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REVIEW

$^{13}C/^{12}C$ fractionation by marine diatoms

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ABSTRACT: The $^{13}C/^{12}C$ distributions in plants record the integrated pattern of photosynthetic carbon acquisition. Most planktonic marine algae are small, relatively rare and hard to separate from background particles, so that very few investigations have used $\delta^{13}C$ to study algal photosynthesis in the sea. Diatoms are perhaps the best studied group among the marine microalgae, and this review summarizes current knowledge of effects of temperature, salinity, pH, growth rate and CO_2 concentrations on diatom $\delta^{13}C$. External CO_2 concentrations have the strongest effect demonstrated thus far on $^{13}C/^{12}C$ distributions and isotope fractionation, with the largest isotopic discriminations found in high CO_2 conditions. Both models and culture studies suggest that $\delta^{13}C$ values record the balance between algal growth demand versus supply of inorganic carbon in seawater, and that carbon isotope studies can be used to probe the diversity of algal carbon acquisition strategies in the sea.

KEY WORDS: Diatoms · $^{13}C/^{12}C$ Isotope fractionation $CO_2(aq)$ · Biomarkers

INTRODUCTION

Stable carbon isotopic investigations of terrestrial plants are now far advanced, in large part because it is easy to collect the 100 to 1000 µg of material necessary for analysis. An impressive amount of literature concerning $\delta^{13}C$ in terrestrial plants shows that these measurements can be used to distinguish C_3, C_4 and CAM plants, establish variations in water use efficiency, and show limits of physiological response to environmental change (see reviews by O'Leary 1988, Farquhar et al. 1989, Lajtha & Marshall 1994). These terrestrial studies promise a rich dividend to physiologists working with other groups of plants, and the purpose of this review is to stimulate an interest in carbon isotope distributions in the marine microalgae.

There are 3 reasons for renewed interest in the $\delta^{13}C$ of microalgae. First, recent culture studies confirm that large (5 to 10‰) differences can be commonly observed both within and between species of microalgae (Wong & Sackett 1978, Hinga et al. 1994), so that $\delta^{13}C$ should be a sensitive indicator of metabolic strategies and performance among the plankton. Second, new techniques are becoming available for separating either whole cells (Olson & Fry unpubl.) or biomarker compounds (Hayes et al. 1990) from seawater so that microalgal samples can be obtained from a variety of interesting field conditions for comparison with culture results. A final impetus for fresh interest in this topic is the observation that over geological time scales, the average $\delta^{13}C$ value of marine phytoplankton has varied by at least 5‰ (Dean et al. 1986), possibly in response to variations in past CO_2 levels. The suggestion that algal $\delta^{13}C$ can be used as a CO_2 paleobarometer (Popp et al. 1989, Rau et al. 1989, Hollander & Mackenzie 1991, Singer & Shemesh 1995) relevant to global change research has brought renewed attention to the study of $^{13}C/^{12}C$ variations in microalgae.

This review is restricted to diatoms and complements broader reviews summarizing $\delta^{13}C$ variations in aquatic algae (Descolas-Gros & Fontugne 1990, Raven 1992, Goericke et al. 1994) and marine particulate organic material (Jeffry et al. 1983, Sackett 1991, Goericke & Fry 1994). The 2 main foci of this review are culture and field results for diatoms. Because it continues to be difficult to collect microalgae from the field, and no technique is yet available to analyse isotopic compositions of individual phytoplankters, I emphasize culture studies as tools for developing and testing models of

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algal isotopic fractionation. Mechanics of making the requisite DIC (dissolved inorganic carbon) and algal measurements in culture work for correct calculation of $^{13}C/^{12}C$ photosynthetic fractionation are reviewed in some detail before discussing the still relatively few laboratory and field results. A concluding section discusses new ways to more routinely sample microalgal $\delta^{13}C$ values in aquatic environments.

ISOTOPE TERMINOLOGY

Photosynthetic carbon fixation occurs with a strong fractionation such that $^{13}CO_2$ is fixed at slower rates than $^{12}CO_2$. Respiration seems to involve very little or no fractionation (O'Leary 1981, Laws et al. 1995). Consequently, isotope fractionation studies dealing with plants largely focus on photosynthetic withdrawal of carbon from the DIC pool during uptake and fixation into cellular biomass. A first approximation of fractionation that, as will be discussed below, is not always a good estimate of the intrinsic fractionation occurring in a system is the observed fractionation between fixed carbon and available DIC. Following conventions established by O'Leary (1981), fractionation or isotopic discrimination can be expressed as a positive number, D, calculated as

$$D = (\delta^{13}C_{DIC} - \delta^{13}C_{FIXED\,C})/(1+ \delta^{13}C_{DIC}/1000) \quad (1)$$

where $\delta^{13}C$ values are measured versus PDB or a working CO_2 gas standard, and DIC can be the total DIC (including CO_2, bicarbonate and carbonate) or the free CO_2 alone, CO_2(aq), that in seawater is only about 1% of the total DIC pool. The remaining DIC in pH 8 seawater is mostly present as bicarbonate. Actual isotopic measurements are made of fixed C and total DIC, while $\delta^{13}C$ of CO_2(aq) can be calculated from $\delta^{13}C$ of total DIC using equations of Mook et al. (1974) When $\delta^{13}C$ of DIC is in the −10 to +10‰ range, Eq. (1) can be simplified with very little loss in accuracy (0.4‰ or less for work with photosynthetic C fixation) to

$$D = \delta^{13}C_{DIC} - \delta^{13}C_{FIXED\,C} \quad (2)$$

since the denominator is very nearly 1 (0.99 to 1.01).

