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Using a phytoplankton growth model to predict the fractionation of stable carbon isotopes

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In the preceding paper in this issue, a phytoplankton growth model based on an analogy with chemical kinetics (the CR model) was re-derived, and a comparison made with the growth rate of cultured phytoplankton assemblages extracted from temperate lakes. In this paper, further derivation of the CR model leads to the same model of carbon isotope fractionation used by Rau et al. (Mar. Ecol. Prog. Ser., ***133****, 275–285, 1996). Both the CR and Rau et al. models are compatible with the observation that isotope fractionation during phytoplankton growth, ϵ_p, is proportional to the growth rate, μ, divided by the extracellular carbon concentration, C. The CR model is then used to explain the observation that the initial slope of ϵ_p divided by μ/C is a negative linear function of the cellular carbon to surface area ratio of the phytoplankton cell. The CR model applies the same model framework to predict both phytoplankton growth and stable isotope fractionation. The ability of the CR model to predict two measurable phenomena increases its usefulness, and also our confidence in its predictive capabilities.*

INTRODUCTION

The fractionation of stable isotopes in aquatic environments has been used extensively to track the movement of elements through aquatic food webs [e.g. (Stapp *et al.*, 1999*;* Zanden *et al.*, 1999)] and to understand ecological processes (Lajtha and Michener, 1994). In particular, the changing rate of stable isotope fractionation of biological and physical processes due to changing environmental conditions has received much attention (Popp *et al.*, 1989, 1999). Much of this research has been motivated by a realization that stable isotopes can be used to reconstruct paleo-oceanographic environments, and therefore for model assessment of coupled global ocean–atmosphere models (von Blackenburg, 1999).

Many laboratory studies have been conducted to determine the rate of stable carbon isotope fractionation during phytoplankton growth (Rau *et al.*, 1996; Laws *et al.*, 1997; Popp *et al.*, 1998, 1999; Burkhardt *et al.*, 1999a). As a result of these studies, fractionation rates are understood to be a function of growth rate and ambient CO_2 concentration (Rau *et al.*, 1996; Laws *et al.*, 1997) and cell size (Popp *et al.*, 1998; Burkhardt *et al.*, 1999a). Furthermore, a comparison of samples from five open-ocean stations with laboratory cultures suggests that stable isotope fractionation can be used to infer *in situ* growth rates (Bidigare *et al.*, 1997).

However, a recent set of experiments (Burkhardt *et al.*, 1999b) have demonstrated that the isotope fractionation rate is dependent on the growth-rate-limiting resource. Burkhardt *et al.* (Burkhardt *et al.*, 1999b) found that, under nitrate limitation, fractionation was a function of growth rate [in agreement with (Rau *et al.*, 1996; Laws *et al.*, 1997; Popp *et al.*, 1998, 1999; Burkhardt *et al.*, 1999a)], but under light limitation, fractionation was virtually independent of growth rate. This led Burkhardt *et al.* to conclude 'a general relationship between ϵ_p and $\mu/[CO_{2(aq)}]$ may not exist. These results suggest that *in situ* growth rates of phytoplankton cannot be estimated from a ϵ_p versus $\mu/[CO_2(aq)]$ relationship' (Burkhardt *et al.*, 1999b).

Phytoplankton growth models may offer a solution to the problem of the fractionation rate varying dependent on the growth-rate-limiting resource. Many phytoplankton growth models have been developed which determine growth rate as a function of more than one possible growth-rate-limiting resource (Baird *et al.*, 2001). In this paper, however, we show that phytoplankton growth models that are either always proportional to the maximum growth rate, or that use the Droop model relating internal

concentrations to growth rates [the vast majority of phytoplankton growth models (Baird *et al.*, 2001)], do not reproduce under nutrient limitation the dependence of carbon isotope fractionation, ϵ_p, on the extracellular carbon concentration and growth rate.

In this paper, a model of phytoplankton growth based on an analogy with chemical kinetics (the CR model), first derived in Baird and Emsley (Baird and Emsley, 1999), and further developed in Baird *et al.* (Baird *et al.*, 2001), is extended to consider stable carbon isotope fractionation. The derivation arrives at the same relationship that Rau *et al.* found between stable isotope fractionation, growth rate and extracellular carbon concentration under nitrate-limited growth (Rau *et al.*, 1996). The significance of this result for both assessing the CR model and in the prediction of stable isotope fractionation under varying growth-rate-limiting resources is discussed.

