

The effect of environmental variables on ^{13}C discrimination by two marine phytoplankton

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ABSTRACT: The effects that environmental variables, other than dissolved CO_2 , have on ^{13}C discrimination by marine phytoplankton were studied. Two selected species showed different responses. The $\delta^{13}\text{C}$ values of the marine diatom *Phaeodactylum tricornutum* grown in a range of temperatures with a constant dissolved $[\text{CO}_2]$ were more negative as the growth temperature decreased. $\delta^{13}\text{C}$ values of *P. tricornutum* showed little response to changes in growth photon flux density (except at low PFD $<10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and growth pH. $\delta^{13}\text{C}$ values of the prymnesiophyte *Emiliana huxleyi* were more negative with decreased growth temperature, more negative with decreased growth PFD and less negative with decreased growth pH. The distinct effects which environmental variables have on the $\delta^{13}\text{C}$ values of 2 contrasting algal species suggest that species-specific responses to environmental change may be important in understanding the variability of $\delta^{13}\text{C}$ values found in particulate organic carbon obtained from oceanic and coastal waters. The role of environmental variables in hindcasting dissolved $[\text{CO}_2]$ is discussed.

KEY WORDS: ^{13}C discrimination · *Emiliana huxleyi* · *Phaeodactylum tricornutum* · Temperature · pH · Photon flux density

INTRODUCTION

In global terms nearly all of the stable isotopes of carbon are in the form of ^{12}C (98.89%) and the remainder is in the form of ^{13}C (1.11%). The $^{13}\text{C}/^{12}\text{C}$ ratio of a carbon compound is influenced by physical as well as biological processes. The differences in $^{13}\text{C}/^{12}\text{C}$ ratio of carbon compounds are very small so the notation of $\delta^{13}\text{C}$ has been adopted. In this way the $^{13}\text{C}/^{12}\text{C}$ of a sample is compared to a standard by the following expression,

$$\delta^{13}\text{C}(\text{‰}) = \frac{(^{13}\text{C}/^{12}\text{C}_{\text{sample}}) - (^{13}\text{C}/^{12}\text{C}_{\text{standard}})}{(^{13}\text{C}/^{12}\text{C}_{\text{standard}})} \times 1000 \quad (1)$$

where the $^{13}\text{C}/^{12}\text{C}_{\text{sample}}$ refers to the unknown sample and $^{13}\text{C}/^{12}\text{C}_{\text{standard}}$ to CaCO_3 from the Pee-Dee Belemnite. As $\delta^{13}\text{C}$ values become more negative the proportion of ^{12}C will increase and the sample will be said to be 'lighter'.

The 2 most important steps in the assimilation and fixation of CO_2 during photosynthesis are the movement by diffusion of CO_2 from the bulk phase to the site of carboxylation and the carboxylation process by the enzyme ribulose biphosphate carboxylase oxygenase (RUBISCO). Each process discriminates against ^{13}C ; diffusion by 0.7‰ and carboxylation by RUBISCO by 28‰ (Raven et al. 1993). Reported $\delta^{13}\text{C}$ values for photosynthetic marine organisms range from -34 to -5‰. The reported $\delta^{13}\text{C}$ values for one particular species, the marine diatom *Skeletonema costatum*, range from -29‰ (Wong & Sackett 1978) to -16‰ (Descolas-Gros & Fontugne 1985) with a number of values in between. An explanation for such a wide range of reported values for a single species is not yet apparent. It is of considerable interest to cell physiologists to understand the processes which influence the $\delta^{13}\text{C}$ of marine phytoplankton as it may be a useful tool in the study of aquatic photosynthesis. Possibly more important is the current interest in the stable isotope analysis of sediment cores which are being used to hindcast atmospheric CO_2 partial pressures and temperatures (Rau et al. 1989).

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Central to the geochemical models used for hind-casting is the notion that the $\delta^{13}\text{C}$ value of a sample is primarily a reflection of the dissolved CO_2 concentration when the organic carbon was synthesized by photosynthesis. A recent paper by Thompson & Calvert (1994) suggests that factors such as irradiance, day length, pH and N source influence the $\delta^{13}\text{C}$ of *Thalassiosira pseudonana*. In this paper additional evidence is presented to support the suggestion that environmental variables, other than dissolved CO_2 concentration, can influence the $\delta^{13}\text{C}$ values of organic carbon in marine phytoplankton.

