Does growth rate affect ketone unsaturation and intracellular carbon isotopic variability in *Emiliania huxleyi*?

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Abstract. We present the first results of U^K_{37} and $\varepsilon_{\text{alkenone}}$ from *Emiliania huxleyi* grown in chemostat cultures at constant temperature but at variable growth rates. A small change in U^K_{37} with growth rate was found for a noncalcifying strain of *E. huxleyi*, whereas results from a calcifying strain showed no systematic variation between U^K_{37} and growth rate. We conclude that nutrient-limited growth rate effects will not produce serious errors in paleotemperature determinations using U^K_{37} or $U^{K'}_{37}$. However, U^K_{37} values for the two strains differed significantly and apparent temperatures calculated using unsaturation ratios obtained from chemostat experiments are very different from those derived from batch culture experiments. Results of isotopic analyses of alkenones and bulk cells indicate no effect of growth rate on $\varepsilon_{\text{alkenone}}$ ($\varepsilon_{\text{alkenone}}$ = 4.2 ± 0.2‰). Constancy of $\varepsilon_{\text{alkenone}}$ values is a requirement for environmental and paleoenvironmental interpretations. These results indicate that isotopic analyses of alkenones are ideal markers for paleo-[CO_2(aq)] determinations.

1. Introduction

Structural and isotopic characterization of marine organic matter can provide important insights into the conditions under which carbon fixation occurs. To define sources of suspended or sedimentary organic matter, geochemists commonly use structural identification of biological compounds or biomarkers. Some biomarkers are very specific with regard to their algal source and can provide important environmental and paleoenvironmental information. In particular, long-chain alkenones (C_{37}, C_{38}, and C_{39}) are known to be produced in open ocean waters only by *Emiliania huxleyi* and the closely related *Gephyrocapsa* [*Volkman*, 1980a, b; *Marlowe et al.*, 1990; *Brassell*, 1993; *Conte et al.*, 1995, *Volkman et al.*, 1995]. Results of analysis of alkenones have become important paleoceanographic tools because these compounds are well preserved in sediments and the unsaturation ratios of the C_{37} alkenones (U^K_{37} and $U^{K'}_{37}$) [cf. *Brassell et al.*, 1986; *Prahl and Wakeham*, 1987] change as a function of sea surface temperature [see *Brassell*, 1993]. Knowledge of molecular structure when combined with isotopic compositions can yield additional important environmental information on specific organisms or groups of organisms [e.g., *Hayes et al.*, 1987; *Freeman et al.*, 1990; *Jasper and Hayes*, 1990;

Freeman and Wakeham, 1992; *Wakeham et al.*, 1993; *Kenig et al.*, 1994, 1995; *Jasper et al.*, 1994; *Laws et al.*, 1995; *Bidigare et al.*, 1997]. A second important paleoceanographic tool based on analyses of alkenones in sediments is carbon-isotopic fractionation associated with phytosynthesis

$$\varepsilon_P \equiv \left(\frac{\delta_{CO2} + 1000}{\delta_{POM} + 1000} - 1 \right) 1000$$

[cf. *Freeman and Hayes*, 1992] which has been used to infer changes in ancient CO_2(aq) concentrations [*Jasper and Hayes*, 1990; *Jasper et al.*, 1994].

Brassell et al. [1986] demonstrated the paleoceanographic potential of U^K_{37} by showing that it correlated with variations in sea surface temperature (SST) inferred from the $\delta^{18}O$ of calcareous planktonic foraminifera in sediments. Calibration of the relationship between temperature of algal growth and U^K_{37} (and $U^{K'}_{37}$) has been produced using results from laboratory batch culture experiments of *E. huxleyi* [*Prahl and Wakeham*, 1987; *Prahl et al.*, 1988, 1995; *Conte et al.* 1995, 1997], mesocosm experiments [*Conte et al.*, 1994] and results of analyses of surface water and sediment trap samples [*Prahl and Wakeham*, 1987; *Conte et al.*, 1992; *Conte and Eglinton*, 1993; *Sikes and Volkman*, 1993; *Sikes et al.*, 1997] or core tops [*Sikes et al.*, 1991; *Rosell-Melé et al.*, 1995; *Madureira et al.*, 1997]. These relationships have been applied to numerous paleo-SST reconstructions using long-chain alkenones extracted from sediments [e.g., *Brassell et al.*, 1986; *Jasper and Gagosian*, 1989; *McCaffrey et al.*, 1990; *Ten Haven and Kroon*, 1991; *Kennedy and Brassell*, 1992a, b; *Eglinton et al.*, 1992; *Müller et al.*, 1994; *Zhao et al.*, 1995; *Prahl et al.*, 1995].