THEORY

First, we will look at the problems of using existing growth models for predicting stable isotope fractionation, and then derive an extension of the CR model necessary for predicting isotope fractionation under nutrient-limited growth.

Predicting fractionation using existing growth models

Growth models based on extracellular concentrations

Growth models that are based on the extracellular concentrations of nutrient generally take the form:

$$\mu = \mu^{max} \times f(\text{limiting factors}) \quad (1)$$

where μ is the growth rate, μ^{max} is the maximum growth rate and f (limiting factors) is a function describing the effect of the rate-limiting factors on growth. Incorporating fractionation into equation (1) is problematic. Most isotope fractionation occurs during intracellular biochemical reactions. However, biochemical reactions are not explicitly represented in growth models that are linearly dependent on the maximum growth rate. If we assume that the maximum growth rate represents the sum of all biochemical reactions, the fractionation rate due to phytoplankton growth, ϵ_p, can be found from:

$$\left(1 - \frac{\epsilon_p}{1000}\right) = \frac{^{13}\text{Flux}}{^{12}\text{Flux}} = \frac{^{13}\mu^{max} \times {}^{13}f(\text{limiting factors})}{^{12}\mu^{max} \times {}^{12}f(\text{limiting factors})} = \left(1 - \frac{\epsilon_b}{1000}\right)\frac{^{13}f(\text{limiting factors})}{^{12}f(\text{limiting factors})} \quad (2)$$

where ϵ_b is the fractionation associated with biochemical processes. Typically, processes proceed faster for the lighter isotope, so whatever the combination of limiting factors:

$$^{13}f(\text{limiting factors}) \leq {}^{12}f(\text{limiting factors}) \quad (3)$$

From equation (2), the stable isotope fractionation due to phytoplankton growth, ϵ_p, must always be greater than or equal to that associated with biochemical reactions alone (ϵ_b). This is inconsistent with all of the above cited experimental studies, which find fractionation to vary between 0 and ϵ_b. It appears, therefore, that growth models that are based an extracellular concentrations, and that are a linear function of the maximum growth rate, cannot easily be applied to the modelling of stable isotope fractionation.

The Droop growth model (Droop, 1983), which has a changing dependence of growth rate on the maximum growth rate depending on physiological state, has the potential to capture the changing fractionation rates at different growth rates. However, the Droop model is best applied to nutrients that can be stored in much greater quantities than are required for instantaneous growth (Droop, 1983; Baird *et al.*, 2001). While the Droop model may predict fractionation of trace nutrients like phosphate and vitamin B_{12}, it is not able to predict fractionation of carbon isotopes.

Predicting fractionation using the CR growth model

Derivation of the CR model for predicting carbon isotope fractionation

The CR growth model uses the interaction of the rates of nutrient uptake, light capture and intracellular biochemical reactions (from now on, termed organic matter construction) to predict phytoplankton growth rate (Baird *et al.*, 2001). In this paper, the overall rate of carbon isotope fractionation during phytoplankton growth, ϵ_p, will be calculated using a version of the CR model (Baird *et al.*, 2001) with only carbon explicitly modelled. The chemical reaction representing growth [analogous to equation (1) in Baird *et al.* (Baird *et al.*, 2001)] becomes:

$$(C_{m_C} + m_C C \xrightarrow{k_p} 2(C_{m_C}) \quad (4)$$

where C is carbon, m_C is the stoichiometry coefficient quantifying the moles of carbon required to make another phytoplankton cell (mol cell^{-1}), C_{m_C} is the elemental composition of a phytoplankton cell (considering carbon only) and k_p is the rate at which the reaction proceeds (s^{-1}). The growth rate of the phytoplankton cell, μ (s^{-1}), is given by:

$$\mu = \mu^{\max} \frac{R_C}{R_C^{\max}} \tag{5}$$

where $\mu^{\max}$ is the maximum growth rate (s^{-1}) and R_C is the reservoir of carbon within the cell (Baird *et al.*, 2001). For the case of light-saturated growth, it is sufficient to consider R_C as the internal CO_2 concentration (mol $cell^{-1}$) and $R_C^{\max}$ is the maximum value of R_C (see Discussion). The uptake of carbon per cell is given by:

$$\left(\frac{d[CO_2]_{aq}}{dt}\right)_{uptake} = k_C \left(\frac{R_C^{\max} - R_C}{R_C^{\max}}\right) \tag{6}$$