METHODS

Culture of marine phytoplankton. The marine diatom *Phaeodactylum tricornutum* Bohlin (CCAP 1052/1A) and the non-calcifying strain of the prymnesiophyte *Emiliana huxleyi* (Lohmann) Hay & Mohler (CCAP 920/2) were obtained from CCAP, Oban, Scotland, UK. These algae were cultured in 275 cm³ of f/2 enriched seawater media (pH 8.2) in 500 cm³ flasks (Guillard & Ryther 1962). A calcifying strain of *E. huxleyi* (E92) was obtained from the Plymouth Marine Laboratory (PML). This alga was cultured in a weakly enriched f/25 media (pH 8.2) to maintain the culture in a high calcifying state (Dr J. Green pers. comm., PML).

With increasing temperature the dissolved CO_2 concentration decreases under constant atmospheric CO_2 partial pressure (see Rau et al. 1989). To separate the possible effects that temperature may have on algal ^{13}C discrimination from that of dissolved CO_2 concentration, algae were grown over a range of temperatures and aerated with different CO_2 partial pressures so that the dissolved CO_2 concentration was near constant (11.3 mmol m⁻³). A range of CO_2 partial pressures were obtained with a gas blending device made by Dr R. Parsons (Parsons et al. 1992). The partial pressures of CO_2 in the air supply were 24.5, 29.8, 34.7 and 40.0 Pa CO_2 respectively. The dissolved CO_2 concentration was calculated using equations from Stumm & Morgan (1981). Even though the dissolved CO_2 concentration was held constant, the HCO_3^- concentration increased with increasing temperature as the dissociation constants of carbonic acid are temperature sensitive. At 10, 16, 21 and 26°C the HCO_3^- concentrations were 1.467, 1.665, 1.832 and 1.996 mol m⁻³ respectively at pH 8.2. The cultures were maintained in glass-sided constant temperature water baths. Light was provided by fluorescent tubing at a photon flux density (PFD) of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (400 to 700 nm) measured with a Macam PAR Quantum with a cosin sensor. Air was bubbled into the culture at 0.5 dm³ min⁻¹ in order to maintain air equilibrium concentrations of dissolved

inorganic carbon and oxygen (see Johnston & Raven 1992).

To determine the effect growth PFD has on the ^{13}C discrimination of marine phytoplankton, *Phaeodactylum tricornutum* and the calcifying strain of *Emiliana huxleyi* were cultured over a range of PFD. 500 cm³ flasks were placed on a 0.5 cm thick glass sheet sitting above 2 fluorescent tubes as the light source. On top of the glass sheet muslin cloth of various thickness was placed to vary the PFD. The muslin was covered by a sheet of cardboard with rings 12 cm in diameter cut out on to which the culture flasks were placed. The sides of the flasks were jacketed with tubes of black paper and a muslin cover was used to reduce the level of background PFD incident on the flasks. Air was bubbled at a rate of 0.5 dm³ min⁻¹ to maintain air equilibrium concentrations of dissolved inorganic carbon and oxygen. Cultures were maintained in a 16 h light : 8 h dark cycle. The growth temperature was 20°C.

Phaeodactylum tricornutum and both strains of *Emiliana huxleyi* were cultured in aerated media where the pH was controlled with the use of 10 mol m⁻³ Bis Tris Propane (BTP).