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Linear relationships between temperature and U_{37}^K (or $U_{37}^{K'}$) based on batch culture experiments by *Prahl and Wakeham* [1987] and *Prahl et al.* [1988] have been considered standard calibrations and fit well data obtained on near-surface seawater samples from equatorial and midlatitude sites [*Prahl and Wakeham*, 1987] and sediments [*Sikes et al.*, 1991; *Rosell-Melé et al.*, 1995]. However, recent field studies at other sites produced different temperature-U_{37}^K relationships [see *Brassell*, 1993; *Conte and Eglinton*, 1993; *Sikes and Volkman*, 1993]. It has also been shown that batch culture experiments using various strains of *E. huxleyi* produce different temperature-U_{37}^K calibrations [*Brassell*, 1993; *Prahl et al.*, 1995; *Conte et al.*, 1997], and a lack of a significant relationship between U_{37}^K (or $U_{37}^{K'}$) and SST has been reported in areas such as the eastern North Atlantic (for SST < 16°C) [*Conte and Eglinton*, 1993], the Southern Ocean (T < 4°C) [*Sikes and Volkman*, 1993] and the Black Sea [*Freeman and Wakeham*, 1992].

Recently, *Conte et al.* [1995] observed variability in biomarker ratios among replicate batch cultures of the same strain of *E. huxleyi* and *Gephyrocapsa oceanica*. *Conte et al.* [1995, p. 281] concluded that distributions of unsaturated C_{37} and C_{38} methyl-ketones were controlled by "unknown environmental variables in addition to temperature." Changes in growth rate or dissolved organic carbon appeared not to have been the cause of this variability as there was no consistent difference in biomarker ratios between cultures with high and low final cell densities or among cultures grown in different batches of natural seawater. *Conte et al.* [1995] postulated that stage of the growth cycle or growth conditions may have affected alkenone/alkenoate distributions in the cultures. These findings indicated that nontemperature-related factors influence U_{37}^K and suggest a possible influence of nutrients on either selection of genotypes with consistently different temperature-U_{37}^K responses or a nutrient-induced variability in this response for multiclonal populations. The results of *Conte et al.* [1995] as well as recent laboratory batch culture and field work calibrations of *Conte and Eglinton* [1993] and *Sikes and Volkman* [1993], respectively, indicate a need to describe adequately environmental factors other than temperature which affect alkenone ratios.

Jasper and Hayes [1990] were the first to use isotopic analyses of C_{37} alkadienones for paleo-[CO_2(aq)] reconstructions. They examined the isotopic fractionation between foraminiferal carbonates and organic carbon derived from the alkenone-producing haptophytes in a sediment core from the Gulf of Mexico. They found ε_p variability in quantitative agreement with that predicted on the basis of (1) atmospheric pCO_2 concentrations known from the Vostok ice core, (2) equilibration of CO_2 between the atmosphere and the oceanic mixed layer in the Gulf of Mexico, and (3) the [CO_2(aq)]-^{13}C relationship proposed by *Popp et al.* [1989]. A more recent paleo-[CO_2(aq)] reconstruction based on isotopic analyses of alkenones for the central equatorial Pacific was found to be related to the strength of upwelling [*Jasper et al.*, 1994]. Use of compound-specific isotopic analyses requires knowledge of the relationship between the isotopic composition of the compound(s) analyzed and that of the whole organism

$$\varepsilon_{biomarker} = \left(\frac{\delta^{13}C_{organism} + 1000}{\delta^{13}C_{biomarker} + 1000} - 1 \right) 1000$$

