

# A model of photosynthetic $^{13}\text{C}$ fractionation by marine phytoplankton based on diffusive molecular $\text{CO}_2$ uptake

Greg H. Rau<sup>1,2,\*</sup>, Ulf Riebesell<sup>3</sup>, Dieter Wolf-Gladrow<sup>3</sup>

<sup>1</sup>Institute of Marine Sciences, University of California, Santa Cruz, California 95064, USA

<sup>2</sup>Atmospheric and Ecological Sciences Program, Lawrence Livermore National Laboratory, Livermore, California 94550, USA

<sup>3</sup>Alfred Wegener Institute for Polar and Marine Research, Postfach 120161, D-27515 Bremerhaven, Germany

**ABSTRACT:** A predictive model of carbon isotope fractionation ( $\epsilon_p$ ) and abundance ( $\delta^{13}\text{C}_{\text{phyto}}$ ) is presented under circumstances where photosynthesis is strictly based on  $\text{CO}_2(\text{aq})$  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  $\text{CO}_2(\text{aq})$  transport is scaled by the ratio of intracellular to external  $[\text{CO}_2(\text{aq})]$ ,  $c_i/c_e$ . 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  $\text{CO}_2(\text{aq})$ , 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  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  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:**  $\delta^{13}\text{C}$  · Photosynthesis · Isotope fractionation · Phytoplankton ·  $\text{CO}_2$  · Modeling

## INTRODUCTION

The significant variability of the  $^{13}\text{C}/^{12}\text{C}$  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  $\text{CO}_2$  concentration,  $[\text{CO}_2(\text{aq})]$ , and marine organic  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{org}}$ ) and thus the possible use of this latter measure to reconstruct the ambient  $[\text{CO}_2(\text{aq})]$  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  $\text{CO}_2(\text{aq})$  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  $\delta^{13}\text{C}_{\text{org}}$  and  $[\text{CO}_2(\text{aq})]$  (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  $[\text{CO}_2(\text{aq})]$  via a greater preponderance of  $\text{HCO}_3^-$  utilization or non-passive inorganic carbon uptake under conditions of reduced  $[\text{CO}_2(\text{aq})]$ . Significant isotopic effects by these processes have been demonstrated experimentally (e.g. Beardall et al. 1982, Sharkey & Berry 1985). Also, if operating independently of  $[\text{CO}_2(\text{aq})]$ , 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,

\*E-mail: rau4@llnl.gov

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  $[\text{CO}_2(\text{aq})]$  on marine photosynthetic isotope fractionation and hence marine  $\delta^{13}\text{C}_{\text{org}}$  (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  $\text{CO}_2(\text{aq})$  limitation in diatoms, Riebesell et al. (1993) developed a model that is of direct relevance to the abovementioned issues. In particular, this model predicts  $[\text{CO}_2(\text{aq})]$  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  $\text{CO}_2(\text{aq})$  that passively diffuses into autotrophic cells,  $c_r$  places an upper limit on the  $[\text{CO}_2(\text{aq})]$  within the cell,  $c_i$ . This in turn can be used to constrain the  $\delta^{13}\text{C}_{\text{org}}$  of phytoplankton biomass,  $\delta^{13}\text{C}_{\text{phyto}}$ , via an equation derived from Farquhar et al. (1982):

$$\delta^{13}\text{C}_{\text{phyto}} = \delta^{13}\text{C}_{c_e} - \epsilon_d - (\epsilon_f - \epsilon_d) \frac{c_i}{c_e} \quad (1)$$

where  $\delta^{13}\text{C}_{c_e}$  is the  $\delta^{13}\text{C}$  of ambient  $\text{CO}_2(\text{aq})$ ,  $\epsilon_d$  is the isotope fractionation associated with diffusive transport of  $\text{CO}_2(\text{aq})$  in water,  $\epsilon_f$  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  $\text{CO}_2(\text{aq})$  to solve for  $c_i$ , and hence  $\delta^{13}\text{C}_{\text{phyto}}$  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  $\delta^{13}\text{C}_{\text{phyto}}$  using an explicit solution for  $c_i$  when temperature, cell radius, cell growth rate, and cell membrane permeability to  $\text{CO}_2(\text{aq})$  are specified. In this initial application we

input  $c_e$  as an independent variable with a base value of  $12 \times 10^{-3} \text{ mol m}^{-3}$  (12  $\mu\text{M}$ ) (Table 1). However,  $c_e$  could also be dependently determined via the formulation of Weiss (1974) when seawater  $p\text{CO}_2$  and temperature ( $T_C$ ,  $^\circ\text{C}$ ) and salinity ( $S$ ) are given. Similarly, at mean ocean salinities, any one of the variables  $T_C$ ,  $c_e$ , or  $p\text{CO}_2$  could be derived when the other 2 are specified.

While  $\epsilon_f$  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  $\epsilon_d = 0.7\text{‰}$  (O'Leary 1984), and ignore the apparently small temperature sensitivity of this parameter (Schönleber 1976).  $\delta^{13}\text{C}_{c_e}$  is treated as a dependent variable such that:

$$\delta^{13}\text{C}_{c_e} = \delta^{13}\text{C}_{\Sigma\text{CO}_2} + 23.644 - \frac{9701.5}{T_K} \quad (2)$$

following the treatments of Mook et al. (1974) and Freeman & Hayes (1992). For example, our base model specifies  $\delta^{13}\text{C}_{\Sigma\text{CO}_2} = +1.7\text{‰}$ , hence  $\delta^{13}\text{C}_{c_e} = -8.1\text{‰}$  at a base surface ocean temperature  $T_K$  of 290.15 K ( $T_C = 17^\circ\text{C}$ ) (Table 1).