where $[CO_2]_{aq}$ is the extracellular concentration of aqueous CO_2 (mol m^{-3}) and k_C is the maximum uptake rate of carbon as limited by diffusion to the cell surface (mol $cell^{-1}$ s^{-1}). Stable isotope fractionation during phytoplankton growth is a result of the interaction of the fractionating processes involved in growth. As will be shown by the following derivation, the CR growth model can be used to capture the interacting effects of diffusive carbon uptake and organic matter construction on stable isotope fractionation. The resulting equation for isotope fractionation is the same as that derived from mass balances of stable isotopes for terrestrial plants (Farquhar *et al.*, 1982) and marine phytoplankton (Rau *et al.*, 1996).

Calculating stable isotope fractionation of a process

Isotope compositions are specified as δ values (Farquhar *et al.*, 1982):

$$\delta\ (‰) = (Rat_{\text{sample}}/Rat_{\text{standard}} - 1) \times 1000 \tag{7}$$

where Rat_{sample} and Rat_{standard} are the abundance ratios of the heavier to lighter isotope of the sample and the standard, respectively. The isotopic fractionation of a process, $\epsilon_{\text{process}}$, is defined by:

$$\begin{aligned}\epsilon_{\text{process}} &= 1000 \times (1 - Rat_{\text{product}}/Rat_{\text{source}}) \\ &= \frac{\delta_{\text{source}} - \delta_{\text{product}}}{1 + \delta_{\text{source}}/1000}\end{aligned} \tag{8}$$

Phytoplankton growth involves processes with different isotopic fractionation. With a knowledge of the fractionation rate of individual processes, and their position in the chain of reactions that describe phytoplankton growth, the total fractionation rate of phytoplankton growth can be calculated.

Fractionation by molecular diffusion, ϵ_d

The heavier ^{13}C isotope diffuses slower than ^{12}C. Along a concentration gradient from a non-zero concentration to a zero concentration, the rate of isotopic fractionation in sea water is $\epsilon_d = 0.7‰$. That is, the flux due to molecular diffusivity of ^{12}C is 0.07% greater than that of ^{13}C. For a steady concentration gradient from c_e to c_i (where $c_e > c_i$), the flux is determined by the interaction of the rate removing molecules at the c_i boundary, and the diffusion rates of both isotopes. A component of the flux is dependent on the diffusion rate, and the resulting isotopic fractionation is given by (Farquhar *et al.*, 1982):

$$\epsilon_{\text{flux}} = \frac{c_e - c_i}{c_e}\epsilon_d \tag{9}$$

Fractionation by biochemical reactions, ϵ_b

Photosynthetic cells convert CO_2 into glucose. The glucose is then used, with other atoms like nitrogen, phosphorus and iron, to construct more complex organic molecules. The process of converting CO_2 into glucose, facilitated by the enzyme Rubisco, proceeds faster for ^{12}C than ^{13}C. The resulting fractionation, ϵ_{Ru}, varies with algal species. For Rubisco isolated from marine eukaryotic algae, ϵ_{Ru} = 25–28‰ (Goericke *et al.*, 1994). In many cases, Rubisco dominates fractionation by biochemical reactions, and $\epsilon_b = \epsilon_{Ru}$. For the cyanobacteria *Synechococcus* sp., Popp *et al.* suggested using a value of ϵ_b = 17‰ to account for the fractionation associated with β-carboxylase (Popp *et al.*, 1998). If a cell contains equal concentrations of $^{13}CO_2$ and $^{12}CO_2$ (i.e. diffusion to the reaction site is unlimiting), the flux of ^{12}C will be (ϵ_b/10)% greater than that of ^{13}C. If transport to the enzyme site does become limiting, the available concentration of isotopes will no longer be equal, and the enzyme will fractionate carbon at a reduced rate. The overall fractionation rate for the combined process of transport and enzyme reaction will be a result of the interaction of the fractionation effects of both processes. This interaction can be modelled as a mass balance (Farquhar *et al.*, 1982).