[see *Hayes*, 1993; *Summons et al.*, 1994]. *Jasper and Hayes* [1990] and *Jasper et al.* [1994] used a $\Delta\delta_{alkenone}$ ($\Delta\delta_{biomarker} \equiv \delta^{13}C_{organism} - \delta^{13}C_{biomarker} \approx \varepsilon_{biomarker}$) value of 3.8‰ which was based on analyses of material grown in a single laboratory culture of *E. huxleyi* where pertinent details about the growth conditions of that culture are not described. This value is typical of the isotopic difference between whole plant material and extractable lipids [e.g., *Hayes*, 1993]. However, it is important to precisely establish $\varepsilon_{biomarker}$ values and to determine if these values change as a function of algal physiology. This information must be known before quantitative interpretations of changes in the isotopic composition of biomarkers in the geologic record can be made with confidence. The extent to which fluctuations in ε_p are due to variations in $\varepsilon_{alkenone}$ is not currently well known (see discussion given by *Jasper et al.* [1994]).

Recent results of theoretical modeling [e.g., *Francois et al.*, 1993; *Goericke et al.*, 1994; *Rau et al.*, 1996], laboratory studies and field observations [e.g., *Rau et al.*, 1989; *Raven and Johnston*, 1991; *Fry and Wainright*, 1991; *Rau et. al.*, 1992; *Laws et al.*, 1995, 1997; *Bidigare et al.*, 1997; *Popp et al.*, 1997] have indicated that variations in ε_p can result from changes in [CO_2(aq)], algal growth rate, cell surface area to carbon ratio, ε_f (the flux-weighted average of isotope effects associated with all carbon-fixation reactions active in the cell), ability of the cell to actively assimilate inorganic carbon, and cell membrane permeability. Of the physiological factors, *Bidigare et al.* [1997] and *Popp et al.* [1997] argued that the effects of cell size, ε_f, active assimilation of inorganic carbon, and membrane permeability on ε_p are likely to be inconsequential among natural populations of the alkenone-producing haptophytes. Therefore, before [CO_2(aq)] can be estimated accurately from ε_p, variations in algal growth rates (μ) must be accounted for in a reliable fashion [see also, *Laws et al.*, 1995, 1997; *Rau et al.*, 1996; *Popp et al.*, 1997]. Since ε_p accompanying the formation of biomass by alkenone-producing algae in contemporary marine environments varied systematically with the concentration of dissolved phosphate, *Bidigare et al.* [1997] speculated that the Cd/Ca ratio in planktonic foraminifera could be used to estimate near-surface seawater [PO_4] and thus provide a constraint on variations in ε_p due to change in μ. This proposed methodology would provide an independent means of evaluating the effects of algal growth rate and thus lends itself to the determination of paleo-[CO_2(aq)].

Although the linearity of the results presented by *Bidigare et al.* [1997] suggests little variation in $\varepsilon_{alkenone}$ values, few $\varepsilon_{alkenone}$ measurements exist. If changes in the growth rate of the alkenone-producing haptophytes produce variations in $\varepsilon_{alkenone}$, the accuracy of this important paleoceanographic proxy is jeopardized.

Nitrogenous nutrient and light availability are two important variables which regulate rates of algal photosynthesis and growth [*Laws and Bannister*, 1980; *Kiefer and Mitchell*, 1983; *Cullen et al.* 1992]. Variations in these parameters greatly influence the abundance and composition of microalgal lipids [see *Roessler*, 1990]. Although there is no question that the lipid content of algae changes as a function of growth rate, it remains to be determined if U_{37}^K and $U_{37}^{K'}$ change with growth conditions other than the well-established temperature effect. In addition, since the environmental factors which regulate algal growth may alter carbon flow within photosynthetic organisms, compound-specific isotope distributions may also change with growth conditions (see

Hayes [1993] for a discussion of isotope effects in biological reaction networks).

The purpose of this investigation is to determine if changes in the growth rate of *E. huxleyi* affect the use of alkenones for paleoceanographic proxy measurements. To quantify the influence of growth rate on the relationship between unsaturated ketone ratios (U_{37}^K and $U_{37}^{K'}$) and temperature, two strains of *E. huxleyi* were grown in chemostat cultures at constant temperature but at various controlled growth rates. Isotopic analyses of the C_{37} alkenones in these experiments also provided the opportunity to determine if $\varepsilon_{alkenone}$ values vary as a function of algal growth rate.

