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A model of photosynthetic ^{13}C fractionation by marine phytoplankton based on diffusive molecular CO_2 uptake

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ABSTRACT: A predictive model of carbon isotope fractionation (ε_p) and abundance ($\delta^{13}C_{phyto}$) is presented under circumstances where photosynthesis is strictly based on CO_2(aq) that passively diffuses into marine phytoplankton cells. Similar to other recent models, the one presented here is based on a formulation where the expression of intracellular enzymatic isotope fractionation relative to that imposed by CO_2(aq) transport is scaled by the ratio of intracellular to external [CO_2(aq)], c_i/c_e. Unlike previous models, an explicit calculation of c_i is made that is dependent on c_e as well as cell radius, cell growth rate, cell membrane permeability to CO_2(aq), temperature, and, to a limited extent, pH and salinity. This allows direct scaling of c_i/c_e to each of these factors, and thus a direct prediction of ε_p and $\delta^{13}C_{phyto}$ responses to changes in each of these variables. These responses are described, and, where possible, compared to recent experimental and previous modeling results.

KEY WORDS: $\delta^{13}C$ · Photosynthesis · Isotope fractionation · Phytoplankton · CO_2 · Modeling

INTRODUCTION

The significant variability of the $^{13}C/^{12}C$ of marine organic matter and its potential as an indicator or proxy for specific biogeochemical conditions has prompted investigation over the past several decades (e.g. recent reviews by Descolas-Gros & Fontugne 1990, Sackett 1991, Hayes 1993, Goericke et al. 1994). Interest has recently focused on the relationship between molecular CO_2 concentration, [CO_2(aq)], and marine organic $\delta^{13}C$ ($\delta^{13}C_{org}$) and thus the possible use of this latter measure to reconstruct the ambient [CO_2(aq)] in which the organic matter was formed (e.g. Jasper et al. 1994, Rau 1994). Based on theoretical considerations of photosynthetic isotope fractionation as originally formulated by Farquhar et al. (1982), such a link is anticipated when marine organic matter is photosynthetically formed from CO_2(aq) that passively enters autotrophic cells via diffusion (Rau et al. 1992, Francois et al. 1993, Goericke et al. 1994). Indeed, a variety of evidence from laboratory experimentation, the modern ocean, and the sedimentary record often shows a strong inverse correlation between marine $\delta^{13}C_{org}$ and [CO_2(aq)] (e.g. Freeman & Hayes 1992, Rau 1994).

Raven et al. (1993) have pointed out, however, that such influences on isotope abundance may be indirectly linked to [CO_2(aq)] via a greater preponderance of HCO_3^- utilization or non-passive inorganic carbon uptake under conditions of reduced [CO_2(aq)]. Significant isotopic effects by these processes have been demonstrated experimentally (e.g. Beardall et al. 1982, Sharkey & Berry 1985). Also, if operating independently of [CO_2(aq)], these preceding factors plus a variety of other physiological effects such as intracellular carbon demand (Rau et al. 1992, Francois et al. 1993) or growth rate (Fry & Wainright 1991, Laws et al. 1995), carbon fixation pathways (Descolas-Gros & Fontugne 1990, Falkowski 1991), cell size (Fry & Wainright 1991,

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Goericke et al. 1994), and cell membrane permeability (Francois et al. 1993), among other factors, could intervene to modify, weaken, or eliminate the effect of $[CO_2(aq)]$ on marine photosynthetic isotope fractionation and hence marine $\delta^{13}C_{org}$ (e.g. Goericke & Fry 1994). If isotope abundances in phytoplankton and in subsequent consumer, detrital, and sedimentary organic remains are to be interpretable in the context of one or more of the preceding variables, it is essential to know the isotopic sensitivity and response to such factors.

In studying the potential for $CO_2(aq)$ limitation in diatoms, Riebesell et al. (1993) developed a model that is of direct relevance to the abovementioned issues. In particular, this model predicts $[CO_2(aq)]$ at the cell surface (c_r) when cell radius, growth rate, temperature, salinity, and pH are specified. Under conditions when marine photosynthetic organic matter formation is strictly based on $CO_2(aq)$ that passively diffuses into autotrophic cells, c_r places an upper limit on the $[CO_2(aq)]$ within the cell, c_i. This in turn can be used to constrain the $\delta^{13}C_{org}$ of phytoplankton biomass, $\delta^{13}C_{phyto}$, via an equation derived from Farquhar et al. (1982):

$$\delta^{13}C_{phyto} = \delta^{13}C_{c_e} - \varepsilon_d - (\varepsilon_f - \varepsilon_d)\frac{c_i}{c_e} \quad (1)$$

where $\delta^{13}C_{c_e}$ is the $\delta^{13}C$ of ambient $CO_2(aq)$, ε_d is the isotope fractionation associated with diffusive transport of $CO_2(aq)$ in water, ε_f is the isotope fractionation associated with enzymatic, intracellular carbon fixation (see Table 1 for listing of symbols, definitions, and units).

While values for most of these independent variables are known or can be approximated, a principle unknown is c_i. This variable has been only indirectly measured either as a function of intracellular pH (e.g. Badger et al. 1980, Beardall & Raven 1981) or via a rearrangement of Eq. (1) when the remaining variable values are supplied (review by Raven 1993). Instead we use an extension of the Riebesell et al. (1993) model in conjunction with a consideration of cell wall permeability to $CO_2(aq)$ to solve for c_i, and hence $\delta^{13}C_{phyto}$ via Eq. (1). We subsequently quantify model output and sensitivity to changes in the primary independent variables, and compare these to previous models and experimental observations.

MODEL DESCRIPTION

Our approach is to use the Farquhar et al. (1982) formulation (Eq. 1) to determine $\delta^{13}C_{phyto}$ using an explicit solution for c_i when temperature, cell radius, cell growth rate, and cell membrane permeability to $CO_2(aq)$ are specified. In this initial application we input c_e as an independent variable with a base value of 12×10^{-3} mol m^{-3} (12 µM) (Table 1). However, c_e could also be dependently determined via the formulation of Weiss (1974) when seawater pCO_2 and temperature (T_C, °C) and salinity (S) are given. Similarly, at mean ocean salinities, any one of the variables T_C, c_e, or pCO_2 could be derived when the other 2 are specified.

