Alkenone δD as an ecological indicator: A culture and field study of physiologically-controlled chemical and hydrogen-isotopic variation in C₃₇ alkenones

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ABSTRACT: A combined culture and field study was conducted in order to 1) more firmly identify the physiological controls on hydrogen isotopic composition of C₃₇ alkenones produced by open-ocean coccolithophores and 2) determine the degree to which these controls are manifested in a natural water column. Nutrient-limitation experiments in culture, combined with previously published data, show that net fractionation between the growth medium and alkenones (α_{K37}) varies with cellular alkenone content and production rate, and, by extension, growth phase. It is hypothesized that the relationship of α_{K37} with cellular alkenone content and production rate is due to increased use of anabolic NADPH in response to high rates of lipid synthesis. Euphotic zone profiles of δD_{K37} , measured in suspended material from the Gulf of California and Eastern Tropical North Pacific, decreased with depth and light availability, and did not correlate in any

expected way with previously-suggested controls on α_{K37} . It is possible that the field data are driven by behavior in light-limited cells that is not represented by the available, nutrient-limited culture data. If true, δD_{K37} may have utility as an indicator of production depth in settings prone to subsurface production maxima. Relationships between α_{K37} , cell density, and the carbon-isotopic fractionation term ϵ_p , however, suggest that α_{K37} acts as an indicator of growth rate, which in this setting is only partially dependent on light, consistent with our interpretation of the culture data. If this latter interpretation proves correct, δD_{K37} may be a powerful ecological proxy specific to these climatically-important, calcifying, temperature-encoding species.

1. INTRODUCTION:

The hydrogen isotopic composition of plant and algal lipids has received considerable attention from the oceanographic and paleoclimate communities. Lipids produced by photoautotrophs encode the relative 2H (deuterium or D) and 1H abundances of environmental water in their abundant, non-exchangeable hydrogen (Estep and Hoering 1980). This relationship has spurred attempts to develop hydrologic proxies from these compounds (e.g. Estep and Hoering 1981; Sauer et al 2001; Huang et al. 2004). However, it has become clear that the large and variable species-specific physiological controls on the isotopic fractionation between water and lipids make biomarkers with multiple potential sources problematic as climate archives (e.g. Zhang and Sachs 2007). The need for a lipid with a well-defined source organism has, in turn, driven research focus to the hydrogen isotopic systematics of C_{37} alkenones, unsaturated ketones which appear to be energy storage products unique to specific prymnesiophytes (Epstein et al. 2001; Eltgroth et al. 2005). These compounds are largely produced in modern marine settings by the coccolithophores *Emiliania huxleyi* and species of the genus *Gephyrocapsa* (Volkman et al. 1980, 1995; Marlowe et al. 1984). These species are found throughout the world ocean (Winter et

al. 1994; Bollmann 1997) and primarily produce the di- and tri-unsaturated 37-carbon methyl ketones (K37:2 and K37:3). The source-specificity of C₃₇ alkenones has already led to development of their expressed carbon isotopic fractionation as a growth rate and/or pCO₂ proxy (e.g. Pagani 2014). The ratio in which these compounds are produced varies as a function of growth temperature (e.g. Prahl et al. 1988; Volkman et al. 1995), also providing these compounds utility as the $U_{37}^{K'}$ paleo-temperature proxy (e.g. Herbert 2003).

Previous development of these compounds as a temperature proxy, coupled with the potential to determine water-alkenone fractionation factors for the limited source species, makes alkenones an attractive target for development as a sedimentary hydrologic proxy. Attempts to relate the hydrogen isotopic composition of sedimentary alkenones (δD_{K37}) to that of the overlying water (δD_{H_2O}) via laboratory- or field-determined values of the apparent fractionation factor, α_{K37} , have yielded reasonable results (Englebrecht and Sachs 2005). δD_{K37} values versus the SMOW standard are defined (unitless) as:

$$\delta D_{K37} = \frac{D/H_{K37} - D/H_{SMOW}}{D/H_{SMOW}} \quad 1.1$$

and α values are defined as:

$$\alpha_{K37} = \frac{D/H_{K37}}{D/H_{H_2O}} = \frac{\delta D_{K37} + 1}{\delta D_{H_2O} + 1} \quad 1.2$$

It has become clear, however, that α_{K37} is not constant, and may vary significantly in response to a number of physiological factors. In addition to differences between species, such as open-ocean alkenone producers *E. huxleyi* and *G. oceanica* (Schouten et al. 2006) and coastal species *Isochrysis galbana* (M'boule et al. 2014) and *Chrysotila lamellose* (Chivall et al. 2014b), factors suggested to control α_{K37} include growth rate and salinity (Schouten et al. 2006; M'boule et al.

