

NOTES

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Sources of inorganic carbon for marine microalgal photosynthesis: A reassessment of $\delta^{13}\text{C}$ data from batch culture studies of *Thalassiosira pseudonana* and *Emiliania huxleyi*

Abstract—A reevaluation of previously published analyses of stable carbon isotope fractionation by batch cultures of *Thalassiosira pseudonana* and *Emiliania huxleyi* indicates that the Rayleigh distillation model was used to model CO_2 uptake incorrectly. Correct use of the model shows that the relationship between the $\delta^{13}\text{C}$ of the particulate organic carbon (δ_p) and the concentration of the dissolved inorganic carbon (DIC) at the time of harvest can be equally well described by a model assuming bicarbonate or CO_2 uptake. The lack of a correlation between growth rate and δ_p in the *T. pseudonana* results suggests that growth rate and the intracellular CO_2 concentration are directly proportional. Theoretical considerations indicate that the *T. pseudonana* cultures started at a pH of 9.2 would have become CO_2 limited before harvest and that this species must have the ability to utilize bicarbonate when CO_2 becomes limiting. The similarity of the *T. pseudonana* δ_p results from cultures started at pH 8.2 and 9.2 suggests that the form of DIC entering the cells was the same in both sets of experiments. The results are consistent with (1) uptake of bicarbonate or (2) uptake of CO_2 , with external carbonic anhydrase-mediated conversion of bicarbonate to CO_2 supplementing the uncatalyzed supply of CO_2 when the latter becomes limiting. Analysis of the $\delta^{13}\text{C}$ of both particulate organic carbon and coccolith carbon in the case of *E. huxleyi* suggests that the cells were taking up primarily bicarbonate at low growth rates, but that at high growth rates the DIC used for photosynthesis was derived almost entirely from uptake of CO_2 . The DIC utilized for coccolith formation seems to have been substantially diluted by isotopically light DIC derived from respiration at high growth rates.

In two recent papers, Thompson and Calvert (1994, 1995) analyzed the $\delta^{13}\text{C}$ of phytoplankton carbon produced in batch cultures of *Thalassiosira pseudonana* and *Emiliania huxleyi* and concluded that the relationship between the concentration of dissolved inorganic carbon (DIC) and the $\delta^{13}\text{C}$ of the algal carbon strongly suggested that HCO_3^- was the major source of carbon for growth. The data were analyzed with the Rayleigh distillation equation, originally derived by Rayleigh to describe fractional distillation of a mixed liquid. When applied to photosynthetic uptake of DIC, the Rayleigh distillation equation takes the form

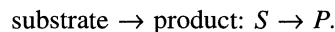
$$\delta_p(t) = \delta_s(0) - \epsilon_{ps} \frac{f \ln(f)}{1-f}, \quad (1)$$

where $\delta_p(t)$ is the $\delta^{13}\text{C}$ of the organic carbon at time t , $\delta_s(0)$ is the $\delta^{13}\text{C}$ of the source carbon at time 0, f is the fraction of substrate remaining after time t , and

$$\epsilon_{ps} = \frac{\delta_p(t) - \delta_s(t)}{1 + [\delta_s(t)/1,000]}, \quad (2)$$

ϵ_{ps} is the isotopic fractionation associated with DIC assimilation and is assumed to remain constant during the course of the reaction.

Strictly speaking, the Rayleigh distillation equation does not apply to the uptake of bicarbonate or aqueous CO_2 , $[\text{CO}_2(\text{aq})]$, because the equations are based on a model (Mariotti et al. 1981) that assumes a single-step unidirectional reaction of the form



An important implication of this model is that $dP/dt = -(dS/dt)$. Thus, the model assumes that $d^{13}\text{C}_p = -d^{13}\text{C}_s$ and $d^{12}\text{C}_p = -d^{12}\text{C}_s$. Because of the dynamics of the carbonate \leftrightarrow bicarbonate \leftrightarrow $\text{CO}_2(\text{aq})$ equilibria, this condition is not satisfied.

However, if the DIC system is at isotopic equilibrium, the Rayleigh distillation model may give an excellent description of experimental data, with f taken to be the fraction of DIC remaining after time t and $\delta_s(0)$ equal to the $\delta^{13}\text{C}$ of the DIC at t of 0. The reason is that at isotopic equilibrium ϵ_{ab} , ϵ_{ac} , and ϵ_{bc} are constant, where the subscripts a , b , and c refer to $\text{CO}_2(\text{aq})$, bicarbonate, and carbonate, respectively. Under these conditions it is straightforward to show that to a high degree of approximation:

$$\begin{aligned} \epsilon_{da} &= \epsilon_{ba}X_b + \epsilon_{ca}X_c \\ \epsilon_{db} &= \epsilon_{ab}X_a + \epsilon_{cb}X_c, \end{aligned} \quad (3)$$

where the subscript d refers to DIC, and X_a , X_b , and X_c are the mole fractions of a , b , and c , respectively. Assuming that the mole fractions of a , b , and c remain constant during the course of the experiment, Eq. 1 will therefore give an excellent description of the experimental data, with the understanding that $\epsilon_{ps} \cong \epsilon_{pa} - \epsilon_{da}$ if $\text{CO}_2(\text{aq})$ is the substrate taken up, and $\epsilon_{ps} \cong \epsilon_{pb} - \epsilon_{db}$ if bicarbonate is the substrate taken up. An important qualification to this argument is that the mole fractions of a , b , and c do not change enough during the course of the experiment to invalidate the assumption that ϵ_{ia} and ϵ_{ib} are approximately constant. Assuming this to be the case, Eq. 1 should give a good fit to experimental data, regardless of which form of inorganic carbon is taken up by the cells, and hence it should be impossible to distinguish between uptake of $\text{CO}_2(\text{aq})$ or bicarbonate based on goodness of fit to plots of $\delta_p(t)$ vs. DIC.