While ε_f may range from 20 to 29‰ (e.g. Goericke et al. 1994), we adopt an intermediate value of 25‰ in our initial base model. We assume $\varepsilon_d = 0.7$‰ (O'Leary 1984), and ignore the apparently small temperature sensitivity of this parameter (Schönleber 1976). $\delta^{13}C_{c_e}$ is treated as a dependent variable such that:

$$\delta^{13}C_{c_e} = \delta^{13}C_{\Sigma CO_2} + 23.644 - \frac{9701.5}{T_K} \quad (2)$$

following the treatments of Mook et al. (1974) and Freeman & Hayes (1992). For example, our base model specifies $\delta^{13}C_{\Sigma CO_2} = +1.7$‰, hence $\delta^{13}C_{c_e} = -8.1$‰ at a base surface ocean temperature T_K of 290.15 K (T_C = 17°C) (Table 1).

The $[CO_2(aq)]$ inside the cell (c_i) is calculated in 2 steps: First, $[CO_2(aq)]$ at the cell surface (c_r) is derived by using the solution of the diffusion-reaction equation in Riebesell et al. (1993). Then c_i is calculated from c_r assuming strict photosynthetic dependence on $CO_2(aq)$ that passively diffuses into the cell via a cell membrane that has specified permeability to $CO_2(aq)$ (see below). c_r is calculated according to

$$c_r = c_e - \frac{Q_r}{4\pi r D_T(1 + r/r_k)} \quad (3)$$

where Q_r is the rate of CO_2 uptake per phytoplankton cell (mol C s^{-1}), r is the radius (in meters) of a sphere whose surface area is equivalent to that of the cell, D_T is the temperature-dependent diffusion coefficient of $CO_2(aq)$ in seawater (m^2 s^{-1}), and r/r_k represents the relative contribution to the CO_2 flux by extracellular spontaneous conversion of HCO_3^- to CO_2 (see Riebesell et al. 1993). It can be shown that diffusional rates/processes about a phytoplankter can be accurately modeled when it is assumed to be a sphere whose surface area is equal to that of the organism, largely irrespective of the cell's actual shape and volume (Wolf-Gladrow & Riebesell unpubl.). Q_r is determined from the carbon content per cell γ_c (mol C) and the instantaneous growth rate μ_i according to

$$Q_r = \gamma_c \mu_i \quad (4)$$

where μ_i (in units of s^{-1}) is related to the specific growth rate μ according to

$$\mu_i = \mu \frac{L + D}{L}$$

Table 1 Listing of symbols, definitions, and base values used in the phytoplankton carbon isotope fractionation model described in the text. Values in parentheses are base values in units commonly encountered in the marine literature. *Indicates independent variables

Variable	Definition	Base value	Units
α	CO_2 solubility	3.62×10^{1}	mol m^{-3} atm^{-1}
b	$= (\varepsilon_p - \varepsilon_f)c_e$ (c_e in µM)	-127.2	‰ µM
c_e	Ambient $[CO_2(aq)]$*	12×10^{-3}	mol m^{-3} (= 12 µM)
c_i	Intracellular $[CO_2(aq)]$	6.64×10^{-3}	mol m^{-3} (= 6.64 µM)
c_f	$[CO_2(aq)]$ at cell surface	9.87×10^{-3}	mol m^{-3} (= 9.87 µM)
$\delta^{13}C$	$^{13}C/^{12}C$ relative to PDB standard	–	per mil, ‰
$\delta^{13}C_{phyto}$	$\delta^{13}C$ of bulk phytoplankton biomass	-22.2	per mil, ‰
$\delta^{13}C_{\Sigma CO_2}$	$\delta^{13}C$ of total dissolved inorganic carbon*	1.7	per mil, ‰
$\delta^{13}C_{c_e}$	$\delta^{13}C$ of c_e	-8.1	per mil, ‰
$\delta^{13}C_{c_i}$	$\delta^{13}C$ of c_i	2.8	per mil, ‰
$\Delta^{13}C$	$= \delta^{13}C_{\Sigma CO_2} - \delta^{13}C_{phyto}$	23.9	per mil, ‰
D_T	Temperature-sensitive diffusivity of $CO_2(aq)$ in seawater	1.45×10^{-9}	m^2 s^{-1}
ε_d	Diffusive isotope fractionation of $CO_2(aq)$ in seawater*	0.7	per mil, ‰
ε_f	Enzymatic isotope fractionation associated with intracellular C fixation*	25	per mil, ‰
ε_p	$= \delta^{13}C_{c_e} - \delta^{13}C_{phyto}$	14.1	per mil, ‰
E_d	Activation energy (diffusion)*	19510	J mol^{-1}
E_k	Activation energy (reaction)*	6.28×10^{4}	J mol^{-1}
γ_c	Carbon content per cell	1.76×10^{-11}	mol C
k'	Rate coefficient	3.41×10^{-2}	s^{-1}
k_1	Rate coefficient	8500	m^3 mol^{-1} s^{-1}
k_2	Rate coefficient	3×10^{-5}	s^{-1}
μ	Specific growth rate = (Cell doubling time)$^{-1}$*	1.16×10^{-5}	s^{-1} (= 1 d^{-1})
μ_i	Instantaneous cell growth rate (= 2μ if light:dark = 12 h:12 h)	2.31×10^{-5}	s^{-1} (equivalent to μ = 1 d^{-1})
ν_{fw}	Dynamic viscosity of freshwater	8.9×10^{-6}	kg m^{-1} s^{-1}
ν_{sw}	Dynamic viscosity of seawater	9.5×10^{-6}	kg m^{-1} s^{-1}
P	Cell wall permeability to $CO_2(aq)$*	10^{-4}	m s^{-1}
pH	$-\log_{10}[H^+]$*	8.2	–
Q_f	$CO_2(aq)$ uptake rate per cell	4.06×10^{-16}	mol C $cell^{-1}$ s^{-1}
Q_s	$CO_2(aq)$ uptake rate per unit cell surface area	3.23×10^{-7}	mol C m^{-2} s^{-1}
r	Surface area equivalent cell radius*	10^{-5}	m (= 10 µm)
r_k	Reacto-diffusive length	2.06×10^{-4}	m
R	Gas constant*	8.3143	J K^{-1} mol^{-1}
S	Salinity*	35	psu
T_C	Temperature*	17	°C
T_K	Temperature (= T_C + 273.15)*	290.15	K
V	Cell volume	4188.8	μm^3

with L and D representing the duration of light and dark periods, respectively. In the base model it was assumed that $L = D$ and therefore $\mu_i = 2\mu$. The specific growth rate μ is conventionally defined as the inverse of cell doubling time. The cell carbon content γ_c (mol C) is calculated from cell volume V (in μm^3) assuming the volume-to-carbon content relationship of Strathmann (1967)

$$\gamma_c = 3.154 \times 10^{-14} V^{0.758} \quad (5)$$

It should be noted that cell volume-to-carbon content relationships in marine phytoplankton can vary significantly (see Montagnes et al. 1994). For comparison of model predictions with experimental data we therefore suggest direct measurement of phytoplankton cell carbon content. Also, if the cell can be assumed to be a sphere as is initially the case in our model, then $V = \pi r^3 4/3$. For cells that significantly deviate from a spherical shape, a separate means of V estimation is required.