In this paper, I mainly report fractionation versus total DIC rather than versus the isotopic composition of CO_2(aq), partly because it is often not clear whether phytoplankton use only CO_2 or a combination of CO_2 and bicarbonate, and partly because $\delta^{13}C$ values of DIC are measured parameters, while $\delta^{13}C$ values of CO_2 are calculated from the $\delta^{13}C_{DIC}$ values. Reporting D versus total DIC also makes it fairly simple to compare measured fractionation with marine $\delta^{13}C$ values reported in the literature, since the reference material in both cases has near-zero values. Specifically, the value of the marine carbonate standard, a fossil Belemnite shell collected from the PeeDee formation of South Carolina (Craig 1953), is assigned a 0‰ value, while DIC measured in surface waters of the modern ocean averages about 1.5‰.

ISOTOPE FRACTIONATION DURING AQUATIC PHOTOSYNTHESIS

The overall fractionation observed between DIC and photosynthetically fixed C reflects 3 major components that can be sequentially considered starting with the external DIC pool and ending with carboxylation (Fig. 1).

(1) *DIC dynamics.* The external DIC pool contains free CO_2 that is lighter than the bulk DIC pool by typically 8 to 12‰ in the marine environment (Mook et al. 1974). An equilibrium isotope effect in the hydration/dehydration reactions between bicarbonate and CO_2 concentrates isotopically light carbon in the CO_2

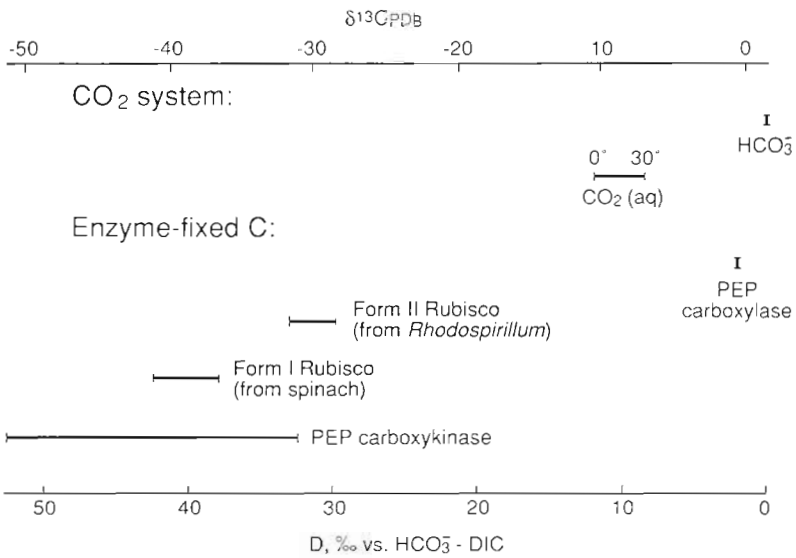

Fig. 1. $\delta^{13}C$ for components of the marine inorganic carbon system and isotopic discrimination (D, ‰) expected for various steps in photosynthetic carbon fixation. A temperature-dependent equilibrium isotope effect results in the isotopic difference between bicarbonate and CO_2(aq), while kinetic isotope effects result in enzyme fixed C having lower $^{13}C/^{12}C$ values than their substrates (bicarbonate substrate for PEP carboxylase, CO_2(aq) substrate for the other enzymes). D is plotted increasing to the left to show the close correspondence between D and conventional marine $^{13}C/^{12}C$ measurements reported as $\delta^{13}C_{PDB}$ (top axis)

in a temperature sensitive manner, with CO_2 values 12‰ lower than those of bicarbonate at 0°C and 8.4‰ lower at 30°C (Mook et al. 1974). The consequence of this chemical equilibrium is that cells using CO_2 should have $\delta^{13}C$ values 8 to 12‰ lower than cells using bicarbonate.

(2) *Transport steps.* During the fixation process, DIC is transported across various compartment boundaries (cell walls, cytoplasmic barriers, etc). Laboratory experiments show that isotopic fractionation associated with steps such as CO_2 diffusion in aqueous media are small, <1‰ (O'Leary 1981).

(3) *Carboxylation reactions.* A third step is the actual carboxylation which can be catalyzed by several enzymes (Fig. 1). Carboxylation by Rubisco similar to that isolated from spinach is most common, and a 29‰ isotopic fractionation versus CO_2(aq), or 37‰ at 30°C versus total DIC, is expected (Roeske & O'Leary 1984, Guy et al. 1987). A smaller fractionation of 17.8‰ occurs with Rubisco isolated from photosynthetic bacteria (Form II Rubisco from *Rhodospirillum rubrum*, Roeske & O'Leary 1985, Robinson & Cavanaugh 1995), although some photosynthetic bacteria use Form I Rubisco instead of or in addition to Form II Rubisco. The possible occurrence of Form II Rubisco among marine diatoms is currently under investigation (C. Cavanaugh pers. comm.).

In addition to Rubisco, β-carboxylases play a more minor role in C uptake, usually during dark anaplerotic reactions and reactions associated with dark ammonium uptake (Descolas-Gros & Fontugne 1985, Guy et al. 1989). Large differences in fractionation are however expected for the 2 major β-carboxylases, with a small (2‰) fractionation versus bicarbonate being typical of PEP carboxylase, but a much larger (24 to 40‰) fractionation versus CO_2 pertaining to PEP carboxykinase (O'Leary 1981, Arnelle & O'Leary 1992; Fig. 1). PEP carboxykinase but not PEP carboyxlase activity is common in diatoms (Descolas-Gros & Oriol 1992).

Models developed for terrestrial photosynthesis show that it is the interaction between transport and carboxylation steps that typically determines the observed overall fractionation between DIC and fixed C (Fig. 2). The extreme cases involving low and high rates of photosynthesis can be understood intuitively. If carboxylation reactions are relatively slow, and most DIC entering cells freely leaves again so that internal and external DIC concentrations are similar, maximum fractionation typical of the CO_2 saturated carboxylase is expected. However, at high rates of photosynthesis, if all DIC entering cells is later carboxylated, there is no opportunity for isotopic fractionation or discrimination at the carboxylation step since all DIC is used, regardless of isotopic composition. Because the fractionation during transport is small while the fractionation in carboxylation is potentially large, observed fractionation should theoretically vary from near-zero when transport steps are limiting to about 40‰ versus DIC when carboxylation is slow and occurs via a spinach-type Rubisco (Fig. 2).

