Cenozoic climates have varied across a variety of time-scales, including slow, unidirectional change over tens of millions of years, as well as severe, geologically abrupt shifts in Earth’s climatic state. Establishing the history of atmospheric carbon dioxide is critical in prioritizing the factors responsible for past climatic events, and integral in positioning future climate change within a geological context. One approach in this pursuit uses the stable carbon isotopic composition of marine organic molecules known as alkenones. The following report represents a summary of the factors affecting alkenone carbon isotopic compositions, the underlying assumptions and accuracy of short- and long-term CO$_2$ records established from these sedimentary molecules, and their implications for the controls on the evolution of Cenozoic climates.

**Keywords:** atmospheric carbon dioxide; alkenones; carbon isotope ratios; Cenozoic

### 1. Introduction

The identification of patterns and forcing mechanisms of ancient climates is a fundamental component of Earth system research. In general, the primary mechanisms driving long-term Cenozoic climate variability include orogenesis (Ruddiman *et al.* 1989, 1997), alterations in ocean circulation driven by relatively subtle changes in surface water density, and/or long-term, tectonically induced changes in basinal topography (Kennett 1977) and variations in atmospheric ‘greenhouse’ gases, notably carbon dioxide and methane concentrations (Berner & Kothavala 2001). Of these factors, the evolution of the partial pressure of atmospheric carbon dioxide is widely credited for the expression of long-term climate change over the past 65 Myr (Raymo *et al.* 1988; Cerling *et al.* 1997). However, other compelling arguments have been presented that call on the gradual, tectonically mediated reorganization of ocean circulation as the principal mechanism responsible for the history of Cenozoic ice accumulation (Kennett 1977; Keigwin 1982).

In lieu of direct measurements, palaeoatmospheric carbon dioxide concentrations and trends have been established in a variety of ways (Arthur *et al.* 1985, 1991; Vincent & Berger 1985; Compton *et al.* 1990; Cerling *et al.* 1997; Raymo 1994; Freeman & Hayes 1992; Pagani *et al.* 1999a; Pearson & Palmer 2000; van der Burg *et al.* 1993). One approach that shows great promise in establishing an accurate

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history of pCO$_2$ uses the stable carbon isotopic composition of sedimentary organic molecules known as alkenones. Alkenones are long-chained (C$_{37}$–C$_{39}$) unsaturated ethyl and methyl ketones primarily synthesized within the ocean mixed layer by a few species of haptophyte algae (Conte et al. 1994). Of these molecular forms, C$_{37}$ diunsaturated alkenones (figure 1a) have been extensively studied in both the laboratory and the field (Bidigare et al. 1997; Popp et al. 1998), and commonly applied in the evaluation of ancient pCO$_2$ (Jasper & Hayes 1990; Jasper et al. 1994; Pagani et al. 1999a, b).

Common in surface marine sediments, alkenones are found throughout Palaeogene and Neogene deposits, with the oldest occurrence currently identified in a Mid-Albian black shale (Farrimond et al. 1986). The dominant alkenone producers in modern oceans include *Emiliania huxleyi* and *Gephyrocapsa oceanica*. However, *E. huxleyi* is known from the Late Pleistocene to the present (McIntyre 1970), whereas *G. oceanica* extends to the Pliocene (Hay 1977). Examination of older sediments containing both alkenones and nannofossils has narrowed the probable source of alkenones in the sedimentary record to microalgae within the family Noelaerhabdaceae (Marlowe et al. 1990; Volkman 2000).

Alkenones, and various other lipids, are commonly separated from bulk organic matter using traditional adsorption chromatographic techniques. In this process, the total lipid extract is passed through a column packed with solid particles (such as silica) using a series of increasingly polar organic solvents. Adsorption of the solute onto the surface of the particles, and equilibration between the adsorbed state and solution, leads to the partitioning of molecules into compound classes. Once alkenones are identified by gas chromatography–mass spectrometry (GC-MS), or by comparison of elution times with a known standard, compound-specific carbon isotope analyses are performed using a gas chromatograph–combustion system interfaced to an isotope-ratio-monitoring mass spectrometer (GC-IRMS) (Merritt et al. 1995).

2. Variables affecting the stable isotopic composition of marine algae

(a) Evidence from culture experiments

The alkenone–CO$_2$ method is founded on the observation that the stable carbon-isotope composition of marine algae ($\delta^{13}$C$_{org}$) contains information that is relevant to the concentration of dissolved carbon dioxide during algal production (Wong &
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Sackett 1978; Rau et al. 1989, 1992). However, other factors including cellular growth rate (Rau et al. 1992; Francois et al. 1993; Laws et al. 1995; Bidigare et al. 1997), cell geometry (Popp et al. 1998), as well as the mechanisms used to supply and transport inorganic carbon to the site of carbon fixation, substantially affect algal carbon isotopic compositions. For example, many microalgae appear to have the ability to actively increase both intercellular and extracellular $\text{CO}_2$ concentrations (Sharkey & Berry 1985; Falkowski 1991; Raven & Johnston 1991), and employ a variety of enzymes during carbon fixation (see Goericke et al. 1994). These variables can greatly affect the relative expression of $\delta^{13}\text{C}_{\text{org}}$ between different classes of microalgae. As a result, not all algae, or their molecular components, can be used as reliable $\text{CO}_2$ tracers.

