Physiological impacts on alkenone paleothermometry

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[1] We conducted isothermal (15°C) batch culture experiments with the coccolithophorid *Emiliania huxleyi* (strain NEPCC 55a) to evaluate the extent to which nutrient and light stress contribute to variability in the alkenone unsaturation index $U^{K'}_{37}$. Alkenone content and composition were constant throughout exponential growth in both experiments when nutrients (nitrate and orthophosphate) were replete. Stationary phase (nutrient-starved) cells continued to produce alkenones, amassing concentrations (ΣAlk) $\geq$ 3 times higher than those dividing exponentially (1.5–2 pg cell^{-1}), and the $U^{K'}_{37}$ of "excess" alkenone dropped by 0.11 units. In contrast, 5 days of continuous darkness resulted in a 75% decrease in cellular ΣAlk and a significant $U^{K'}_{37}$ increase (+0.11 units). Given an established 0.034 unit/°C response for exponentially growing cells of this strain, the observed range of $U^{K'}_{37}$ variability at 15°C corresponds to an uncertainty of ±3.2°C in predicted growth temperature. This level of variability matches that of the global $U^{K'}_{37}$ annual mean sea surface temperature calibration for surface marine sediments, begging the question: What is the physiological condition of alkenone-producing cells exported to marine sediments? Comparison of our laboratory results for a strain of *E. huxleyi* isolated from the subarctic Pacific Ocean with depth profiles for alkenones in surface waters from two contrasting sites in the northeast Pacific Ocean suggests that the answer to this question depends on the ocean regime considered, a possibility with significant bearing on how stratigraphic $U^{K'}_{37}$ records in marine sediments are to be interpreted paleoceanographically. *INDEX TERMS:* 4267 Oceanography: General: Paleoceanography; 4808 Oceanography: Biological and Chemical: Chemical tracers; 4850 Oceanography: Biological and Chemical: Organic marine chemistry; 4855 Oceanography: Biological and Chemical: Plankton; 4863 Oceanography: Biological and Chemical: Sedimentation

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1. Introduction

[2] Unsaturation ratios ($U^{K'}_{37}$) in long-chain alkenones from haptophyte algae serve stratigraphically in marine sediments as a paleorecord for sea surface temperature (SST) [*Brassell et al.*, 1986]. Validated globally by a strong statistical correlation between $U^{K'}_{37}$ measures in surface sediments and overlying annual mean SST [*Muller et al.*, 1998], this method has now been employed to yield paleotemperature records for the Holocene [e.g., *Kennedy and Brassell*, 1992], Pleistocene [e.g., *Bard et al.*, 1997; *Lyle et al.*, 1992; *Rostek et al.*, 1993; *Sachs and Lehman*, 1999], and Miocene [e.g., *Pagani et al.*, 1999] periods. However, the statistical variability of the $U^{K'}_{37}$-SST calibration adds uncertainty to its paleothermometric accuracy [*Prahl et al.*, 2000]. A combination of environmental and biological factors almost certainly contributes at some level to this variability. Although the $U^{K'}_{37}$ value appears resistant to diagenetic change [*Grimalt et al.*, 2000], not all algae capable of alkenone biosynthesis [*Brassell*, 1993] and of potentially contributing a signal to sediments display identical temperature responses for $U^{K'}_{37}$ [*Conte et al.*, 1998]. Also, in addition to these apparent genetic differences, physiological concerns within a given genotype are also now evident [*Conte et al.*, 1998; *Epstein et al.*, 1998].

[3] Here we present results from two types of batch culture experiments with a specific strain of major alkenone producer, *Emiliania huxleyi*, whose calibration curve represents global data remarkably well [*Muller et al.*, 1998]. These results show how physiological factors such as nutrient and light availability impact $U^{K'}_{37}$ ratios recorded in growing and stationary phase cells. Our laboratory-controlled experiments were designed to illuminate the first-order impact of physiology on biomarker synthesis by alkenone-producing haptophytes in natural waters and the integrity of thermometric interpretations based on the analysis of alkenone unsaturation patterns ($U^{K'}_{37}$) in sedimentary samples. Nutrient stress like that imposed on cells by our first experiment can be encountered at the end of batch-culture-like algal blooms stimulated by the upwelling of fertile waters along coastal

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margins. Light-limited growth conditions, potentially as extreme as those posed by the second experiment, are encountered by algal cells residing in deep chlorophyll maxima prevalent throughout the open ocean [e.g., *Longhurst and Harrison*, 1989] or transported to depth by deep mixing events along margins [e.g., *Murphy and Cowles*, 1997]. Although our findings by no means undermine the utility and value of alkenone paleothermometry, they do highlight a clear need to carefully consider such nonthermal environmental variables when stratigraphic $U_{37}^{K'}$ profiles from different sedimentary regimes are specifically interpreted, particularly in context with other paleoceanographic proxies [*Mix et al.*, 2000].