2014), growth phase (Wolhowe et al. 2009; Chivall et al. 2014b), and temperature (Wolhowe et al. 2009; Zhang et al. 2009b). There is an additional ‘temperature effect’ in that a temperature-dependant isotopic offset exists between K37:2 and K37:3 ($\alpha_{K37:3-K37:2}$; D’Andrea et al. 2007; Schwab and Sachs 2009, 2011; van der Meer et al. 2013).

The ‘salinity effect’, which has been hypothesized to act via changes in composition of the intracellular water pool (Sachse and Sachs 2008; M’boule et al. 2014), has been the best-studied physiological influence on α_{K37} (e.g. Schouten et al. 2006). As salinity appears to have an ‘amplifying’ effect on the association of more negative (D-depleted) δD_{K37} values with fresher (lower δD_{H2O}) waters (Schouten et al. 2006; Sachse and Sachs 2008), the ‘salinity effect’ has been a factor in multiple studies of δD_{K37} as a hydrological proxy in environments such as estuaries, inland seas, and areas of high fresh-water discharge (Pahnke et al. 2007; van der Meer et al. 2008; Schwab and Sachs 2011). Though shifts between low-salinity/coastal and marine species assemblages may be a concern (Kasper et al. 2015), the impact of physiological influences on δD_{K37} in these settings is theoretically minimal relative to the impacts of high salinity contrasts (~ten to hundreds of salinity units) and high- δD_{H2O} contrasts (~ten to hundreds of per mil).

While some promising work has related δD_{K37} to open-ocean salinity (Kasper et al. 2014), the other physiological controls on α_{K37} have the potential to make δD_{K37} a poor hydrologic proxy in locations with single-digit salinity variations and accordingly-low (<10‰) δD_{H2O} variations (Wolhowe et al. 2009). This statement, though, can be reversed and posed as a question: in places with low variability in δD_{H2O} and salinity, can δD_{K37} , responding instead to changes in α_{K37} , be used as a sedimentary proxy for the physiological condition of alkenone-producing coccolithophores? A collection of purely empirical relationships between α_{K37} and growth rate, temperature, growth phase, etc. presents too many degrees of freedom if we are to interpret δD_{K37}

without a host of ancillary measurements. In order to answer this question, then, we must better understand how these physiological factors mechanistically impact δD_{K37} .

In the following work, we detail two steps taken towards making this determination. They are:

- 1) to present results of dilute batch culture experiments, in comparison with previously published data, in order to evaluate the consistency of the purported growth rate, temperature, and growth phase effects on α_{K37} in *G. oceanica* and *E. huxleyi* (Section 3.1);
- 2) to present novel δD_{K37} data from marine samples collected in the Gulf of California and Eastern Tropical North Pacific, and to evaluate whether the interpretive framework we develop from our culture data extends to natural coccolithophore populations (Section 3.3).

We compare α_{K37} to both environmental (temperature, nutrients, light) and physiological (alkenone production rate and cellular concentration, growth rate, species composition, carbon isotopic composition) parameters to determine the potential for future sedimentary proxy work employing ecology/physiology- α_{K37} dynamics. We hypothesize that the nutrient-depleted upper water column found throughout the Gulf of California and ETNP in summer will express low α_{K37} values, consistent with nutrient-stressed culture experiments, while higher values will be observed near the nitracline.