Mass spectrometric analysis. It is important when using open systems of algal culture that the $\delta^{13}\text{C}$ value of the source CO_2 remains constant. To this end cylinders of air (BOC) were initially used but the concentration and $\delta^{13}\text{C}$ value of the CO_2 was found to be too variable. For this study the source CO_2 was taken from the top of the Biological Sciences department, Dundee. pCO_2 was analysed with an IRGA and the $\delta^{13}\text{C}$ value of source CO_2 was trapped with a prep line (Griffiths et al. 1990) and analysed on a Europa Anca mass spectrometer. All the cultures were maintained in exponential growth and at least 3 subcultures were made before samples were taken for $\delta^{13}\text{C}$ analysis. *Phaeodactylum tricornutum* was harvested when the cell density reached 0.5×10^6 cells cm⁻³ and *Emiliana huxleyi* was harvested at a cell density of 0.7×10^6 cells cm⁻³. Volumes of culture media were gently centrifuged and the concentrated cells transferred to small tin boats, volume 45 mm³ (Fisons), and freeze dried. The $\delta^{13}\text{C}$ value of organic carbon from the calcifying strain of *E. huxleyi* was obtained by acidifying the concentrated cells in the tin boats to remove the inorganic carbon from the sample. The samples were combusted with a Carlo Erba NA 1500 Series II CHN analyzer. The $\delta^{13}\text{C}$ value of the CO_2 was analyzed on an ISOGAS SIRA II isotope ratio mass spectrometer. The overall precision of the combustion and mass spectrometric analysis (1 SD) was 0.08‰ (n = 6).

Growth rate determination. Cell counts were performed daily using an Improved Neubauer haemocytometer. A minimum of 400 cells were counted for each

sampling and growth rates calculated as described by Geider et al. (1985).

Statistical analysis. The effects of 2 environmental growth treatments, temperature and PFD, on the $\delta^{13}\text{C}$ value were determined using 1-way analysis of variance with growth temperature and growth PFD as the main treatment. Where appropriate, subsequent multiple comparisons were done using the t-method (Sokal & Rohlf 1981). Unless otherwise stated the level of significance was set at $p = 0.05$.

RESULTS

In a closed system or poorly aerated system the removal of ^{12}C from the dissolved inorganic carbon pool will result in decreasing $\delta^{13}\text{C}$ values in the organic carbon as the source inorganic carbon becomes 'enriched' with ^{13}C (Johnston & Raven 1992, Thompson & Calvert 1994). To overcome this all of the cultures in this study were aerated. Fig. 1 shows that with increasing cell density the $\delta^{13}\text{C}$ values of *Phaeodactylum tricornutum* remained unchanged. $\delta^{13}\text{C}$ analysis of the air gave a $\delta^{13}\text{C}$ value of -7.98‰ (± 0.12 ; $n = 5$)

Temperature

In order to be able to analyze the $\delta^{13}\text{C}$ data from natural populations of marine phytoplankton from a range of temperatures in terms of dissolved CO_2 concentration (Rau et al. 1992, Francois et al. 1993) it is necessary to demonstrate that the relationship between $\delta^{13}\text{C}$ and dissolved CO_2 is not affected by temperature *per se*.

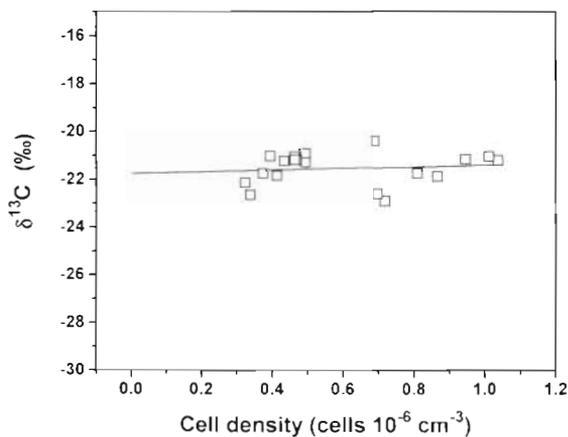


Fig. 1. *Phaeodactylum tricornutum*. Relationship between $\delta^{13}\text{C}$ and cell density for *P. tricornutum* grown between 30 and $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD at 20°C . The solid line represents the line of best fit from regression analysis $r^2 = 0.67$ and intercept = -21.76 ($n = 19$)