In fitting their experimental data, Thompson and Calvert

correctly equated $\delta_s(0)$ to the $\delta^{13}\text{C}$ of the DIC at $t = 0$ when testing the assumption that the cells were taking up bicarbonate, and they obtained a good fit to their experimental data. However, when testing the assumption that the cells were taking up $\text{CO}_2(\text{aq})$, they incorrectly equated $\delta_s(0)$ to the $\delta^{13}\text{C}$ of the $\text{CO}_2(\text{aq})$ at $t = 0$, and this fact accounts for the poor fit they obtained in their experimental results when assuming $\text{CO}_2(\text{aq})$ uptake. It should be clear from Eq. 1 that as $f \rightarrow 0$, $\delta_p(t) \rightarrow \delta_s(0)$. Because of the dynamics of the inorganic carbon system, the isotopic composition of the organic carbon will approach the isotopic composition of the initial DIC as the DIC is exhausted, regardless of whether bicarbonate or $\text{CO}_2(\text{aq})$ was the form of inorganic carbon taken up. Hence, if Thompson and Calvert had applied the Rayleigh distillation model correctly, it would have been impossible for them to distinguish between uptake of $\text{CO}_2(\text{aq})$ and uptake of bicarbonate based on their analysis.

However, before concluding that it is impossible to determine the source of inorganic carbon from the data presented by Thompson and Calvert, it is appropriate to ask whether a closer examination of the data and use of a more rigorous theoretical model might permit a distinction to be made between uptake of $\text{CO}_2(\text{aq})$ and bicarbonate. Fitting the data to the Rayleigh distillation model requires assuming no change in the mole fractions of a , b , and c during each experiment. The pH changes reported by Thompson and Calvert are clear evidence that this assumption was violated. The changes amounted to as much as 0.9 pH unit.

The extent to which violation of this assumption might permit a distinction between $\text{CO}_2(\text{aq})$ and bicarbonate utilization can be determined by numerically integrating the appropriate set of differential equations describing the assimilation of inorganic carbon by phytoplankton in a closed batch culture system. The relevant equations are

$$^{12}\text{C}_p(t + \Delta t) = ^{12}\text{C}_p(t) + \Delta^{12}\text{C}_p \quad (4)$$

$$^{13}\text{C}_p(t + \Delta t) = ^{13}\text{C}_p(t) + \Delta^{12}\text{C}_p(^{13}\text{C}_s/^{12}\text{C}_s)/\alpha_{ps} \quad (5)$$

$$\text{DI}^{12}\text{C}(t + \Delta t) = \text{DI}^{12}\text{C}(t) - \Delta^{12}\text{C}_p \quad (6)$$

$$\text{DI}^{13}\text{C}(t + \Delta t) = \text{DI}^{13}\text{C}(t) - \Delta^{12}\text{C}_p(^{13}\text{C}_s/^{12}\text{C}_s)/\alpha_{ps}, \quad (7)$$

where $^{13}\text{C}_s/^{12}\text{C}_s$ is the $^{13}\text{C}/^{12}\text{C}$ ratio in the source carbon, either bicarbonate or $\text{CO}_2(\text{aq})$, and $\alpha_{ps} = 1 + \epsilon_{ps}/1,000$. We assumed the initial DIC concentration to be $2,076.5 \mu\text{mol kg}^{-1}$ and the initial pH to be 8.2, as in Thompson and Calvert (1994). The DIC system was assumed to be in both chemical and isotopic equilibrium at each time. Following Thompson and Calvert, we calculated the equilibrium concentrations of bicarbonate, carbonate, and $\text{CO}_2(\text{aq})$ by using the apparent dissociation constants of carbonic acid in seawater given by Mehrbach et al. (1973). The $^{13}\text{C}/^{12}\text{C}$ ratios in the bicarbonate, carbonate, and $\text{CO}_2(\text{aq})$ were calculated by using Eq. 3, the $^{13}\text{C}/^{12}\text{C}$ ratio of the DIC (Eq. 6, 7), and ϵ_{ba} of 9.30 and ϵ_{ca} of 7.73 (Deines et al. 1974). $\Delta^{12}\text{C}_p$ was chosen so that the total decrease in DIC at each time step, i.e. $\Delta^{12}\text{C}_p + \Delta^{12}\text{C}_p(^{13}\text{C}_s/^{12}\text{C}_s)/\alpha_{ps}$, was equal to 0.1% of the DIC at time t .

Figure 1 shows the results of this exercise assuming (1) that bicarbonate was the source carbon with a discrimination factor of 20.0‰ and (2) that $\text{CO}_2(\text{aq})$ was the source carbon with a discrimination factor of $20.0 - \epsilon_{ba}$ ($20.0 - 9.3 =$