2. Materials and Methods

Two strains of the marine haptophyte (sensu prymnesiophyte) *E. huxleyi* (calcifying strain PLY B92/11 obtained from J. C. Green, Plymouth Marine Laboratory, and noncalcifying strain BT6, CCMP373 obtained from the Center for Culture of Marine Phytoplankton, West Boothbay Harbor, Maine) were grown at constant temperature (18°C) in a nitrate-limited chemostat culture system described in detail by *Laws and Bannister* [1980] and *Laws and Wong* [1978] and similar to that used by *Laws et al.* [1995]. In this experimental system, growth rate is accurately controlled ($\mu \pm 0.01$ d^{-1}). The light required by the culture for photosynthesis was provided continuously by a bank of daylight fluorescent lamps at an intensity of 20 mol quanta m^{-2} d^{-1}. Samples were collected at specific growth rates ranging from 0.2 to 0.6 d^{-1}. Samples of phytoplankton particulate organic matter were taken for analyses after a minimum of about four doubling times after a steady state growth rate had been reached, so that more than 93% of the biomass harvested had been grown under conditions of constant dissolved inorganic carbon (DIC) concentration and isotopic composition [*Laws et al.*, 1995, 1997]. These experiments were part of a larger ongoing study examining the effects of algal physiology on carbon isotopic fractionation in marine microalgae. Consequently, strains were initially chosen to address specific questions regarding their isotopic biogeochemistry rather than their biochemical composition.

Discussion of isotopic results as well as detailed descriptions of the collecting and processing of algae and water samples and the calculations are given by *Laws et al.* [1995, 1997], *Bidigare et al.* [1997] and *Popp et al.* [1997].

The rationale for growing cultures in chemostats as opposed to batch culture is severalfold. As the name implies, a chemostat is a system whose chemical characteristics do not change with time. In a batch culture, phytoplankton metabolic processes alter the chemistry of the growth medium. In addition, to obtain reasonable cell densities in batch cultures, all nutrients are supplied in excess and at high concentrations at the beginning of the experiment. Situations such as this rarely occur in nature. With chemostats a rigorous control over growth rates can be achieved. In a nutrient-limited chemostat the growth rate of the phytoplankton is controlled by the rate at which fresh medium is pumped into the growth chamber. This pumping rate also controls the rate at which cells are removed from the growth chamber since the volume of the growth chamber is constant. The phytoplankton population must grow at least as fast as the pumping rate to avoid washing out of the growth chamber because of the continuous removal of cells via an overflow line. The phytoplankton, however, can grow no faster than the medium pumping rate since fresh medium is the only source of the limiting nutrient (in this case, nitrate) needed to produce additional biomass. At steady state the phytoplankton are therefore constrained to grow at precisely the growth rate determined by the medium pumping rate and the volume of the growth chamber. The uncertainty in this growth rate is determined mainly by the day-to-day variability in the medium pumping rate, which is controlled by a peristaltic pump. This day-to-day variability is never >2% of the growth rate and under most conditions is <1% [Laws *et al.*, 1995].

Samples (500 mL) for alkenone analysis were filtered onto precombusted Whatman GF/F filters, immediately frozen in liquid N_2, and stored frozen until extraction. Filters were extracted with dichloromethane/methanol (2:1) and extracts were saponified using aqueous 0.5N KOH/MeOH. Neutral lipid fractions were recovered by extraction with hexane, and alkenones were isolated by adsorption chromatography on silica gel [*Freeman and Wakeham*, 1992]. Purity of the alkenones was checked by gas chromatography-mass spectrometry.