The  $[\text{CO}_2(\text{aq})]$  inside the cell ( $c_i$ ) is calculated in 2 steps: First,  $[\text{CO}_2(\text{aq})]$  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  $\text{CO}_2(\text{aq})$  that passively diffuses into the cell via a cell membrane that has specified permeability to  $\text{CO}_2(\text{aq})$  (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  $\text{CO}_2$  uptake per phytoplankton cell ( $\text{mol C s}^{-1}$ ),  $r$  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  $\text{CO}_2(\text{aq})$  in seawater ( $\text{m}^2 \text{ s}^{-1}$ ), and  $r/r_k$  represents the relative contribution to the  $\text{CO}_2$  flux by extracellular spontaneous conversion of  $\text{HCO}_3^-$  to  $\text{CO}_2$  (see Riebesell et al. 1993). It can be shown that diffusional rates/processes about a phytoplankton 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  $\gamma_c$  ( $\text{mol C}$ ) and the instantaneous growth rate  $\mu_i$  according to

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

where  $\mu_i$  (in units of  $\text{s}^{-1}$ ) is related to the specific growth rate  $\mu$  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
$\alpha$	CO <sub>2</sub> solubility	$3.62 \times 10^1$	mol m <sup>-3</sup> atm <sup>-1</sup>
$b$	$= (\epsilon_p - \epsilon_i)c_e$ ( $c_e$ in $\mu\text{M}$ )	-127.2	‰ $\mu\text{M}$
$c_e$	Ambient [CO <sub>2</sub> (aq)]*	$12 \times 10^{-3}$	mol m <sup>-3</sup> (= 12 $\mu\text{M}$ )
$c_i$	Intracellular [CO <sub>2</sub> (aq)]	$6.64 \times 10^{-3}$	mol m <sup>-3</sup> (= 6.64 $\mu\text{M}$ )
$c_s$	[CO <sub>2</sub> (aq)] at cell surface	$9.87 \times 10^{-3}$	mol m <sup>-3</sup> (= 9.87 $\mu\text{M}$ )
$\delta^{13}\text{C}$	<sup>13</sup> C/ <sup>12</sup> C relative to PDB standard	-	per mil, ‰
$\delta^{13}\text{C}_{\text{phyto}}$	<sup>13</sup> C of bulk phytoplankton biomass	-22.2	per mil, ‰
$\delta^{13}\text{C}_{\Sigma\text{CO}_2}$	<sup>13</sup> C of total dissolved inorganic carbon*	1.7	per mil, ‰
$\delta^{13}\text{C}_{c_e}$	<sup>13</sup> C of $c_e$	-8.1	per mil, ‰
$\delta^{13}\text{C}_{c_i}$	<sup>13</sup> C of $c_i$	2.8	per mil, ‰
$\Delta^{13}\text{C}$	$= \delta^{13}\text{C}_{\Sigma\text{CO}_2} - \delta^{13}\text{C}_{\text{phyto}}$	23.9	per mil, ‰
$D_T$	Temperature-sensitive diffusivity of CO <sub>2</sub> (aq) in seawater	$1.45 \times 10^{-9}$	m <sup>2</sup> s <sup>-1</sup>
$\epsilon_{\text{cl}}$	Diffusive isotope fractionation of CO <sub>2</sub> (aq) in seawater*	0.7	per mil, ‰
$\epsilon_i$	Enzymatic isotope fractionation associated with intracellular C fixation*	25	per mil, ‰
$\epsilon_{\text{p}}$	$= \delta^{13}\text{C}_{c_e} - \delta^{13}\text{C}_{\text{phyto}}$	14.1	per mil, ‰
$E_{\text{d}}$	Activation energy (diffusion)*	19510	J mol <sup>-1</sup>
$E_{\text{k}}$	Activation energy (reaction)*	$6.28 \times 10^4$	J mol <sup>-1</sup>
$\gamma_c$	Carbon content per cell	$1.76 \times 10^{-11}$	mol C
$k'$	Rate coefficient	$3.41 \times 10^{-2}$	s <sup>-1</sup>
$k_1$	Rate coefficient	8500	m <sup>3</sup> mol <sup>-1</sup> s <sup>-1</sup>
$k_2$	Rate coefficient	$3 \times 10^{-5}$	s <sup>-1</sup>
$\mu$	Specific growth rate (= (Cell doubling time) <sup>-1</sup> *	$1.16 \times 10^{-5}$	s <sup>-1</sup> (= 1 d <sup>-1</sup> )
$\mu_i$	Instantaneous cell growth rate (= $2\mu$ if light:dark = 12 h:12 h)	$2.31 \times 10^{-5}$	s <sup>-1</sup> (equivalent to $\mu = 1$ d <sup>-1</sup> )
$\nu_{\text{fw}}$	Dynamic viscosity of freshwater	$8.9 \times 10^{-6}$	kg m <sup>-1</sup> s <sup>-1</sup>
$\nu_{\text{sw}}$	Dynamic viscosity of seawater	$9.5 \times 10^{-6}$	kg m <sup>-1</sup> s <sup>-1</sup>
$P$	Cell wall permeability to CO <sub>2</sub> (aq)*	$10^{-4}$	m s <sup>-1</sup>
pH	$-\log_{10} [\text{H}^+]$ *	8.2	-
$Q_r$	CO <sub>2</sub> (aq) uptake rate per cell	$4.06 \times 10^{-16}$	mol C cell <sup>-1</sup> s <sup>-1</sup>
$Q_n$	CO <sub>2</sub> (aq) uptake rate per unit cell surface area	$3.23 \times 10^{-7}$	mol C m <sup>-2</sup> s <sup>-1</sup>
$r$	Surface area equivalent cell radius*	$10^{-5}$	m (= 10 $\mu\text{m}$ )
$r_k$	Reacto-diffusive length	$2.06 \times 10^{-4}$	m
$R$	Gas constant*	8.3143	J K <sup>-1</sup> mol <sup>-1</sup>
$S$	Salinity*	35	psu
$T_C$	Temperature*	17	°C
$T_K$	Temperature (= $T_C + 273.15$ )*	290.15	K
$V$	Cell volume	4188.8	$\mu\text{m}^3$

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  $\mu$  is conventionally defined as the inverse of cell doubling time. The cell carbon content  $\gamma_c$  (mol C) is calculated from cell volume  $V$  (in  $\mu\text{m}^3$ ) 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  $V = \pi r^3 / 3$ . 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

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

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

$$\text{p}K_w = \frac{3441.0}{T_K} + 2.241 - 0.09415\sqrt{S}$$

( $S$  = 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  $E_d = 19510 \text{ J mol}^{-1}$  and the gas constant  $R = 8.3143 \text{ J K}^{-1} \text{ mol}^{-1}$ . 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  $\nu_{fw}$  and  $\nu_{sw}$  are the dynamic viscosities of freshwater and seawater, respectively (Wolf-Gladrow & Riebesell unpubl.). Temperature dependence of  $k_1$  is given by

$$k_1(T_k) = k_1(T_{k0}) e^{-\left(\frac{E_k}{RT_k}\right)} e^{-\left(\frac{E_k}{RT_{k0}}\right)} \quad (10)$$

where the temperature  $T_{k0} = 298.15 \text{ K}$  (25°C), and the activation energy  $E_k = 6.28 \times 10^4 \text{ J mol}^{-1}$  (15 kcal mol<sup>-1</sup>) (Stumm & Morgan 1981). For  $k_2$  we use the same temperature dependence.