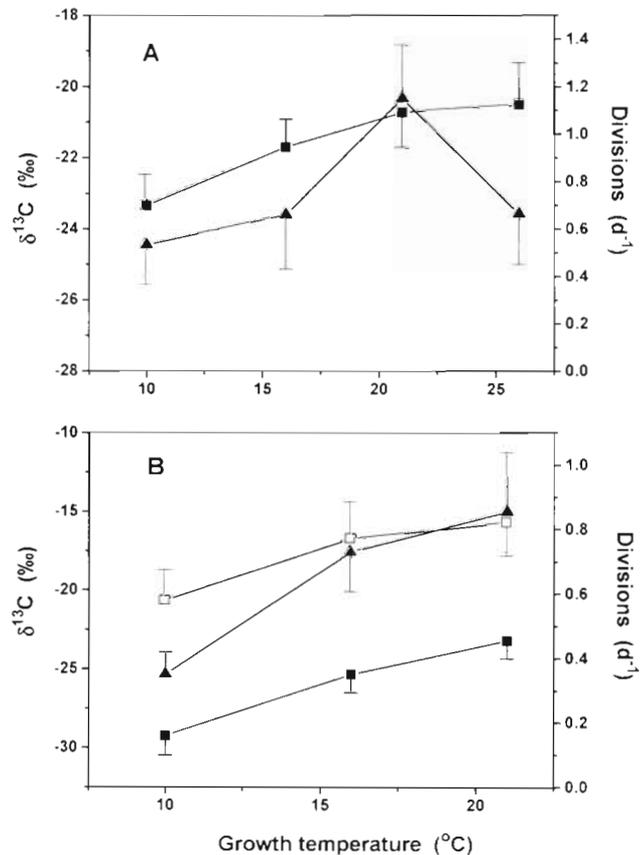


Fig. 2. *Phaeodactylum tricornutum* and *Emiliana huxleyi*. The relationship between $\delta^{13}\text{C}$ organic carbon (■), whole cell carbon (□), growth rates (▲) and growth temperature of (A) *P. tricornutum* and (B) calcifying strain of *E. huxleyi* grown in $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD and 16 h light : 8 h dark cycle. Each point is the mean of 6 samples and the vertical bars denote SD

Fig. 2A shows the $\delta^{13}\text{C}$ values of *Phaeodactylum tricornutum* cultured between 10 and 26°C with a uniform dissolved CO_2 concentration. The $\delta^{13}\text{C}$ values were least negative (-20.5‰) at 26°C and became increasingly more negative as the temperature decreased, reaching a value of -23.3‰ at 10°C . There was a significant treatment effect of temperature on the $\delta^{13}\text{C}$ values ($p = 0.0005$) based on 1-way ANOVA. The mean $\delta^{13}\text{C}$ value at 10°C was found to be significantly different from the remaining 3 values which were a distinct homogenous group based on the multiple range test. The growth rates showed that *P. tricornutum* had a well-defined growth temperature optimum of 21°C (Fig. 2A)

The $\delta^{13}\text{C}$ values of the organic carbon of the calcifying strain of *Emiliana huxleyi* also became more negative with decreasing temperature, falling from -24.8‰ at 21°C to -29.4‰ at 10°C (Fig. 2B). There was a significant treatment effect of temperature on the $\delta^{13}\text{C}$ values ($p < 0.0001$) based on 1-way ANOVA.

The mean of each $\delta^{13}\text{C}$ value of organic carbon was found to be a distinct homogeneous group based on the multiple range test. Also shown in Fig. 2B is the $\delta^{13}\text{C}$ of the whole cell carbon of *E. huxleyi* which is less negative than the organic carbon as this alga possesses a substantial quantity of inorganic carbon in the form of calcite which is used in the formation of coccoliths. The whole cell carbon $\delta^{13}\text{C}$ values showed the same relationship with growth temperature as the organic carbon. The largest difference in the $\delta^{13}\text{C}$ value of whole cell carbon and organic carbon was at 10°C , which suggests that the amount of calcification was greatest at this temperature (Sikes & Wilbur 1982). The growth rates did not show a optimal temperature over the temperatures tested.