2. Methods

2.1. Culture Conditions

[4] NEPCC 55a, the subarctic Pacific (50°11′N, 144°57′W) strain of *Emiliania huxleyi* used to establish the commonly employed $U_{37}^{K'}$-growth temperature calibration [*Prahl and Wakeham*, 1987], was cultured in 500 mL Pyrex flasks in a constant temperature water bath (15°C) using Cool-White fluorescent lights (~50 μE m^{-2} sec^{-1}; 12:12 daily light cycle) and a standard growth media derivative (f/20-Si, see http://ccmp.bigelow.org/CI/CI_01e.html). NEPCC 55a was obtained as CCMP1742 from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton. Culture flasks were stirred by gentle swirling at the same time each day and immediately subsampled using a sterile pipette for daily determination of cell density, nitrate and orthophosphate concentrations, cellular organic carbon content, and cellular alkenone content and composition.

[5] At each time point, cell density was determined microscopically using a haemocytometer. Cells in known volumes of culture were also filtered using gentle vacuum onto two precombusted (450°C, 8 hour), 25 mm diameter glass fiber filters (GFF). Each GFF was wrapped in aluminum foil and stored frozen (−80°C) until needed for organic chemical analysis. A volume of filtrate from each sample was also collected in an acid-cleaned, 25-mL polyethylene vial and stored frozen until needed for nutrient analysis.

2.2. Chemical Analyses

[6] One GFF was fumed overnight with concentrated hydrochloric acid to remove any trace of inorganic carbon and was then analyzed for organic carbon content using a standard high-temperature combustion method [*Verardo et al.*, 1990]. The other filter was extracted ultrasonically three successive times using a 75:25 mixture of methylene chloride and methanol (20 mL each). Upon the addition of water (10 mL), combined extracts (60 mL total) were partitioned into hexane. After drying over granular, anhydrous sodium sulfate, the hexane solution was then concentrated by rotary evaporation and transferred into a solvent-cleaned, 1 dram borosilicate glass vial with a Teflon-lined cap. Upon evaporation of the solvent under a gentle stream of prepurified N_2 the resultant total lipid residues were derivatized using bis-trifluorotrimethylacetamide with 1% trimethylchlorosilane (Sigma Chemicals) and analyzed for alkenone content and composition using capillary gas chromatography with flame ionization detection (GC-FID). All reported alkenone concentrations have been corrected for recovery efficiency (typically 80–90%) of an internal standard (nonadecan-2-one) added to each sample prior to extraction. Further details of the alkenone work-up and GC-FID analysis procedure are presented by *Prahl et al.* [1988]. Frozen filtrate from each sampling time point was thawed and analyzed for nitrate and orthophosphate concentration by routine, autoanalyzer-based, colorimetric methods [*Strickland and Parsons*, 1972].

3. Results

[7] Two different types of isothermal (15°C) batch culture experiments were conducted with NEPCC 55a. Alkenone content and composition were tracked (1) in cells dividing exponentially until growth was limited by nutrient depletion (nutrient stress experiment) and (2) in cells shifted at midexponential growth to prolonged, continuous darkness (light limitation experiment).

[8] Cells at the start of both experiments grew exponentially at a rate of ~0.35 divisions d^{-1} (Figures 1a and 1b). In the nutrient stress experiment, cells maintained this growth rate until they stripped the medium of its limiting nutrient, orthophosphate. Cells stopped dividing 1 day after orthophosphate was depleted but continued to consume nitrate for another day until it was also exhausted (Figure 1a). The molar ratio for nitrate to orthophosphate drawdown during the exponential growth period was 15.1:1, essentially Redfield stoichiometry.