The CO<sub>2</sub> 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<sub>2</sub>(aq) permeability (in  $\text{m s}^{-1}$ ; 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),

$$\delta^{13}\text{C}_{\text{phyto}} = \delta^{13}\text{C}_{c_e} - \epsilon_f + (\epsilon_f - \epsilon_d) \frac{Q_s}{c_e} \left( \frac{r}{D_T(1+r/r_k)} + \frac{1}{P} \right) \quad (13)$$

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

$$\epsilon_p = \epsilon_f + \frac{b}{c_e} \quad (14)$$

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

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

Significant variations in  $b$  ( $-109 \pm 14$  to  $-164 \pm 7$ ‰  $\mu\text{M}$ ) 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<sub>2</sub>(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<sub>2</sub>(aq) diffusion. In the sections that follow we show that reasonable base values for these independent variables (Table 1) produce realistic  $\delta^{13}\text{C}_{\text{phyto}}$  and  $\epsilon_p$ . We then show the sensitivity of  $\delta^{13}\text{C}_{\text{phyto}}$  and  $\epsilon_p$  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  $\delta^{13}\text{C}_{\text{phyto}}$  and  $\epsilon_p$ . 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  $\delta^{13}\text{C}_{\text{phyto}}$  of  $-22.2$ ‰, a value well within the  $\delta^{13}\text{C}_{\text{org}}$  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  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  to changes in each individual factor.

For example, by allowing  $\mu$  in our base model to increase from 0 to 2.3 d<sup>-1</sup>, (i.e.  $\mu_1$  to increase from 0 to  $5.3 \times 10^{-5} \text{ s}^{-1}$ ) an  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  change of approximately 25‰ is elicited (Fig. 1D). The linearity of this isotopic response to  $\mu$  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<sub>2</sub>(aq)] ( $c_e$ ,  $c_r$