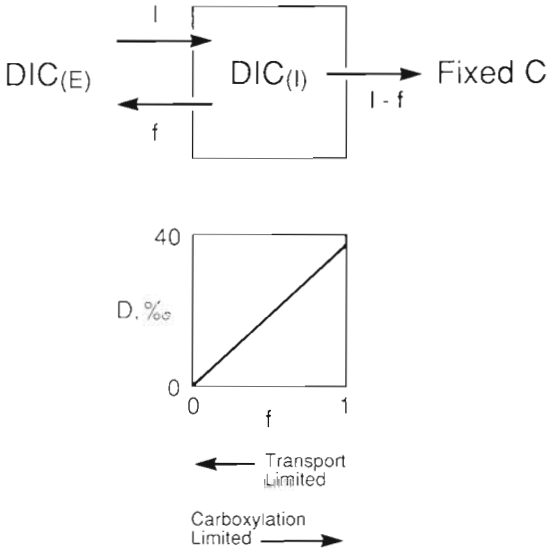

Fig. 2. Model of DIC uptake and fixation by diatoms, with isotopic discrimination (D, ‰) as a function of f, the cell leakiness of DIC from internal pools back to external pools (modified from O'Leary 1981, 1988). Photosynthetic discrimination is largest when equilibration between external and internal DIC pools is nearly complete (f approaches 1), and carboxylation is relatively slow

Overall, model results show that smaller fractionations indicate transport-limited uptake, while larger fractionations indicate carboxylation-limited uptake. Experimental work with freshwater microalgae shows good agreement with these expectations in that induction of active transport systems associated with declining DIC levels result in smaller fractionations (Sharkey & Berry 1985), while high activities of carbonic anhydrase that increase availability of CO_2(aq) for carboxylation result in larger fractionations (Guy et al. 1989).

A more precise formulation of the models emphasizes the role of DIC loss from cells; the larger fractionations associated with carboxylation are increasingly expressed the more internal DIC concentrations approach extracellular concentrations, which occurs when DIC leaks out of cells as fast as it enters. This leakiness, the fraction (f) of DIC that is lost from cells, can also be used to scale the observed fractionations (Fig. 2). Different strategies cells employ in regulating f (Burns & Beardall 1987) may explain much of the $\delta^{13}C$ variability observed among the microalgae (Fogel et al. 1992, Fogel & Cifuentes 1993).

MECHANICS OF MEASURING ISOTOPIC FRACTIONATION USING ALGAL CULTURES

Two kinds of culture studies are most useful, those that investigate the maximum fractionation attained by microalgae, and those that carefully simulate growth conditions in seawater. The studies of maximum fractionation set a limit expected of the carboxylation reactions while the 'normal growth' experiments in seawater are often more diagnostic of transport limitation.

These 2 types of experiments require different measurements. The maximum fractionation experiments need to be conducted in CO_2-rich conditions. Two studies have shown that comparable results are obtained in cultures bubbled with 5% CO_2 and in closed vessel cultures containing 5 to 50× normal seawater DIC concentrations of 10 to 100 mM bicarbonate (Abelson & Hoering 1961, Degens et al. 1968a). Other experiments that used a 5% CO_2 gas stream to repress active transport of DIC also showed large fractionations expected for carboxylation reactions (Beardall et al. 1982, Sharkey & Berry 1985). While these experiments suggest use of 5% CO_2 should be sufficient to insure full expression of the fractionation intrinsic to CO_2-saturated carboxylases, the measured isotope values are usually 24 to 27‰ versus CO_2 rather than the full 29‰ measured for Rubisco in *in vitro* experiments (Roeske & O'Leary 1984). Part of this discrepancy may be due to culture conditions. Pardue et al. (1976) documented smaller fractionations even in CO_2-rich cultures (3.6% CO_2) when cell densities were high, and recommended that cells be harvested at low densities in studies of maximal fractionations. The single report showing the full expected 29‰ fractionation from cultures involved addition of carbonic anhydrase to experimental suspensions of freshwater algae at pH 7 with <1 mM DIC (Guy et al. 1989). Besides high CO_2 levels, carbonic anhydrase additions as well as low cell densities and some attention to the importance of PEP carboxylase (Guy et al. 1989) may prove necessary to probe the maximal fractionations microalgae can express in laboratory cultures.

Experiments that simulate normal growth conditions in seawater require different conditions, namely 2 mM bicarbonate at about pH 8.0. Cells can be grown from a small inoculum in stirred, closed vessels, then harvested when they are still at low density but after at least 5 doublings to allow turnover and dilution of the initial inoculum (Hinga et al. 1994). Experiments are evaluated using measurements of temperature, pH, cell $\delta^{13}C$, $\delta^{13}C_{DIC}$, and the fractional conversion of DIC to cell biomass (r). While temperature, pH and cell $\delta^{13}C$ are relatively easy to measure, measurement of $\delta^{13}C_{DIC}$ requires special apparatus to sparge CO_2 out of culture medium for $\delta^{13}C$ analysis (Kroopnick 1974, Guy et al. 1989, Laws et al. 1995). $\delta^{13}C_{DIC}$ can also be assessed with a gas equilibration technique (Miyajima et al. 1995). Because autoclaving, bubbling with air or allowing diffusive entry of laboratory air into static cultures can change $\delta^{13}C_{DIC}$ in cultures, direct DIC measurements are necessary when trying to estimate DIC-cell isotopic fractionations (Johnston & Raven 1992). In general, pH, alkalinity, and DIC concentrations should be measured or calculated in these experiments—a fairly sophisticated approach to the inorganic carbon system is needed to accurately assess isotopic fractionation.