2. METHODS:

2.1 Cultures:

Six calcifying strains of *E. huxleyi* isolated from the Mediterranean Sea (RCC1812, RCC1813, RCC1817, RCC1827, RCC1830 and RCC1833; Roscoff Culture Collection, <http://roscoff-culture-collection.org>) were batch cultured in nutrient replete (f/2, ~740 μ M nitrate,

~35 μM phosphate) and low phosphate (f/2 modified for ~0.2 μM initial phosphate) media under 400 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ PAR (16:8 light:dark) at 20°C and a salinity of 32 (Table 1; reported salinity values were measured individually after sampling, see Section 2.4.2). Low-phosphate cultures depleted their phosphate in ~5 days and were harvested after 3 or 4 days in stationary phase at a cell density of ~100,000 cells ml^{-1} . Nutrient-replete cultures were harvested (still in log phase) at the same approximate cell density. A strain of *E. huxleyi* isolated from the Norwegian Sea (B92/11; Plymouth Marine Lab, <http://www.mba.ac.uk/culture-collection>) was cultured at 15°C under analogous conditions (phosphate replete and reduced-phosphate f/2 treatments) and harvested at the same cell densities. Additionally, this strain underwent a nitrate limited treatment (f/2 modified for ~2 μM initial nitrate). Duplicate cultures were conducted for each set of conditions. Samples (300 ml filtered onto pre-combusted glass fiber filters) were oven-dried and stored at room temperature until analysis. These cultures are described in more detail, along with discussion of their calcification behavior, in Langer et al. (2013; for B92/11) and Oviedo et al. (2014; for RCC1812-1833).

2.2 LVF Samples:

In situ large volume filters (LVFs) were collected during ‘GoCal4’, a 2008 cruise to the Gulf of California and adjacent eastern tropical north Pacific (ETNP). Alkenone-producers *E. huxleyi* and *Gephyrocapsa spp.* are the predominant species of coccolithophores in this setting (Ziveri et al. 1995; Thunell et al. 1996; Ziveri and Thunell 2000; Malinverno et al. 2008). This cruise and the stations in question (Table 2) are documented fully in White et al. (2013) and Wolhowe et al. (2014).

At seven of the Gulf of California and ETNP stations (Table 2), WTS-LV *in situ* pumps (McLane Research Laboratories) were deployed by hydrowire to sample alkenones in suspended

particulate matter (SPM) for δD_{K37} analysis. These samples were collected at two or three depths between 10 and 57m by filtering ~340-570 L onto 142 mm pre-combusted type A/E glass fiber filters ($> 1 \mu m$ fraction) over the course of ~2-4 hours. Samples from other depths at these stations were acquired by using filtered material from similar pump deployments made for U/Th analysis (~50% filter remaining after U/Th sub-sampling). The additional samples were collected on pre-combusted QM-A quartz filters ($\sim 1 \mu m$ to $53 \mu m$ size fraction), but otherwise were handled identically. All filters were frozen at $\sim -80^{\circ}C$ until analysis. Filter type/size cutoff did not appear to impact the resulting isotopic data.

2.3 Chemical Analysis:

Alkenone quantification followed the procedures outlined in Wolhowe et al. (2014 and references therein). Briefly, lipids were extracted from the filters using a Dionex ASE-200 automated solvent extraction system (Walinsky et al. 2009) and the resulting total extractable lipids (TEL) partitioned into hexane. In the case of the field samples, alkenone fractions were isolated from the TEL by liquid column chromatography on silica gel (Prahl and Pinto 1987). The alkenone fractions (field) or TELs (culture) were then saponified in 1 M KOH in ethanol to remove alkenoates (Christie 2003) and characterized/quantified by capillary gas chromatography with flame ionization detection (GC-FID; Walinsky et al. 2009). Reported alkenone concentrations have been corrected for recovery (typically 80-90%) of hexatriacontan-2-one (K36:0) added to each sample prior to ASE extraction. Due to a lack of true sample replication for the field samples, a conservative uncertainty of 10% (as per Popp et al. 2006) has been assumed in the quantification of K37:2 and K37:3.

For the culture experiments, organic carbon was determined by acid-fuming filter cakes to remove inorganic carbon, followed by high-temperature combustion elemental analysis. Details

are provided in Langer et al. (2013) and Oviedo et al. (2014). Typical uncertainty in cell-carbon analysis was $\leq 5\%$.