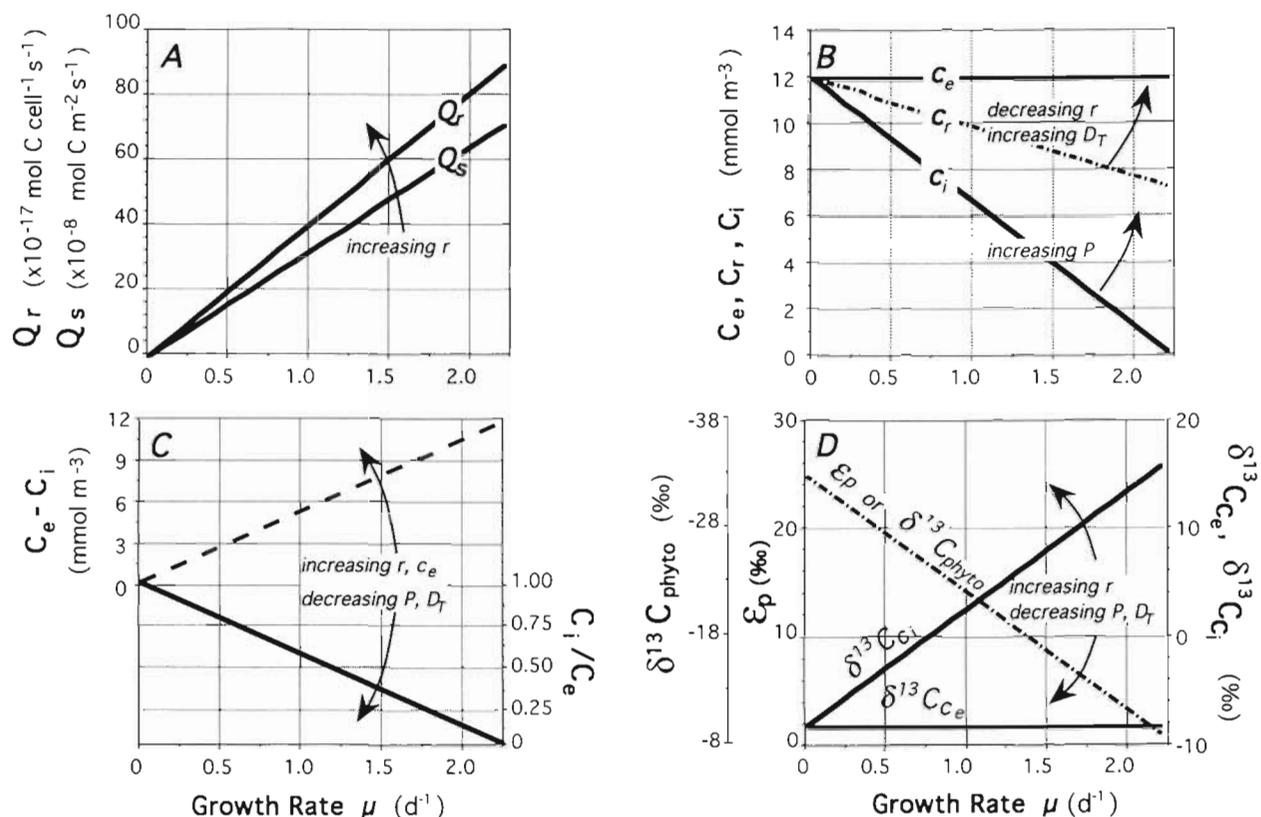


Fig. 1 Model response to changing growth rate,  $\mu$ , when all other independent variables are held at their base value (see Table 1). (A) Carbon flux per cell ( $Q_r$ ) and per cell surface area ( $Q_s$ ). Arrow denotes effect on line slope of changing cell radius,  $r$ . (B) [CO<sub>2</sub>(aq)] 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  $c_i$ , and between  $c_r$  and  $c_i$  at any given  $\mu$ . (C)  $c_e - c_i$  and  $c_i/c_e$  relative to  $\mu$ . Arrows denote effect of changing  $r$ ,  $c_e$ ,  $D_T$ , or  $P$ . (D)  $\delta^{13}\text{C}_{c_e}$ ,  $\delta^{13}\text{C}_{c_i}$ ,  $\epsilon_p$ , and  $\delta^{13}\text{C}_{\text{phyto}}$  versus  $\mu$  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  $\mu$  also increases with increasing cell radius,  $r$ . The divergence between  $c_e$  and  $c_i$  is caused solely by the limitations imposed on the rate of CO<sub>2</sub>(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  $\delta^{13}\text{C}_{c_i}$  increase with  $\mu$  being the sole cause of the  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  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  $\mu$  (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  $\epsilon_p \geq 0$  and  $\delta^{13}\text{C}_{\text{phyto}} \geq \delta^{13}\text{C}_{c_e} - \epsilon_d$  according to our

model. Under conditions depicted in Fig. 1 this means that  $\mu$  cannot exceed approximately 2.3 d<sup>-1</sup>;  $c_i$  demand outstrips supply at higher growth rates. Clearly this particular  $\mu_{\text{max}}$  will decrease as  $r$  and hence  $V$  and  $\gamma_c$  increase. It is conceivable that under conditions specified in our base model, as this  $\mu_{\text{max}}$  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  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  to  $\mu$  as the above  $\mu_{\text{max}}$  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  $\epsilon_f$  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

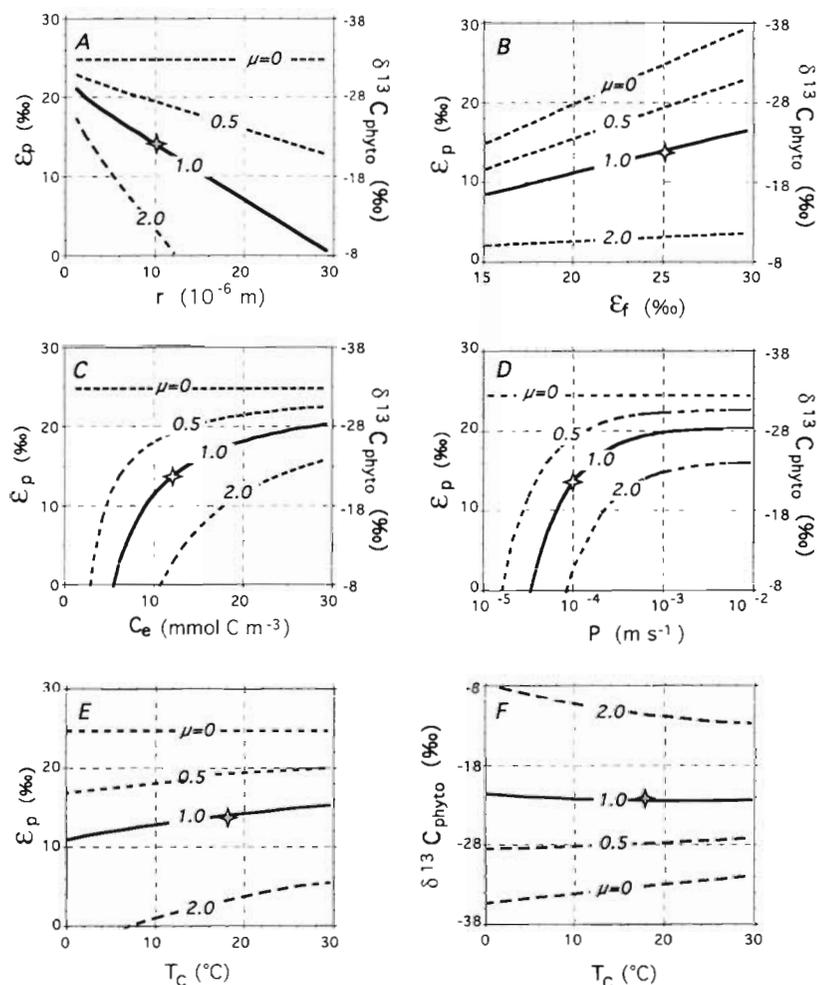


Fig. 2. Base model response of  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  to changes in growth rate,  $\mu$ , and: (A) cell radius,  $r$ ; (B) enzymatic fractionation associated with carbon fixation,  $\epsilon_f$ ; (C) external  $[\text{CO}_2(\text{aq})]$ ,  $c_e$ ; (D) cell wall permeability to  $\text{CO}_2(\text{aq})$ ,  $P$ ; (E) and (F) ambient temperature,  $T_c$ . For reference, stars denote  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  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  $\mu$  values, and observe the modeled  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  responses.

**Cell radius effects.** At  $r > 2 \times 10^{-6}$  m ( $>2$   $\mu\text{m}$ ) our model predicts a nearly linear decrease of  $\epsilon_p$  (or increase in  $\delta^{13}\text{C}_{\text{phyto}}$ ) with increasing  $r$ . The slope of these responses, however, significantly increases with increasing  $\mu$  (Fig. 2A). The effect of increasing cell radius increases the cell mass elaborated per unit of  $\mu$ , 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  $\delta^{13}\text{C}_{\text{phyto}}$  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  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  responses at  $r$  below about  $5 \times 10^{-6}$  m (5  $\mu\text{m}$ ) (Fig. 2A). This model prediction might be tested experimentally if appropriate controls on  $r$ ,  $c_e$ ,  $\mu$  etc. were enforced in a laboratory setting

#### Enzymatic fractionation effects.

There is uncertainty as to appropriate  $\epsilon_f$  values to apply to phytoplankton (e.g. 20 to 29‰; Goericke et al. 1994). This stems from uncertainty in the  $\epsilon_f$  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,  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  linearly change as  $\epsilon_f$  increases, but with an isotopic sensitivity to changing  $\epsilon_f$  that decreases as  $\mu$  increases (Fig. 2B). This decreasing sensitivity occurs because the importance of  $\epsilon_f$  decreases relative to  $\epsilon_d$  as the disequilibrium between  $c_e$  and  $c_i$  is exacerbated by increasing  $\mu$ . Note that when all other factors are held at their base value,  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  readily fall within the range of observed marine  $\delta^{13}\text{C}_{\text{org}}$  values (approx. -35 to -15‰) when  $\epsilon_f$  is allowed to range within the presumed limits of 20 to 29‰. That is, this treatment does not further constrain what representative  $\epsilon_f$  values might be. This, however, will be explored in greater detail with experimental data below.