[9] In the light limitation experiment the growth rate of ~0.35 divisions d^{-1} was maintained until the cells were placed in continuous darkness, upon which cell division ceased after the first day (Figure 1b). When cells were reexposed after 5 days to their prior daily light-dark cycle, they resumed growth but at <50% of the initial rate. Nitrate and orthophosphate uptake was evident in nondividing cells throughout the darkness phase of the experiment (Figure 1b). Nutrient uptake in darkness occurred in the molar proportion of ~16.6:1, only slightly higher than in cells growing exponentially under lighted conditions.

[10] In both experiments, total alkenone concentrations (ΣAlk, pg $cell^{-1}$) were relatively constant in exponentially growing cells, but absolute concentrations differed between experiments, with those in the nutrient stress experiment containing ~40% more (compare Figures 1c and 1d). Upon nutrient depletion, cellular alkenone concentration increased steadily by a factor of ~3 over an 8-day period (Figure 1c). This increase was accompanied by a systematic decline in the alkenone unsaturation index $U_{37}^{K'}$ ([K37:2]/([K37:2] + [K37:3])), a trend driven by selective accumulation of the tri-unsaturated (K37:3) relative to the di-unsaturated (K37:2) C_{37} methyl ketone (Table 1). Upon exposure to continuous darkness, cellular alkenone concentration decreased steadily by ~80% over the 5-day period (Figure 1d). This decrease was accompanied by a pronounced increase in $U_{37}^{K'}$, a trend driven by selective loss of K37:3 (Table 1). Upon reinstating a daily light-dark cycle, cells rapidly accumulated alkenones and, after 3 days, displayed a concentration and $U_{37}^{K'}$ like that observed