Two criteria need to be satisfied for $\delta^{13}\text{C}_{\text{org}}$ to act as a potential $\text{CO}_{2\text{aq}}$ proxy:

1. the inorganic carbon used during carbon fixation is in the form of aqueous carbon dioxide ($\text{CO}_{2\text{aq}}$), and

2. the supply of $\text{CO}_{2\text{aq}}$ used during photosynthesis arrives from the ambient environment to the site of carboxylation by simple diffusion.

If these conditions are realized, then the magnitude of total carbon isotope discrimination, which occurs during photosynthesis ($\varepsilon_p$) can be expressed as a function of the isotope fractionations associated with carbon transport and fixation, as well as the extracellular and intercellular concentrations of $\text{CO}_{2\text{aq}}$ (Farquhar et al. 1982):

$$\varepsilon_p = \varepsilon_t + (\varepsilon_f - \varepsilon_t)(C_i/C_e),$$

(2.1)

where $\varepsilon_f$ and $\varepsilon_t$ are constants representing the carbon isotope fractionations associated with carbon fixation and diffusive transport, and $C_e$ and $C_i$ are the extracellular and intercellular concentrations of $\text{CO}_2$, respectively.

Initial research focused on the influence of $C_e$ on $\delta^{13}\text{C}_{\text{org}}$ (Degens et al. 1968; Wong & Sackett 1978). More recently it has become evident that the intercellular pool of carbon dioxide ($C_i$) exerts considerable control over $\varepsilon_p$. Because quantification of intercellular $\text{CO}_2$ is difficult and generally elusive, attempts have been made to recast equation (2.1) in terms of the physiological variables that exert control over $C_i$. If one accepts that the specific growth rate ($\mu$) of an algal cell (i.e. the net flux of $\text{CO}_{2\text{aq}}$ divided by the carbon per cell) is related to $C_i$ by the relationship (Rau et al. 1992; Francois et al. 1993; Laws et al. 1995, 1997)

$$\mu = \frac{k_1 C_o - k_2 C_i}{C},$$

(2.2)

where $C$ is the carbon content of the cell, and $k_1$ and $k_2$ are rate constants for the diffusion of $\text{CO}_{2\text{aq}}$ into and out of the cell; in which case, equation (2.1) can be recast as

$$\varepsilon_p = \varepsilon_t + (\varepsilon_f - \varepsilon_t) \left(1 - \frac{\mu C}{k C_e}\right).$$

(2.3)

This formulation reasonably assumes that for a diffusive model of carbon uptake, the resistance to diffusion of $\text{CO}_{2\text{aq}}$ into and out of the cell is equivalent (i.e. $k_1 = k_2 = k$). It follows that the rate of diffusion for $\text{CO}_{2\text{aq}}$ will be proportional to the permeability of the cell membrane, which is arguably related to cellular surface area (Laws et al. Phil. Trans. R. Soc. Lond. A (2002))
Furthermore, one anticipates that the carbon content of the cell will be proportional to the biovolume of the micro-organism (Verity et al. 1993; Popp et al. 1998). Further model simplifications are applied in field-based research, where physiological parameters are difficult to quantify. For these data, equation (2.3) is commonly reduced to

\[ \varepsilon_p = \varepsilon_f - (b/C_e), \]  

(2.4)

where \( b \) represents an integration of all the physiological variables, such as growth rate and cell geometry, affecting the total carbon isotope fractionation during photosynthesis (Jasper et al. 1994; Bidigare et al. 1997).

Chemostat incubations for the alkenone-producing haptophyte algae *Emiliania huxleyi* (Bidigare et al. 1997) and diatoms *Phaeodactylum tricornutum* (Laws et al. 1997) and *Porosira glacialis* (Popp et al. 1998) provide supporting evidence for the theoretical model described in equation (2.3) (figure 2a). These three experiments, performed under nitrate-limited conditions, demonstrate that \( \varepsilon_p \) varies linearly in respect to the ratio of \( \mu/\text{CO}_2\text{aq} \), each with different slopes and identical y-intercepts (ca. 25\%, see below). The y-intercept represents the value of \( \varepsilon_f \) as \( \mu/\text{CO}_2\text{aq} \) approaches zero (see equation (2.3)). Moreover, differences in slopes can be normalized by accounting for differences in cell geometries, specifically the ratio of volume to surface area (Popp et al. 1998) (figure 2b). Although these experimental results are consistent with a diffusive model of carbon uptake, linear relationships between \( \varepsilon_p \) and \( \mu/\text{CO}_2\text{aq} \) can also be explained by models incorporating the effects of active carbon uptake (Popp et al. 1998).