2.4 Isotopic Analysis:

δD_{K37} and δD_{H2O} analysis was performed using a ThermoQuest-Finnigan Delta Plus XL isotope ratio mass spectrometer. H_3^+ correction factors (Sessions et al., 1999) were determined daily. Values ranged between ~ 2 and ~ 4 ppm nA^{-1} , but did not vary by more than ~ 0.5 within a given batch of analyses. All δD values are reported in per mil (‰) versus Standard Mean Ocean Water (SMOW).

2.4.1 Compound-Specific δD Analysis:

δD_{K37} was determined by gas chromatography-thermal conversion-isotope ratio monitoring mass spectrometry (GC-TC-IRMS). Samples were dissolved in the volume of toluene necessary to analyze ~ 500 ng of the minor (or sole, in cases where $U_{37}^{K'} \approx 1$) C_{37} component in a $2 \mu L$ GC injection. Samples, coinjected with $1 \mu L$ of an n-alkane working standard (400 ng $nC_{36} \mu L^{-1}$ and 410 ng $nC_{37} \mu L^{-1}$), were chromatographically separated using an HP6890 GC equipped with a cool on-column injector and a SGE BPX5 column (30 m x 0.32 mm i.d., $1.0 \mu m$ film; #054153). The GC was operated at a constant helium carrier gas flow rate (2.5 mL min^{-1}). Separations were achieved by temperature programming (80 - $270^\circ C$ at $10^\circ C$ min^{-1} , 270 - $320^\circ C$ at $5^\circ C$ min^{-1} , 43 min hold at $320^\circ C$) and alkenones eluted after approximately 50 minutes. The alumina pyrolysis reactor (32 cm x 0.5 mm i.d.; Thermo-Finnigan GC-TC interface) was maintained at $1410^\circ C$. Upon initial use, $1 \mu L$ injections of isooctane were passed through the reactor for pre-conditioning (graphitization) until measured standard values stabilized. δD_{K37} values were

calculated versus the coinjected nC_{36} , nC_{37} , and C_{36} methyl ketone (K36:0) reference materials. 'Known' values of these working references were determined by daily standardization through co-injection with 'Mixture B', a 15 compound mixture of n-alkanes with known δD values, obtained from A. Schimmelmann (Indiana University; <http://mypage.iu.edu/~aschimme/compounds.html>). The standard errors of the mean (SEM) of the working reference materials ($n = 68$) were 0.7‰ (nC_{36}), 0.6‰ (nC_{37}), and 0.6‰ (K36:0). All δD_{K37} values are reported as the mean of two or three replicate analyses of the same sample; conservative uncertainties of 5‰ were assumed for samples where replication was not possible (indicated in Tables 1, 2). The mean standard deviation for replicate analyses was 3.0‰ for the LVF samples and 1.9‰ for the culture samples.

2.4.2 Water δD Analysis:

Culture media δD_{H_2O} was determined by H_2 equilibration following the methods of Horita et al. (1989) and Coplen et al. (1991). For each sample, 2.5 mL of filtered media and a set of platinum "Hokko bead" catalysts (Isoprime) were placed in a 20 mL glass reservoir interfaced to the dual inlet of the mass spectrometer (manifold of 18 samples + standards in a given run). The reservoirs were lowered into an 18°C water bath, evacuated, and the headspace filled to 250 mbar with H_2 of $\delta D_{H_2} \sim 124$ ‰. The isotopic composition of the fill gas was determined to have no measurable effect on the final equilibrated values due to its small contribution to the hydrogen mass balance. The samples were shaken for ten hours to allow for isotopic equilibration between H_2O and H_2 . The gas in each reservoir was then admitted to the dual inlet through a liquid N_2 water trap. Each sample was measured eight times versus a monitoring gas, yielding a mean analytical precision (1σ) of 0.1‰. Each run contained two reservoirs each of two working standards (-0.9 and -69.1‰) to which this monitoring gas was referenced, as well as at least one

sample from each previous run to monitor drift. The working standards, in turn, were calibrated against the GISP, SLAP, and VSMOW2 standards. The SEM of the working reference materials ($n = 6$) was 0.3 and 0.1‰, respectively. The mean standard deviation of all sets of repeated samples was 0.5‰.