#### $[\text{CO}_2(\text{aq})]$ 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  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  response to  $c_e$  increases, as is again the case in our model (Fig. 2C).  $\epsilon_p$  asymptotes to  $\epsilon_f$  (or  $\delta^{13}\text{C}_{\text{phyto}}$  to  $\delta^{13}\text{C}_{c_e - \epsilon_f}$ ) 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  $\text{m}^{-3}$  (10  $\mu\text{M}$ ) and/or at high  $\mu$ . The convexity of the relationship between  $\epsilon_p$  and  $c_e$  decreases as  $\mu$  increases (Fig. 2C). The isopleths in Fig. 2C produced by changing  $\mu$  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  $\mu$  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<sub>2</sub> permeability effects.** Trends in  $\delta^{13}\text{C}_{\text{phyto}}$  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<sub>2</sub> permeability (or resistance to CO<sub>2</sub> 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  $3500 \times 10^{-6} \text{ m s}^{-1}$ ; Raven 1993), our model suggests that values  $\ll 10^{-4} \text{ m s}^{-1}$  are not representative of marine phytoplankton relying on diffusive CO<sub>2</sub> uptake because such values produce unrealistically low  $\epsilon_p$  (high  $\delta^{13}\text{C}_{\text{phyto}}$ ) (Fig. 2D).  $P$  values of  $10^{-6}$  to  $10^{-5} \text{ m s}^{-1}$  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<sub>2</sub> 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  $\epsilon_p$  (Fig. 2E), but with a sensitivity that increases somewhat with decreasing temperature and increasing  $\mu$ . This temperature sensitivity is caused principally by  $T_c$ -affected changes in the diffusivity of CO<sub>2</sub>(aq),  $D_T$ , that in turn influences  $c_e$  and  $c_i$  disequilibrium at any given  $\mu$  as described earlier. An additional effect imparted on  $\delta^{13}\text{C}_{\text{phyto}}$  (but not  $\epsilon_p$ ) is the  $T_c$  effect on  $\delta^{13}\text{C}_{c_e}$  at equilibrium with the specified  $\delta^{13}\text{C}_{\Sigma\text{CO}_2}$  base value of +1.7‰. The size of this effect alone, about 0.1‰ per degree  $T_c$ , can be seen in the ' $\mu = 0$ ' isopleth in Fig. 2F. Note, however, that this isotopic trend is attenuated and eventually reversed in our modeled  $\delta^{13}\text{C}_{\text{phyto}}$  as  $\mu$  increases (Fig. 2F). Again this reflects the increasing  $c_i$  and  $\delta^{13}\text{C}_{c_i}$  sensitivity to  $D_T$  which eventually overrides direct temperature effects on  $\delta^{13}\text{C}_{c_e}$  as  $\mu$  increases. It also must be pointed out that due to air/seawater exchange, the constant  $c_e = 12 \times 10^{-3} \text{ mol m}^{-3}$  (12  $\mu\text{M}$ ) assumed in this treatment is unlikely to be maintained in a natural setting, and would tend to vary from  $>20 \times 10^{-3} \text{ mol m}^{-3}$  to  $<8 \times 10^{-3} \text{ mol m}^{-3}$  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  $\epsilon_p$  and

$\delta^{13}\text{C}_{\text{phyto}}$  via  $r_k$  (Eqs. 7 & 8). As shown by Riebesell et al. (1993),  $r_k$  becomes significant in calculating  $c_i$  only at very large cell radii ( $\geq 10 \mu\text{m}$ ). Thus, in the base model condition, changing pH from 6 to 9 or changing  $S$  from 10 to 40 psu results in changes in  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  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  $\Sigma\text{CO}_2$  has been ignored. Were this to be included in our model, significant  $S$  and especially pH effects on  $\epsilon_p$  and  $\delta^{13}\text{C}_{\text{phyto}}$  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<sub>2</sub> 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  $\Delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\Sigma\text{CO}_2} - \delta^{13}\text{C}_{\text{phyto}}$ ) (Fig. 3). Similarly, converting our base model output to  $\Delta^{13}\text{C}$  units produces a linear trend with  $\mu$ , but whose slope (sensitivity) with  $\mu$  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  $\mu\text{M}$  and found a general lack of isotopic response to this variable. In contrast, under such circumstances our model predicts  $\Delta^{13}\text{C}$  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  $\delta^{13}\text{C}_{\text{phyto}}$  with  $\mu$  could merely reflect changes in isotope fractionation with changing phytoplankton species that are independent of  $\mu$ , i.e. fast-growing diatoms with  $\mu$  values  $>1$  (div.)  $\text{d}^{-1}$  and slow-growing flagellates with  $\mu <1$  (div.)  $\text{d}^{-1}$ .

We found that imposing  $\epsilon_f = 18\text{‰}$  produced the best fit of the model to observations. If we ignore the potential complications mentioned above, such an unexpectedly low value for  $\epsilon_f$  would imply that non-Rubisco carboxylation was prevalent, that  $^{13}\text{C}$ -enriched  $\text{HCO}_3^-$

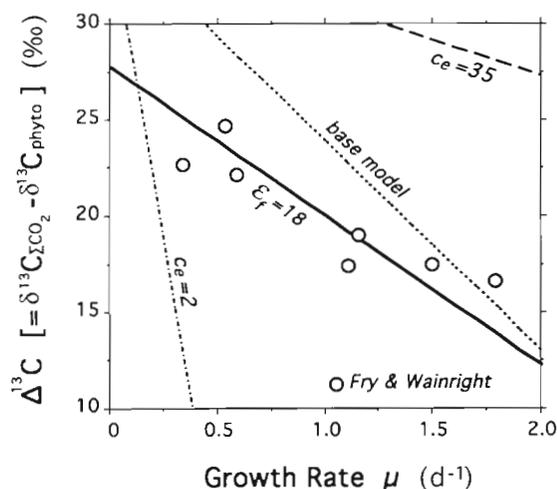


Fig. 3. Response of  $\Delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\Sigma\text{CO}_2} - \delta^{13}\text{C}_{\text{phyto}}$ ) to phytoplankton growth rate ( $\mu$ ) as reported by Fry & Wainright (1991). Solid line denotes response of  $\Delta^{13}\text{C}$  as predicted by base model described in text. Dotted or dashed lines indicate model responses when selected base values are changed as shown