Photon flux density

When cultured in PFD between 15 and $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ the $\delta^{13}\text{C}$ value of *Phaeodactylum tricornutum* did not change, averaging a value of -21.1‰ (Fig. 3A). At

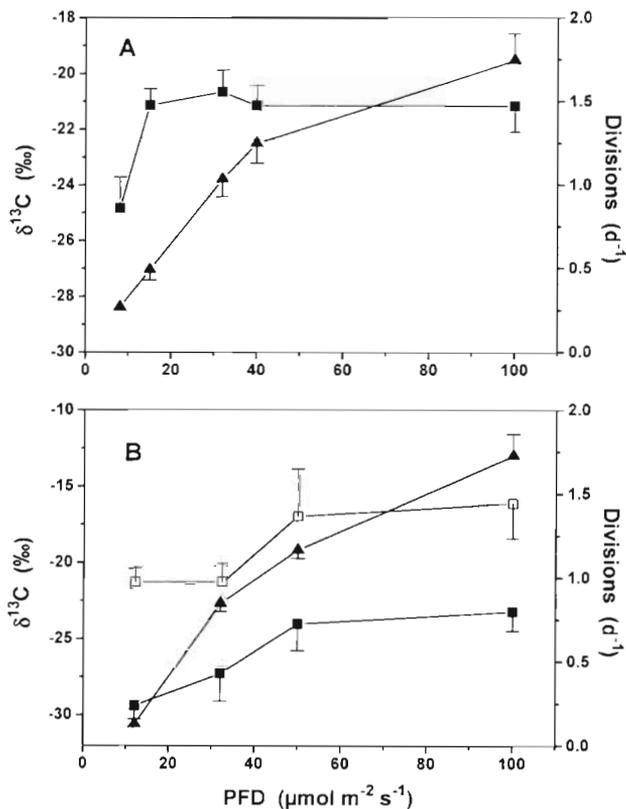


Fig. 3. *Phaeodactylum tricornutum* and *Emiliana huxleyi*. Relationship between $\delta^{13}\text{C}$ organic carbon (■), whole cell carbon (□), growth rates (▲) and growth PFD in (A) *P. tricornutum* and (B) the calcifying strain of *E. huxleyi* grown at 20°C and 16 h light : 8 h dark cycle. Each point is the mean of 6 samples and the vertical bars denote SD

a low PFD, $8 \mu\text{mol m}^{-2} \text{s}^{-1}$, the $\delta^{13}\text{C}$ value of *P. tricornutum* was more negative, -24.8‰ . There was a significant treatment effect of temperature on the $\delta^{13}\text{C}$ values ($p = 0.0005$) based on 1-way ANOVA. The mean $\delta^{13}\text{C}$ value at $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ was found to be significantly different from the remaining 4 values which were a distinct homogeneous group based on the multiple range test. The growth response was consistent with the previous report of Geider et al. (1985) with PFD saturation at around $100 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The $\delta^{13}\text{C}$ values of organic carbon from the calcifying strain of *Emiliana huxleyi* became more negative with decreasing growth PFD (Fig. 3B). At $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ the $\delta^{13}\text{C}$ value was -23.7‰ and fell to -29.6‰ at $12 \mu\text{mol m}^{-2} \text{s}^{-1}$. There was a significant treatment effect of growth PFD on the $\delta^{13}\text{C}$ values organic carbon ($p = 0.0037$) and whole cell carbon ($p = 0.0052$) based on 1-way ANOVA. Two distinct homogeneous groups were observed (12 to $32 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 50 to $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) based on multiple range test.

pH

Growth of *Phaeodactylum tricornutum* in media with a range of different pH values had little effect on either $\delta^{13}\text{C}$ values or growth rates (Fig. 4A). The average $\delta^{13}\text{C}$ value was -20.1‰ and the growth rates average was 1.47 divisions d^{-1} . The non-calcifying strain of *Emiliana huxleyi* did not to grow well above pH 8.2. With decreasing pH the $\delta^{13}\text{C}$ value became less negative reaching a maximum at pH 7.3 of -21.1‰ (Fig. 4B). Not enough organic carbon was available for $\delta^{13}\text{C}$ analysis from cultures above pH 8.2. This is in contrast with the calcifying strain of *E. huxleyi* which was unable to grow well below pH 7.8 (Fig. 4C). Cultures grown at pH 7.25 and 7.6 lost their coccoliths and did not replicate. Above pH 7.8, the growth rates were 0.9 divisions d^{-1} or greater. The $\delta^{13}\text{C}$ values of the organic carbon became more negative with increasing pH while the whole cell carbon showed a reduced but similar response (Fig. 4C). The largest $\delta^{13}\text{C}$ difference between whole cell carbon and organic carbon was at pH 8.3, which indicates that the degree of calcification was greatest at this pH.