Mass balance

A mass balance similar to that used by Farquhar *et al.* (Farquhar *et al.*, 1982) for terrestrial plants, and applied to marine phytoplankton by Rau *et al.* (Rau *et al.*, 1996), is used. Two simplifying assumptions used by Farquhar *et al.* make this derivation simpler (Farquhar *et al.*, 1982). First, as the maximum possible fractionation, ϵ_b, is <3%, assume that the ratio of the isotopes inside the cell, $^{13}R_C/^{12}R_C$, and the ratio of the maximum carbon isotopes within the cell, $^{13}R_C^{\max}/^{12}R_C^{\max}$, are both equal to the ratio in the surrounding fluid, $^{13}C/^{12}C$:

$$\frac{^{13}R_C}{^{12}R_C} \approx \frac{^{13}R_C^{\max}}{^{12}R_C^{\max}} \approx \frac{^{13}C}{^{12}C} \tag{10}$$

Secondly, since the natural abundance of ^{12}C makes up 98.9% of the total of all carbon isotopes, assume that the

concentration of ^{12}C is equal to the total concentration of all carbon isotopes:

$$^{12}C = C \tag{11}$$

The maximum supply rate of carbon, k_C, is a function of the diffusion shape factor for the cell, ψ (m cell^{-1}) (Baird and Emsley, 1999), the molecular diffusivity of the carbon isotope, D (m^2 s^{-1}), the rate of conversion of HCO_3^- to CO_2 in the boundary layer, and, in the case of passive diffusion, the cell wall permeability, $\mathcal{P}$ (m s^{-1}). Since the carbon molecules must diffuse through the boundary layer and then the cell membrane (i.e. not simultaneously), an effective conductance, υ (m^3 s^{-1}), of carbon transport through the boundary layer and cell membrane can be defined:

$$\frac{1}{\upsilon} = \frac{1}{\psi D (1 + r/r_k)} + \frac{1}{A_s \mathcal{P}} \tag{12}$$

where A_s is the surface area of the cell (m^2), r is the radius of a sphere with the same surface area as the cell (m) and $r_k \approx 2.06 \times 10^{-4}$ m is the reacto-diffusive length for the conversion of HCO^-_3 to CO_2 (Rau *et al.*, 1996). The difference in effective conductance of the ^{13}C and ^{12}C isotopes is given by:

$$^{13}\upsilon = \left(1 - \frac{\epsilon_d}{1000}\right) {}^{12}\upsilon \tag{13}$$

The supply rate of carbon isotopes ^{13}C and ^{12}C, $^{13}\mathcal{J}$ and $^{12}\mathcal{J}$, respectively (mol s^{-1}), assuming a dependence on the internal reservoir of carbon of the cell given by equation (6), can be written:

$$^{13}\mathcal{J} = {}^{13}\upsilon {}^{13}C\,(1 - {}^{13}R^*) \tag{14}$$

$$^{12}\mathcal{J} = {}^{12}\upsilon {}^{12}C\,(1 - {}^{12}R^*) \tag{15}$$

where $R^* = R_C / R_C^{max}$. In the case of passive uptake of CO_2, R^* is equivalent to c_i/c_e in the formulation of Farquhar *et al.* (Farquhar *et al.*, 1982) and Rau *et al.* (Rau *et al.*, 1996).

The fluxes of ^{13}C and ^{12}C can also be determined based on the growth rate [from equations (4) and (5)]:

$$^{13}\mathcal{J} = {}^{13}\mu^{max} m_C \, {}^{13}R^* \tag{16}$$

$$^{12}\mathcal{J} = {}^{12}\mu^{max} m_C \, {}^{12}R^* \tag{17}$$

where m_C is the stoichiometry coefficient for carbon. The difference in biochemical rates of the two isotopes is given by:

$$^{13}\mu^{max} = \left(1 - \frac{\epsilon_d}{1000}\right) {}^{12}\mu^{max} \tag{18}$$

Equations (16) and (17) do not balance loss and gain terms to intracellular reserves. An additional term is required to account for the sharing of internal resources amongst offspring. This additional term is small for stored intracellular aqueous carbon. For example, using the Rau *et al.* (Rau *et al.*, 1996) model's base values, intracellular dissolved carbon = $\mu c_i V = 4.85 \times 10^{-20}$ mol C cell^{-1} compared to 1.76×10^{-11} mol C cell^{-1} for the carbon held as structural material. For isotopes of nutrients such as nitrate, which are actively taken up and are stored in quantities of the same order of magnitude as they are held in organic matter, the sharing amongst offspring is an important term.