The reacto-diffusive length r_k is given as

$$r_k = \sqrt{\frac{D_T}{k'}} \quad (6)$$

with

$$k' = k_1[OH^-] + k_2 \quad (7)$$

where k_1 = 8500 l mol^{-1} s^{-1}, k_2 = 0.03 s^{-1} (both at 25°C) and $[OH^-]$ is the hydroxyl ion concentration. The hydroxyl and hydrogen ion concentrations are related by $[OH^-] = K_w/[H^+]$ with the hydrogen ion concentration $[H^+] = 10^{-pH}$, the ion product of water $K_w = 10^{-pK_w}$ and

$$pK_w = \frac{3441.0}{T_K} + 2.241 - 0.09415\sqrt{S}$$

(S = salinity in psu). Both D_T and r_k vary with temperature.

An approximation for the temperature dependence of D_T is given by Jähne et al. (1987), who measured molecular diffusivity in freshwater in the temperature range from 5 to 35°C and applied an Arrhenius fit to the data with

$$D_T = 5.019 \times 10^{-6} e^{-\left(\frac{E_d}{RT_K}\right)} \quad (8)$$

where the activation energy E_d = 19510 J mol^{-1} and the gas constant R = 8.3143 J K^{-1} mol^{-1}. To correct for differences in the dynamic viscosity of freshwater and seawater (Li & Gregory 1974), Eq. (8) is multiplied with

$$\frac{\nu_{fw}}{\nu_{sw}} = 0.9508 - 7.389 \times 10^{-4} T_C \quad (9)$$

where ν_{fw} and ν_{sw} are the dynamic viscosities of freshwater and seawater, respectively (Wolf-Gladrow & Riebesell unpubl.). Temperature dependence of k_1 is given by

$$k_1(T_K) = k_1(T_{K_o}) \frac{e^{-\left(\frac{E_k}{RT_K}\right)}}{e^{-\left(\frac{E_k}{RT_{K_o}}\right)}} \quad (10)$$

where the temperature T_{K_o} = 298.15 K (25°C), and the activation energy $E_k = 6.28 \times 10^4$ J mol^{-1} (15 kcal mol^{-1}) (Stumm & Morgan 1981). For k_2 we use the same temperature dependence.

The CO_2 flux into the cell per unit surface area of cell membrane, Q_s, equals $Q_r/4\pi r^2$ and is proportional to the concentration gradient between c_i and c_r according to

$$Q_s = P(c_r - c_i) \quad (11)$$

The proportionality coefficient P is called membrane CO_2(aq) permeability (in m s^{-1}; see Nobel 1983 for a discussion of the permeability concept). Solving for c_i using Eqs. (3) & (11) yields

$$c_i = c_r - \frac{Q_s}{P} = c_e - Q_s\left(\frac{r}{D_T(1 + r/r_k)} + \frac{1}{P}\right) \quad (12)$$

Inserting into Eq. (1),

$$\delta^{13}C_{phyto} = \delta^{13}C_{c_e} - \varepsilon_f + (\varepsilon_f - \varepsilon_d)\frac{Q_s}{c_e}\left(\frac{r}{D_T(1 + r/r_k)} + \frac{1}{P}\right) \quad (13)$$

Defining ε_p as the overall expression of photosynthetic isotope fractionation, equivalent to $\delta^{13}C_{c_e} - \delta^{13}C_{phyto}$, a general form of the relation between ε_p and c_e, again based on the Farquhar et al. (1982) model, is (Jasper et al. 1994):

$$\varepsilon_p = \varepsilon_f + \frac{b}{c_e} \quad (14)$$

A rearrangement of Eq. (13) offers an explicit solution to b such that

$$b = -(\varepsilon_f - \varepsilon_d)Q_s\left(\frac{r}{D_T(1 + r/r_k)} + \frac{1}{P}\right) \quad (15)$$

Significant variations in b (−109 ± 14 to −164 ± 7‰ µM) have been observed in empirical fits to field and experimental data (Jasper et al. 1994, Laws et al. 1995). The influences on b by growth rate, membrane CO_2(aq) permeability, cell radius, and boundary layer thickness have also been qualitatively explored by Francois et al. (1993) and Goericke et al. (1994). However, our model offers the first quantitative formulation of these factors in solving for b, and also adds a consideration of temperature-sensitive CO_2(aq) diffusion. In the sections that follow we show that reasonable base values for these independent variables (Table 1) produce realistic $\delta^{13}C_{phyto}$ and ε_p. We then show the sensitivity of $\delta^{13}C_{phyto}$ and ε_p to changes in each of the primary independent variables, compare these model responses and sensitivities to previously published experimental results, and compare our model behavior to earlier models of $\delta^{13}C_{phyto}$ and ε_p. Comparisons between our model predictions and observations in the modern ocean and the sedimentary record are planned as a separate paper.

RESULTS AND DISCUSSION

Specifying a base condition for all independent variables in our model (Table 1) yields a $\delta^{13}C_{phyto}$ of −22.2‰, a value well within the $\delta^{13}C_{org}$ range observed in the ocean (approx. −35 to −15‰; e.g. Rau et al. 1989). While this outcome is encouraging, it does not by itself represent a very compelling validation of our model; any number of other parameter values could lead to the same result. It does, however, provide a starting point from which isotopic responses to changes in these independent variables can be investigated. That is, by allowing only one independent variable to change while holding all others constant at their base value, we can explore the sensitivities of our modeled ε_p and $\delta^{13}C_{phyto}$ to changes in each individual factor.