Table 1. Specific Growth Rate, Cell Counts, Abundance of $C_{37:2}$ and $C_{37:3}$ Alkenones, $C_{37:3}/C_{37:2}$, and U_{37}^K for Chemostat Experiments with *Emiliania huxleyi* (Strains BT6 and B92/11) All Grown at 18°C

μ, d^{-1}	Cell Counts, cells ml^{-1}	C/cell, pg cell^{-1}	$[C_{37:2}]$, mg gC^{-1}	$[C_{37:3}]$, mg gC^{-1}	$\left[\dfrac{C_{37:3}}{C_{37:2}}\right]$	U_{37}^K
BT6 (Noncalcifying)						
0.20	1,522,000	7.87	1.86	3.83	2.06	0.327
0.30	1,379,000	7.45	1.13	2.44	2.16	0.316
0.40	1,285,000	9.06	1.40	3.33	2.39	0.295
0.50	1,085,000	9.58	0.99	2.68	2.72	0.269
0.50	890,000	10.75	0.38	0.86	2.29	0.304
0.60	968,000	10.65	0.70	1.94	2.78	0.265
B92/11 (Calcifying)						
0.30	532,000	10.50	1.61	1.75	1.09	0.479
0.40	482,000	748	1.78	1.74	0.98	0.506
0.50	382,000	6.07	1.18	1.09	0.93	0.519
0.60	516,000	7.90	1.80	1.79	0.99	0.502

Alkenone unsaturation ratios were determined by gas chromatography equipped with an FID. U^{K}_{37} was originally defined by *Brassell et al.*[1986] as $([C_{37:2}] - [C_{37:4}])/([C_{37:2}] + [C_{37:3}] + [C_{37:4}])$ and simplifies, in the absence of $C_{37:4}$, to $[C_{37:2}]/([C_{37:2}] + [C_{37:3}])$, the so-called U^{K}_{37} parameter [*Prahl and Wakeham*, 1987]. BT6 did not produce significant quantities of the tetraunsaturated C_{37} alkenone under the growth conditions used in this study. Consequently, U^{K}_{37} is used to describe results in the present report.

Reproducibility in alkenone unsaturation ratio analyses was determined using results from strain BT6. Two separate chemostat experiments were performed with strain BT6 at $\mu = 0.5$ d^{-1} (but with different $[CO_2(aq)]$) [see *Bidigare et al.*, 1997, Table 2] and carried through the extraction, chromatography, and quantitation separately. The U^{K}_{37} values differed by 0.034 (Table 1), and thus we take our uncertainty conservatively as ±0.035. For comparison, uncertainties ranging from ±0.02 to ±0.03 are reported by *Prahl and Wakeham* [1987].

Isotope-ratio-monitoring gas chromatography-mass spectro-metry (Delta-S irmGCMS; Ultra-1, 50 m) was used to determine δ values for alkenones (values of δ refer to $\delta^{13}C$ relative to the Pee Dee belemnite (PDB) standard). The compound-specific isotopic results reported in this study were collected using techniques described by *Hayes et al.* [1990], *Merritt and Hayes* [1994], and *Merritt et al.* [1995]. Isotopic analyses were performed in duplicate or triplicate when possible in order to calculate meaningful uncertainties. Values of $\epsilon_{alkenone}$ for the C_{37} alkadienones and alkatrienones were determined using a weighted mean $(= \Sigma(\delta_i/\sigma_i^2)/\Sigma(1/\sigma_i^2))$ which gives greater weight to compound-specific isotopic analyses with lower uncertainty [*Laws*, 1997]. The uncertainty associated with the calculated weighted mean $(= [\Sigma(1/\sigma_i^2)]^{-0.5})$ does not, however, provide a realistic estimate of a single measurement of $\epsilon_{alkenone}$. The uncertainty in this number is conservatively ±0.2‰ which was calculated as (standard deviation of the mean) x $n^{0.5}$ [*Laws*, 1997]. Accuracy of compound-specific isotopic analyses was assessed through analyses of internal standards in which the δ value was known.