Due to the presence of salinity effects on the catalyzed equilibrium fractionation between H_2O and H_2 (Martineau et al. 2011), a salinity correction was empirically determined. A series of 15 artificial seawater standards with salinities ranging from 0 to 40 were made from distilled water of -80‰. Salinities were determined via AgCl titration (Adkins and Schrag 2003). A δD correction of ~ 0.06 ‰ relative to fresh water, per salinity unit, was determined from equilibration of these standards. The salinity of samples and isotope working standards was measured prior to equilibration to determine the necessary degree of correction. As all samples had salinities of ~ 32 , no sample had an applied correction greater than ~ 2 ‰.

2.4.3 Estimation of Environmental δD_{H_2O} :

Water samples corresponding to the LVF samples from the Gulf of California were not available. However, given the narrow range of salinity measurements in our study area and the covariation of salinity and δD_{H_2O} in marine settings (e.g. Craig and Gordon 1965; Rohling 2007), we are able to reasonably estimate water composition for the purposes of sample comparison within the LVF data set. First, a regional $\delta^{18}O$ vs. salinity relationship ($\delta^{18}O = 0.56S - 19.23$; $R^2 = 0.86$) was determined from all data points in the NASA GISS ^{18}O database from <100 m between 15 to $35^\circ N$ and 95 to $150^\circ W$ with associated salinity measurements ($n = 19$). Assuming a meteoric relationship between $\delta^{18}O$ and δD ($\delta D = 8\delta^{18}O + 10$), these points generate a δD vs. S relationship of $\delta D = 4.5S - 143.8$. This equation and the salinity fields reported in Wolhowe et al. (2014) were used to estimate δD_{H_2O} at the depth of each LVF. As the full range of salinity

associated with the LVF data set was 33.47 to 35.05, estimated δD_{H_2O} ranged from 6.5 to 13.6‰ (Table 2). We assume a generous uncertainty in these values of 7.1‰, the range of the individual estimates.

3. RESULTS AND DISCUSSION

3.1 Results of Culture Experiments

The alkenone signatures in cultured Mediterranean and Norwegian strains of *E. huxleyi* responded, both chemically and isotopically, as expected from studies of log- and stationary-phase alkenone-producers (Table 1). Log-phase cells (growth rates between 0.99 and 1.32 d⁻¹) had low per-cell alkenone quotas (q_{K37}), averaging 0.96 pg cell⁻¹ for the Mediterranean strains and 0.46 pg cell⁻¹ for the Norwegian strain. Stationary-phase cells, in contrast, exhibited increased quotas, averaging 3.8 pg cell⁻¹ for the P-limited Mediterranean strains and 2.9 and 1.3 pg cell⁻¹, respectively, for the P-limited and N-limited treatments of the Norwegian strain. These phase-dependant changes in alkenone abundance echo previous results (Prahl et al. 2003, Chivall et al. 2014a). As seen previously (Wolhowe et al. 2009 and Chivall et al. 2014b), these changes were accompanied by shifts to more D-depleted isotopic compositions (Table 1). The δD_{K37} of the Mediterranean strains were ~20‰ lighter in stationary phase than in log phase. While the δD_{K37} of the N-limited Norwegian strain was ~4‰ lighter than in log phase, the P-limited treatment was lighter by more than 50‰.

Combining these results with previously-published culture data (Schouten et al. 2006; Wolhowe et al. 2009; M'boule et al. 2014), a body of data from experiments with the major oceanic alkenone-producers, within a narrow range of growth salinities, is available with which we may evaluate isotopic and chemical trends. One consistent observation is that for log-phase cells, an inverse correlation exists between α_{K37} values and q_{K37} (Figure 1A; $R^2 = 0.54$). Results

for stationary-phase samples either fall along the log-phase α_{K37} vs. q_{K37} trajectory or appear to lose functionality at values higher than $\sim 3 \text{ pg cell}^{-1}$. This decrease with q_{K37} is exhibited over a temperature range of 15 to 25°C, by multiple strains of both *E. huxleyi* and *G. oceanica*, by both calcifying and non-calcifying cultures (indicated in Table 1), and by log-phase, late-log ‘transition phase’ and stationary-phase cells. A functional relationship between α_{K37} and q_{K37} , breaking down for cells that are no longer at steady state with respect to their alkenone stores (i.e. accumulating alkenone stores with time in stationary phase; Prahl et al. 2003; Wolhowe et al. 2009; Chivall et al. 2014a) suggests an underlying functional relationship between α_{K37} and alkenone production rate. For samples where production rate data is available, a correlation is in fact observed (Figure 1B; $R^2 = 0.68$).