For example, by allowing μ in our base model to increase from 0 to 2.3 d^{-1}, (i.e. μ_i to increase from 0 to 5.3×10^{-5} s^{-1}) an ε_p and $\delta^{13}C_{phyto}$ change of approximately 25‰ is elicited (Fig. 1D). The linearity of this isotopic response to μ within models based on the Farquhar et al. (1982) formulation (Eq. 1) has been previously noted (Goericke et al. 1994, Laws et al. 1995). The reason for this effect is that increasing growth rate also increases carbon demand and hence carbon flux into the cell ($Q_{r,s}$; Fig. 1A). This in turn promotes an increasing difference (mass disequilibrium) among external, cell surface, and intracellular [CO_2(aq)] (c_e, c_r

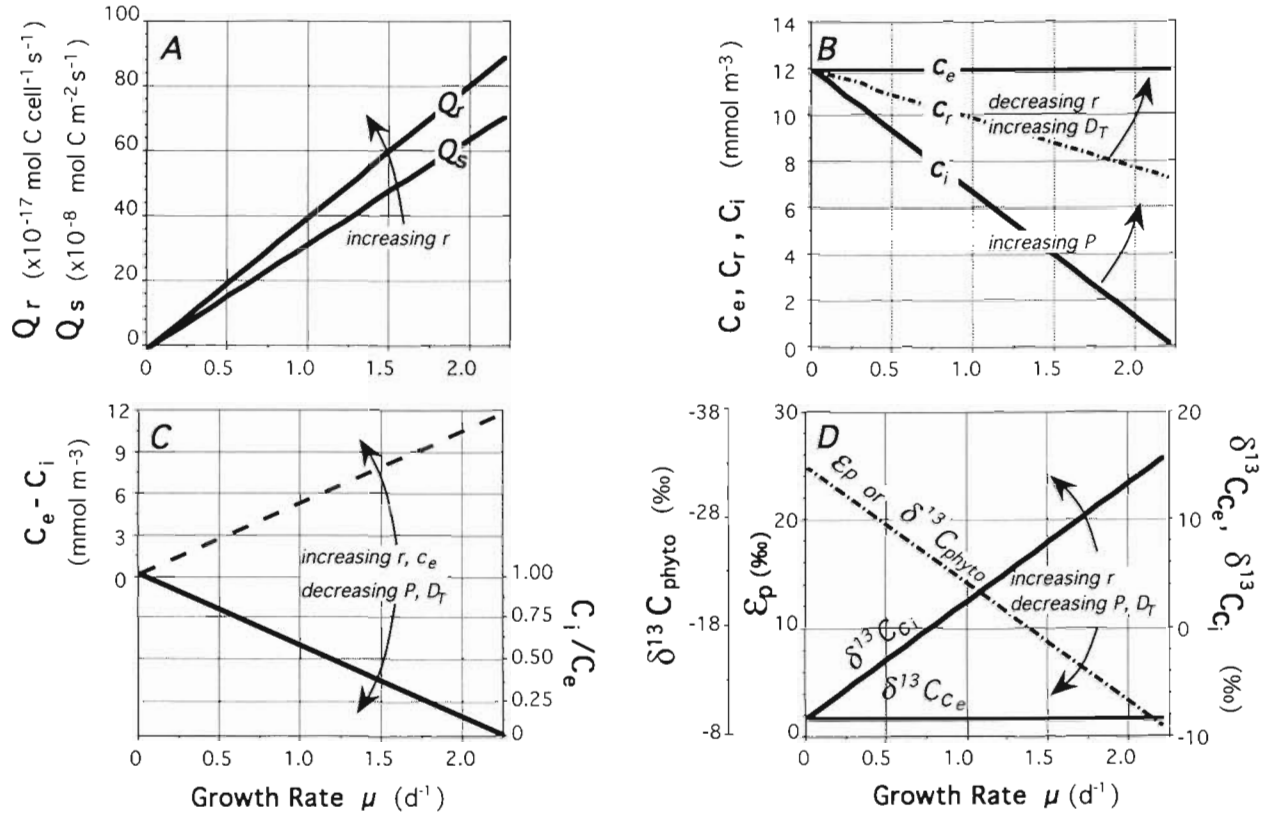

Fig. 1 Model response to changing growth rate, μ, when all other independent variables are held at their base value (see Table 1). (A) Carbon flux per cell (Q_r) and per cell surface area (Q_s). Arrow denotes effect on line slope of changing cell radius, r. (B) [CO_2(aq)] external to the cell (c_e), at the cell surface (c_r), and within the cell (c_i). Arrows denote effect of changing r, diffusivity (D_T) or permeability (P) in decreasing the difference between c_e and c_r, and between c_r and c_i at any given μ. (C) $c_e - c_i$ and c_i/c_e relative to μ. Arrows denote effect of changing r, c_e, D_T, or P. (D) $\delta^{13}C_{c_e}$, $\delta^{13}C_{c_i}$, ε_p, and $\delta^{13}C_{phyto}$ versus μ based on model described in text. Effects of increasing r or c_e, or decreasing D_T or P are indicated with arrows

and c_i; Fig. 1B). As denoted by arrows in Fig. 1A and B, the sensitivity of Q (and in turn c_r and c_i) to μ also increases with increasing cell radius, r. The divergence between c_e and c_r is caused solely by the limitations imposed on the rate of CO_2(aq) diffusivity, D_T, relative to carbon demand, Q. Similarly, c_i diverges from c_r due to the limitations exerted by cell wall permeability, P, relative to Q. That is, $c_i \rightarrow c_r \rightarrow c_e$ as D_T and $P \rightarrow \infty$ and/or $Q \rightarrow 0$.

The isotopic consequences of the preceding *mass* disequilibrium between c_e and c_i is that an *isotopic* disequilibrium between c_e and c_i is also imparted, the $\delta^{13}C_{c_i}$ increase with μ being the sole cause of the ε_p and $\delta^{13}C_{phyto}$ trends depicted in Fig. 1D. Parameters most often used to scale this isotopic response are either c_i/c_e (e.g. Eq. 1) or $c_e - c_i$ (e.g. Rau et al. 1992, Francois et al. 1993). Because our model offers a direct calculation of c_i, we can for the first time quantitatively predict both c_i/c_e and $c_e - c_i$ response to μ (or to any other of the model's independent variables) (e.g Fig. 1C).

Since neither c_e, c_r, nor c_i can be less than zero, at all times $\varepsilon_p \geq 0$ and $\delta^{13}C_{phyto} \geq \delta^{13}C_{c_e} - \varepsilon_d$ according to our model. Under conditions depicted in Fig. 1 this means that μ cannot exceed approximately 2.3 d^{-1}; c_i demand outstrips supply at higher growth rates. Clearly this particular μ maximum will decrease as r and hence V and γ_c increase. It is conceivable that under conditions specified in our base model, as this μ_{max} is approached in nature compensatory cell physiology may be induced (such as active inorganic carbon 'pumping' or use of alternate carbon substrates; e.g. Sharkey & Berry 1985) whose isotopic consequences are beyond the scope of our model. In any case under controlled experimentation, deviations from the linear response of ε_p and $\delta^{13}C_{phyto}$ to μ as the above μ_{max} is approached might be used either as a test of our model or of the assumptions about the test organism's carbon sources and physiology.