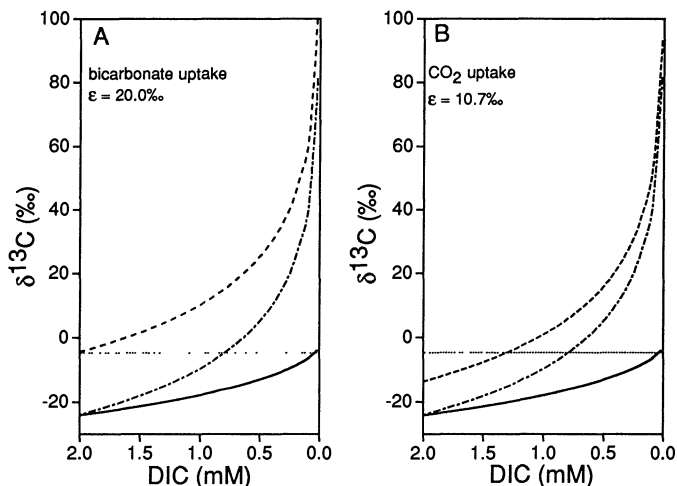


Fig. 1. Relationship between the $\delta^{13}\text{C}$ of the accumulated POC (solid curve), source carbon (dashed curve), and incremental POC (dash-dot curve) in a closed seawater batch culture system with an initial pH of 8.2 and DIC of $2,076.5 \mu\text{mol kg}^{-1}$ at 18°C and salinity of 33‰ obtained by integrating Eq. 10–13 and assuming (A) bicarbonate uptake with $\epsilon_{pb} = 20.0\text{‰}$ and (B) $\text{CO}_2(\text{aq})$ uptake with $\epsilon_{pa} = 10.7\text{‰}$. The dotted line is the $\delta^{13}\text{C}$ of the DIC initially, -4.9‰ .

10.7‰). The model results are virtually indistinguishable with respect to the predicted relationship between δ_p and DIC. Once again, the implication is that there is no way to distinguish between uptake of bicarbonate and $\text{CO}_2(\text{aq})$ based on plots of δ_p vs. DIC.

To explore this issue further, we numerically integrated Eq. 4–7 to the final DIC concentration at the time of harvest for all the *T. pseudonana* experiments reported by Thompson and Calvert (1994). In nine of those experiments, the initial pH and DIC were 9.2 and $2,066 \mu\text{mol kg}^{-1}$, respectively. In the other 30 experiments, the initial pH and DIC were 8.2 and $2,076.5 \mu\text{mol kg}^{-1}$. We varied ϵ_{ps} to give the best least-squares fit to the measured δ_p values. The results of this exercise are shown in Fig. 2. The best fit to the data was obtained assuming either bicarbonate to be the source of inorganic carbon with an ϵ_{ps} of 20.0‰ or $\text{CO}_2(\text{aq})$ to be the source of the inorganic carbon with an ϵ_{ps} of $20.0 - 9.3 = 10.7\text{‰}$. The results were virtually identical for the two scenarios. The standard deviation of the difference between the measured and calculated δ_p values was 1‰.

From this analysis one clearly cannot distinguish between uptake of bicarbonate and uptake of $\text{CO}_2(\text{aq})$ based on nothing more than goodness-of-fit to plots of δ_p vs. DIC. The assumptions of bicarbonate or $\text{CO}_2(\text{aq})$ uptake will give equally good fits to the data if one chooses the right value of ϵ_{ps} .

An estimation of whether phytoplankton are using primarily bicarbonate or primarily $\text{CO}_2(\text{aq})$ as a source of inorganic carbon must therefore be based on considerations other than goodness of fit to DIC vs. δ_p data. Several aspects of the results reported by Thompson and Calvert (1994) and shown in Fig. 2 may bear on the issue of bicarbonate vs. $\text{CO}_2(\text{aq})$ uptake. First, there is no correlation between growth rate and the measured δ_p values. For example, in the case of

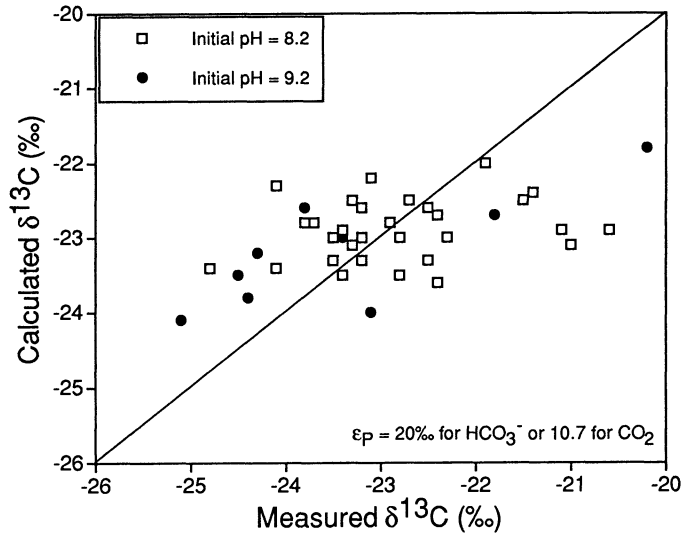


Fig. 2. Experimental values of $\delta^{13}\text{C}_{\text{POC}}$ at the time of harvest for *T. pseudonana* cultures started at a pH of 8.2 (*) and 9.2 (+) vs. values calculated by integrating Eq. 10–13 assuming uptake of $\text{CO}_2(\text{aq})$ with $\epsilon_{ps} = 20.0 - 9.3 = 10.7\%$. The straight line corresponds to equality of the calculated and experimental values. Results obtained by assuming uptake of bicarbonate with $\epsilon_{pb} = 20.0\%$ differed from the values plotted here by at most 0.1‰.

eight cultures grown on continuous light and started at a pH of 8.2, growth rates ranged from 0.23 to 1.76 d^{-1} , but the δ_p values ranged from only -24.1 to -21.1% , and the correlation coefficient between growth rate and δ_p was only -0.28 , which was statistically insignificant. Second, virtually the same ϵ_{ps} described the results of the cultures started at pH 8.2 and 9.2. Third, there is no significant correlation between the measured and calculated δ_p values.