rather than  $\text{CO}_2(\text{aq})$  was an important substrate for phytoplankton biomass production, and/or that non-diffusive  $\text{CO}_2(\text{aq})$  transport was in operation in these experiments. Use of  $\text{HCO}_3^-$  might also explain the apparent lack of experimental  $\Delta^{13}\text{C}$  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  $\epsilon_p$  with increasing  $c_e$  (Fig. 4). Using their prescribed  $T_c$ ,  $\mu$ ,  $\delta^{13}\text{C}_{c_e}$ , and using  $r = 3.25 \mu\text{m}$  (appropriate for their experimental organism *Skeletonema costatum*), our model similarly predicts nonlinear  $\epsilon_p$  increases with  $c_e$ , but with absolute  $\epsilon_p$  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  $\epsilon_t$ , 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  $\epsilon_p$  to non-uniformly and inappropriately change across  $c_e$  (Fig. 4). Such a low  $\epsilon_t$  value again suggests non-Rubisco fixation, non-diffusive transport, or non- $\text{CO}_2(\text{aq})$  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  $\epsilon_p$  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  $c_e$  cells predicted by

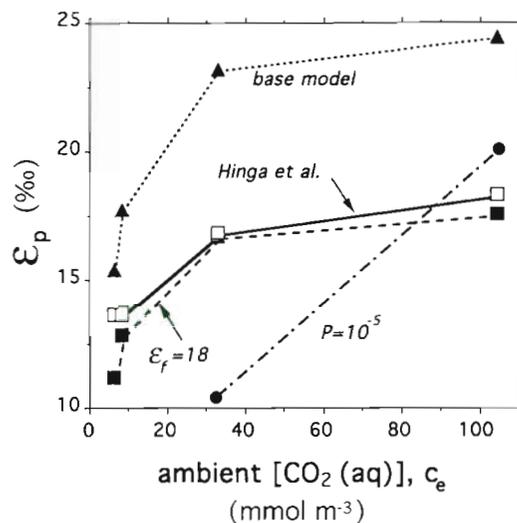


Fig. 4. Response of phytoplankton isotope fractionation,  $\epsilon_p$ , to changes in ambient  $[\text{CO}_2(\text{aq})]$ ,  $c_e$ , as observed by Hinga et al. (1994) (solid line) and as modeled (dotted lines).  $\mu = 0.83 \text{ d}^{-1}$  and  $c_e$  is calculated from pH and  $\Sigma\text{CO}_2$  data presented in Table 1 of Hinga et al. (1994) using the dissociation constants of Mehrbach et al. (1973). Modeled  $\epsilon_p$  trends were produced by driving the base model with the preceding  $c_e$  and with temperature and  $\mu$  as reported by Hinga et al. (1994). Also shown are the model's responses when cell wall  $\text{CO}_2(\text{aq})$  permeability,  $P$ , or enzymatic isotope fractionation,  $\epsilon_t$ , are altered as noted. Comparisons between modeled  $\epsilon_p$  and observations from 3 other experiments conducted by Hinga et al. (1994) at different growth rates were very similar to comparisons shown here

our model provides no additional clues as to the cause of these experimental results. Hinga et al. (1994) suggested that pH may influence  $\epsilon_p$  by reducing  $\text{HCO}_3^-$  transport across the cell membrane at both high and low pH, affecting both the cell's  $c_i$  and  $\delta^{13}\text{C}_{c_i}$ . If real, such a mechanism is beyond the scope of our current model.

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

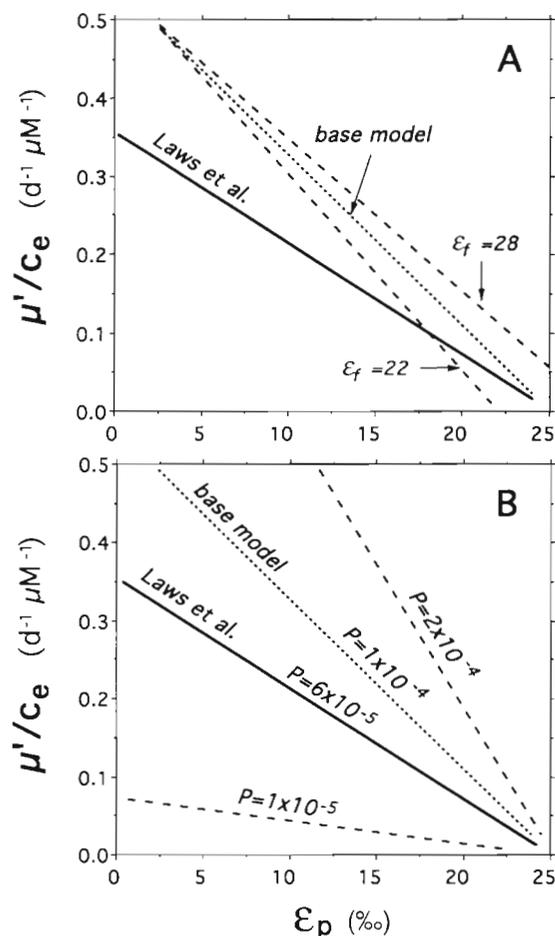


Fig. 5. Relationship between phytoplankton isotope fractionation,  $\epsilon_p$ , and the ratio of relative growth rate to ambient  $[\text{CO}_2(\text{aq})]$ ,  $\mu'/c_e$ , as reported by Laws et al. (1995) (solid line). Dotted lines denote relationship predicted by the base model or where either (A) enzymatic isotope fractionation,  $\epsilon_f$ , or (B) cell wall  $\text{CO}_2(\text{aq})$  permeability,  $P$ , are changed from their base value as indicated. Base model response when  $P = 0.6 \times 10^{-4} \text{ m s}^{-1}$  overlaps the trend of Laws et al. (1995)