Other physiological variables previously shown to describe variability in α_{K37} do not appear as robust. Comparing α_{K37} to growth rate (μ) for all log-phase samples, weak correlations are observed within individual data sets (Norwegian *E. huxleyi* data from this work; *G. oceanica* data from Schouten et al. 2006) but no overall correlation with μ is seen (Figure 1C). Likewise, while the new Norwegian and Mediterranean *E. huxleyi* data are roughly consistent with the correlations previously observed between α_{K37} and growth temperature (Wolhowe et al. 2009; Schouten et al. 2006), the range of α_{K37} values at a given temperature is large relative to that of the full data set (Figure 1D).

3.2 Interpretation of Culture Data

3.2.1. Alkenone Production Rates and Potential Mechanism of Growth Rate Control

A relationship of α_{K37} with alkenone or lipid production rate, regardless of cause, may explain the previously-observed inverse correlation of α_{K37} and log-phase growth rate. As described by Halsey et al. (2011), phytoplankton produce proportionally more lipids, relative to

other biochemical classes, at higher growth rates. This has been observed explicitly for alkenone-producers *I. galbana* (Sukerik and Livine 1991) and *E. huxleyi* in culture (Fernández et al. 1996) and implicitly for *E. huxleyi* in the field (significant increase in per-cell lipid content associated with a subsurface production maximum; Fernández et al. 1994). Increased lipid loading is explained by Halsey et al. (2011) in terms of greater demand for polar structural lipids at higher growth rates – neutral storage lipids such as alkenones are not explicitly discussed. However, Fernández et al. (1994) reported approximately identical proportions of carbon incorporation into polar and neutral (predominantly alkenones in *E. huxleyi*; Volkman et al. 1986; Eltgroth et al. 2005) lipids across low- and high-productivity depths despite a ~2-fold difference in lipids as a percent of carbon. Thus, it appears reasonable to extend the relationship between lipid content and growth rate to alkenones.

If carbon-normalized lipid production rate, and thus lipid content, generally increase at higher growth rates, and if α_{K37} decreases at higher production rates (Figure 1B), it would explain both the observed inverse correlation of net fractionation on growth rate (Schouten et al. 2006; M'boule et al 2014) and the lack of reproducibility of this response between cultures (Figure 1C). There are presumably numerous factors (light intensity, nutrient availability, strain and species) that one may expect to alter the response of q_{K37} to growth rate. For example, while individual *E. huxleyi* and *G. oceanica* culture experiments express increasing q_{K37} with increasing growth rate (where q_{K37} is available, Table 1), there is no overall correlation observed between these two measures. Unfortunately, the only other literature reporting both α_{K37} and q_{K37} values (Chivall et al. 2014b) concerns coastal, low-salinity species, and is not directly comparable due to the use of salinity treatments and a lack of variability in growth rate. We suggest that q_{K37} values be reported in subsequent literature on this topic as, for cells at steady state, this term also allows assessment of production rate.