Active carbon uptake and/or other carbon-concentrating mechanisms have been detected in various algae. Laws et al. (1997) demonstrated that under extremely low [CO\(_{2\text{aq}}\)] (less than 4 \( \mu \text{mol kg}^{-1} \)) the relationship between \( \varepsilon_p \) and \( \mu/\text{CO}_2\text{aq} \) for *P. tricornutum* sharply deviates from linearity (figure 2c). Nonlinearity is consistent with either active uptake of inorganic carbon or CO\(_2\) augmentation through the extracellular enzymatic conversion of HCO\(_3^-\) to CO\(_2\text{aq}\) (Laws et al. 1997). It is possible, however, that different growth and environmental conditions trigger different carbon uptake pathways, as well as carbon isotopic responses (Laws et al. 2000). For example, strains of *E. huxleyi*, *P. tricornutum* and *P. glacialis* grown in dilute batch cultures, under nitrate-replete conditions and varying light:dark cycles, demonstrate that although \( \varepsilon_p \) varies with respect to CO\(_{2\text{aq}}\) and \( \mu/\text{CO}_2\text{aq} \), these relationships differ from those established in nutrient-limited, predominantly continuous-light, chemostat incubations (Burkhardt et al. 1999; Riebesell et al. 2000). Relative to chemostat cultures, dilute batch cultures result in substantially lower absolute \( \varepsilon_p \) values, and different, as well as nonlinear, species-specific slopes for \( \varepsilon_p \) versus \( \mu/\text{CO}_2\text{aq} \) (figure 3).

In palaeo-CO\(_2\) reconstructions, the value of \( \varepsilon_f \) (carbon isotope fractionation factor attributed to carboxylation) applied becomes increasingly important as the magnitude of \( \varepsilon_p \) increases. Although recent chemostat experiments suggest that a value of 25\% is an appropriate value for \( \varepsilon_f \) for a variety of microalgae (figure 2a), a wider range is possible. For example, a value of 29\% is widely accepted for the *in vitro* fractionation factor of Rubisco (ribulose-1,5-biphosphatate carboxylase/oxygenase) with respect to aqueous CO\(_2\) (Roeske & O’Leary 1984; Raven & Johnston 1991). However, a smaller *in situ* fractionation associated with carbon fixation in algae is commonly observed and generally attributed to the effects of other enzymes, such as
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Figure 2. Comparison of εₚ with µ/[CO₂aq]. (a) Chemostat incubations (from Popp et al. 1998). All experiments were conducted under nitrate-limited, continuous-light conditions, except for P. tricornutum, which includes both continuous-light and 12 h : 12 h light:dark cycles. Diamonds, P. tricornutum (Laws et al. 1995); squares (calcifying, closed squares; non-calcifying, open squares) E. huxleyi (Bidigare et al. 1997); circles, P. glacialis (Popp et al. 1998). (b) Comparison of εₚ with µ/[CO₂aq] (volume/surface area) (from Popp et al. 1998). (c) Effect of low [CO₂aq] on P. tricornutum. Closed squares, greater than 10 µmol kg⁻¹; open squares, less than 7 µmol kg⁻¹ (Laws et al. 1995, 1997).

the β-carboxylase, phosphoenolpyruvate-carboxylase (Farquhar & Richards 1984). Goericke et al. (1994) calculated a range of 25–28‰ for εₜ in algae with C₃-type metabolisms, considering a 2–10% contribution of β-carboxylation to the total carboxylation. Given our limited knowledge of ancient algal physiologies, this broader, more conservative range of εₜ should be applied in palaeo-pCO₂ reconstructions.

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In summary, the stable carbon isotopic composition of marine algae is a function of the carbon isotopic composition of inorganic dissolved carbon, surface-water \([\text{CO}_2\text{aq}]\), cellular growth rate, cellular geometry and possibly irradiance and nitrate concentration (Riebesell et al. 2000; Eek et al. 1999). Clearly, care must be taken in the interpretation of \(\delta^{13}\text{C}_{\text{org}}\). Furthermore, bulk sedimentary marine organic carbon potentially contains significant contributions from terrestrial sources (Pagani et al. 2000b). Thus, when establishing isotopic records from bulk sedimentary organic matter, one can only be confident of analysing carbon derived exclusively from marine organic matter, free from biases associated with selective preservation, by using molecular markers from known marine sources (Hayes et al. 1990; Freeman et al. 1990). Therefore, by restricting analysis to alkenone carbon isotope chemistry, and, by extension, the limited population of alkenone-producing organisms, the effect of cell geometry and other physiological effects are arguably mitigated. If variation in growth rate is constrained, alkenone \(\varepsilon_p\) values presumably provide information that is specific to surface-water \([\text{CO}_2\text{aq}]\) and \(p\text{CO}_2\).

It is important to recognize that the carbon isotopic composition of alkenones is distinct from bulk haptophyte organic matter due to additional isotopic fractionations that occur during lipid biosynthesis. Therefore, a correction is applied to alkenone \(\delta^{13}\text{C}\) in order to establish \(\varepsilon_p\) values that represent the isotopic difference between the algal biomass and the inorganic carbon used during photosynthesis. Cultures performed under various experimental conditions indicate that the isotopic difference between alkenone and bulk \(\delta^{13}\text{C}\) (\(\Delta\delta\)) has a narrow range (ca. \(-4.2 \pm 1\%e\)) for two alkenone-producing algae (see Laws et al. (2000) for a review). Given the available data, \(\Delta\delta\) is assumed invariant in palaeo-pCO\(_2\) studies (Pagani et al. 1999a). However, it is possible that secular changes in the relative ratio of proteins, carbohydrates and lipids in alkenone-producing organisms can impact the value of \(\Delta\delta\).
Given the experimental range for $\Delta\delta$, such variability will have little impact on palaeo-pCO$_2$ interpretations when the value of $\varepsilon_p$ is low, but becomes increasingly important as $\varepsilon_p$ approaches maximum theoretical values.