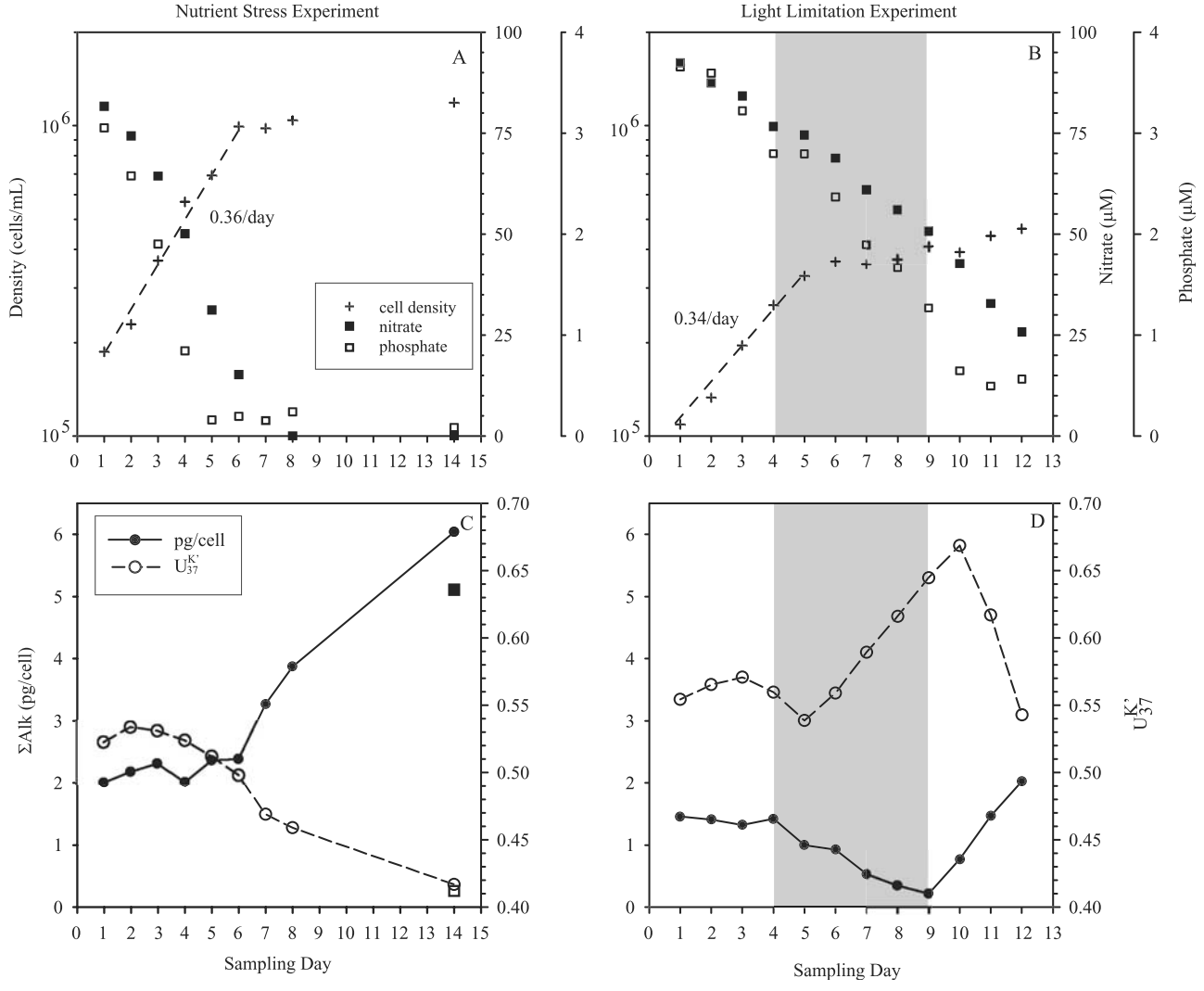

Figure 1. Batch culture experiments conducted with *E. huxleyi* strain NEPCC 55a to test the effect of (a, c) nutrient depletion (nutrient stress experiment) and (b, d) darkness (light limitation experiment) on cellular alkenone content and composition. The shaded areas in Figures 1b and 1d indicate the 5-day period in the light limitation experiment when the culture flask was covered with sufficient aluminum foil to exclude all light but not to impede gaseous exchange between the culture media and the atmosphere. Figures 1a and 1b display growth curves for each experiment. Dashed lines identify the portion of each curve used to calculate exponential growth rates (~0.35 divisions d^{-1}). Data depicting the drawdown of nitrate and orthophosphate concentration in each experiment are also plotted. Figures 1c and 1d illustrate how total $C_{37\text{-}39}$ alkenone concentration (ΣAlk, pg $cell^{-1}$) and values for the unsaturation index, $U_{37}^{K'}$, evolved during the course of each experiment. The solid and open squares in Figure 1c depict $C_{37\text{-}39}$ alkenone concentration and $U_{37}^{K'}$, respectively, measured in stationary phase cells from day 14 of the nutrient stress experiment that were subsequently put into continuous darkness for 5 days prior to sampling for analysis.

in exponentially dividing cells at the start of the experiment (Figure 1d).

[11] The two types of experiments described above have been repeated now several times using culture medium with different starting proportions of nitrate to orthophosphate (F. G. Prahl et al., unpublished data, 2001, 2002). The salient features of these experiments are the same as what has just been described. Detailed results from the complete set of experiments will be discussed more specifically in a future manuscript.

4. Discussion

[12] Results from our batch culture experiments add to the growing body of evidence that nutrient or light stress can affect cellular burdens and isomeric composition of long-

Table 1. Summary of Results From the Nutrient Stress and Light Limitation Experiments Conducted With Strain NEPCC 55a of *E. huxleyi* Grown in Laboratory-Controlled Batch Cultures

Experiment	Sampling Day	POC, pg cell^{-1}	ΣAlk,[a] % of C	K37:2, pg cell	K37:3, pg cell	K37,[b] %	K38m,[b] %	K38e,[b] %	K39,[b] %
Nutrient stress	1	9.6	16	0.607	0.664	64	13	20	2.6
Nutrient stress	2	10.1	17	0.660	0.754	64	13	20	2.2
Nutrient stress	3	8.9	21	0.675	0.763	62	14	22	2.0
Nutrient stress	4	8.1	20	0.625	0.686	65	12	21	2.1
Nutrient stress	5	9.6	19	0.748	0.784	65	12	21	2.2
Nutrient stress	6	9.4	20	0.764	0.758	63	12	22	2.4
Nutrient stress	7	11.9	22	1.06	0.942	61	13	23	2.8
Nutrient stress	8	12.8	24	1.26	1.07	60	14	24	3.3
Nutrient stress	14	12.1	39	1.98	1.42	56	15	26	4.2
Nutrient stress (5 days darkness)	19	8.9	46	1.63	1.14	53	15	28	4.3
Light limitation (light)	1	7.3	15	0.392	0.488	62	12	20	5.7
Light limitation (light)	2	6.4	17	0.412	0.535	68	11	20	1.6
Light limitation (light)	3	6.2	17	0.378	0.502	67	11	20	1.9
Light limitation (light to dark)	4	7.7	15	0.413	0.526	67	11	20	2.0
Light limitation (dark)	5	5.9	13	0.298	0.348	65	12	21	1.7
Light limitation (dark)	6	5.6	13	0.242	0.306	61	12	23	3.1
Light limitation (dark)	7	6.3	7	0.122	0.174	56	14	28	2.4
Light limitation (dark)	8	6.5	4	0.068	0.109	51	14	32	3.1
Light limitation (dark to light)	9	5.3	4	0.039	0.071	45	14	35	5.6
Light limitation (light)	10	8.0	8	0.141	0.284	55	15	25	4.3
Light limitation (light)	11	8.9	13	0.330	0.531	59	15	23	3.2
Light limitation (light)	12	9.6	17	0.536	0.636	58	15	23	3.7