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

We view (1) the general linear response of  $\epsilon_p$  to  $\mu'/c_e$  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  $\text{HCO}_3^-$  conversion to  $\text{CO}_2(\text{aq})$  (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  $\text{pCO}_2$  reported since temperature and salinity were not listed. Additionally, specific concentrations of  $\text{CO}_2(\text{g})$  were bubbled into the cultures that had a  $\delta^{13}\text{C}$  that was some 20‰ below that initially present in the seawater cultures. Since  $\delta^{13}\text{C}_{\Sigma\text{CO}_2}$  and hence  $\delta^{13}\text{C}_{c_e}$  were not monitored, it is not known to what extent changes in  $\delta^{13}\text{C}_{c_e}$  during the course of the experiments contributed to the observed  $\delta^{13}\text{C}_{\text{phyto}}$  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  $\epsilon_p$  values they reported for their treatments, however, were not directly determined, using instead initial  $\delta^{13}\text{C}_{\Sigma\text{CO}_2}$ , terminal  $\delta^{13}\text{C}_{\text{phyto}}$ , 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  $\text{HCO}_3^-$  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  $\epsilon_p$  based on the Farquhar et al. (1982) formulation can be reduced to the form  $\epsilon_p = \epsilon_f + b/c_e$  (Jasper et al. 1994). Rearrangement of the treatments offered by Rau et al. (1992) and Francois et al. (1993) show that  $b = -(c_e - c_i)(\epsilon_f - \epsilon_d)$ , 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  $\mu$ ,  $r$ ,  $c_e$ ,  $D_T$ , and  $P$ . It nevertheless should be obvious that the response of  $\epsilon_p$  to changes in  $\epsilon_f$ ,  $\epsilon_d$ ,  $c_i$  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)  $b = -(\epsilon_f)Q_s B/D_T$ , with  $B$  stated to represent 'boundary layer thickness' Ignoring the minor contribution made by  $\epsilon_d$

(as did Goericke et al. 1994) and eliminating the usually very small  $r/r_k$ , our model's solution for  $b = -(\epsilon_f) Q_s [(r/D_T) + 1/P]$ . 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  $2.45 \times 10^{-5}$  m (24.5  $\mu$ m).

Laws et al. (1995) also derived an explicit solution for  $\epsilon_p$  (their Eq. 4) with values equivalent to our  $\epsilon_d$ ,  $\epsilon_f$ ,  $c_e$ , and  $P$ . However, our treatment takes into consideration the effects of temperature-sensitive  $\text{CO}_2$  diffusivity relative to phytoplankton  $\text{CO}_2$  demand in affecting the  $[\text{CO}_2(\text{aq})]$  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  $\mu$ . When such circumstances are violated, however, our model anticipates significant deviations in  $\epsilon_p$  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  $\text{CO}_2(\text{aq})$ -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- $\text{CO}_2(\text{aq})$  substrates and non-diffusive  $\text{CO}_2(\text{aq})$  transport may also be required for such a model to be applicable to a broad range of marine autotrophs and environments.

**Acknowledgements.** We thank J. Bijma for facilitating electronic file transfers over the Internet and B. Jähne and 3 anonymous referees for helpful comments. G.H.R. was supported by grants from NOAA's Paleoclimatology Program and from the Lawrence Livermore National Laboratory's Directed Research and Development Program (Environmental Sciences/DOE Contract W-7405-Eng-48). This is publication number 955 of the Alfred Wegener Institute for Polar and Marine Research.

#### LITERATURE CITED

- Badger MR, Kaplan A, Berry JA (1980) Internal inorganic carbon pool of *Chlamydomonas reinhardtii*: evidence for a carbon dioxide-concentrating mechanism. *Plant Physiol* 66:407–413
- Beardall J, Griffiths H, Raven JA (1982) Carbon isotope discrimination and the  $\text{CO}_2$  accumulating mechanisms in *Chlorella emersonii*. *J exp Bot* 23:729–737
- Beardall J, Raven JA (1981) Transport of inorganic carbon and the ' $\text{CO}_2$  concentrating mechanism' in *Chlorella emersonii* (Chlorophyceae). *J Phycol* 17:134–141
- Descolas-Gros C, Fontugne M (1990) Stable carbon isotope fractionation by marine phytoplankton during photosynthesis. *Plant Cell Environ* 13:207–218
- Eppley RW (1972) Temperature and phytoplankton growth in the sea. *Fish Bull US* 70:1063–1085
- Falkowski PG (1991) Species variability in the fractionation of  $^{13}\text{C}$  and  $^{12}\text{C}$  by marine phytoplankton. *J Plankton Res* 13: 21–28
- Farquhar GD, O'Leary MH, Berry JH (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust J Plant Physiol* 9:121–137
- Francois R, Altabet MA, Goericke R, McCorkle DC, Brunet C, Poisson A (1993) Changes in the  $\delta^{13}\text{C}$  of surface water particulate organic matter across the subtropical convergence in the S.W. Indian Ocean. *Global biogeochem Cycles* 7:627–644
- Freeman KH, Hayes JH (1992) Fractionation of carbon isotopes by phytoplankton and estimates of ancient  $\text{CO}_2$  levels. *Global biogeochem Cycles* 6:185–198
- Fry B, Wainright SC (1991) Diatom sources on  $^{13}\text{C}$ -rich carbon in marine food webs. *Mar Ecol Prog Ser* 76:149–157
- Gimmler H, Hartung W (1988) Low permeability of the plasma membrane of *Dunaliella parva* for solutes. *J Plant Physiol* 133:165–172
- Gimmler H, Weiss C, Baier M, Hartung W (1990) The conductance of the plasmalemma for  $\text{CO}_2$ . *J exp Bot* 41:785–795
- Goericke R, Fry B (1994) Variations of marine plankton  $\delta^{13}\text{C}$  with latitude, temperature, and dissolved  $\text{CO}_2$  in the world ocean. *Global biogeochem Cycles* 8:85–90
- Goericke R, Montoya J P, Fry B (1994) Physiology of isotope fractionation in algae and cyanobacteria. In: Lajtha K, Michener B (eds) *Stable isotopes in ecology*. Blackwell Scientific, Boston, p 187–221
- Guy RD, Fogel ML, Berry JA (1993) Photosynthetic fractionation of the stable isotopes of oxygen and carbon. *Plant Physiol* 101:37–47
- Hayes JM (1993) Factors controlling  $^{13}\text{C}$  contents of sedimentary compounds: principles and evidence. *Mar Geol* 113:111–125
- Hinga KR, Arthur MA, Pilson MEQ, Whitaker D (1994) Carbon isotope fractionation by marine phytoplankton in culture: the effects of  $\text{CO}_2$  concentration, pH, temperature, and species. *Global biogeochem Cycles* 8:91–102
- Jähne B, Heinz G, Dietrich W (1987) Measurement of the diffusion coefficients of sparingly soluble gases in water. *J geophys Res* 92:10767–10776
- Jasper JP, Hayes JM, Mix AC, Prah FG (1994) Photosynthetic fractionation of  $^{13}\text{C}$  and concentrations of dissolved  $\text{CO}_2$  in the central equatorial Pacific during the last 255,000 years. *Paleoceanography* 9:781–798
- Laws EA, Popp BN, Bidigare RR, Kennicutt MC II, Macko SA (1995) Dependence of phytoplankton carbon isotopic composition on growth rate and  $[\text{CO}_2(\text{aq})]$ : theoretical considerations and experimental results. *Geochim cosmochim Acta* 59:1131–1138
- Li YH, Gregory S (1974) Diffusion of ions in sea water and deep-sea sediments. *Geochim cosmochim Acta* 38:703–714
- Mehrbach C, Culbertson CH, Hawley JE, Pytkowicz RM (1973) Measurements of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18:897–907
- Montagnes DJS, Berges JA, Harrison PJ, Taylor FJR (1994) Estimating carbon, nitrogen, protein, and chlorophyll a from volume in marine phytoplankton. *Limnol Oceanogr* 39:1044–1060