Finally, changes in the partial pressure of atmospheric oxygen (pO$_2$) can also play a role in the relative magnitude of $\varepsilon_p$ over geologic time. Recent experiments indicate that rather large changes in pO$_2$ can potentially impact the $\delta^{13}$C of marine algae (Berner et al. 2000). This effect is potentially driven by an increase in photorespiration rates due to the oxygenase function of Rubisco, leading to an increase in fixation rates of $^{13}$C-depleted respired CO$_2$. Although these results point to an additional control on the $\delta^{13}$C of marine algae, it is unlikely to alter the interpretation of Cenozoic $\varepsilon_p$, trends because the change in isotopic fractionation attributable to large variations in pO$_2$ (ca. $-1.4\%e$ for P. tricornutum) under moderately low CO$_2$ conditions is relatively small.

A more comprehensive review of the issues discussed above can be found in Laws et al. (2000).

(b) Data from natural haptophyte populations

Extensive alkenone carbon isotopic measurements from natural haptophyte populations have been performed across an array of oceanic environments (Bidigare et al. 1997, 1999; Popp et al. 1999; Eek et al. 1999; Laws et al. 2000). These data provide evidence for a robust relationship between the physiological-dependent term $b$ (see equation (2.4)) and the concentration of reactive soluble phosphate (figure 4). Given our understanding of the factors controlling $\delta^{13}C_{org}$, it is likely that differences in relative growth rates are responsible for this relationship. Although phosphate is a major limiting nutrient, it is unlikely that [PO$_4^{3-}$] alone is responsible for the variability in growth rates inferred from variation in $b$. Instead, it is assumed that the availability of one or more trace elements, which display phosphate-like distributions in the ocean (i.e. Se, Co, Ni), is ultimately influencing the growth characteristics of these populations (see Bidigare et al. 1997; Laws et al. 2000 and references cited therein).

An alternative explanation is that the apparent correlation of $b$ with [PO$_4^{3-}$] is the result of chemical oceanography, and not growth-related factors. Because the term $b$ is a function of CO$_2$ (i.e. $b = (\varepsilon_t - \varepsilon_p)CO_2$), the relationship displayed in figure 4 could ultimately reflect a $b$ versus [CO$_{2aq}$] correlation through the natural association of phosphate with dissolved carbon dioxide. Bidigare et al. (1997) explored this possibility in their original dataset ($n = 39$) and found that the correlation between [PO$_4^{3-}$] and [CO$_{2aq}$] ($r^2 = 0.65$) was poor relative to that of $b$ versus [PO$_4^{3-}$] ($r^2 = 0.95$). On this basis, they concluded that a $\varepsilon_p$-[PO$_4^{3-}$] relationship was robust. However, the most recent dataset ($n = 109$) reveals a similarly high correlation between [PO$_4^{3-}$] and [CO$_{2aq}$] ($r^2 = 0.80$) and $b$ versus [PO$_4^{3-}$] ($r^2 = 0.78$) (figure 5). Nonetheless, given the apparent association of [PO$_4^{3-}$] with [CO$_{2aq}$], there is no correlation when $\varepsilon_p$ is compared with 1/[CO$_{2aq}$] (see equation (2.3)), while a clearer relationship between $\varepsilon_p$ and [PO$_4^{3-}$] ($r^2 = 0.23$) is evident (figure 6). Popp et al. (1999) emphasized this conclusion by suggesting that the effect of [PO$_4^{3-}$] on $\varepsilon_p$ is readily apparent if one restricts analysis to samples with a relatively invariant range of [CO$_{2aq}$] (i.e. 12–16.5 µmol kg$^{-1}$). Moreover, correlation of $\varepsilon_p$ with [PO$_4^{3-}$] improves when [PO$_4^{3-}$] is normalized to [CO$_{2aq}$] (figure 7).

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In an attempt to explain data that deviate from the global \( b \) versus \([PO_4^{3-}]\) regression, Eek et al. (1999) argued that the \( \varepsilon_p \) versus \([PO_4^{3-}]/[CO_{2aq}]\) relationship is robust when the concentration of NO\(_3^+ + NO_2^- ([N])\) is greater than zero, but fails when \([N] = 0\). While data from some low-[\(N\)] environments (i.e. Hawaiian Ocean Time Series, Bermuda Atlantic Time Series) lend support to this supposition, low-[\(N\)] data from the Equatorial Indian Ocean and the Arabian Sea do not. To date, the totality of factors responsible for the scatter in the available data for both \( \varepsilon_p \) versus \([PO_4^{3-}]/[CO_{2aq}]\) and \( b \) versus \([PO_4^{3-}]\) remains elusive.

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Figure 5. Plot of [CO₂aq] versus [PO₄³⁻]. The dashed line denotes geometric mean regression.

Figure 6. (a) Plot of εp versus 1/[CO₂aq]. (b) Plot of εp versus [PO₄³⁻]. The dashed line denotes geometric mean regression.

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Clearly, if distinct regional differences exist between alkenone carbon isotope compositions and ocean chemistry, it questions the validity of a global $\varepsilon_p$–$\text{CO}_2$ calibration based on $[\text{PO}_4^{3-}]$. Furthermore, the results of culture experiments raise critical questions regarding the effects of light- versus nutrient-limited growth, as well as the validity of the diffusion model of carbon uptake (equations (2.1), (2.3)). For example, the depth of haptophyte production within subtropical gyres may exceed 100 m, placing a portion of alkenone synthesis within the limits of the photic zone (Ohkouchi et al. 1999). If light-limited growth greatly affects alkenone-derived $\varepsilon_p$ values in the natural environment, one would anticipate large errors between alkenone-based $[\text{CO}_2\text{aq}]$ and water-column $[\text{CO}_2\text{aq}]$ across well-stratified, nutrient-poor, subtropical regimes.