Solving for $^{13}\mathcal{J}$ from equations (14) and (16):

$$^{13}\mathcal{J} = \frac{{}^{13}\upsilon {}^{13}C}{1 + \dfrac{{}^{13}\upsilon {}^{13}C}{{}^{13}\mu^{max} m_C}} \tag{19}$$

and similarly for $^{12}\mathcal{J}$. The overall rate of carbon fractionation for phytoplankton growth is found from the ratio of fluxes of the ^{13}C and ^{12}C isotopes:

$$\frac{{}^{13}\mathcal{J}}{{}^{12}\mathcal{J}} = \underbrace{\frac{{}^{13}\upsilon {}^{13}C}{{}^{12}\upsilon {}^{12}C\left(1 + \dfrac{{}^{13}\upsilon {}^{13}C}{{}^{13}\mu^{max} m_C}\right)}}_{\text{diffusive fractionation}} + \underbrace{\frac{\dfrac{{}^{12}\upsilon {}^{12}C}{{}^{12}\mu^{max} m_C}}{{}^{12}\upsilon {}^{12}C\left(1 + \dfrac{{}^{13}\upsilon {}^{13}C}{{}^{13}\mu^{max} m_C}\right)}}_{\text{enzymic fractionation}} \tag{20}$$

where the terms for diffusive and enzyme fractionation can be separated. The term for diffusive fractionation can be reduced to:

$$\frac{{}^{13}\upsilon {}^{13}C}{{}^{12}\upsilon {}^{12}C\left(1 + \dfrac{{}^{13}\upsilon {}^{13}C}{{}^{13}\mu^{max} m_C}\right)} = \frac{{}^{13}\upsilon {}^{13}C}{{}^{12}\upsilon {}^{12}C}\left(1 - {}^{13}R^*\right) \tag{21}$$

The most convenient measure of fractionation is relative to the fluid surrounding the cell. So dividing equation (21) by $^{13}C/^{12}C$, substituting ϵ_d from equation (13) and given from equation (10) that $(1 - {}^{13}R^*) \approx (1 - {}^{12}R^*) \approx (1 - R^*)$:

$$\frac{{}^{13}\upsilon {}^{13}C}{{}^{12}\upsilon {}^{12}C\left(1 + \dfrac{{}^{13}\upsilon {}^{13}C}{{}^{13}\mu^{max} m_C}\right)} = \epsilon_d (1 - R^*) \tag{22}$$

The enzyme fractionation term in equation (20) can be reduced to:

$$\frac{\frac{{}^{12}\upsilon\,{}^{12}C}{{}^{12}\mu^{\max}{}_{mf}}}{{}^{12}\upsilon\,{}^{12}C\left(1+\frac{{}^{13}\upsilon\,{}^{13}C}{{}^{13}\mu^{\max}{}_{mf}}\right)} = \frac{{}^{13}\mu^{\max}{}_{mf}}{{}^{12}\mu^{\max}{}_{mf}}\,{}^{12}R^{*} = \epsilon_b\,{}^{12}R^{*} \quad (23)$$

So the fractionation of carbon isotopes relative to the surrounding fluid during phytoplankton growth, ϵ_p, becomes:

$$\epsilon_p = \epsilon_d\,(1 - R^*) + \epsilon_b R^* \quad (24)$$

Equation (24) is mathematically similar to the formulation presented by Farquhar *et al.* (Farquhar *et al.*, 1982) for carbon fractionation in terrestrial plants, which Rau *et al.* (Rau *et al.*, 1996) applied to marine phytoplankton, and can be written:

$$\epsilon_p = \epsilon_d\frac{c_e - c_i}{c_e} + \epsilon_b\frac{c_i}{c_e} \quad (25)$$

where c_i is the partial pressure of CO_2 the leaf stomata (Farquhar *et al.*, 1982) or the internal concentration of $CO_{2(aq)}$ in phytoplankton cells (Rau *et al.*, 1996) and c_e is the extracellular concentration of CO_2. To our knowledge, this is the first example in aquatic autotrophs where a single model framework has been used to predict both growth and stable isotope fractionation.