- Mook WG, Bommerson JC, Staverman WH (1974) Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth Planet Sci Lett* 22:169–176
- Morel FMM, Reinfelder JR (1995) Growth limits on phytoplankton. *Nature* 373:28
- Morel FMM, Reinfelder JR, Roberts SB, Chamberlain CP, Lee JG, Yee D (1994) Zinc and carbon co-limitation of marine phytoplankton. *Nature* 369:740–742
- Nobel PS (1983) *Biophysical plant physiology and ecology*. Freeman, San Francisco
- O'Leary MH (1984) Measurement of the isotope fractionation associated with diffusion of carbon dioxide in aqueous solution. *J Phys Chem* 88:823–825
- Rau GH (1994) Variations in sedimentary organic  $\delta^{13}\text{C}$  as a proxy for past changes in ocean and atmospheric  $[\text{CO}_2]$ . In: Zahn R, Kaminski M, Labeyrie LD, Pedersen TF (eds) *Carbon cycling in the glacial ocean: constraints on the ocean's role in global climate change*. Springer, Berlin, p 307–322
- Rau GH, Takahashi T, Des Marais DJ (1989) Latitudinal variations in plankton  $\delta^{13}\text{C}$ : implications for  $\text{CO}_2$  and productivity in past oceans. *Nature* 341:516–518
- Rau GH, Takahashi T, Des Marais DJ, Repeta DJ, Martin JH (1992) The relationship between  $\delta^{13}\text{C}$  of organic matter and  $[\text{CO}_2(\text{aq})]$  in ocean surface water: data from a JGOFS site in the northeast Atlantic Ocean and a model. *Geochim cosmochim Acta* 56:1413–1419
- Raven JA (1993) Carbon: a phycocentric view. In: Evans GT, Fasham MJR (eds) *Towards a model of ocean biogeochemical processes*. Springer-Verlag, Berlin, p 123–152
- Raven JA, Geider RJ (1988) Temperature and algal growth. *New Phytol* 110:441–461
- Raven JA, Johnston AM, Turpin DH (1993) Influence of changes in  $\text{CO}_2$  concentration and temperature on marine phytoplankton  $^{13}\text{C}/^{12}\text{C}$  ratios: an analysis of possible mechanisms. *Global planet Change* 8:1–12
- Riebesell U, Wolf-Gladrow D (1995) Growth limits on phytoplankton. *Nature* 373:28
- Riebesell U, Wolf-Gladrow D, Smetacek V (1993) Carbon dioxide limitation of marine phytoplankton growth rates. *Nature* 361:249–251
- Roeske CA, O'Leary MH (1984) Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose biphosphate. *Biochemistry* 23:6275–6284
- Roeske CA, O'Leary MH (1985) Carbon isotope effects on carboxylation of ribulose biphosphate catalyzed by ribulosebiphosphate carboxylase from *Rhodospirillum rubrum*. *Biochemistry* 24:1603–1607
- Romanek CS, Grossman EL, Morse JW (1992) Carbon isotope fractionation in synthetic aragonite and calcite: effects of temperature and precipitation rate. *Geochim cosmochim Acta* 56:419–430
- Round FE, Crawford RM, Mann DG (1990) *The diatoms*. Cambridge University Press, Cambridge
- Sackett WM (1991) A history of the  $\delta^{13}\text{C}$  composition of oceanic plankton. *Mar Chem* 34:153–156
- Schönleber G (1976) Messungen der Isotopentrennung bei der Diffusion von  $^{13}\text{CO}_2$  und  $^{12}\text{CO}_2$  in Wasser. MS thesis, Institut für Umweltphysik, Universität Heidelberg
- Sharkey TD, Berry JA (1985) Carbon isotope fractionation in algae as influenced by inducible  $\text{CO}_2$  concentrating mechanism. In: Lucas WJ, Berry JA (eds) *Inorganic carbon uptake by aquatic photosynthetic organisms*. American Society of Plant Physiologists, Rockville, MD, USA, p 389–401
- Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol Oceanogr* 12:411–418
- Stumm W, Morgan JJ (1981) *Aquatic chemistry*. John Wiley & Sons, New York
- Thompson PA, Calvert SE (1994) Carbon-isotope fractionation by a marine diatom: the influence of irradiance, daylength, pH, and nitrogen source. *Limnol Oceanogr* 39:1835–1844
- Thompson PA, Calvert SE (1995) Carbon isotope fractionation by *Emiliania huxleyi*. *Limnol Oceanogr* 40:673–679
- Weiss RF (1974) Carbon dioxide in water and seawater: the solubility of a non-ideal gas. *Mar Chem* 2:203–215

This article was submitted to the editor

Manuscript first received: June 7, 1995

Revised version accepted: October 20, 1995