3. Accuracy of alkenone-based CO$_2$ records

In an effort to test the precision of the alkenone–CO$_2$ approach, Pagani et al. (2002) established pre-industrial water-column $[\text{CO}_2\text{aq}]$ from sedimentary alkenone $\delta^{13}$C values across a North Pacific transect (along 175° E from 45° N to 15° S) (figure 8). Sedimentary alkenone-based CO$_2$aq estimates ([CO$_2$aq]$_{\text{alk}}$) were established by first identifying the depth of alkenone production at each site as defined by $U_{37}K'$ temperature estimates (Ohkouchi et al. 1999) and seasonal temperature–depth relationships established by the Northwest Pacific Carbon Cycle Study (Tsubota et al. 1999). The production depths inferred by these records were then used to designate $\delta^{13}$C$_{\text{CO}_2\text{aq}}$ values and $[\text{PO}_4^{3-}]$ required in the calculation of $\varepsilon_p$ and $[\text{CO}_2\text{aq}]_{\text{alk}}$.

The results of this work provide critical evidence supporting the efficacy of the alkenone CO$_2$ approach. Pre-industrial alkenone-based $[\text{CO}_2\text{aq}]$ across the subtropics (between 40° N and 20° N) deviate from water-column concentrations consistent with a 30% anthropogenic increase in pCO$_2$ over the past 150 years. When the effects of anthropogenic $[\text{CO}_2\text{aq}]$ are removed from the modern signal, alkenone CO$_2$aq estimates accurately reproduce pre-industrial water-column concentrations with the majority of reconstructed $[\text{CO}_2\text{aq}]$ falling within 20% of modelled pre-industrial water-column concentrations (figure 9).

The relative accuracy of the alkenone–CO$_2$ method across the subtropics, where the lowest $[\text{CO}_2\text{aq}]$ and potentially the deepest production depths occur (Ohkouchi et al. 1999) suggests that either the occurrence of light-limited growth and active carbon uptake are negligible or that these processes have negligible effects on carbon isotopic compositions of haptophytes in the natural environment. In either case, these results demonstrate that the alkenone approach can be used to accurately reconstruct water-column CO$_2$ when phosphate concentrations and temperatures are well constrained.

4. Ancient alkenone-based pCO$_2$ records

(a) Neogene research

The alkenone methodology has been applied to evaluate palaeo-oceanographic dynamics and surface-water $[\text{CO}_2\text{aq}]$ during the Pleistocene (Jasper & Hayes 1990; Jasper et al. 1994), Late Quaternary surface-water pCO$_2$ in the South Atlantic (Andersen et al. 1999), and global Miocene pCO$_2$ trends (Pagani et al. 1999a, b).

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In general, ancient $\varepsilon_p$ records are established by measuring the carbon isotopic composition of diunsaturated alkenones and coeval near-surface-dwelling planktonic foraminifera. The $\delta^{13}C$ values of foraminiferal carbonate are then used to estimate the $\delta^{13}C$ of $CO_{2aq}$ in equilibrium with calcite. Site selection for Miocene research focused on several low-growth, oligotrophic environments. In this way, the range of phosphate concentrations available for algal production was narrowed, allowing for more accurate $[CO_{2aq}]$ estimates. Furthermore, the low air-to-sea gas disequilibrium characteristic of these oceanic settings reduces uncertainty in the calculation of $pCO_2$. Miocene $\varepsilon_p$ records from four ocean localities are shown in figure 10. When compared with modern $\varepsilon_p$ values, Miocene values fall on the low end of the spectrum suggesting either the untenable conclusion that haptophyte growth rates were anomalously high in low productive waters or that $CO_2$ concentrations were similar to or, more likely, below modern levels (figure 11).

Temporal differences in the pattern of Miocene $\varepsilon_p$ values between sites can be explained in terms of regional differences in algal growth rates. It follows that sustained changes in regional growth rates imply substantial changes in physical oceanographic conditions. For example, rapid change in $\varepsilon_p$ values (ca. 20 Ma) at Site 516 in the Southwest Atlantic was interpreted as reflecting substantial changes in surface-water nutrient concentrations. Arguments were presented that linked the timing of these changes to the opening of the Drake Passage, the onset of deep-water flow through the passage, and subsequent invigoration of the Antarctic Circumpolar Current and Antarctic Intermediate Water production (Pagani et al. 2000a).