Fractionation in a continuous culture

In a continuous culture at steady state, the phytoplankton growth rate ($\mu = \mu^{max}R^*$) is equal to the dilution rate, $\mathcal{D}$. The value of R^* required in equation (24) is given by:

$$\mu^{max}R^*m_C = k_C\,(1 - R^*) = \mathcal{D}m_C \quad (26)$$

ϵ_p can be found by substituting R^* into equation (24). This is numerically the same as the Rau *et al.* model of fractionation at a constant growth rate (Rau *et al.*, 1996). Given that $k_C = \upsilon C$ [equation (15)], R^* is given by:

$$R^* = 1 - \frac{\mathcal{D}_{mf}}{\upsilon C} \quad (27)$$

Substituting equation (27) into equation (24):

$$\epsilon_p = \epsilon_b - (\epsilon_b - \epsilon_d)\frac{\mathcal{D}_{mf}}{\upsilon C} \quad (28)$$

Popp *et al.* observed that at low values of μ/C there was a linear relationship between ϵ_p and μ/C (Popp *et al.*, 1998). At steady state in a continuous culture, $\mu = \mathcal{D}$, so ϵ_p versus μ/C will be a line beginning at ϵ_b, with a slope equal to $-(\epsilon_b - \epsilon_d)\,m_C/\upsilon$, where m_C is the stoichiometry coefficient of the carbon in the phytoplankton cell, or cellular carbon, and υ (is the effective conductivity, and is proportional to the surface area (Wolf-Gladrow and Riebesell, 1997). Therefore, equation (28) would suggest that the slope of ϵ_p versus μ/C should itself be a negative linear function of cellular carbon to surface area ratio. Popp *et al.* have found a negative linear relationship (r^2 = 0.99, n = 4) between the slope of ϵ_p versus μ/C and the ratio of cellular carbon to surface area (Popp *et al.*, 1998).

Comparison with laboratory experiments

Figure 1 plots ϵ_p against μ/C of four phytoplankton species as measured experimentally, and determined using the CR model. It is difficult, however, to use this comparison to verify the fractionation predicted by the growth model, because the value of the cell wall permeability, $\mathcal{P}$ (an important parameter in the determination of the conductivity, υ), was not determined during the experimental procedure. To illustrate the behaviour of the model, cell wall permeability is calculated for each species from the measured slope of ϵ_p versus μ/C (Figure 1). The calculation is given by:

$$\mathcal{P} = \frac{1}{A_c\left[\frac{(\epsilon_b - \epsilon_d)\,\mu/C}{-\epsilon_b - \epsilon_d)\,m_C} + \frac{1}{(b-1-\epsilon/\epsilon_b)\,D}\right]} \quad (29)$$

The values of $\mathcal{P}$ calculated for each species, given in Figure 1, appear reasonable [Rau *et al.* (Rau *et al.*, 1996) use a value of 10^{-4} m s^{-1}]. The high value of $\mathcal{P}$ for *Synechococcus* sp. suggests that the resistance to transport through the cell membrane is relatively small. A definite test of the above model of stable isotope fractionation would require independent determination of $\mathcal{P}$ for each species.

DISCUSSION

The extension of the CR model to carbon isotope fractionation explains the interaction of diffusive uptake of CO_2 and biochemical reactions in the overall fractionation rate of the growing phytoplankton cell, arriving at the same set of equations as Rau *et al.* (Rau *et al.*, 1996). The CR model captures the relationship between growth rate, extracellular CO_2 concentration, cell size and stable isotope fractionation rate observed in a number of continuous cultures (Rau *et al.*, 1996; Laws *et al.*, 1997; Popp *et al.*, 1998), and known to be applicable in some open-ocean marine locations (Bidigare *et al.*, 1997).

As acknowledged in Baird *et al.* (Baird *et al.*, 2001), the CR model captures only the basic (or first-order) aspects of phytoplankton growth. The CR model is also not expected to capture all the complexities of carbon isotope fractionation. Two deviations between observations and