3. Results and Discussion

3.1. Variations in Unsaturation Ratios

Since changes in growth conditions have the potential to produce variability in alkenone unsaturation, we determined the U^{K}_{37} ratio as a function of nutrient-limited growth rate μ in experiments using a noncalcifying and a calcifying strain of *E. huxleyi* (Table 1 and Figure 1). A linear relationship with the slope significantly different from zero (at the 95% confidence interval) was found between the U^{K}_{37} ratio and μ for noncalcifying strain BT6 (Figure 1). However, the entire range of values of U^{K}_{37} are within the uncertainties of the measurement. No correlation between the U^{K}_{37} index and μ (slope not significantly different from zero at 95% CI) was found for calcifying strain B92/11 (Table 1 and Figure 1). The implication of our results is that variations in alkenone ratios due to nutrient-limited growth rate effects will not produce serious errors in paleotemperature determinations using U^{K}_{37} or $U^{K'}_{37}$. For example, if the U^{K}_{37}-μ relationship for the noncalcifying BT6 is accepted, our results yield a calculated temperature change of ~3.5°C over a threefold
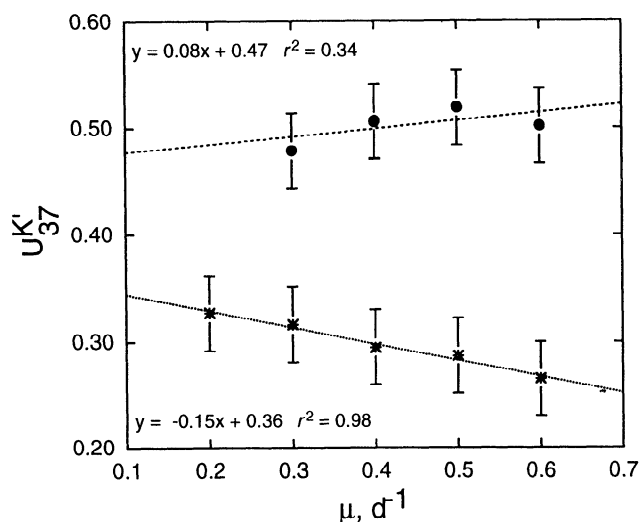

Figure 1. Relationship between U^{K}_{37} and growth rate for *Emiliania huxleyi*. The noncalcifying strain BT6 is shown as asterisks, and the calcifying strain B92/11 is shown as solid circles. Error bars are equal to ±0.035 (see text). Data for BT6 at $\mu = 0.5$ d^{-1} are the average for two experiments (see Table 1). Regression lines are model I least squares fit to the data.

change in growth rate. The growth rate of natural populations of *E. huxleyi* is notoriously difficult to determine [see *Bidigare et al.*, 1997], although threefold changes in the growth rate of haptophyte algae have been observed [e.g., *Latasa et al.*, 1997; *Gieskes and Kraay*, 1989].

In nutrient-replete and light-saturated environments, the growth rate of algae is correlated with temperature [e.g., *Eppley*, 1972; *Goldman and Carpenter*, 1974]. The implication of this relationship is that when temperature and growth rate are positively correlated, increasing temperature will also increase the growth rate. The net effect on U^{K}_{37} will therefore be the combined effects of changes in both growth rate and temperature. If U^{K}_{37} is negatively correlated with growth rate at a fixed temperature and positively correlated with temperature at a fixed growth rate, allowing both temperature and growth rate to increase will result in a smaller change in U^{K}_{37} than would be the case if only temperature or only growth rate were varied. The result will be that the slope of a plot of U^{K}_{37} versus temperature will be smaller than would be the case if growth rate were held constant. Since the relationship between temperature and growth rate may differ between populations of *E. huxleyi* isolated from different oceanographic regions, the different slopes of the regression lines relating U^{K}_{37} to temperature for strains of *E. huxleyi* isolated from various marine waters [*Prahl et al.*, 1995; *Conte et al.*, 1997] may be due in part to differences in the relationship between temperature and growth rate in these different strains.

The U^{K}_{37} values from *E. huxleyi* grown in chemostat cultures differed markedly from those grown in batch cultures at the same temperature. The relationship between U^{K}_{37} and temperature has been examined with noncalcifying strain BT6 in batch cultures grown on a 14:10 L:D cycle ($U^{K}_{37} = 0.017T - 0.195$) [*Prahl et al.*, 1995; F. Prahl, personal communication, 1995]. Calculated temperatures using the equation of *Prahl et al.* [1995] yield temperatures for chemostat samples of 27.1°-30.7°C, more than 9°C higher than the actual growth temperature. U^{K}_{37} values for

the calcifying strain B92/11 grown in replicate batch culture at 15°C (U^K_{37} = 0.417 and 0.510) [*Conte et al.*, 1995; M. Conte, personal communication, 1997] bracket results from chemostat culture at 18°C (Table 1). These results indicate that the temperature- U^K_{37} relationship is different in chemostat and batch cultures.