As already alluded to, one can anticipate that the preceding model response to growth rate might change significantly when such factors as r, c_e, T_C, P, and ε_f are allowed to change from their base values. To quantitatively explore these effects we singly allow each of these parameters to vary across a prescribed

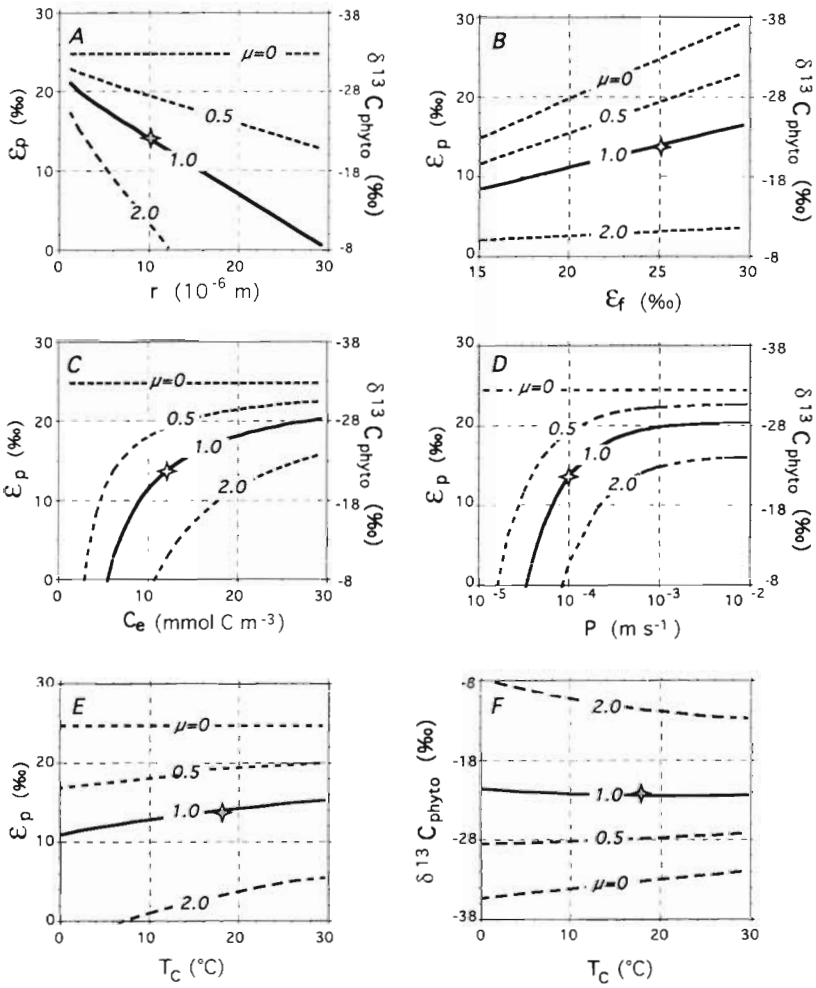

Fig. 2. Base model response of ε_p and $\delta^{13}C_{phyto}$ to changes in growth rate, μ, and: (A) cell radius, r; (B) enzymatic fractionation associated with carbon fixation, ε_f; (C) external $[CO_2(aq)]$, c_e; (D) cell wall permeability to $CO_2(aq)$, P_i; (E) and (F) ambient temperature, T_C. For reference, stars denote ε_p and $\delta^{13}C_{phyto}$ values when all independent variables are at their base value (see Table 1). Solid lines denote base model response when x-axis parameter alone is varied

range under selected μ values, and observe the modeled ε_p and $\delta^{13}C_{phyto}$ responses.

Cell radius effects. At $r > 2 \times 10^{-6}$ m (>2 μm) our model predicts a nearly linear decrease of ε_p (or increase in $\delta^{13}C_{phyto}$) with increasing r. The slope of these responses, however, significantly increases with increasing μ (Fig. 2A). The effect of increasing cell radius increases the cell mass elaborated per unit of μ, thus increasing Q and therefore increasing mass and isotopic disequilibrium among c_e, c_r, and c_i as described above. Previous studies (e.g. Fry & Wainright 1991, Goericke et al. 1994) have also seen or predicted positive effects on $\delta^{13}C_{phyto}$ with increasing r, although Laws et al. (1995) calculated that this effect should be relatively small in natural populations. One possibly unexpected result from our model is the non-linearity of the ε_p and $\delta^{13}C_{phyto}$ responses at r below about 5×10^{-6} m (5 μm) (Fig. 2A). This model prediction might be tested experimentally if appropriate controls on r, c_e, μ etc. were enforced in a laboratory setting

Enzymatic fractionation effects. There is uncertainty as to appropriate ε_f values to apply to phytoplankton (e.g. 20 to 29‰; Goericke et al. 1994). This stems from uncertainty in the ε_f associated with a purely Rubisco-based fixation system (e.g. Roeske & O'Leary 1984, 1985, Guy et al. 1993), and to the uncertainty in the relative proportion of carbon fixation that occurs by non-Rubisco routes (e.g. Descolas-Gros & Fontugne 1990, Raven et al. 1993). As might be expected, ε_p and $\delta^{13}C_{phyto}$ linearly change as ε_f increases, but with an isotopic sensitivity to changing ε_f that decreases as μ increases (Fig. 2B). This decreasing sensitivity occurs because the importance of ε_f decreases relative to ε_d as the disequilibrium between c_e and c_i is exacerbated by increasing μ. Note that when all other factors are held at their base value, ε_p and $\delta^{13}C_{phyto}$ readily fall within the range of observed marine $\delta^{13}C_{org}$ values (approx. −35 to −15‰) when ε_f is allowed to range within the presumed limits of 20 to 29‰. That is, this treatment does not further constrain what representative ε_f values might be. This, however, will be explored in greater detail with experimental data below.

$[CO_2(aq)]$ effects. Earlier adaptations of the Farquhar et al. (1982) model to marine phytoplankton (e.g. Rau et al. 1992, Francois et al. 1993) predict a hyperbolic ε_p and $\delta^{13}C_{phyto}$ response to c_e increases, as is again the case in our model (Fig. 2C). ε_p asymptotes to ε_f (or $\delta^{13}C_{phyto}$ to $\delta^{13}C_{c_e} - \varepsilon_f$) as c_e increases. Under the circumstances depicted in Fig. 2C, the isotopic response is most sensitive to changes in c_e when c_e is below about 10^{-3} mol C m^{-3} (10 μM) and/or at high μ. The convexity of the relationship between ε_p and c_e decreases as μ increases (Fig. 2C). The isopleths in Fig. 2C produced by changing μ are directly analogous to those produced in earlier models as a function of intracellular carbon demand represented by $c_e - c_i$ (Rau et al. 1992, Francois et al. 1993). As shown previously (Fig 1C) it is now possible to quantitatively interrelate μ and $c_e - c_i$. In a later section we will further compare our model's c_e

response to previous models and to experimental results.