A more general concern involves correcting fractionation estimates based on how much DIC is consumed by cells during growth in experimental vessels. The basic problem is that as cells consume DIC, they withdraw light carbon, leading to progressive changes in the residual DIC pool. As the culture grows, it becomes

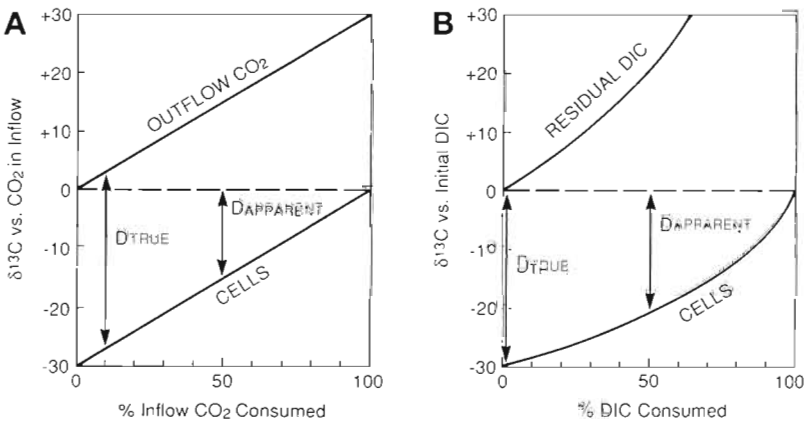

Fig. 3. $\delta^{13}C$ fractionation between DIC and cells growing in 2 types of cultures. (A) In open-system cultures continuously bubbled with a CO_2-containing gas stream, the isotopic discrimination factor, D, is the difference between effluent CO_2 and cells. (B) In closed systems where DIC is initially added and the culture vessel then sealed, D is most simply measured when very little DIC has been consumed. Common mistakes that can result in severe underestimation of D, shown as $D_{APPARENT}$ at 50% DIC consumption in both panels, include measuring the difference between cells and influent CO_2 rather the difference between cells and effluent CO_2 in bubbled cultures (A) and measuring the difference between cells and initial DIC rather than correcting for the extent of DIC consumption in closed-culture experiments (B). Modified from Mariotti et al. (1981) and Hayes (1983)

less and less appropriate to calculate fractionation using initial values for DIC, and in fact if initial values are used, fractionation occurring in the cultures can be seriously underestimated. Fig. 3 shows this problem for 2 common types of cultures, those bubbled with CO_2-containing gas, and cultures in which bicarbonate is added to closed flasks.

The bubbled cultures are open systems, and fractionation is most easily observed as the isotopic difference between cells and outflow CO_2 rather than cells and inflow CO_2 (Fig. 3A). Near-complete consumption of CO_2 by dense cultures will result in cell values approaching that of inflow CO_2, and generally comparing cell-$\delta^{13}C$ values to those of inflow CO_2 will give an erroneous apparent fractionation that underestimates the true fractionation driving isotopic changes in the system (Fig. 3A). Near-complete consumption of inflow CO_2 by dense cultures could explain some reports of near-zero fractionations for cultures bubbled with air (Calder & Parker 1973, Takahashi et al. 1991).

It is important to realize that the model shown in Fig 3, developed for work with freshwater algae at pH 5.6 where DIC pools are small (ca 10 µM DIC all as free CO_2, Sharkey & Berry 1985), is too simple for aerated cultures of marine microalgae in which pH value is >7. Algal growth in seawater (pH 8, 2000 µM DIC) typically leads to increasing pH, and this in turn results in increased uptake of CO_2 from the influent bubbling stream directly into the DIC pool; cellular uptake is no longer the only sink for C in the culture. The consequence is that since there are now 3 rather than 2 products (effluent CO_2, cellular C, and DIC, rather than effluent CO_2 and cellular C) in the system, the simple model of Fig. 3 no longer applies. Depending on how cultures are buffered and aerated, a more complex and time-dependent modeling approach may be needed to analyze these higher pH marine experiments, and many past experiments that have employed CO_2 bubbling under intermediate and high pH conditions are difficult to interpret because experimenters did not follow the detailed DIC dynamics. These more complex effects involving strong variations in DIC dynamics are apparent in some recent studies using batch cultures aerated with CO_2 (Johnston & Raven 1992, Laws et al. 1995).

Keeping track of changes in DIC and $\delta^{13}C$-DIC is also important for closed cultures maintained on a bicarbonate source. In closed cultures, $\delta^{13}C$ of cells will again approach that of initial DIC if cell densities increase to the point that all DIC is fixed into cellular biomass (Fig. 3B). The appropriate measurement of fractionation is made with the following equation (adapted from Mariotti et al. 1981 by substituting 'D' for '$-\varepsilon$' and '$1-r$' for 'f'):

$$D = (\delta^{13}C_{\text{INITIAL DIC}} - \delta^{13}C_{\text{CELLS}})(r)/[\ln(1-r)][r-1] \quad (3)$$

where r = fraction of reacted DIC ranging from 0 to 1. When r is small (in the 0.02 to 0.10 range), the correction for DIC consumption amounts to increasing the observed difference between DIC and cells by a only a small amount, 2 to 6%, so that most studies conducted with closed-culture systems harvest cells when less than 10% of DIC has been consumed. However, when cultures are allowed to grow further, this DIC-related correction increases, and generally using the simple difference between initial DIC and cells, uncorrected for the fraction of reaction, leads to an apparent fractionation smaller than the true fractionation driving isotopic changes in the system (Fig. 3B).

A second method of measuring fractionation in closed-system cultures deserves mention here. Rapid sample analysis is increasingly possible with automated mass spectrometers interfaced to elemental analyzers and gas headspace analyzers (e.g. Flynn & Davidson 1993). Analysis of algal or DIC samples collected sequentially from cultures can lead to very precise estimates of isotopic fractionation (Guy et al. 1989), provided r is measured from DIC decreases or POC increases (assuming little DOC excretion) at the times of sample collection. Fractionation is calculated as the slope of best fit linear regressions using (x, y) data of the form $[(1-r)(\ln(1-r)),\ \delta^{13}C]$ for a time sequence of algal samples or $[-\ln(1-r),\ \delta^{13}C]$ for a time sequence of DIC samples. Finally, the interested reader should consult Mariotti et al. (1981), Hayes (1983), and O'Leary et al. (1986) for further treatment of how to measure and calculate isotopic fractionations.