Miocene atmospheric carbon dioxide concentrations were estimated (figure 12) from $\varepsilon_p$ values by applying phosphate concentrations typical of these environments, and temperatures derived from $\delta^{18}O$ values from shallow-dwelling foraminifera. The results suggest that during much of the Miocene, the maximum $pCO_2$ (less than 290 ppmv) was far lower than modern levels, falling within a range similar to Pleistocene glacial/interglacial concentrations (Pagani et al. 1999a). These results are further supported by a recent convergence of $CO_2$ estimates derived from distinctly different approaches (Pearson & Palmer 2000; van der Burg et al. 1993; Royer et al. 2001). Moreover, recent efforts have provided new data that extend $\varepsilon_p$ records from the Miocene into the Pleistocene (M. Pagani, K. H. Freeman & M. A. Arthur 2002, unpublished data). The magnitude of Pliocene/Pleistocene $\varepsilon_p$ values are indistinguishable from Miocene values (table 1, figure 10), providing evidence that the factors controlling the isotopic expression of alkenones (i.e. $CO_2$, growth rate) at these localities have remained remarkably stable for the past 25 Myr.

Low Neogene $CO_2$ concentrations are particularly surprising in light of the climatic and ecological events that occurred during this time. The early Miocene is characterized as an interval of expansive high-latitude warmth, leading to deep-water temperatures ca. 6 $^\circ$C warmer than today (Shackleton & Kennett 1975; Savin et al. 1975). In contrast, the middle Miocene is known for rapid high-latitude cooling and East Antarctic ice-sheet expansion (Shackleton & Kennett 1975; Miller et al. 1987; Wright et al. 1992; Flower & Kennett 1993). Although the late Miocene is not recognized for distinctive changes in global climates, it is acknowledged as a period of important ecological change. Carbon isotopic evidence from palaeosol carbonates and fossil tooth enamel support a rapid, predominantly low-latitude expansion of $C_4$ grasses between ca. 8 and 4 Ma (Cerling et al. 1997). This floral shift is argued to have resulted from a decline in carbon dioxide across a critical threshold (ca. 500–
Figure 7. Plot of $\varepsilon_p$ versus $[\text{PO}_4^{3-}]/[\text{CO}_{2aq}]$. The dashed line represents geometric regression ($r^2 = 0.31$). The solid line represents polynomial regression ($r^2 = 0.42$).

Figure 8. Stable carbon isotopic compositions of Holocene-age, diunsaturated alkenones from a North Pacific transect (175° E) (Pagani et al. 2002). Samples were collected with a box corer during the RV Hakurei Maru cruises in 1992/1993, frozen, and subsequently analysed for $U^{137}$ temperature values (Ohkouchi et al. 1999).
Figure 9. Per cent deviation of alkenone-based $[\text{CO}_2\text{aq}]$ from modelled pre-industrial $[\text{CO}_2\text{aq}]$.

(a) Per cent deviation assuming that haptophyte production occurred in the autumn (August and September). (b) Per cent deviation assuming that haptophyte production occurred in the spring (April, May and June). Alkenone-based $[\text{CO}_2\text{aq}]$ were calculated from reconstructed $\varepsilon_P$ values. $\varepsilon_P = [(\delta_{\text{CO}_2\text{aq}} + 1000)/(\delta_{\text{org}} + 1000) - 1]10^3$, where $\delta_{\text{org}} \approx \delta^{13}\text{C}_{\text{alk}} + 4$ and $\delta_{\text{CO}_2\text{aq}}$ represents $\delta^{13}\text{C}$ values of pre-industrial $\text{CO}_2\text{aq}$. Pre-industrial $\delta_{\text{CO}_2\text{aq}}$ values were calculated from pre-industrial $\delta_{\text{DIC}}$ by adjusting modern $\delta_{\text{DIC}}$ values by ca. 1% between 20° and 40° N (Lynch-Stieglitz et al. 1995). Modern $\delta_{\text{DIC}}$ values were established using data from the World Ocean Circulation Experiment (WOCE P13) and Kroopnick (1985). Maximum alkenone-based $[\text{CO}_2\text{aq}]$ calculated using an $\varepsilon_{\text{ef}} = 27\%$ and the equation, $[\text{CO}_2\text{aq}] = (4.14[\text{PO}_4^{3-}] + 125.48[\text{PO}_4^{3-}] + 107.85)/(27 - \varepsilon_{\text{ef}})$. Minimum $[\text{CO}_2\text{aq}]$ calculated using an $\varepsilon_{\text{ef}} = 25\%$ and the equation, $[\text{CO}_2\text{aq}] = (116.12[\text{PO}_4^{3-}] + 81.5)/(25 - \varepsilon_{\text{ef}})$. Haptophyte production depths and seasonal phosphate concentrations were established using seasonal phosphate–temperature–depth relationships established by NOPACCS and UBC temperature values. Pre-industrial, water-column $[\text{CO}_2\text{aq}]$ were estimated by removing anthropogenic $[\text{CO}_2\text{aq}]$ from modern $[\text{CO}_2\text{aq}]$. The concentration of anthropogenic $\text{CO}_2\text{aq}$ at each site was simulated from a modified version of the Geophysical Fluid Dynamics Laboratory’s Modular Ocean Model (Duffy & Caldeira 1997; Pacanowski et al. 1991).

600 ppmv), where the $C_4$ photosynthetic pathway is favoured over $C_3$ photosynthesis. However, $p\text{CO}_2$ estimates lend no support to this supposition (Pagani et al. 1999b) (figure 13).

An interval of global warmth returns in the early to middle Pliocene. Maximum temperatures were established by ca. 3 Ma with high-latitude North Atlantic temperatures as much as 6 °C higher than today (Dowsett & Poore 1991; Cronin 1991). Global cooling followed the moderate climate of the middle Pliocene as major ice-sheet expansion in the Northern Hemisphere occurred between 3.1 and 2.5 Ma (Shackleton et al. 1984; Tiedemann et al. 1994).