[a] ΣAlk ≡ total concentration of C_{37}, C_{38}, and C_{39} methyl and ethyl ketones expressed as a percentage of total cellular organic carbon; calculation made assuming the mass of each compound is ~80% carbon by weight.

[b] %K37, %K38m, %K38e, %K39 ≡ concentration of C_{37} methyl, C_{38} methyl, C_{38} ethyl, and C_{39} ethyl ketones as a percentage of total alkenone concentration, respectively.

chain alkenones in *E. huxleyi*. Buildup of alkenone concentration in nutrient-stressed cells has been reported previously in other strains [*Conte et al.*, 1998; *Epstein et al.*, 1998]. Significant change in $U^{K'}_{37}$ has also been noted during this concentration buildup, although the shift reported by *Epstein et al.* [1998] was opposite in direction to what *Conte et al.* [1998] documented and our work now shows. The cause for the apparent biosynthetic difference between strains is unclear and needs further investigation.

[13] Recently, loss of alkenone concentration in cells kept in continuous darkness has also been reported for NEPCC 55a and another *E. huxleyi* strain [*Epstein et al.*, 2001]. Unlike the case for our light limitation experiment, however, Epstein et al. focused on cells that had been in stationary growth for at least a week and had no nitrate or orthophosphate left in their medium. The magnitude of loss of cellular alkenone concentration was comparable to what we observed (i.e., ~80%) but occurred over a period of time 4 times longer. Although selective loss of K37:3 was observed, the degree of selectivity was much less, resulting in a $U^{K'}_{37}$ shift of only 0.04 units. In our nutrient stress experiment with NEPCC 55a we reexamined the effect of darkness on alkenone content and composition of stationary phase cells. We found that the contrasts just described are reproducible. Stationary phase cells from day 14 that were kept for 5 additional days in total darkness prior to analysis showed a more minor loss of alkenone content (~15%) with negligible impact on $U^{K'}_{37}$ (Figure 1c, compare corresponding solid and open symbols).

[14] We also evaluated how alkenone contribution to total cellular carbon (ΣAlk as percent of C, Table 1) varied as a function of nutrient stress and light limitation. As documented in prior work with *E. huxleyi* [*Conte et al.*, 1998; *Prahl et al.*, 1988], alkenones comprised a significant fraction of total cellular carbon in exponentially growing cells (i.e., 15–20%, Table 1). Under nutrient stress, when stationary phase cells concentrated even more of these lipids (Figure 1c), their contribution to total cellular carbon more than doubled, reaching 39% in the final day of our first experiment (Table 1). Under light deprivation, when nondividing, nutrient replete cells appear to consume these biochemicals (Figure 1d), their contribution to total cellular carbon is reduced significantly, reaching 4% on the fifth day of darkness in our second experiment (Table 1). Notably, alkenone contribution to total cellular carbon was not reduced, but rather increased somewhat, in the case where nutrient-limited, stationary phase cells from day 14 of our first experiment were subjected to darkness for 5 additional days prior to sampling and analysis (Table 1). For physiological reasons yet to be specifically determined, the effect of darkness on alkenone content and composition appears greatest for nutrient replete, exponentially growing cells.