The lack of correlation between the δ_p values and growth rate is especially noteworthy, since theoretical considerations would lead one to expect a correlation, regardless of whether the algae were taking up bicarbonate or $\text{CO}_2(\text{aq})$. Assuming that isotope discrimination effects due to respiration and photorespiration are negligible, Francois et al. (1993) have shown that ϵ_{ps} should be related to growth rate through the equation

$$\epsilon_{ps} = \epsilon_1 + \frac{\epsilon_2 - \epsilon_{-1}}{1 + (\mu C/k_{-1} C_i)}, \quad (8)$$

where μ is the growth rate, C is the carbon cell quota, k_{-1} is the permeability of the cell membrane to CO_2 , C_i is the concentration of CO_2 in the cytoplasm, and ϵ_1 , ϵ_{-1} , and ϵ_2 are the isotopic discriminations associated with uptake of inorganic carbon (either bicarbonate or CO_2) through the membrane into the cell, diffusion of CO_2 back into the surrounding water, and enzymatic carboxylation to produce phytoplankton biomass, respectively. Thus, one would expect to see a negative correlation between ϵ_{ps} and μ and hence a positive correlation between δ_p and μ , regardless of whether the cells were taking up $\text{CO}_2(\text{aq})$ or bicarbonate.

The absence of such a correlation can be explained if one assumes that μ and C_i are proportional to each other. Is there any reason to think that this might be the case? It is well

known that the primary carboxylating enzyme ribulose biphosphate carboxylase oxygenase (Rubisco) has a low affinity for CO_2 , with reported half-saturation constants for marine microalgae being on the order of 100 μM (Glover 1989). If C_i is less than $\sim 100 \mu\text{M}$, it is therefore quite possible that the rate of carboxylation and hence the growth rate of the cell would be roughly proportional to C_i . Assuming this to be the case, Eq. 8 implies that there would be no correlation between ϵ_{ps} and μ , regardless of whether bicarbonate or $\text{CO}_2(\text{aq})$ were the form of carbon taken up by the cell.

Models of diffusive uptake of $\text{CO}_2(\text{aq})$ predict a positive correlation between ϵ_{ps} and $\text{CO}_2(\text{aq})$ (Goericke et al. 1994; Laws et al. 1995). Because the $\text{CO}_2(\text{aq})$ concentration in the *T. pseudonana* cultures started at pH 9.2 was several orders of magnitude lower than the $\text{CO}_2(\text{aq})$ concentration in the cultures started at pH 8.2 (Thompson and Calvert 1994; Fig. 2B), the fact that virtually the same ϵ_{ps} gives a good fit to both datasets (Fig. 2) suggests that the *T. pseudonana* cells were not fixing $\text{CO}_2(\text{aq})$ that had entered the cells by passive diffusion from the bulk medium. Alternative scenarios include (1) active transport of bicarbonate, (2) active transport of CO_2 , or (3) conversion of bicarbonate to CO_2 by means of an extracellular carbonic anhydrase followed by transport of the CO_2 into the cell via either active transport or passive diffusion. With respect to the question of the source of inorganic carbon, it seems appropriate to ask two questions: (1) Will the alga use bicarbonate when the supply of $\text{CO}_2(\text{aq})$ in the medium is adequate to meet its needs? (2) Can the alga use bicarbonate when the supply of $\text{CO}_2(\text{aq})$ in the medium is inadequate to meet its needs? Rotatore et al. (1995) and Colman and Rotatore (1995), for example, have convincingly shown that the diatom *Phaeodactylum tricornutum* has the ability to actively take up bicarbonate. Their experiments, however, were conducted at chlorophyll *a* (Chl *a*) concentrations of 15–20 $\mu\text{g ml}^{-1}$, 2–3 orders of magnitude higher than the Chl *a* concentrations in Thompson and Calvert's batch cultures and at least 3 orders of magnitude higher than typical Chl *a* concentrations found in the marine environment, even under bloom conditions (Yoder et al. 1994). At such high phytoplankton concentrations the uncatalyzed conversion of bicarbonate to $\text{CO}_2(\text{aq})$ fails to meet the requirements of the algae for inorganic carbon at moderate to high growth rates, and in the absence of an external carbonic anhydrase (CA) the algae could achieve rapid growth only by actively taking up bicarbonate. Even under these extreme conditions, "The measured CO_2 uptake rates . . . accounted for 50% of the total DIC uptake at HCO_3^- - CO_2 equilibrium" (Rotatore et al. 1995, p. 913). Rotatore et al. (1995, p. 914) concluded, "The cells therefore appear to take up CO_2 preferentially from the medium."

The dynamics of the bicarbonate \leftrightarrow $\text{CO}_2(\text{aq})$ reaction raise two questions with respect to the issue of inorganic carbon uptake. First, is the uncatalyzed rate of conversion of bicarbonate to $\text{CO}_2(\text{aq})$ sufficient to meet the needs of the algae? Second, is the chemical and isotopic disequilibrium between bicarbonate and $\text{CO}_2(\text{aq})$ large enough that conclusions based on equilibrium model calculations are seriously in error?