Low $p\text{CO}_2$ over the past 25 Myr has important implications for climate research. First, if alkenone-based $\text{CO}_2$ estimates are valid, then modern levels are higher than...
they have been for at least the past 25 Myr. This suggests that the history of Neogene climate is inadequate as a reference for future changes in climate as CO$_2$ continues to rise. In addition, if one assumes that pCO$_2$ is the principal driver of Neogene climate, it is difficult to reconcile large-scale variations in temperatures, ice-volume and ecology with apparently small fluctuations in atmospheric carbon dioxide. Rather, it appears that other factors such as tectonically induced changes in ocean circulation and orogenesis were critical agents of climate change during this time (Pagani et al. 1999a, b). The opening of the Drake Passage, subsidence of the Greenland–Scotland Ridge, the restriction of flow across the Indian Ocean, cessation of flow through the eastern and western Mediterranean Sea, and the closure of the Panamanian Isthmus would have substantially altered ocean circulation, forcing changes in the hydrologic cycle and ocean heat transport. It is probable that the climatic effects of these processes were amplified under low levels of atmospheric carbon dioxide. Indeed, one would anticipate that the character of global climates would remain relatively stable and buffered from change under extreme greenhouse conditions, whereas under a low CO$_2$ atmosphere, Earth’s climates are likely to be more sensitive and responsive to changes in physical oceanography and mountain-building events.

Although the ability to accurately reconstruct Holocene water-column CO$_2$ is encouraging and supports the efficacy of the alkenone approach for core-top sediments (figure 9), the underlying assumptions applied in more ancient CO$_2$ research are difficult, if not impossible, to verify. As previously discussed, these assumptions include the long-term endurance of a diffusive model of carbon uptake, the $b$–phosphate–micronutrient relationship, and algal cell geometries. One could argue that alkenone-producing algal physiologies, and their carbon isotopic compositions, evolved in close association with the evolution of climate and, thus, account for con-
sistently low Neogene $\varepsilon_p$ values. Such arguments are warranted given the apparent ability of some extant haptophytes to actively transport inorganic carbon (Burns & Beardall 1987; Raven & Johnston 1991), and/or use carbonic anhydrase (Quiroga & Gonzalez 1993; Nimer et al. 1994), an enzyme that catalyses the reversible hydration of carbon dioxide (Tsuzuki & Miyachi 1989). The long-term selection of carbon concentrating mechanisms (CCMs) would clearly affect alkenone carbon isotopic compositions and distort CO$_2$ estimates. However, their prevalence would imply carbon limitation in Miocene oligotrophic environments and thus also support low surface-water CO$_2$ concentrations. Further, it is unlikely that the selection of an energetically expensive CCM would be widespread under high pCO$_2$.

Other considerations, such as secular changes in the $b$–phosphate–micronutrient relationship and algal cell geometries, are difficult to prove, but could account for low Miocene $\varepsilon_p$ values under high-CO$_2$ conditions.

(b) Palaeogene records

An effort to extend alkenone-based $\varepsilon_p$ records into the Palaeogene is ongoing and preliminary results appear to alleviate some of the concerns discussed above. The first phase of this work is focused on the Eocene/Oligocene (E/O) transition, which represents a critical climatic step away from the pervasive warmth of the Eocene (Zachos et al. 1994). Rapid and severe changes occurred at the E/O boundary, with the deep ocean cooling ca. 4 °C in less than 350 kyr (Zachos et al. 1996) in association
Figure 12. Maximum $p$CO$_2$ estimates calculated from $\varepsilon_p$ records shown in figure 12. [CO$_{2aq}$] derived by applying a range of values for $\varepsilon_t$ (25–27%$e$). $p$CO$_2$ calculated from [CO$_{2aq}$] by applying Henry’s law and temperatures derived from coeval planktic foraminifera $\delta^{18}$O (Pagani et al. 1999a). $p$CO$_2$ values represented by the right edge of the shaded band are calculated using a value of 27%$e$ for $\varepsilon_t$, [PO$_4^{3-}$] = 0.3 $\mu$mol l$^{-1}$, and an equation for the physiological-dependent term $b$ calculated using the upper 95% confidence limit from the global dataset derived from all available data ($b_{(27%e)} = 4.14[PO_4^{3-}]^2 + 125.48[PO_4^{3-}] + 107.85$). Values on the left edge of the shaded band are calculated using a value of 25%$e$ for $\varepsilon_t$, [PO$_4^{3-}$] = 0.2 $\mu$mol l$^{-1}$, and an equation for the physiological-dependent term $b$ calculated using the geometric mean regression from the global dataset ($b_{(25%e)} = 116.12[PO_4^{3-}] + 81.5$). ((a) Site 588, Southwest Pacific; (b) Site 608, North Atlantic; (c) Site 516, Southwest Atlantic; and (d) Site 730, Northwest Indian.)

with a massive expansion of continental ice sheets on Antarctica (Robert & Kennett 1997). Although episodic glaciations are apparent during the late Eocene (Browning et al. 1996), the E/O transition represents Earth’s first clear step into the ‘icehouse’ conditions of the Cenozoic.