DISCUSSION

If the analysis of stable isotope data from sediment cores is to be of any benefit then an understanding of the processes which influence the $^{13}\text{C}/^{12}\text{C}$ ratio of marine phytoplankton is required. A number of studies have shown a good relationship between dissolved CO_2 concentration and $\delta^{13}\text{C}$ values of particulate

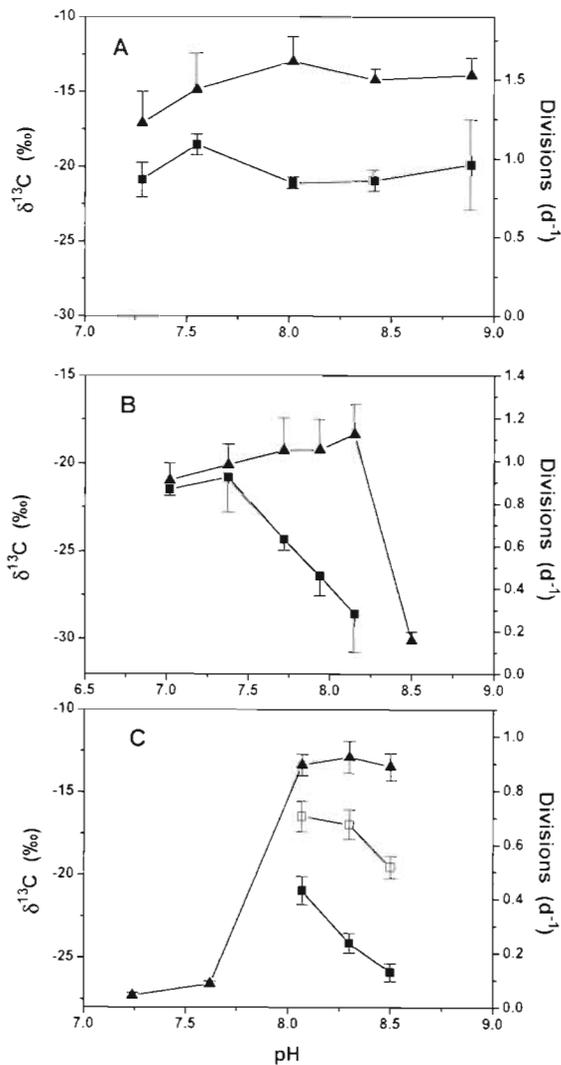


Fig. 4. *Phaeodactylum tricornutum* and *Emiliana huxleyi*. The relationship between $\delta^{13}\text{C}$ organic carbon (■), whole cell carbon (□), growth rate (▲) and growth pH in (A) *Phaeodactylum tricornutum*, (B) *E. huxleyi*, non-calcifying strain and (C) calcifying strain grown at 20°C , $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD and 16 h light : 8 h dark cycle. Each point is the mean of 4 samples and the vertical bars denote SD

organic carbon (POC) from natural populations (Rau et al. 1989, Francois et al. 1993). There are, however, some anomalous data that do not fit the models that have been developed to describe such relationships (Francois et al. 1993, Hinga et al. 1994). These models require that all phytoplankton react to changes in CO_2 concentration in the same way and that other environmental parameters are not overly significant. Recent work by Thompson & Calvert (1994) on *Thalassiosira pseudonana* suggests that irradiance rather than dissolved CO_2 concentration primarily influences the $\delta^{13}\text{C}$ of marine phytoplankton. The results presented in this paper would seem to confirm some of the conclusions

based on a single species of Thompson & Calvert (1994) that temperature, PFD and pH were all found to influence the $\delta^{13}\text{C}$ values of the algae investigated. The finding that 2 algae respond in different ways could account for some of the anomalous $\delta^{13}\text{C}$ POC data from natural phytoplankton data. The diatom *Phaeodactylum tricornutum* is not commonly found in natural situations but can be considered to be representative of benthic diatoms. It has been shown to have the physiological characteristics of an efficient HCO_3^- user (Patel & Merrett 1986, Burns & Beardall 1987). The calcifying strain of *Emiliana huxleyi* is thought to be a HCO_3^- user (see Brownlee et al. 1994). For 2 algae which are thought to have similar inorganic carbon sources they appear to differ remarkably in the effect environmental variables have on their $\delta^{13}\text{C}$ values.