To explore these questions, we adopted a second finite

difference model in which the concentration of CO_2 was assumed to be governed by the equations (Johnson 1982)

$$\frac{\Delta(\text{CO}_2)}{\Delta t} = (k_d a_{\text{H}} + k_{\text{HCO}_3^-})(\text{HCO}_3^-) - (k_{\text{CO}_2} + k_{\text{OH}^-} K_w / a_{\text{H}})(\text{CO}_2) - U, \quad (9)$$

where a_{H} is the hydrogen ion activity, k_d , $k_{\text{HCO}_3^-}$, k_{CO_2} , and k_{OH^-} are rate constants, and K_w is the thermodynamic dissociation constant of water. U is the uptake rate of inorganic carbon by the algae and was set equal to $\mu \times \text{POC}$. In accord with Riley and Skirrow (1965, p. 253) and Johnson (1982), the ionization reactions $\text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+$ and $\text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-} + \text{H}^+$ were assumed to be extremely rapid and hence at both chemical and isotopic equilibrium at all times.

To model the rate of change of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ using Eq. 9, it was necessary to know the kinetic fractionation factors associated with the hydration of CO_2 and dehydration of bicarbonate, α_1 and α_{-1} , respectively. Values of α_1 and α_{-1} at 24°C in freshwater are 1.0069 and 1.0147, respectively (Marlier and O'Leary 1984). Corresponding values in seawater have not been reported, but the ratio α_{-1}/α_1 must equal $\alpha_{ba} = 1 + \epsilon_{ba}/1,000$. Assuming that $\alpha_{ba} = 1.0093$ (Deines et al. 1974) at 18°C, we assumed $\alpha_{-1} = 1.0157$ and $\alpha_1 = 1.0157/1.0093 = 1.0063$. The fractionation associated with algal uptake was treated exactly as in Eq. 4–7. We integrated Eq. 9 using a Δt of 10 s and assuming an initial phytoplankton inoculum consisting of 0.01 mM particulate organic carbon (POC) with a δ_p of -23‰ . The integration was terminated when the DIC concentration had been reduced to the DIC concentration at the time of harvest in the *T. pseudonana* batch cultures.

The results of this exercise showed that for all of the cultures started at pH 8.2 the supply of CO_2 from the uncatalyzed dehydration of bicarbonate was more than adequate to meet the needs of the algae. The discrepancy between the final δ_p calculated from the equilibrium and nonequilibrium models was in all cases $<0.2\text{‰}$, in accord with the calculations of Goericke et al. (1994), who concluded (p. 195), "Biological activity does not change the isotopic equilibrium appreciably in a closed system under normal conditions . . . in seawater. Expected perturbations of the isotopic equilibrium are less than $\pm 0.2\text{‰}$ since rates of carbon uptake are usually orders of magnitude lower than rates of hydration and dehydration." The results were different for the cultures started at pH 9.2. At the highest reported growth rate, 1.74 d^{-1} , the uncatalyzed dehydration of bicarbonate was insufficient to allow the algae to attain their final biomass of 0.26 mM POC. Interestingly, however, it was possible for the algae to achieve a final biomass of 0.21 mM POC at a growth rate of 1.72 d^{-1} . In this second case, however, there was a 1% discrepancy between the final δ_p calculated from the equilibrium and nonequilibrium models, and at a growth rate of 1.1 d^{-1} there was a similar discrepancy of 0.37%.

The conclusion is that for almost all of the experiments reported by Thompson and Calvert (1994), the supply of CO_2 from the uncatalyzed dehydration of bicarbonate would have been sufficient to meet the needs of the algae throughout the batch culture cycle. A similar analysis of the *E. huxleyi* data leads to the same conclusion, with the effect of

lower temperature (10°C vs. 18°C) on bicarbonate \leftrightarrow $\text{CO}_2(\text{aq})$ kinetics being more than offset by the lower maximum growth rate of the algae and the fact that DIC concentrations were reduced to only ~ 1.8 mM when the cells were harvested. However, isotopic disequilibria in the cases of the *T. pseudonana* cultures started at pH 9.2 were probably sufficient to create discrepancies of ~ 0.5 – 1.0‰ with δ_p values estimated from equilibrium theory. However, since the standard deviation of the δ_p values from replicated cultures in these experiments was also 0.5– 1.0‰ , such discrepancies might be difficult to distinguish from the noise in the data.

A second consideration with respect to CO_2 supply is the rate of diffusion of CO_2 from the bulk medium to the cell surface through the boundary layer around each cell. This problem has been considered in some detail by Pasciak and Gavis (1975) and Riebesell et al. (1993). For a cell of spherical geometry, equation 3 of Riebesell et al. (1993) can be used to calculate the diffusive flux of CO_2 through the boundary layer required to support a given growth rate. Conservatively ignoring any conversion of bicarbonate to CO_2 within the boundary layer, the ratio of the CO_2 concentration at the cell surface, C_s , to the equilibrium $\text{CO}_2(\text{aq})$ concentration in the bulk medium, C_e , is given by the equation