CO_2 permeability effects. Trends in $\delta^{13}C_{phyto}$ similar to those elicited by changes in c_e (Fig. 2C) are again affected by changing P in our model (Fig. 2D). The similarity in isotopic response to each of these factors can be anticipated because both affect the communication and hence the disequilibrium between c_e and c_i. This isotopic effect of cell wall CO_2 permeability (or resistance to CO_2 transport across the cell wall) was first pointed out by Francois et al. (1993). While there is a considerable range in P values reported for plasma membranes (2 to 3500 $\times 10^{-6}$ m s^{-1}; Raven 1993), our model suggests that values $\ll 10^{-4}$ m s^{-1} are not representative of marine phytoplankton relying on diffusive CO_2 uptake because such values produce unrealistically low ε_p (high $\delta^{13}C_{phyto}$) (Fig. 2D). P values of 10^{-6} to 10^{-5} m s^{-1} have been reported for a few species of unicellular green algae (Gimmler & Hartung 1988, Gimmler et al. 1990), but these values appear much too low to allow passive CO_2 influx at rates required to meet typical photosynthetic carbon demand. While no direct measurements for representative marine phytoplankton species are available, isotope-model-dependent inferences of P values have been conducted (e.g Raven 1993), as will also be explored below with recent experimental data.

Temperature effects. Allowing T_C to increase from 0 to 30°C results in relatively small model-predicted changes in ε_p (Fig 2E), but with a sensitivity that increases somewhat with decreasing temperature and increasing μ. This temperature sensitivity is caused principally by T_C-affected changes in the diffusivity of CO_2(aq), D_T, that in turn influences c_e and c_i disequilibrium at any given μ as described earlier. An additional effect imparted on $\delta^{13}C_{phyto}$ (but not ε_p) is the T_C effect on $\delta^{13}C_{c_e}$ at equilibrium with the specified $\delta^{13}C_{\Sigma CO_2}$ base value of +1.7‰. The size of this effect alone, about 0.1‰ per degree T_C, can be seen in the 'μ = 0' isopleth in Fig. 2F. Note, however, that this isotopic trend is attenuated and eventually reversed in our modeled $\delta^{13}C_{phyto}$ as μ increases (Fig. 2F). Again this reflects the increasing c_i and $\delta^{13}C_{c_i}$ sensitivity to D_T which eventually overrides direct temperature effects on $\delta^{13}C_{c_e}$ as μ increases. It also must be pointed out that due to air/seawater exchange, the constant $c_e = 12 \times 10^{-3}$ mol m^{-3} (12 µM) assumed in this treatment is unlikely to be maintained in a natural setting, and would tend to vary from $> 20 \times 10^{-3}$ mol m^{-3} to $< 8 \times 10^{-3}$ mol m^{-3} across the temperature gradient specified above (e.g. Rau et al. 1989). Additionally, phytoplankton growth rates are unlikely to be independent of T_C (Eppley 1972, Raven & Geider 1988).

Other factors. In the current version of the model, salinity (S) and pH only affect the calculation of ε_p and $\delta^{13}C_{phyto}$ via r_k (Eqs. 7 & 8). As shown by Riebesell et al. (1993), r_k becomes significant in calculating c_r only at very large cell radii (≥10 µm). Thus, in the base model condition, changing pH from 6 to 9 or changing S from 10 to 40 psu results in changes in ε_p and $\delta^{13}C_{phyto}$ of less than 1‰ (not shown). However, because we have treated c_e as a fully independent variable in this model version, the large effect of pH (and to a lesser extent S) on c_e via equilibration within ΣCO_2 has been ignored. Were this to be included in our model, significant S and especially pH effects on ε_p and $\delta^{13}C_{phyto}$ via large changes in c_e would be elicited (e.g. Fig. 2C).

Comparison to experimental results

We can further test the validity of the abovementioned model responses by comparison to experimental observations. However, it will become evident that most previous experimentation has inadequately measured or controlled one or more variables relevant to our model. Thus, it may be unclear if deviations between our model predictions and previous experimental observations reflect an error in our model, a violation of model assumptions by the test organism (e.g. non-passive CO_2 uptake), inadequate controls/measures of relevant experimental variables, or some combination of the preceding. A new and carefully designed experimental effort that rectifies these previous shortcomings may ultimately be needed to convincingly test the isotopic relationships postulated by our model.

Fry & Wainright (1991) reported significant, roughly linear growth rate effects on $\Delta^{13}C$ ($\delta^{13}C_{\Sigma CO_2} - \delta^{13}C_{phyto}$) (Fig. 3). Similarly, converting our base model output to $\Delta^{13}C$ units produces a linear trend with μ, but whose slope (sensitivity) with μ is somewhat steeper than that observed by Fry & Wainright (1991) (Fig. 3). These researchers conducted their experiments under widely varying c_e ranging from 2 to 35 µM and found a general lack of isotopic response to this variable. In contrast, under such circumstances our model predicts $\Delta^{13}C$ values and trends that differ substantially from those seen by Fry & Wainright (1991) (Fig. 3). It is possible, however, that since species composition was not controlled in these experiments, the observed trend in $\delta^{13}C_{phyto}$ with μ could merely reflect changes in isotope fractionation with changing phytoplankton species that are independent of μ, i.e fast-growing diatoms with μ values >1 (div.) d^{-1} and slow-growing flagellates with μ <1 (div.) d^{-1}.