Possibly the most interesting data from this paper is the effect of growth PFD on the $\delta^{13}\text{C}$ values of marine phytoplankton. There are few data in the literature on the effect of growth PFD on microalgal $\delta^{13}\text{C}$ values. In *Phaeodactylum tricornutum* there was little effect (except at low PFD $< 10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and in the calcifying strain of *Emiliana huxleyi* the $\delta^{13}\text{C}$ values became more negative as the growth PFD decreased. A similar pattern was shown by Takahashi et al. (1991) working on *Chlamydomonas reinhardtii*. This is contrary to the findings of Thompson & Calvert (1994), who showed that in general ^{13}C discrimination increased with increased growth PFD in *Thalassiosira pseudonana*. Their data are complex as the level of discrimination fell in cultures grown between 25 and $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to those grown in 0 to $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ and then rose to higher value still in the 50 to $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ cultured algae. Similarly, Thompson & Calvert (1994) found no clear relationship between ^{13}C discrimination and day length. The possession of an inorganic carbon concentrating mechanism does appear to be linked with low levels of ^{13}C discrimination at high PFD. It is notable that in marine macroalgae there is a group of rhodophytes that occupy low light environments which have been shown to be dependent on CO_2 diffusion for photosynthetic carbon acquisition (Johnston et al. 1992, Maberly et al. 1992, Kübler & Raven 1994) and these algae have very negative $\delta^{13}\text{C}$ values, $< -30\%$. No such low values have been reported for marine phytoplankton from natural samples. Beardall (1991) showed that the cyanobacterium *Anabaena variabilis* increased its affinity for inorganic carbon when growth PFD increased which would be consistent with decreased ^{13}C discrimination at high PFD. Decreased ^{13}C discrimination at high light has also been reported for the seagrasses *Posidonia oceanica* (Cooper & De Niro 1989) and *Thalassia testudinum* (Durako & Hall 1992). It remains to be investigated why the response of $\delta^{13}\text{C}$ values of *P. tricornutum* is so

small while the growth rates varied with growth PFD. It is possible that the change in $\delta^{13}\text{C}$ values may indicate a change in the CO_2 concentrating mechanism (Burns & Beardall 1987, Beardall 1991).

Some of the experimental findings of this study may seem unusual. For example the increasingly more negative $\delta^{13}\text{C}$ values observed with decreasing growth temperature. This is consistent with reports but it is generally thought to be due to the increase in CO_2 solubility as temperature declines in the literature. In this study atmospheric CO_2 concentrations were altered to offset the temperature solubility effect and maintain a constant dissolved CO_2 concentration. It should be noted that at a constant dissolved CO_2 concentration the temperature effect on the dissociation constants of carbonic acid results in increased HCO_3^- concentrations with increasing temperature. Results from the pH study suggest that ^{13}C discrimination increases as pH increases for *Emiliania huxleyi* but has little effect on *Phaeodactylum tricornutum* $\delta^{13}\text{C}$ values. The CO_2 concentration would have been the same for all cultures, as they were aerated and kept at a low cell density, but the concentration of HCO_3^- and CO_3^{2-} would have increased with increasing pH. If the level of ^{13}C discrimination was related to dissolved CO_2 concentration alone, then the $\delta^{13}\text{C}$ value would not be expected to change with pH. The fact that it does in the temperature and pH experiments could be associated with the increased total inorganic carbon ([TIC]) or a pH effect.

The findings that species-specific differences and environmental variables other than dissolved CO_2 concentration can influence the $\delta^{13}\text{C}$ values of marine phytoplankton may help to explain some of the anomalous data found in the analysis of $\delta^{13}\text{C}$ POC values. Considerably more work is needed before a full understanding of the relationships between $\delta^{13}\text{C}$ values, $[\text{CO}_2]_{\text{aq}}$, ([TIC]), temperature, PFD, pH, nutrient limitation and growth rates is achieved.

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