$$\frac{C_s}{C_e} = 1 - \frac{C_p \mu}{4\pi r D C_e}, \quad (10)$$

where D is the diffusion coefficient for $\text{CO}_2(\text{aq})$, r is the cell radius, and C_p is the carbon content of each phytoplankton cell. For *T. pseudonana*, r and C_p are ~ 2.2 μm and 6 pg cell^{-1} , respectively. Corresponding figures for *E. huxleyi* are 2.5 μm and 4.6 pg cell^{-1} (Montagnes et al. 1994). We assume that $D = 1.38 \times 10^{-4}$ $\text{m}^2 \text{d}^{-1}$ (Riebesell et al. 1993). Noting that for carbon 1 $\mu\text{M} = 12 \times 10^9$ pg m^{-3} , $C_p/4\pi r D = 0.048$ and 0.042 $\text{d } \mu\text{M}$ for *T. pseudonana* and *E. huxleyi*, respectively. The C_s/C_e ratio required to support a given growth rate is therefore $1 - [0.048(\mu/C_e)]$ for *T. pseudonana* and $1 - [0.042(\mu/C_e)]$ for *E. huxleyi*. For the *T. pseudonana* cultures started at pH 8.2, C_e never dropped below ~ 1.0 μM (Thompson and Calvert 1994; Fig. 2B). Because the growth rates of these cultures never exceeded 2.0 d^{-1} , it is fair to say that a C_s/C_e ratio of no less than $1 - (0.048)(2) = 0.90$ could have produced enough diffusion of CO_2 through the boundary layer around the cells to provide the inorganic carbon needed for photosynthesis. The C_s/C_e ratio could have been even higher in the case of the *E. huxleyi* cultures, since the maximum growth rate was only 0.36 d^{-1} and DIC concentrations were reduced by only $\sim 7\%$. Based on this analysis, it does not appear that diffusion of CO_2 through the boundary layer around the cells should have been a limiting factor for the *E. huxleyi* cultures or the *T. pseudonana* cultures started at a pH of 8.2.

The *T. pseudonana* cultures started at a pH of 9.2 are a different matter, since the initial C_e was ~ 0.8 μM and in some cases the final C_e was only ~ 0.01 μM (Thompson and Calvert 1994; Fig. 2B). Because C_s cannot be negative, diffusion of CO_2 through the boundary layer could not have provided the inorganic carbon needed for photosynthesis if $\mu/C_e > 1/0.048$ ($= 21 \text{ d}^{-1} \mu\text{M}^{-1}$). Analysis of the final pH

and DIC results indicates that μ/C_e was $>21 \text{ d}^{-1} \mu\text{M}^{-1}$ by the time of harvest for all of the *T. pseudonana* cultures started at pH 9.2. The implication of this analysis is that while diffusion of CO_2 through the boundary layer should not have been limiting initially for the *T. pseudonana* cultures started at pH 9.2, it would have become limiting in all cases toward the end of the incubations.

We conclude that the supply of CO_2 should have been more than adequate to meet the needs of the *E. huxleyi* cultures and of the *T. pseudonana* cultures started at pH 8.2. However, sometime before harvest the supply of CO_2 would have become limiting for the *T. pseudonana* cultures started at pH 9.2, and the cells would have been forced to utilize bicarbonate in some way to sustain the reported growth rates. There are basically two ways the cells could have utilized bicarbonate. First, they could have speeded up the transformation of bicarbonate to CO_2 through an external CA. The existence of an external CA has been demonstrated in several marine phytoplankton by Burns and Beardall (1987) and Colman and Rotatore (1995). If such a mechanism were operative, "Isotope fractionation by carbonic anhydrase, which for the catalysed conversion of HCO_3^- to CO_2 is 10.1‰ . . . would largely cancel the initial difference in $\delta^{13}\text{C}$. . . between HCO_3^- and CO_2 . The $\delta^{13}\text{C}$ signal resulting from carbonic anhydrase-mediated HCO_3^- uptake and CO_2 uptake would essentially be indistinguishable" (Riebesell and Wolf-Gladrow 1995). Note, however, that the fractionation associated with CA activity in microalgae may differ from the figure of 10.1‰ cited by Riebesell and Wolf-Gladrow (1995), which comes from a study of bovine CA (Paneth and O'Leary 1985).

The second mechanism would be active uptake of bicarbonate followed by conversion of bicarbonate to CO_2 within the cell. If such uptake were in fact the dominant mechanism by which the cells acquired inorganic carbon, the phytoplankton carbon would indeed be isotopically heavier than if the cells were taking up only CO_2 . The similarity of the δ_p results for the *T. pseudonana* cultures started at pH 8.2 and 9.2 suggests that the same form of inorganic carbon entered the cells in both cases. This implies that either (1) the cells were using bicarbonate in both sets of experiments and that the bicarbonate was dehydrated internally or (2) the cells used both $\text{CO}_2(\text{aq})$ and bicarbonate, but the bicarbonate was dehydrated by an external CA and entered the cells as CO_2 .

Perhaps more thought-provoking than the *T. pseudonana* results are the ϵ_{ps} values calculated by Thompson and Calvert (1995) for *E. huxleyi*. The values are positively correlated with growth rate and equal 25‰ (assuming bicarbonate uptake) at the maximum measured growth rate of 0.35 d^{-1} . This result is very inconsistent with expectations based on supply/demand considerations (Francois et al. 1993).

Because all the *E. huxleyi* cultures were started at a pH of 8.13 and because the maximum growth rate was only 0.36 d^{-1} , there is no reason to think that the supply of $\text{CO}_2(\text{aq})$ would have been limiting for photosynthesis. In other words, the uncatalyzed conversion of bicarbonate to CO_2 and the diffusion of CO_2 through the boundary layer around the cell would have been more than adequate to supply the CO_2 needed by the algae. The forms of carbon used for photosynthesis and calcification by *E. huxleyi* have been studied