Some of the variation between culturing methods may be due to the growth conditions in the different experimental systems. In nutrient-limited chemostats, the growth rate of phytoplankton is constant. On the other hand, in batch culture experiments, cell density changes constantly, and nutrient concentrations are in excess during log-phase growth, when most cultures have been harvested for analyses [*Prahl and Wakeham*, 1987; *Conte et al.*, 1995]. Recently, *Conte et al.* [1995, 1997], *Epstein et al.* [1996], and B. L. Epstein et al. (The effect of nutrient concentration on alkenone-based sea surface temperature estimates, submitted to *Paleoceanography*, 1997) documented large changes in U^K_{37} values between cells harvested in log-phase and stationary-phase growth conditions in batch cultures. Although our experiments suggest that growth rates in the range 0.2-0.6 d^{-1} do not produce large variations in U^K_{37} for *E. huxleyi*, we cannot eliminate the possibility that alkenone unsaturation ratios become sensitive to growth rates at μ < 0.2 d^{-1}. *E. huxleyi* growth rates in the range 0.2-0.6 d^{-1} are, however, typical of those estimated in the contemporary ocean [see *Bidigare et al.*, 1997]. If alkenone production and unsaturation are sensitive to growth conditions, then questions concerning alkenone production become environmental rather than experimental: namely, are the alkenones which are preserved in marine sediments biosynthesized by alkenone-producing haptophytes which are in nutrient-replete conditions such as when phytoplankton are in log-phase growth or nutrient-deplete conditions typical of stationary-phase growth or steady state growth?

Our results confirm previous laboratory findings indicating that different strains of *E. huxleyi* can produce different U^K_{37} values when grown at the same temperature [*Brassell*, 1993; *Prahl et al.*,

1995; *Conte et al.*, 1995, 1997]. Although both strains of *E. huxleyi* were grown in chemostat cultures at the same temperature, U^K_{37} values measured for calcifying strain B92/11 are nearly twice as large as those determined for noncalcifying strain BT6, indicating that unsaturation ratios can be strain dependent (Figure 1 and Table 1). If the various strains of *E. huxleyi* present in nature have different temperature- U^K_{37} relationships, the general use of this paleotemperature proxy will be compromised. Calibrations based on field sampling that include multiclonal assemblages of *E. huxleyi* [*Medlin et al.*, 1996] growing under natural environmental conditions may turn out to be more robust than culture-based calibrations. In this context it is interesting to note that the original field calibration of *Prahl and Wakeham* [1987] is remarkably similar to the more recent compilation described by *Brassell* [1993].

3.2. Variations in Isotopic Ratios

Results of isotopic analyses of *E. huxleyi* grown in chemostat cultures under various nutrient-limited growth conditions indicate that there is no effect of growth rate on $\varepsilon_{alkenone}$ for calcifying and noncalcifying strains of *E. huxleyi* (Table 2). In addition, these results are in close agreement with the $\Delta\delta_{alkenone}$ value of 3.8‰ measured by *Jasper and Hayes* [1990]. Application of theoretical, field, and laboratory results to environmental interpretations based on values of ε_P in modern and ancient oceans requires knowledge of isotopic fractionation during lipid synthesis because variability in $\varepsilon_{alkenone}$ will produce uncertainty in ε_P. Our results indicate that uncertainties in ε_P due to changes in $\varepsilon_{alkenone}$ are minor. In their evaluation of isotopic measurements of alkenones as indicators of growth rates of alkenone-containing haptophytes, *Bidigare et al.* [1997] determined using a propagation of error calculation the uncertainties in growth rate estimates based on ε_P. Our results are in agreement with these authors who found that the greatest variability in ε_P was due to uncertainty in the determination of [CO$_2$(aq)] and not in $\varepsilon_{alkenone}$.

