the algae in our experiments satisfied their carbon requirements by both diffusion and active transport of CO_2(aq) across the plasmalemma. It should be noted that the ability of *E. huxleyi* to actively transport DIC is a matter of an ongoing debate (Raven, 1993; Nimer et al., 1996). Since our permeability calculation for *E. huxleyi* is similar to that for *P. tricornutum* and several authors (Burns and Beardall, 1987; Raven, 1993; Colman and Rotatore, 1995) conclude that *P. tricornutum* has the ability to actively assimilate inorganic carbon, we conclude that, under our experimental conditions, *E. huxleyi* obtained some of its inorganic carbon by active assimilation.

Although equations in the form of Eqn. 2, which have been proposed by several authors (Francois et al., 1993, Goericke et al., 1994; Laws et al., 1995; Rau et al., 1996), provide reasonable empirical descriptions of carbon isotopic fractionation in marine microalgae, the underlying assumption of passive diffusion of CO_2(aq) into the cell may not be strictly valid under most natural conditions. But even if active transport supplies a portion of the DIC requirements of the cell, this process does not appear to affect the linearity of the ϵ_P vs. μ/CO_2 relationship under the growth and CO_2(aq) conditions found in most of the contemporary ocean.

4. CONCLUSIONS

Further studies are required to clearly separate the effects of growth rate, cell geometry, and [CO_2(aq)] on ϵ_P. Although results of our experiments suggest that CO_2(aq) does not cross the plasmalemma by passive diffusion alone, but rather is supplemented by an active transport mechanism, the inescapable conclusion is that ϵ_P nonetheless varies as a linear function of growth rate, [CO_2(aq)] and the cellular-carbon-to-surface-area ratio under most natural conditions. One implication of this conclusion is that active uptake of inorganic carbon is proportional to intracellular carbon demand or growth rate. Our results are consistent with transport of CO_2(aq) (not bicarbonate) across the plasmalemma and indicate that the flux must be proportional to cell surface area over a considerable range of cell sizes. Our results suggest that carbon isotopic fractionation in marine phytoplankton can be quantified by knowing only (1) ϵ_f, (2) growth rate, (3) [CO_2(aq)], and (4) cellular carbon-to-surface area ratio or volume-to-surface area ratio. Furthermore, our results imply that the poor correlation between [CO_2(aq)] and carbon isotopic fractionation determined from isotopic analyses of bulk marine suspended organic matter in the contemporary ocean (e.g., Goericke and Fry, 1994; Francois et al., 1993; Popp et al., 1997) can be explained by natural variations in growth rate, the source of Rubisco and algal cell geometry.

Our findings have implications for estimating reliably paleo-[CO_2(aq)] levels from ϵ_P based on analyses of bulk organic matter or biomarkers (e.g., Arthur et al., 1985; Jasper and Hayes, 1990; Rau et al., 1991; Freeman and Hayes, 1992; Jasper et al., 1994). Workers wishing to determine paleo-[CO_2(aq)] based on stable carbon isotopic compositions of organic matter and biomarkers must have some means of estimating (or constraining) the size, shape, and growth rate of the organisms producing the organic matter analyzed before an empirical relationship such as Eqn. 2 can be applied. We suggest that the results shown in Fig. 2d provide a preliminary calibration for eukaryotic microalgae. Although some scatter in these data may result from surface irregularities or micropores present on the surface of some of these algae, this calibration may be used when growth rates can be estimated (perhaps using a T-μ relationship, e.g., Eppley, 1972; see Eqn. 3 in Popp et al., 1997) and cell size and geometry can be constrained. Refinements to this calibration will require further study of carbon-volume relationships and of ϵ_P vs. μ/CO_2 relationships on algae with well characterized cellular properties.

We suggest that future field studies include estimates of cell size and geometry. Cell geometry can only be quantitatively constrained when the source of the phytoplankton carbon analyzed is known. One example in which this can be accomplished is through isotopic analyses of the matrix-bound organic materials in the tests of fossil diatoms (Shemesh et al., 1992; Singer and Shemesh, 1995). In this case, the origins of the matrix organic materials analyzed and cell geometry can be constrained using the morphology of the diatom frustrule. Isotopic analyses of alkenones in sediments provides another way to constrain the size and shape of the source organism. Biomarkers in the form of long-chain (C_{37}, C_{38}, and C_{39}) alkenones are known to be produced in open-oceanic waters only by *E. huxleyi* and the closely related *Gephyrocapsa oceanica* (Marlowe et al., 1990; Brassell, 1993). Thus variations in cellular-carbon-to-surface area ratio should be relatively small because of limited variability in cell size and shape. Recently, Bidigare et al. (1997) showed that ϵ_P of alkenone-producing algae in natural marine environments varied systematically with the concentration of dissolved phosphate. These authors suggested that where both Cd/Ca and the isotopic composition of C_{37} alkadienones can be determined, it may be possible to use relationships between [PO_4] and Cd/Ca ratios in shells of planktonic foraminifera to constrain growth rate variations and accurately estimate paleo-[CO_2(aq)].

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