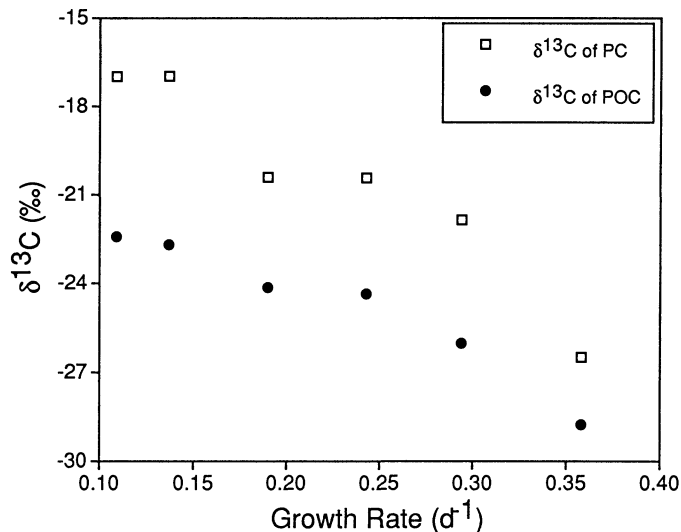


Fig. 3. $\delta^{13}\text{C}$ values for particulate organic carbon (POC) and particulate carbon (PC) reported by Thompson and Calvert (1995) for *E. huxleyi* as a function of growth rate.

by Sikes et al. (1980) by using $^{14}\text{CO}_2$ and $\text{H}^{14}\text{CO}_3^-$ as tracers and taking advantage of the relatively slow kinetics of the $\text{CO}_2(\text{aq}) \leftrightarrow$ bicarbonate reaction. The results of the $^{14}\text{CO}_2$ tracer experiments clearly demonstrated that the cells were taking up CO_2 and incorporating it into organic matter. The results of the $\text{H}^{14}\text{CO}_3^-$ tracer studies clearly showed that bicarbonate was the form of carbon used for coccolith formation and further demonstrated that the CO_2 resulting from carbonate deposition supplemented the CO_2 taken up from the medium for photosynthesis.

Coccolith formation in *E. huxleyi* takes place in a special intracellular compartment that has two morphologically distinct, lumenally connected parts that were called the reticular body and the coccolith room by Van Emborg (1989). Mineralization is restricted to the coccolith room. Because the CO_2 produced by CaCO_3 deposition becomes available for photosynthesis, it is apparent that there is some exchange of inorganic carbon between the coccolith room and the intracellular pool of DIC used for photosynthesis. Assuming that there is little fractionation associated with the uptake of bicarbonate and deposition of CaCO_3 , one might expect that the $\delta^{13}\text{C}$ of coccolith carbon would be similar to that of the external bicarbonate. However, if there is an exchange of DIC between the chloroplast and coccolith room, it is possible that the bicarbonate used for coccolith formation may become isotopically heavy due to the 25–28‰ fractionation associated with Rubisco.

Figure 3 shows the $\delta^{13}\text{C}$ values for POC and particulate carbon (PC) reported by Thompson and Calvert (1995) for *E. huxleyi* as a function of growth rate. The data follow a systematic pattern with apparently little noise. From these results and the fact that the coccolith carbon accounted for ~16% of the PC (Thompson and Calvert 1995, table 1), we have calculated in Fig. 4 the $\delta^{13}\text{C}$ of the coccolith carbon (δ_{cc}) and the difference between δ_{cc} and δ_p .

At the two lowest growth rates δ_{cc} was 16–17‰ heavier than the external bicarbonate. The explanation for this result

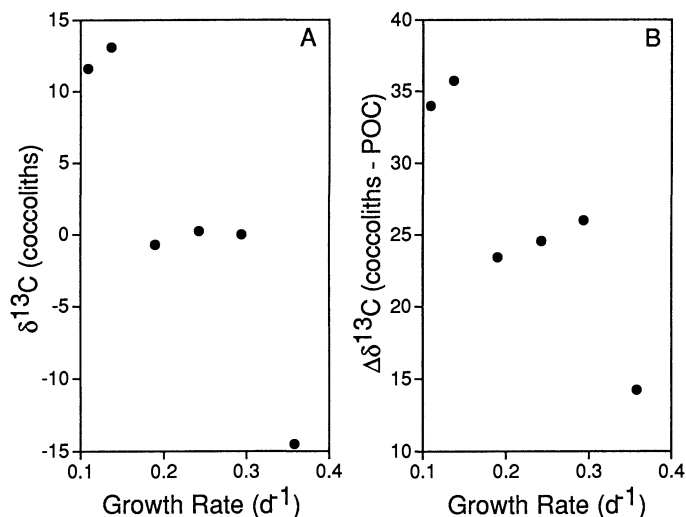


Fig. 4. (A) $\delta^{13}\text{C}$ of the coccolith carbon ($\delta^{13}\text{C}_{\text{CC}}$) and (B) the difference between $\delta^{13}\text{C}_{\text{CC}}$ and $\delta^{13}\text{C}_{\text{POC}}$ for *E. huxleyi* as a function of growth rate.

would seem to be that at these two growth rates much of the carbon used for coccolith formation was derived from isotopically heavy DIC resulting from Rubisco fractionation. Consistent with this explanation is the 35‰ difference between δ_{cc} and δ_{p} . If the CO_2 used for photosynthesis and the bicarbonate used for coccolith formation were drawn from a chemically and isotopically homogeneous intracellular pool, the bicarbonate would be $\sim 10\%$ heavier than the CO_2 , and the POC produced from the CO_2 would be 25–28‰ lighter than the CO_2 .