We found that imposing ε_f = 18‰ produced the best fit of the model to observations. If we ignore the potential complications mentioned above, such an unexpectedly low value for ε_f would imply that non-Rubisco carboxylation was prevalent, that ^{13}C-enriched HCO_3^-

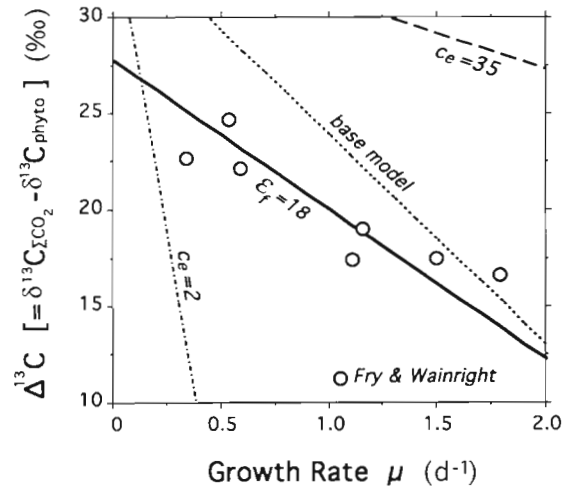

Fig. 3. Response of $\Delta^{13}C$ ($\delta^{13}C_{\Sigma CO_2} - \delta^{13}C_{phyto}$) to phytoplankton growth rate (O) as reported by Fry & Wainright (1991). Solid line denotes response of $\Delta^{13}C$ as predicted by base model described in text. Dotted or dashed lines indicate model responses when selected base values are changed as shown

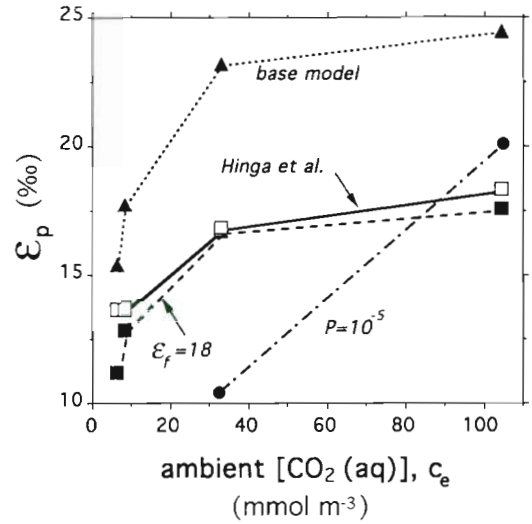

Fig. 4. Response of phytoplankton isotope fractionation, ε_p, to changes in ambient [CO_2(aq)], c_e, as observed by Hinga et al. (1994) (solid line) and as modeled (dotted lines). μ = 0.83 d^{-1} and c_e is calculated from pH and ΣCO_2 data presented in Table 1 of Hinga et al. (1994) using the dissociation constants of Mehrbach et al. (1973). Modeled ε_p trends were produced by driving the base model with the preceding c_e and with temperature and μ as reported by Hinga et al. (1994). Also shown are the model's responses when cell wall CO_2(aq) permeability, P, or enzymatic isotope fractionation, ε_f, are altered as noted. Comparisons between modeled ε_p and observations from 3 other experiments conducted by Hinga et al. (1994) at different growth rates were very similar to comparisons shown here

rather than CO_2(aq) was an important substrate for phytoplankton biomass production, and/or that non-diffusive CO_2(aq) transport was in operation in these experiments. Use of HCO_3^- might also explain the apparent lack of experimental $\Delta^{13}C$ response to changes in c_e relative to that predicted by our model. For these and the above reasons it is difficult to evaluate the accuracy of our model predictions based on comparisons to these particular experimental results.

Hinga et al. (1994) experimentally found significant, nonlinear increases in ε_p with increasing c_e (Fig. 4). Using their prescribed T_C, μ, $\delta^{13}C_{c_e}$, and using r = 3.25 µm (appropriate for their experimental organism *Skeletonema costatum*), our model similarly predicts nonlinear ε_p increases with c_e, but with absolute ε_p values that are consistently higher by 5 to 10 ‰ than those reported by Hinga et al. (1994) (Fig. 4). Of the 2 independent variables in our model not prescribed or measured by Hinga et al. (1994), P and ε_f, we found that a reduction in the latter base value from 25 to 18‰ produced the best fit to observations (Fig. 4). Changing P caused modeled ε_p to non-uniformly and inappropriately change across c_e (Fig. 4). Such a low ε_f value again suggests non-Rubisco fixation, non-diffusive transport, or non-CO_2(aq) substrates may be relevant to interpreting these experimental results, and may therefore question their relevance to our model simulations.

In their experimental cultures, Hinga et al. (1994) also observed significant ε_p sensitivity to pH, independent of those imparted by changes in c_e. The previously stated lack of significant, c_e-independent pH effects in all but exceptionally large cells predicted by our model provides no additional clues as to the cause of theses experimental results. Hinga et al. (1994) suggested that pH may influence ε_p by reducing HCO_3^- transport across the cell membrane at both high and low pH, affecting both the cell's c_i and $\delta^{13}C_{c_i}$. If real, such a mechanism is beyond the scope of our current model.

Laws et al. (1995) reported consistent interrelationships among growth rate, ε_p and c_e using field and experimental (chemostat) observations. Note that ε_p was defined by these researchers as $1000(\delta^{13}C_{c_i} - \delta^{13}C_{phyto})/(1000 + \delta^{13}C_{phyto})$ which yields values that are little different from the ε_p calculated by our model under the range of $\delta^{13}C_{c_i}$ and $\delta^{13}C_{phyto}$ considered here. These differences are ignored in the following comparisons. By measuring the population growth constant μ' (which is related to specific growth rate μ according to $\mu' = \mu/1.443$), Laws et al. (1995) found a negative linear relationship between μ'/c_e and ε_p that differs only slightly from our base model response (Fig. 5). Using a cell volume V = 100 µm^3 (Laws et al. 1995) and a surface equivalent cell radius of the experimental organism *Phaeodactylum tricornutum* r = 4.3 µm (calculated for a fusiform cell represented by ellipsoidal geometry with r_1 = 12 µm, r_2 = 2 µm, and r_3 = 1 µm; Round et al.