I have elaborated these technical points about how to measure fractionation in this review because many of the older studies that did not include careful measurements of the DIC or CO_2 system may have underestimated fractionation. For example, it is difficult to interpret results for the diatom *Skeletonema costatum* grown on air (Degens et al. 1968a) because CO_2 concentrations in the air streams exiting cultures were not reported. Also results for *S. costatum* from some closed-system cultures (Descolas-Gros & Fontugne 1985) and semi-closed systems (Falkowski 1991) may be biased since DIC changes were not measured. Results for *S. costatum* from these 3 studies (Degens et al. 1968a, Descloas-Gros & Fontugne 1985, Falkowski 1991) all show minimal fractionations (D = 10 to 12‰ versus DIC) under conditions when DIC consumption could have been extensive, so that measurements may have reflected changes in DIC chemistry rather than algal fractionation (Fig. 3). For these reasons, these estimates must be considered with caution at this time.

PATTERNS AND CONTROLS OF $^{13}C/^{12}C$ FRACTIONATION IN DIATOMS

Laboratory cultures

While there are uncertainties about results from some of the older studies, several studies have produced reliable and interesting results. Two independent high-quality studies performed with diatoms have assayed $^{13}C/^{12}C$ fractionation for *Skeletonema costatum* grown in stirred closed cultures maintained near a pH 8 similar to that of seawater (Wong & Sackett 1978, Hinga et al. 1994). Results show that fractionation depends on DIC levels when expressed as total DIC (Fig. 4). Smallest fractionations calculated relative to DIC were 21 to 25‰ and occurred near the 2 mM DIC concentration of surface seawater (Fig 4); these smaller fractionations are similar to those observed for field collections of *S. costatum* from nearshore blooms (Fig. 4). Maximal fractionations near 30‰ occurred at DIC concentrations 5 to 10× higher than the 2 mM seawater value (Fig. 4). Salinity, temperature and growth rate affected observed fractionations less than DIC levels (Wong & Sackett 1978, Hinga et al. 1994), although the 10× range examined for total DIC concentrations was larger than ranges examined for the other parameters.

I note here that some data for *Skeletonema costatum* has been excluded from Fig. 4, especially results showing small fractionations (D = 10 to 12‰ versus DIC) found in several studies (Degens et al. 1968a, Des-Colas-Gros & Fontugne 1985, Falkowski 1991) when measurements may have reflected DIC changes rather than algal physiology (see above), and also 2 larger fractionations of 31 to 33‰ reported by Hinga et al. (1994) and used in that paper to make extensive corrections to measured data. The 2 larger fractionations came from 2 cultures in experimental run 12, and were not duplicated in other runs in which growth conditions were largely similar (run 7, pH 8.41 and 8.45 cultures and run 10, pH 8.13 and 8.28 cultures; Table 1, Hinga et al. 1994). Because the large fractionations measured in run 12 seem outliers in the overall data set, I have not included them in this analysis (Fig. 4). By discounting the importance of these 2 points, I have also chosen to not follow the pH normalizations made by Hinga et al. (1994), and have worked instead with their measured raw data. Summarizing, a conservative view of the fractionation measurements made thus far for *S. costatum* shows that isotopic fractionation in photosynthesis varies between 17 and 30‰ versus DIC (Fig. 4), with both smaller estimates to 10‰ and larger estimates to 33‰ present in the literature.

Recent models emphasize both the external CO_2 availability and the internal cell demand for CO_2 in accounting for $\delta^{13}C$ values in algae (Rau et al. 1989, 1992, Francois et al. 1993, Goericke et al. 1994). Fig. 4 shows a DIC-dependent transition from low to high fractionation that is in reasonable accord with model predictions, although a wide range of growth rates was not tested in conjunction with the DIC manipulation experiments. Interestingly, larger fractionations observed in the 5 to 10 mM DIC range for *Skeletonema costatum* grown at 9°C versus 15 and 25°C (Fig. 4) would be consistent with a growth rate effect superimposed on the DIC treatments. Although