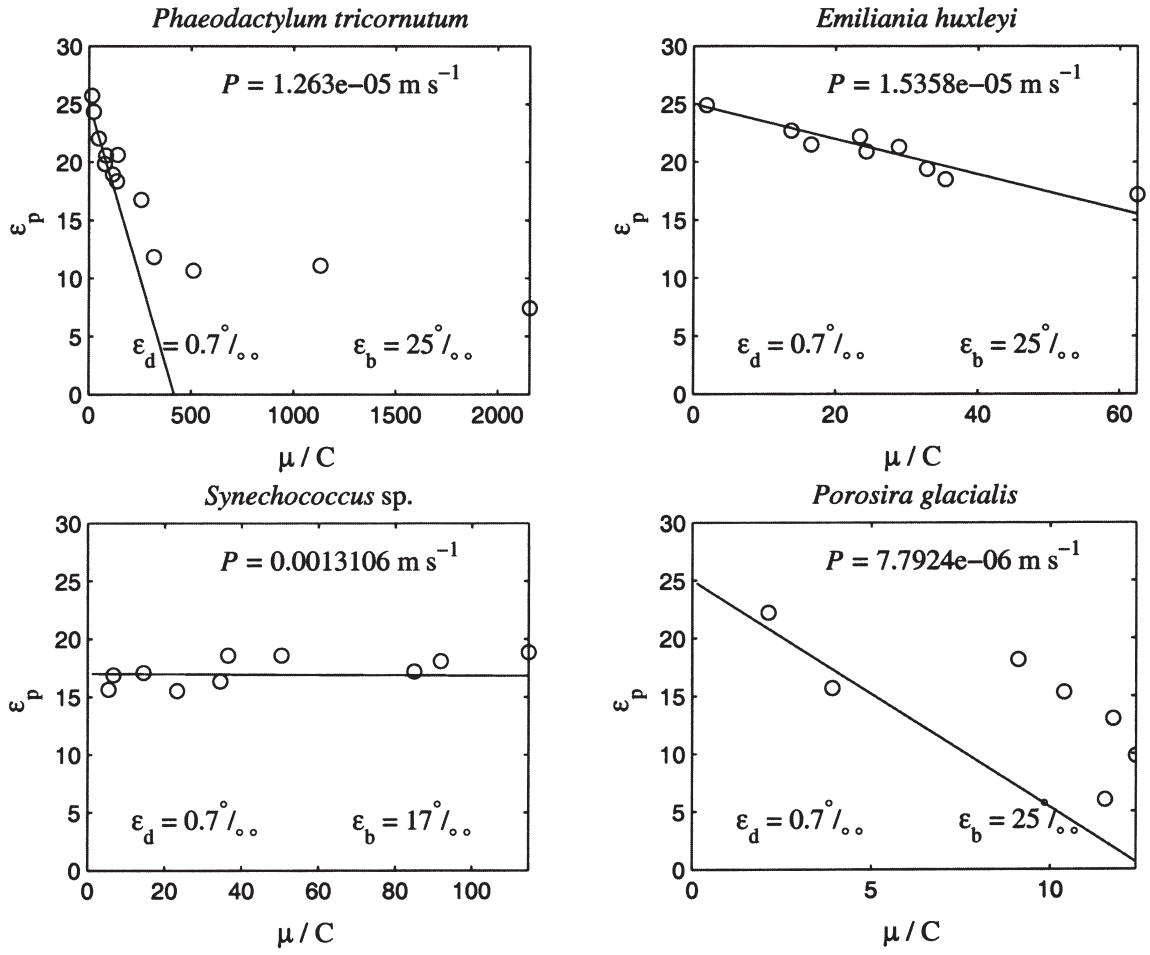

Fig. 1. Measured (○) and predicted [using equation (28)] (–) carbon isotope fractionation for phytoplankton cells, ϵ_p (‰), at a given μ/C (mol CO_2^{-1} m^3 day^{-1}) for *P. tricornutum* (Laws *et al.*, 1997), *Emiliana huxleyi* (Bidigare *et al.*, 1997) *Synechococcus* sp. (Popp *et al.*, 1998) and *P. glacialis* (Popp *et al.*, 1998). The carbon per cell (m_C) for the four species can be found in Table 2 of Popp *et al.* (Popp *et al.*, 1998). For an oceanic value of $CO_{2(aq)}$ of $\approx 20 \times 10^{-3}$ mol m^{-3}, and growth rates between 0 and 2.0 day^{-1}, μ/C varies between 0 and 100. The very high values for *Phaeodactylum* which do not fit the model line are well outside the likely oceanic range.