Table 2. Isotopic Analyses of Whole Cells and Alkenones for Chemostat Experiments with *Emiliania huxleyi* (Strains BT6 and B92/11)

μ, d^{-1}	δ_{cells}	$\delta_{37:2}$[a]	$\varepsilon_{cell-37:2}$[b]	$\delta_{37:3}$[a]	$\varepsilon_{cell-37:3}$[b]
BT6 (Noncalcifying)					
0.2	-44.7	-49.2±0.3	4.7	-48.6±0.2	4.1
0.3	-41.8	-46.6±0.2	5.0	-46.0±0.3	4.4
0.4	-38.6	-42.9*	4.5	-42.5±0.1	4.1
0.5	-46.6	-50.9±0.2	4.5	-50.4±0.1	4.0
0.5	-36.4	-40.9*	4.7	-40.3±0.2	4.1
0.6	-34.8	-39.1±0.1	4.5	38.9±0.1	4.3
B92/11 (Calcifying)					
0.3	-29.2	-33.9±0.2	4.9	-34.1±0.1	5.1
0.4	-27.7	-31.7±0.2	4.1	-32.5±0.8	5.0
0.5	-27.6	-31.1±0.1	3.6	-31.4±0.1	3.9
0.6	-27.7	-31.7±0.1	4.1	-32.9±0.2	5.4
		Grand mean[c]	4.24±0.05	Grand mean[c]	4.30±0.04

[a]Mean and standard deviation of two or more replicates; assumed to be ± 0.5‰ for single analysis (denoted by an asterisk).

[b]Fractionation between whole cells and alkenones was determined using $\varepsilon = 1000\{[(\delta^{13}C_{bulk\ cells} + 1000)/(\delta^{13}C_{alkenone} + 1000)] -1\}$.

[c]Weighted mean (all data) = $\Sigma(\delta_i/\sigma_i^2)/\Sigma 1/\sigma_i^2)$; SD (all data) = $[\Sigma(1/\sigma_i^2)]^{-0.5}$; weighting of results was based on the reproducibility of CSIA analyses of alkenones from a single experiment.

Since carbon isotopic fractionation by marine phytoplankton depends on concentrations of $CO_2(aq)$ and a variety of physiological factors, use of ε_P as a proxy for paleo-$[CO_2(aq)]$ requires that uncertainty in those physiological factors be minimized. The effects of physiological factors on ε_P can be minimized only when the source of organic matter can be constrained. Because alkenones have a limited source in oceanic waters and because growth rates of the alkenone-containing haptophytes may be independently constrained, alkenone-based ε_P measurements have the potential to provide the best means of estimating paleo-$[CO_2(aq)]$ [see Bidigare et al., 1997].

4. Conclusions

Although a correlation between U_{37}^K and specific growth rate was found with our chemostat cultures using a noncalcifying strain of Emiliania huxleyi (strain BT6), the range of variation due to changes in growth rate is within the analytical uncertainty of the measurement. Combined with experimental results from a calcifying strain of E. huxleyi (strain B92/11), we conclude that variations in alkenone unsaturation ratios due to nutrient-limited growth rate effects are insignificant and will not produce serious errors in paleotemperature determinations using U_{37}^K or $U_{37}^{K'}$. However, U_{37}^K values from the two strains grown in chemostat culture differ significantly despite identical growth temperatures. The different slopes of regression lines relating alkenone

unsaturation ratios to temperature obtained with different populations of E. huxleyi from different geographical regions [e.g., Brassell, 1993] remain a source of uncertainty in using U_{37}^K or $U_{37}^{K'}$ for the determination of paleotemperatures.

Results of isotopic analyses of alkenones indicate that there is no effect of growth rate on $\varepsilon_{alkenone}$ for calcifying and noncalcifying strains of E. huxleyi. Constancy of $\varepsilon_{biomarker}$ values is a requirement for the use of any isotopic biomarker for environmental and paleoenvironmental interpretations. Paleo-$[CO_2(aq)]$ estimations using ε_P based on isotopic analyses of alkenones are ideal because the source of the compound is well constrained and because algal physiological effects are either minimized or can be estimated on the basis of other paleoproxy measurements [see Bidigare et al. [1997]. We suggest the adoption of $\varepsilon_{alkenone}$ values of 4.2‰ when calculating the isotopic composition of E. huxleyi biomass from isotopic analyses of alkenones.

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