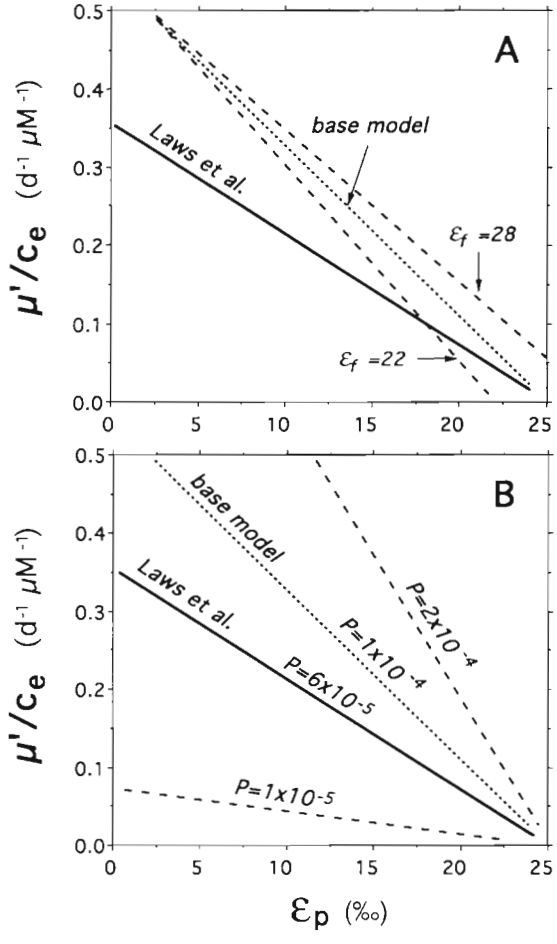

Fig. 5. Relationship between phytoplankton isotope fractionation, ε_p, and the ratio of relative growth rate to ambient $[CO_2(aq)]$, μ'/c_e, as reported by Laws et al. (1995) (solid line). Dotted lines denote relationship predicted by the base model or where either (A) enzymatic isotope fractionation, ε_f, or (B) cell wall $CO_2(aq)$ permeability, P, are changed from their base value as indicated. Base model response when $P = 0.6 \times 10^{-4}$ m s^{-1} overlaps the trend of Laws et al. (1995)

1990) we find that a decrease in P from the base model value of 1×10^{-4} to 0.6×10^{-4} m s^{-1} yields an ε_p trend with μ'/c_e that is virtually identical to the observed trend (Fig. 5B). A separate adjustment to ε_f did not produce a line of comparable slope to observations (Fig. 5A).

We view (1) the general linear response of ε_p to μ'/c_e observed by Laws et al. (1995) and (2) our model's ability to replicate this trend using 'reasonable' parameter values as evidence that the basic Farquhar et al. (1982) model and the refinements we have added are highly relevant to the marine environments and organisms studied by Laws et al. (1995). We also point out that the minor model fitting (via P adjustment) we conducted might prove useful as a tool for determining P for a wide range of marine autotrophs when grown under otherwise well-controlled laboratory settings.

We are aware of at least 2 other experimental data sets that are potentially relevant to our model. The results of Morel et al. (1994) are significant in showing isotopic effects of carbon limitation in the Zn-controlled presence and absence of carbonic anhydrase-catalyzed HCO_3^- conversion to $CO_2(aq)$ (but see also Riebesell & Wolf-Gladrow 1995 and Morel & Reinfelder 1995). Unfortunately, it is not possible to reconstruct c_e in this study from the pCO_2 reported since temperature and salinity were not listed. Additionally, specific concentrations of $CO_2(g)$ were bubbled into the cultures that had a $\delta^{13}C$ that was some 20‰ below that initially present in the seawater cultures. Since $\delta^{13}C_{\Sigma CO_2}$ and hence $\delta^{13}C_{c_e}$ were not monitored, it is not known to what extent changes in $\delta^{13}C_{c_e}$ during the course of the experiments contributed to the observed $\delta^{13}C_{phyto}$ variations, separate from the isotopic effects imparted by the controlled variables.

In other studies, Thompson & Calvert (1994, 1995) examined the effect of irradiance, day length, pH, and nitrogen source on carbon isotope fractionation by the diatom *Thalassiosira pseudonana* and the coccolithophorid *Emiliania huxleyi*. The ε_p values they reported for their treatments, however, were not directly determined, using instead initial $\delta^{13}C_{\Sigma CO_2}$, terminal $\delta^{13}C_{phyto}$, and an assumed correction for closed-system effects imparted during the course of each experiment. More importantly, the authors believed that their experimental organisms used HCO_3^- as a significant carbon source. These circumstances make the results of Thompson & Calvert (1994, 1995) inapplicable to our model.

Comparisons to other models

As previously shown (Eq. 14), models of ε_p based on the Farquhar et al. (1982) formulation can be reduced to the form $\varepsilon_p = \varepsilon_f + b/c_e$ (Jasper et al. 1994). Rearrangement of the treatments offered by Rau et al. (1992) and Francois et al. (1993) show that $b = -(c_e - c_i)(\varepsilon_f - \varepsilon_d)$, which is again the case in our model. The only difference between these earlier models and the one presented here is that we provide a specific solution for c_i based on the primary variables μ, r, c_e, D_T, and P. It nevertheless should be obvious that the response of ε_p to changes in ε_f, ε_d, c_i and c_e will be the same in all these models when a common set of values for these latter 4 parameters are used.

Another theoretical solution for b has been offered by Goericke et al. (1994) where (using our symbols) $b = -(\varepsilon_f) Q_s B/D_T$, with B stated to represent 'boundary layer thickness' Ignoring the minor contribution made by ε_d

(as did Goericke et al. 1994) and eliminating the usually very small r/r_k, our model's solution for $b = -(\varepsilon_f)\ Q_s\ [(r/D_T) + 1/P]$. This implies that B/D_T of Goericke et al.'s model is functionally equivalent to $[(r/D_T) + 1/P]$ in our model, and further suggests $B \approx r + D_T/P$. Whereas no means of scaling B was offered by Goericke et al. (1994), our model gives an explicit solution such that in its base condition it predicts a B of 2.45×10^{-5} m (24.5 µm).

Laws et al. (1995) also derived an explicit solution for ε_p (their Eq. 4) with values equivalent to our ε_d, ε_f, c_e, and P. However, our treatment takes into consideration the effects of temperature-sensitive CO_2 diffusivity relative to phytoplankton CO_2 demand in affecting the $[CO_2(aq)]$ at the cell surface, c_r. Laws et al. (1995) as well as Francois et al. (1993) and Goericke et al. (1994) in effect assumed $c_e = c_r$ in their treatments, which is indeed likely to be well-approximated under conditions of high c_e, high T_C, small r, and low μ. When such circumstances are violated, however, our model anticipates significant deviations in ε_p from that predicted by these earlier studies.

In conclusion, by merging the concepts of Farquhar et al. (1982) and Riebesell et al. (1993) we have described a model of $CO_2(aq)$-based marine photosynthetic carbon isotope fractionation as a function of a set of important environmental and biological variables. While comparisons to existing experimental data appear to substantiate some of the modeled relationships, new and well-controlled experiments are apparently needed to rigorously test many of the relationships and isotopic effects predicted by this study. Including a consideration of non-$CO_2(aq)$ substrates and non-diffusive $CO_2(aq)$ transport may also be required for such a model to be applicable to a broad range of marine autotrophs and environments.

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