[15] Alkenones are typically biosynthesized by *E. huxleyi* and selected other haptophytes as a set of C_{37}-C_{38} methyl and C_{38}-C_{39} ethyl ketones [*Brassell*, 1993]. In order to illuminate the effect of physiological factors such as nutrient stress and light limitation on alkenone biosynthesis we formulated four compositional indices, i.e., %K37, %K38m, %K38e, and %K39. The compositional index %K37 is defined by the relative abundance of C_{37} to total C_{37}, C_{38}, and C_{39} alkenones. The indices %K38m, %K38e, and %K39 are defined accordingly for the C_{38} methyl, C_{38} ethyl, and C_{39} alkenones, respectively. An examination of the data in Table 1 shows that the pronounced alkenone concentration increase in illuminated, nutrient-limited, stationary phase cells and its decrease in light-deprived,

nutrient replete stationary phase cells has a similar consequence on %K37: a decrease in its value. The cause for the decrease is not the same for the two cell types, however: In the case of nutrient stress the decrease results from selective biosynthetic production of C_{38} methyl/ethyl and C_{39} ethyl ketones, while in the case of light limitation the decrease results from selective metabolic removal of the C_{37} methyl ketones (Table 1).

4.1. Physiological Function of Alkenones

[16] The observations from our batch culture experiments are consistent with the suggested role of alkenones as cellular energy stores [*Epstein et al.*, 2001; *Pond and Harris*, 1996]. *E. huxleyi* does not biosynthesize significant levels of triacylglycerides (TAG) [*Volkman et al.*, 1986], the neutral lipids that other algal phyla typically accumulate when cells are nutrient stressed [*Lombardi and Wangersky*, 1991; *Parrish and Wangersky*, 1987]. On this basis, *Epstein et al.* [2001] speculated that alkenones in *E. huxleyi* are biochemical surrogates for TAG. However, the analogy may not be exact, as diatoms accumulate TAG as conspicuous droplets in transmission electron microscopy (TEM) images [*Sicko-Goad et al.*, 1988]. Review of TEM literature revealed no report of such features in *E. huxleyi* images [*Pienaar*, 1994; *van der Wal et al.*, 1985]. The apparent absence of such a feature is quite noteworthy given that $C_{37\text{-}39}$ alkenones are major contributors to cellular carbon in these organisms, comprising 10–20% of that in exponentially growing cells [*Conte et al.*, 1998; *Prahl et al.*, 1988] and up to 40% of that in nutrient-stressed, stationary phase cells (Table 1).

[17] On the basis of the response of alkenone unsaturation to temperature, *Brassell et al.* [1986] originally conjectured that these biomarkers serve a structural role in the cell by helping in some way to regulate membrane fluidity [*Harwood and Russell*, 1984]. Our results argue that alkenones are not used strictly for purposes of cellular architecture and biophysical maintenance of membrane properties. Nonetheless, they also do not discount the possibility that these compounds are associated in some way with a particular membrane in cellular substructure (e.g., chloroplasts). Clearly, more laboratory-controlled study is warranted to elucidate specifically how alkenones are contained and how they function physiologically within the cells of the select group of haptophytes capable of this biosynthesis [*Brassell*, 1993].

4.2. Oceanographic Evidence for Physiological Uncertainty in Paleothermometer Calibration

[18] Despite well-documented genetic variation in $U^{K'}_{37}$-T calibration among *E. huxleyi* strains [*Conte et al.*, 1998], a remarkable linear correlation exists between nearly global $U^{K'}_{37}$ measures in marine surface sediments and annual mean SST in overlying waters that is statistically indistinguishable from the $U^{K'}_{37}$-growth temperature calibration for NEPCC 55a [*Muller et al.*, 1998] (Figure 2). Our data support prior studies [*Conte et al.*, 1998; *Epstein et al.*, 1998] which suggest that physiological factors such as nutrient and light availability could contribute to the apparent variability in the core top data set. Although maintained isothermally, the observed 0.11 unit change in each