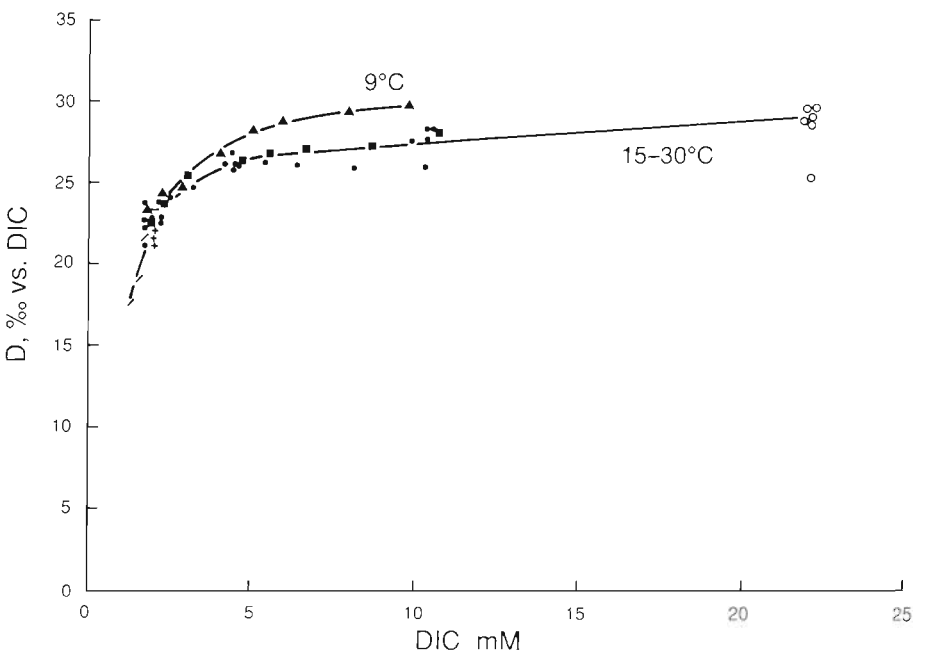

Fig. 4. $^{13}C/^{12}C$ fractionation versus DIC by the diatom *Skeletonema costatum* as a function of DIC concentration. (▲, ●, ■) 9, 15, and 25°C cultures respectively (Hinga et al. 1994, data from their Table 2), (○) 18 to 30°C cultures (Wong & Sackett 1978), +, –, /: field collections of >50% *S. costatum* (Gearing et al. 1984, +; Hedges et al. 1988, –; Fogel et al. 1992, /)

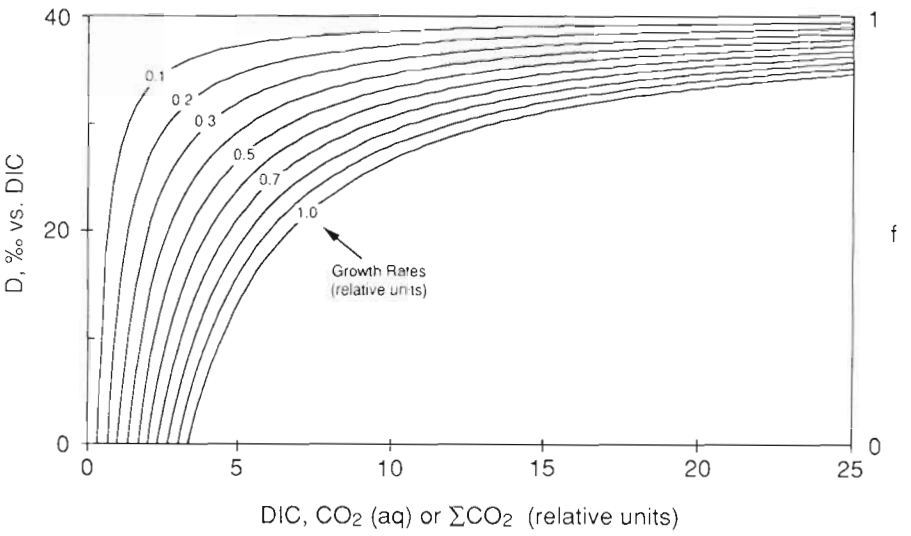

Fig. 5. Model results for carbon isotope discrimination as a function of DIC concentration and growth rate (from Goericke et al. 1994). The discrimination factor (*D*, left axis) increases as cell leakiness (*f*, right axis, see also Fig. 2) increases

growth rates were unfortunately not measured in the 9°C experiments (Hinga et al. 1994), much slower growth rates are often observed in low-temperature *S. costatum* cultures (Curl & McLeod 1961, Sakshaug 1977), and these slower growth rates are predicted to result in larger fractionations such as that observed at 9°C.

The experimental results are however not in complete agreement with model results. Models predict that there should be an increasing range of fractionation at low DIC concentrations based on even slight differences in growth rate; when CO_2 is in short supply, small variations in cell demand should result in large variations in fractionation (Fig. 5). The experimental data instead seem to indicate a narrowing of fractionations when DIC concentrations decline below 3 mM (Fig. 4). The model may be incomplete in that it considers only diffusive entry of CO_2 into cells (Goericke et al. 1994); induction of active DIC uptake at relatively low DIC levels could lead to the relatively narrow range of observed fractionations at seawater DIC concentrations (Fig. 4). Active DIC uptake could also account for the observation that $^{13}C/^{12}C$ fractionation in *Skeletonema costatum* did not strongly depend on CO_2(aq) concentrations over the 8 to 22 µM range of CO_2(aq) concentrations normally found in seawater (Fig. 6). These findings are similar to those recently obtained for *Thalassiosira pseudonana* (Thompson & Calvert 1994) where fractionation also appeared independent of CO_2(aq) concentrations. Physiological studies of internal DIC pools (Burns & Beardall 1987) are needed in conjunction with the growth experiments to really understand details of isotopic fractionation by these diatoms.

Maximal fractionation has been estimated for a dozen diatom species besides *Skeletonema costatum* (Fig. 7). Fractionations range from 22 to 32‰ versus total DIC, with pennate diatoms having a 4‰ average smaller fractionation than centric diatoms (Wong & Sackett 1978). These fractionations are 13 to 24‰ when expressed relative to $\delta^{13}C$ of CO_2(aq), and thus substantially smaller than the 29‰ expected for Rubisco isolated from spinach (O'Leary 1988). These smaller fractionations may indicate (1) use of a Form II Rubisco similar to that observed in photosynthetic bacteria (Robinson & Cavanaugh 1995), (2) some participation of PEP carboxylase during growth (although

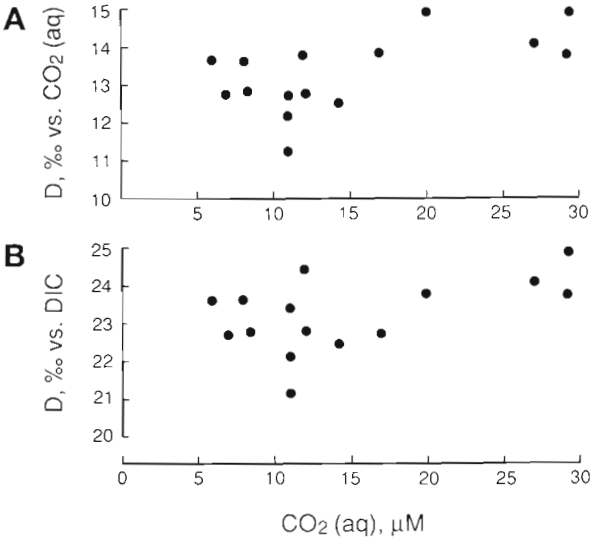

Fig. 6. $^{13}C/^{12}C$ discrimination by *Skeletonema costatum* in seawater cultures calculated relative to the isotopic composition of (A) total DIC or (B) CO_2(aq), versus CO_2(aq) concentrations. Data are from Hinga et al. (1994), and represent all cultures with DIC concentrations near those of seawater (1× and 2× treatments). Temperatures and pH ranged from 9 to 25°C and 7.9 to 8.5, respectively in these cultures. Data were taken from Table 2 and Figs. 1 & 3a of Hinga et al. (1994), were not corrected for pH and show the original measured data