Alkenone $\delta^{13}$C values were measured at the E/O boundary from two ocean sites (DSDP Sites 277 and 516) encompassing a range of growth environments (figure 14a). Compared with modern and Miocene data, E/O $\delta^{13}$C values are considerably lower, providing evidence that ancient alkenone $\delta^{13}$C values can indeed be much more depleted in $^{13}$C than Miocene or modern values.

While it is clear that growth rate and CO$_2$ exert considerable influence on alkenone $\delta^{13}$C, variability in $\varepsilon_p$ in the modern ocean appears to be primarily a function of [PO$_4^{3-}$] and not [CO$_{2aq}$] (figure 6). This suggests that while the modern range of $\varepsilon_p$ is controlled by differences in haptophyte growth rates, the maximum and minimum limits of variability are dictated by the present range of surface-water [CO$_{2aq}$]. It follows that if [CO$_{2aq}$] was higher during the Palaeogene, the limits of $\varepsilon_p$ would be shifted toward higher values relative to the modern. Therefore, these results provide
Table 1. DSDP Site 588
(Data from M. Pagani, K. H. Freeman & M. A. Arthur (2002, unpublished data); ‘NA’ denotes ‘not available’.)

<table>
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<th>age (Ma)</th>
<th>$\delta^{13}$C_{alk}</th>
<th>$\varepsilon_p$</th>
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</tr>
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Figure 13. Late Miocene pCO$_2$ estimates from Site 588 in relation to the expansion of C$_4$ grasses (from Pagani et al. 1999b). See the caption for figure 12 for equations and variables used to calculate pCO$_2$.

Figure 14. (a) Compilation of $\delta^{13}C$ values of sedimentary diunsaturated alkenones. The dashed lines capture the range of alkenone values collected from water-column samples (E/O values from M. Pagani, J. C. Zachos & K. H. Freeman 2002, unpublished data). (b) Estimates of Eocene/Oligocene $pCO_2$. Values from Site 277 are estimated using the equation $[CO_{2aq}] = (128.96[PO_4^{3-}] + 101.37)/(27 - \varepsilon_p)$, $[PO_4^{3-}] = 1.6 \text{ mmol l}^{-1}$, temperature $= 10 ^\circ C$. Values from Site 516 are estimated using $[PO_4^{3-}] = 0.3 \text{ mmol l}^{-1}$, temperature $= 25 ^\circ C$. E/O values are not in stratigraphic order.

Preliminary evidence for higher $pCO_2$ conditions at the E/O boundary relative to the present.

Carbon dioxide concentrations can be estimated from these data if we apply the modern $b$ versus phosphate relationship, reasonable $[PO_4^{3-}]$, and further assume that the $\delta^{13}C$ of $CO_{2aq}$ at each site was $-10^\%$ (relatively negative compared with modern values). Using these assumptions, minimum estimates of $pCO_2$ calculated from these
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5. Conclusions

Alkenones are distinct molecular markers derived from limited species of haptophyte microalgae in the modern ocean, and to the best of our knowledge, were produced by similar organisms in the geological past. Culture experiments performed on alkenone-producing organisms are limited, but show a distinct relationship between cellular growth rate, carbon dioxide concentration, and the total stable carbon isotope fractionation that occurs during photosynthesis ($\varepsilon_p$). Furthermore, extensive field measurements have unveiled a significant correlation between the concentration of soluble phosphate and $\varepsilon_p$. This chemical–physiological association has provided a mathematical relationship that allows for the calculation of ancient [CO$_2$] if values of $\varepsilon_p$ are known and phosphate concentrations are reasonably constrained. As is the case with all CO$_2$ proxies, the validity of ancient pCO$_2$ records is only as robust as the validity of the underlying assumptions of the technique. Although the alkenone
approach is demonstrably accurate in the reconstruction of Holocene water-column $[\text{CO}_2]_{\text{aq}}$, the integrity of similar assumptions in more ancient sediments is difficult to confirm. Thus, unravelling the history of atmospheric carbon dioxide will ultimately require relative agreement of $p\text{CO}_2$ records derived from disparate approaches.

The available data suggest that ancient alkenone-based $\varepsilon_p$ values encompass a broad range. Low $\varepsilon_p$ values, similar to and lower than today’s, characterize sediments encompassing the past 25 Myr, whereas much higher values have recently been recorded across the Eocene/Oligocene boundary. If the temporal expression of these records results from the evolution of carbon dioxide concentrations, and not algal physiologies, then it suggests that $p\text{CO}_2$ was at least 2–5 times higher at the end of the Eocene and declined to near modern levels by the beginning of the Miocene.

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References

Andersen, N., Müller, P. J., Kirst, G. & Schneider, R. R. 1999 The $\delta^{13}\text{C}$ signal in C$_{37:2}$ alkenones as a proxy for reconstructing Late Quaternary $p\text{CO}_2$ in surface waters from the South Atlantic. In Proxies in paleoceanography: examples from the South Atlantic (ed. G. Fischer & G. Wefer), pp. 469–488. Springer.


Phil. Trans. R. Soc. Lond. A (2002)
The alkenone–$\text{CO}_2$ proxy and ancient atmospheric $\text{CO}_2$ 629


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Zachos, J. C., Quinn, T. M. & Salamy, K. A. 1996 High-resolution (10$^4$ years) deep-sea foraminiferal stable isotope records of the Eocene–Oligocene climate transition. Paleoceanography 11, 251–266.