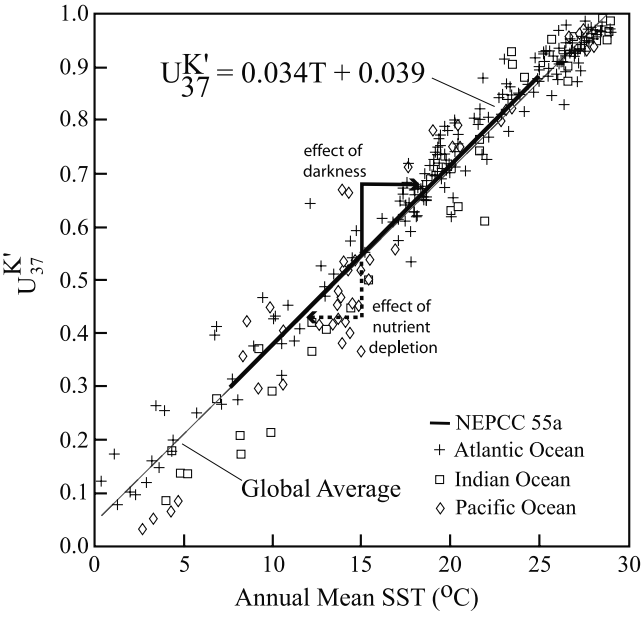

Figure 2. Graph displaying the complete data set used for the global core top calibration of $U^{K'}_{37}$ versus annual mean SST [*Muller et al.*, 1998]. Comparison shows that the best linear fit of these data (shaded dashed line, ''global average'' defined by $U^{K'}_{37} = 0.033T + 0.069$ from *Muller et al.* [1998]) is virtually indistinguishable from the original batch culture calibration of *E. huxleyi* strain NEPCC 55a (bold line, $U^{K'}_{37} = 0.034T + 0.039$ from *Prahl et al.* [1988]). The bold and dotted arrows depict quantitative effects of nutrient depletion and darkness on $U^{K'}_{37}$, respectively, documented in the present study by our isothermal batch culture experiments with NEPCC 55a.

of our experiments corresponds to a perceived ~3°C change in growth temperature if translated using the established calibration for NEPCC 55a ($U^{K'}_{37} = 0.034T + 0.039$ [*Prahl et al.*, 1988]). Superimposing the similar magnitude but opposite quantitative effects of nutrient stress and light limitation on $U^{K'}_{37}$ documented by our experiments on the plot in Figure 2 shows that potentially all of the variability in the core top calibration could be accounted for by algal physiology.

[19] This prospect begs an answer to the following biological oceanographic question: What is the physiological condition of cells exporting alkenones to marine sediments? The answer to this question is most relevant to paleoceanographic temperature reconstructions based on $U^{K'}_{37}$ [*Conte et al.*, 1998] and may depend on what particular ocean location is under consideration [e.g., *Conte et al.*, 2001]. Comparison of depth profiles for alkenone content and composition in suspended particulate material from surface waters collected at two different northeast Pacific oceanographic sites provides support, albeit yet circumstantial, for a geographically dependent answer to this question.

[20] Open ocean waters in the temperate northeast Pacific (~40°N, 132°W) are seasonally stratified, and in summer through early fall they display a subsurface chlorophyll maximum (SCM) at the top of a nutricline within the upper