the model output are worth pointing out. (i) The Laws *et al.* experimental investigation of *Phaeodactylum tricornutum* showed a deviation from the linear relationship between ϵ_p and μ/C at higher values of μ/C (Figure 1) (Laws *et al.*, 1997). As μ/C increases, growth becomes more 'diffusion limited', with the associated decrease in fractionation rates (as $\epsilon_b > \epsilon_d$). The deviation observed by Laws *et al.* is towards higher ϵ_p at a particular μ/C than predicted by the linear relationship (Laws *et al.*, 1997). This represents an uptake rate above that predicted by diffusion-limited uptake. A possible explanation for this phenomenon is the supply of carbon via a route other than passive diffusion of CO_2, such as active uptake of CO_2, or HCO_3^-. In fact, HCO_3^- uptake, rather than a high cell membrane permeability, may also account for the constant fractionation rate of *Synechococcus* sp. (Figure 1) (Tchernov *et al.*, 1997). Evidently, the CR growth model does not incorporate the complex understanding of carbon use by photosynthetic organisms that is being developed (Tchernov *et al.*, 1997). (ii) The observed fractionation of *Porosira glacialis* at high μ/C was above the model line. *Porosira glacialis* is a large diatom, and may be achieving higher than calculated diffusion rates (and therefore more biochemical fractionation) as a result of changes in size, shape, membrane permeability or relative motion with the fluid. These types of adaptations are more available to larger cells.

Of particular concern to modellers of isotope fractionation is the observation of Burkhardt *et al.* that, at a particular growth rate, different relationships between growth rate and fractionation rate can be observed, depending on the growth-rate-limiting resource (Burkhardt *et al.*, 1996b). The empirical relationships of Laws *et al.* (Laws *et al.*, 1997) and Popp *et al.* (Popp *et al.*, 1998), and both the Rau *et al.* (Rau *et al.*, 1996) and simplified CR models

presented here [equation (4)], solve to just one possible fractionation rate at a particular growth rate. In the Introduction to this paper, the need for a coupling of a growth model and an isotope fractionation model to capture the rate-limiting dependence of fractionation was considered. As presented, the CR model does not achieve this. By simplifying the equation of growth to $C_{m_C} + m_C C \xrightarrow{k_2} 2C_{m_C}$, the CR model works only for cases when diffusion of dissolved inorganic carbon interacts directly with growth rate. Burkhardt *et al.* observed that, under light limitation, growth rate does not interact with fractionation (Burkhardt *et al.*, 1999b). To model this under the CR framework, (at least) two coupled chemical reactions of growth would be required: one describing the transport of CO_2 to the photosynthetic apparatus, and a second describing the fixing of carbon using the energy from incident photons.

The CR model framework has demonstrated an ability to predict growth rates under varying growth-rate-limiting resources and temperature (Baird *et al.*, 2001). In this paper, we have shown that the CR model framework is also compatible with isotope fractionation under diffusion limitation. We believe that further theoretical work under the CR model framework is likely to lead to a model that captures the rate-limiting dependence of stable isotope fractionation under a variety of nutrient concentrations, light levels and temperatures.

An important aspect of developing a growth model that predicts stable isotope fractionation is its application to assessing the performance of the growth model in natural environments. Typically, the phytoplankton component of ecosystem models is assessed on the match between measured phytoplankton biomass and model predicted biomass (Fasham *et al.*, 1990; Hurtt and Armstrong, 1999). If a mismatch is found, the model failure could be in any component of the dynamical system (such as grazing rates or other phytoplankton loss terms). Instead, it is preferable to assess the performance of individual components of the system (Sterner and Grover, 1998). Such assessments are usually restricted to the laboratory. In the field, phytoplankton population changes due to growth are hard to interpret from population data which are affected by other processes such as cell sinking, grazing, programmed cell death, etc. In contrast to population dynamics, stable isotope fractionation in phytoplankton cells is independent of phytoplankton loss terms. Using the CR model, stable isotope fractionation therefore offers a direct test of the equations describing growth of phytoplankton, without being obscured by the other processes affecting phytoplankton populations.

In further developing the CR model to predict carbon isotope fractionation, we added only the assumptions of Rau *et al.* (Rau *et al.*, 1996) of cell wall permeability, and a rate of fractionation due to molecular diffusion and the action of Rubisco. The CR model then reduced to the Rau *et al.* model of carbon isotope fractionation (Rau *et al.*, 1996). The CR model was initially derived without consideration of isotope fractionation, but rather an understanding of physical processes involved in phytoplankton growth. Nonetheless, we consider that the same underlying processes control both growth and fractionation. The ability of the CR model to predict the rate of fractionation of stable carbon isotopes, without significant additions to the growth model, is a good test of the modelling approach: a test the CR model passes where other growth models fail. Since the CR model can predict growth rates for varying growth-rate-limiting resources, it is possible that it may achieve the same for stable isotope fractionation. If so, the CR model could be used to infer growth rates from stable isotope signatures. This introduces the possibility that *in situ* growth rates could be determined without sampling of live cells.

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