The extent to which CO_2 was taken up by the cells at these low growth rates may be inferred from an examination of ϵ_{ps} values and the relationship between ϵ_{ps} and growth rate. To calculate ϵ_{ps} at each growth rate, we integrated Eq. 4–7, assuming that 16% of the DIC uptake was used for CaCO_3 production and that the total alkalinity was decreased by twice the rate of formation of CaCO_3 . Based on supply/demand considerations one would expect the degree of fractionation to be highest at low growth rates, the maximum possible value being roughly the fractionation associated with Rubisco, 25–28‰ (Goericke et al. 1994). If we assume that most of the DIC used for photosynthesis entered the cells as CO_2 , the calculated ϵ_{ps} at the two lowest growth rates is $\sim 8.3\%$. If bicarbonate is assumed to be the form of DIC entering the cells, the calculated ϵ_{ps} is 18.3%. The latter figure is much closer to the maximum fractionation of 25–28‰ expected in the limit as $\mu \rightarrow 0$. Between the lowest and highest growth rates, the calculated ϵ_{ps} increases by $\sim 7.3\%$. This result is inconsistent with supply/demand considerations, but would follow logically if the DIC entering the cells became progressively lighter at higher growth rates. A complete shift from bicarbonate uptake to CO_2 uptake would imply a decrease of 10‰ in the $\delta^{13}\text{C}$ of the DIC entering the cells. Because the range of growth rates in this case is only 0.26 d^{-1} , supply/demand considerations would suggest only a small decrease in ϵ_{ps} . Thus a decrease of $\sim 2\text{--}3\%$ in ϵ_{ps} due to increased growth rate combined with a shift

from primarily bicarbonate to primarily CO_2 uptake could account for the apparent net increase of 7.3‰ in ϵ_{ps} .

The decrease in the difference between δ_{cc} and δ_{p} with increasing growth rate suggests that the DIC in the coccolith room became increasingly isolated from the DIC in the chloroplast. This increasing isolation presumably reflects the more rapid turnover rate of these pools at increasing growth rates relative to the rate of intracellular exchange. A surprising result is that at the highest growth rate the coccolith carbon is lighter than the bicarbonate in the external medium by $\sim 10\%$. The most likely explanation for this observation is that the DIC in the coccolith room was diluted with DIC derived from respiration. The latter would be expected to have a $\delta^{13}\text{C}$ similar to that of the POC, which at this growth rate was -29% .

In conclusion, the *E. huxleyi* results suggest that at low growth rates bicarbonate accounted for most DIC entering the cells, and the internal pool of DIC seems to have been isotopically homogeneous. At the highest growth rate most of the carbon used for photosynthesis seems to have entered the cells as CO_2 , a conclusion consistent with the studies of Sikes et al. (1980), and the intracellular DIC used for photosynthesis seems to have been isotopically heavy compared to the DIC used for coccolith formation. The latter appears to have been substantially diluted with isotopically light DIC derived from respiration.

In addition to the interesting physiological information gained from this study, the results have important implications for geochemists, who have often assumed that the $\delta^{13}\text{C}$ of phytoplankton carbon is related to the concentration of $\text{CO}_2(\text{aq})$ (Fry and Wainright 1991; Rau et al. 1992). The rationale for such a relationship is based on the assumption that phytoplankton take up $\text{CO}_2(\text{aq})$ rather than bicarbonate. There is little doubt, however, that coccolithophorids have the ability to take up bicarbonate and that CO_2 derived from the deposition of CaCO_3 is used for photosynthesis (Sikes and Fabry 1994). There is also general agreement that coccolithophorids have the ability to take up CO_2 and that their organic carbon/carbonate carbon ratio exceeds 1 (Sikes and Fabry 1994). What has been unclear is the extent to which coccolithophorids may use CO_2 derived from bicarbonate uptake for photosynthesis, irrespective of CaCO_3 deposition. The *E. huxleyi* results reported by Thompson and Calvert (1995) are particularly intriguing in that they suggest that CO_2 derived from bicarbonate uptake is the primary source of CO_2 for photosynthesis at low growth rates, but that direct uptake of CO_2 becomes the most important source of CO_2 at high growth rates. The reason for this transition is unclear, nor is it known whether other coccolithophorids behave in a similar way. Studies of other coccolithophorids, including both calcifying and noncalcifying strains, should prove informative.

Based on theoretical considerations, the lack of any apparent dependence of ϵ_{ps} on growth rate or $\text{CO}_2(\text{aq})$ in *T. pseudonana* would seem to indicate that growth rate and the internal concentration of CO_2 , C_{i} , are directly proportional to each other. This behavior can be rationalized on the assumption that C_{i} is less than the half-saturation constant for Rubisco, which is on the order of $100 \mu\text{M}$ (Glover 1989). Assuming that this is the explanation, it is unclear whether such behavior will prove typical or atypical of marine phy-

toplankton. Laws et al. (1995), for example, have shown that the diatom *Phaeodactylum tricornutum* shows the pattern between ϵ_{ps} , growth rate, and $\text{CO}_2(\text{aq})$ concentration expected from theoretical considerations based on $\text{CO}_2(\text{aq})$ uptake. Studies on more species will be needed to determine what general conclusions, if any, can be reached concerning the relationship between ϵ_{ps} , growth rate, and $\text{CO}_2(\text{aq})$ concentration in marine phytoplankton. The results of such studies will have important implications, for example, for the use of the $\delta^{13}\text{C}$ of sedimentary organic compounds as indicators of paleo $\text{CO}_2(\text{aq})$ concentrations.

Edward A. Laws

Department of Oceanography
School of Ocean and Earth Science
and Technology
University of Hawaii
Honolulu, Hawaii 96822

Peter A. Thompson

CSIRO, Division of Fisheries
P.O. Box 20, North Beach
6020 WA, Australia

Brian N. Popp

Department of Geology and Geophysics
School of Ocean and Earth Science
and Technology
University of Hawaii

Robert R. Bidigare

Department of Oceanography
School of Ocean and Earth Science
and Technology
University of Hawaii

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