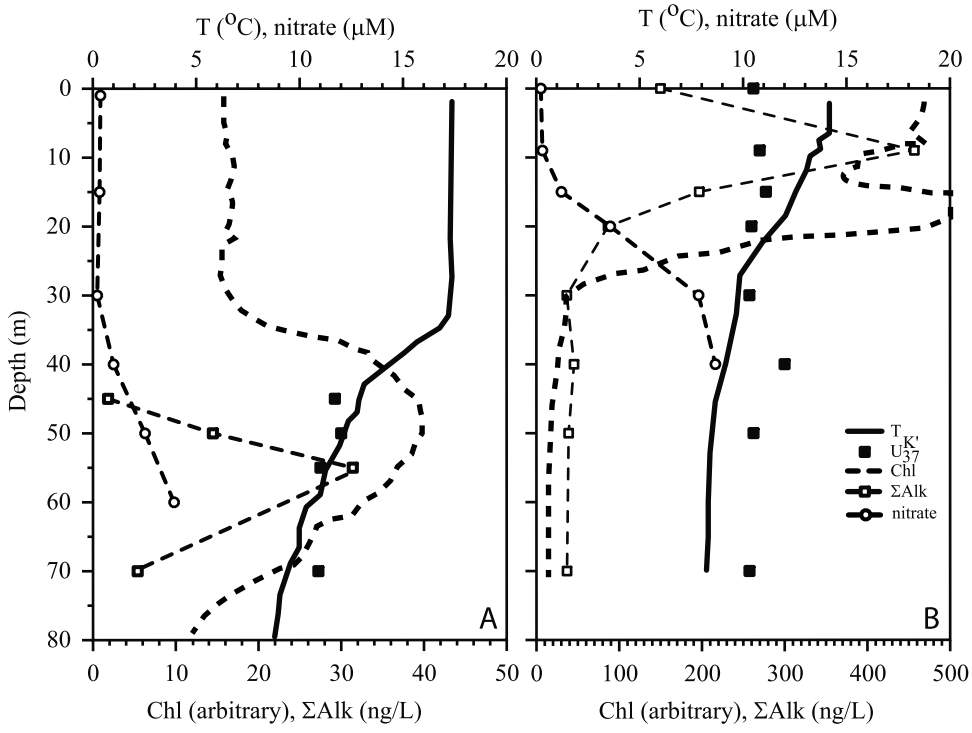

Figure 3. Depth profiles for temperature (solid line), nitrate concentration (open circles), chlorophyll fluorescence (Chl) (dashed line), and total $C_{37\text{-}39}$ alkenone concentration (ΣAlk) (open squares) in suspended particulate materials (SPM), measured in the late 1980s in surface waters at two sites in the northeast Pacific Ocean. (a) Temperate gyre location (~40°N, 132°W) [*Prahl et al.*, 2001] and (b) continental margin location (46°45′N, 125°5′W) off the coast of Washington State (F. G. Prahl et al., unpublished data, 2001, 2002). Solid squares depict growth temperature estimates for alkenone producers at the depth of SPM collection using the established $U^{K'}_{37}$ calibration for NEPCC 55a (i.e., $U^{K'}_{37} = 0.034T + 0.039$ [*Prahl et al.*, 1988]).

thermocline. In September 1989 we found measurable alkenone concentrations at this site only in the SCM, with $U^{K'}_{37}$-based growth temperature estimates in good agreement with actual water temperature measured in this feature (Figure 3a). In contrast, ocean waters along the northeast Pacific continental margin experience seasonal upwelling of nutrients [*Hickey*, 1979] and consequently, episodic phytoplankton blooms throughout summer and early fall [*Landry and Hickey*, 1989]. At one such site off the Washington coast (46°45′N, 125°5′W) in August 1988 we found high alkenone concentrations in near-surface waters containing very high chlorophyll and low nitrate concentrations (Figure 3b). $U^{K'}_{37}$-based growth temperature estimates were ~3°C lower than actual surface water temperatures. We have also made similar observations at continental margin sites in the northeast Pacific off northern California (~40°N) (F. G. Prahl et al., unpublished data, 1989). When these observations are considered in context with findings from our batch culture experiments, alkenone producers at the open ocean site in the temperate northeast Pacific would seem to have a supply of nutrient and light sufficient for perhaps slow but steady state, continuous culture-like growth [e.g., *Popp et al.*, 1998] and thereby export a $U^{K'}_{37}$ signal to the seafloor that predominantly records water temperature at the depth of production. Alkenone signatures in continental margin waters of the northeast Pacific, on the other hand, seem consistent with that of cells experiencing significant nutrient stress under episodic, batch-culture-like growth conditions (e.g., Figures 1a and 1c). If so, the $U^{K'}_{37}$ signal exported to the seafloor at such sites quite significantly underestimates actual growth temperature at the depth of production (i.e., ~3°C). Recognizing whether the bias in the temperature assessment is systematic or variable through time would then pose a significant challenge to paleoceanographic research.

[21] The physiological interpretation applied to these water column profiles is certainly not yet unequivocal and warrants further evaluation. Nonetheless, it is now defensible based on results from our batch culture experiments and the growing body of biogeochemical literature on these molecules, and it is therefore reasonable to pose. If proven in the future to be correct, it bears significantly on how specifically stratigraphic $U^{K'}_{37}$ measurements in marine sediments, particularly those accumulating near ocean margins, can be read as a paleorecord for absolute changes in annual mean SST.

5. Conclusions

[22] The findings from our work with laboratory batch cultures of a single strain of *E. huxleyi* by no means discount the use of $U^{K'}_{37}$ as an insightful paleotemperature

proxy. However, they clearly show that the absolute value for $U^{K'}_{37}$ recorded in sediments may not be set exclusively by growth temperature or by export of a signal from cells necessarily productive at the sea surface. Further research is warranted to refine the understanding of alkenone biosynthesis and its physiological control as well as the understanding of environmental controls on organic matter export from the euphotic zone to sediments. This need for further research is now particularly required if our paleoceanographic goal [*Mix et al.*, 2000] is to use the alkenone fossil record for more than just a measure of relative changes in annual mean SST through time [*Brassell et al.*, 1986; *Muller et al.*, 1998].

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