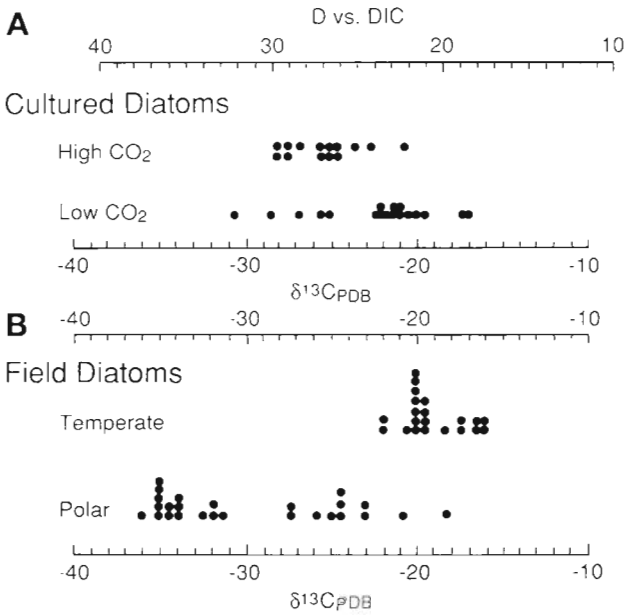

Fig. 7. $^{13}C/^{12}C$ fractionation of diatoms from (A) cultures and (B) the field. Culture results have been plotted with 2 axes, discrimination versus DIC at the top, and expected $\delta^{13}C_{PDB}$ extrapolated to field conditions at the bottom (assuming field $\delta^{13}C_{DIC}$ = 1.5‰). Field results are measured $\delta^{13}C_{PDB}$ values. Symbols show results for separate species (high CO_2 cultures), separate experiments with *Skeletonema costatum* and *Phaeodactylum tricornutum* cultures (low CO_2 cultures), and representative values for studies in which samples of >50% mixed diatoms were measured (field collections). Low CO_2 cultures were those with 2000 to 4000 µM DIC concentrations. Sources for culture studies include: Degens et al. (1968a) (excluding air-grown *S. costatum*), Estep et al. (1978), Wong & Sackett (1978), Johnston & Raven (1992), Hinga et al. (1994) (excluding experimental run 12), Thompson & Calvert (1994), and Laws et al. (1995). Sources for field studies include: Degens et al. (1968b), Deuser (1970), Gearing et al. (1984), Hedges et al. (1988), Duggins et al. (1989), Goering et al. (1990), Fischer (1991), Fry & Wainright (1991), Voss (1991), Fogel et al. (1992), Ostrom (1992), Nakatsuka et al. (1992), Rau et al. (1992), Francois et al. (1993), Simenstad et al. (1993) for Arctic $\delta^{13}C$-DIC measurements, J. Hobbie (unpubl. data) for spring bloom diatoms in the Baltic

most diatoms use PEPCK rather than PEPC; Descolas-Gros & Oriol 1992), (3) that even though some of the fractionation estimates were made with 20 mM bicarbonate cultures at pH 7.6, there may still have been some CO_2 limitation of Rubisco carboxylation reactions. A final possibility is that CO_2(aq) may be enriched in ^{13}C relative to equilibrium expectations in the boundary layer around rapidly growing aquatic plants (Goericke et al. 1994), which would lead to diminished apparent fractionation between total DIC and cells. Concerning this last point, it is intriguing that the largest fractionations measured in cultures occurred under low CO_2 conditions of 2 mM DIC in pH 8 seawater (Laws et al. 1995), possibly because the chemostat culture employed allowed sufficient time for isotopic equilibration in all portions of the DIC-cell system.

Field collections

Many diatoms are large and can be collected from the field in fairly high purity using nets of various mesh sizes. Samples from diatom blooms are most commonly collected for analysis. Field collections are typically mixtures of various diatom species which may differ strongly in isotopic composition (Wong & Sackett 1978), but it is nonetheless interesting to summarize the range of fractionations observed in the field for comparison with culture results.

There is generally good overlap of fractionation estimates from cultures and field collections, although field collections show a broader range than culture estimates of fractionation (Fig. 7). Largest fractionations (up to 35‰ versus DIC) are observed in multiple collections of an Antarctic diatom *Corethron criophilum* with $\delta^{13}C$ values of −31 to −36‰ (Fischer 1991). One possible explanation for these low values would be a high lipid content, since lipids are known to be depleted in ^{13}C versus other cellular components (Degens et al. 1968b). However, examination of C:N ratios and chlorophyll *a*:POC ratios showed that the *C. criophilum* diatoms were not especially enriched in lipids (Fischer 1989, p. 78), confirming results from an earlier study (Sackett et al. 1965) which also found that lipid enrichment was not the cause of low $\delta^{13}C$ in Antarctic diatoms. By comparison to a second diatom species that had a 9‰ smaller fractionation and was collected from the same waters later in the year, Fischer (1991) found that *C. criophilum* had low biomass and growth rate. Fischer (1991) hypothesized that low growth rate led to the low *C. criophilum* values, just as low growth rate in Antarctic macroalgae also led to low $\delta^{13}C$ (Wiencke & Fischer 1990). Fry & Wainright (1991) also hypothesized that the known very low growth rates of Antarctic diatoms (Sommer 1989) could account for the generally low $\delta^{13}C$ values of the Antarctic flora, since maximal fractionations are observed when CO_2 supply much exceeds cellular demand (Fig. 5). Recent experiments with the marine diatom *Phaeodactylum tricornutum* show this expected effect, with fractionations near 33‰ versus DIC for slow-growth cultures (Laws et al. 1995).

At the other end of the fractionation spectrum, the smallest diatom ^{13}C fractionations of 17 to 18‰ versus DIC have been reported from several areas, including Georges Bank and the nearshore Gulf of Mexico (Fry & Wainright 1991), the Delaware Bay estuary (Fogel et al. 1992), and the south Atlantic (François et al. 1993).

Small discriminations in the 16.6 to 19‰ range were also observed by Fry & Wainright (1991) for mixed diatom assemblages enriched from seawater. However, these fractionations are still relatively large versus the near-zero fractionations expected for intensive use of bicarbonate.

The collections made from the nearshore Gulf of Mexico probably come closest to conditions expected for intensive bicarbonate use—bloom diatoms were collected from an offshore low salinity 14 to 18‰ surface plume of Mississippi River water which extended over many square kilometers and consistently had high chlorophyll *a* content (>10 µg l^{-1}) and high pH (8.6 to 9.0). Calculated concentrations of CO_2(aq) which are normally 8 to 20 µM in most surface waters (Rau et al. 1992) would have been <2 µM under prevailing high pH conditions in the bloom, and use of bicarbonate rather than CO_2(aq) might have occurred. Similar fractionations near 17‰ have also been reported for high pH (>9) diatom cultures (Thompson & Calvert 1994).

Maintenance of a 17‰ fractionation under high pH conditions may indicate that diatoms adapt to low CO_2 conditions by increased use of carbonic anhydrase to keep CO_2 levels in a normal range, or adapt by rapidly using CO_2 as it dissociates from bicarbonate, before adequate time for isotopic equilibrium has elapsed. Isotopic disequilibria are not expected to be important when algal densities are low (Goericke et al. 1994), but do occur under some circumstances associated with high pH and high photosynthesis (Herczeg 1987, Herczeg & Fairbanks 1987, McConnaughey 1989a, b). Laboratory experiments show that CO_2 formed from dehydration of bicarbonate is 14.7‰ depleted in ^{13}C (Marlier & O'Leary 1984), a value that is close to minimum fractionation observed for diatoms studied thus far.

FUTURE DIRECTIONS

A combination of culture studies and field observations is necessary to develop a better working understanding of isotopic fractionation in microalgae. Several models of isotopic fractionation now exist (Rau et al. 1992, Raven 1992, Goericke et al. 1994, Laws et al. 1995), and through careful experimentation these models can be tested and refined. There is especially scope for culture studies in which both DIC dynamics and algal growth conditions are carefully monitored. Besides extending initial observations now available for mixed diatoms (Fry & Wainright 1991) and *Skeletonema costatum* (Hinga et al. 1994), there is ample room to study fractionation using non-aerated seawater cultures that more closely simulate natural conditions. These batch cultures involve time-dependent changes in algal growth rates and DIC dynamics that need sequential rather than end-point analysis to convincingly characterize fractionation patterns. However, because both growth rate and DIC chemistry are simultaneously changing in most batch cultures, chemostat cultures conducted at constant pH may be necessary to separate effects of these 2 variables (Laws et al. 1995, B. Popp pers. comm.). Measuring the time-course of isotopic equilibration in the DIC system may prove important in obtaining accurate fractionation estimates in both batch and chemostat work (Guy et al. 1989, B. Popp pers. comm.). Also, further consideration needs to be given to measuring enzymes active at various growth stages (Descolas-Gros & Fontugne 1985, 1990, Fontugne et al. 1991), as well as DIC levels within cells (Burns & Beardall 1987). Because recent research suggests that diatoms may rely exclusively on CO_2 and not use bicarbonate (Riebesell et al. 1993; but see Thompson & Calvert 1994 for an alternate view), further experiments with diatoms may help clarify when and where CO_2(aq) concentrations strongly control observed isotopic fractionations in the sea (Fry & Wainright 1991, Hinga et al. 1994, Goericke & Fry 1994, Laws et al. 1995).

Field measurements of microalgal $\delta^{13}C$ have been limited by the relatively large amount of material needed for isotopic analysis, but some new developments are reducing sample size requirements. For example, a few micrograms of dried material can now be analyzed for $\delta^{13}C$ using automated elemental analyzer/mass spectrometer systems, using low-blank silver boats as sample holders. Small samples picked from nets or sorted with flow cytometers can be analyzed, and results show that algal species picked from the same water sample can differ strongly in $\delta^{13}C$ values (Fry & Wainright 1991, Yoshioka et al. 1994). Another development involves analysis of biomarker lipids that are derived normally from diatoms in the sea. Biomarker compounds that seem largely specific for diatoms include the carotenoid fucoxanthin (R. Goericke unpubl.), the fatty acids 20:5ω3 (Pollero et al. 1979) and n-C21:6ω3 (K. Freeman pers. comm.), the branched isoprenoid diene br25:2 (Nichols et al. 1988) and the sterols 24-methylcholesta-5,24(8)-dien-3β-ol and 24-ethylcholesta-5,22-dien-3β-ol (Volkman 1986). Lipid measurements offer a sensitive way (Hayes et al. 1990) to routinely sample bulk diatom $\delta^{13}C$ from most water samples, and could complement measurements made with sorted species in both marine and freshwater studies (Hollander & Mackenzie 1991, Yoshioka et al. 1994). $\delta^{13}C$ measurements seem a promising technique for probing the balance between carbon uptake and fixation reactions maintained by diatoms and other microalgae in the face of changing environmental conditions.

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