We still require an explanation, though, for why A) why alkenone production rate should correlate with net fractionation and B) low α_{K37} values should be associated with both higher growth rates and with stationary-phase cells that are not growing at all. Wolhowe et al. (2009) suggested that increased production of acetogenic lipids (i.e. alkenones) and decreased production of more D-depleted isoprenoid structural lipids (i.e. Chikaraishi et al. 2004a) might, by mass balance, be expected to result in an isotopically-lighter acetogenic lipid pool. This explanation seems unlikely, however, as Chikaraishi et al. (2004a) suggest the greater D-depletion observed in isoprenoid lipids is due to fractionation during hydrogenation steps. An explanation that has been proposed for the phase-dependence of α_{K37} by Chivall et al. (2014b) is that the balance between photosynthetic rate and the exchange time of intracellular water controls internal δD_{H2O} (and ultimately δD_{K37}) due to the D-enriching effect of NADPH production. I.e., as photosynthetic NADPH production increases, a ‘Rayleigh-like’ decrease in expressed fractionation should occur as either the water pool available for reduction of NADP, or the NADPH pool itself, is more completely consumed. This is a compelling mechanism, as it would provide a way in which the well-established salinity effect (e.g. Schouten et al., 2006; Chivall et al. 2015b; M’boule et al. 2014) could impact α_{K37} (via salinity-driven changes in cell wall permeability altering the residence time of intracellular water). Furthermore, it would explain the inverse correlation of α_{K37} and q_{K37} across growth phases (stationary phase cells express both reduced photosynthetic rate and increased q_{K37}). However, it would not explain the inverse correlations between that have been observed between α_{K37} and growth rate, q_{K37} , or alkenone production rate during log-phase growth. At the higher bulk carbon (or alkenone) production rates that occur in faster-growing cells, the fractionating utilization of the NADPH or water pools should be more complete, increasing δD_{H2O} ; if anything, this would be expected to generate a positive correlation between α_{K37} and q_{K37} or growth rate. Additionally, no correlation is observed

between α_{K37} and estimated cell-specific net organic carbon production or daily irradiance (Table 1), further suggesting any such mechanism does not play a major role in log-phase, light-saturated cells.

A potential, mechanistic explanation for the apparent dependence of α_{K37} on q_{K37} or lipid production rate may be an increased role of reductant (NADPH) derived from the oxidative pentose phosphate (OPP) pathway at higher rates of lipid synthesis. The hydrogen in lipids such as alkenones is derived from NADPH, incorporated during both the formation of glyceraldehyde 3-phosphate and during subsequent chain elongation steps (Chikaraishi et al. 2004a, 2004b). High rates of NADPH production via the OPP pathway, in order to support high rates of lipid synthesis, have been proposed as a mechanism for the rapid turnover of carbohydrates in fast-growing phytoplankton (Halsey et al. 2011; Figure 2A). The redox-active hydrogen that is abstracted from glucose and ribulose 5-phosphate to form NADPH in this pathway (e.g. Fan et al. 2014) may have a very different isotopic composition than that in NADPH produced by the photosystem (Zhang et al. 2009a). Increased OPP activity, and a corresponding shift in the δD of NADPH, was thus invoked by Romero-Viana et al. (2013) as a reason why D-enrichment of alkenones (rather than depletion, as observed here) might occur at higher growth rates.

This hypothesis, however, was based on studies of higher plants with long-lived starch/cellulose pools that were shown to be D-enriched (relative to water, positive δD values), presumably as a result of ongoing heterotrophic (e.g. OPP, glycolysis) processing (e.g. Yakir and DeNiro 1990; Luo and Sternberg 1991). NADPH derived from these D-enriched starch pools appears to lead to less-negative δD of lipids in plants with high respiration rates (Sessions 2006). While this shows that changing levels of anabolic activity can alter the δD of subsequently synthesized lipids, there is no reason to presume that the anabolic NADPH used in alkenone synthesis should be likewise D-enriched relative to primary photosynthate. The relative D/H

composition of photosynthetic versus anabolic NADPH will depend on numerous factors including the A) the initial δD of the substrates (water, sugars), B) the particular iso-enzymes used for OPP activity (which can vary both with growth rate and the organelle in which the activity is taking place; Kruger and von Schaewen 2003) and their characteristic fractionation factors, C) the residence time of the starch pool and its exposure to H-exchanging isomerization reactions and heterotrophic processing (Yakir 1992), and D) the relative flux of sugars into the OPP and glycolysis pathways depending on anabolic/catabolic requirements (Hayes 2002).

Most of these factors are either unknown (B) or unconstrained in the available culture experiments (C, D). However, significant D-enrichment of the OPP substrate, suggested to be responsible for relatively D-enriched anabolic NADPH in previous work, should not occur here. Alkenones appear to be produced in the chloroplast (Eltgroth et al. 2005), where Luo and Sternberg (1991) observed the synthesis of highly D-depleted starch and where Sessions (2006) proposed that synthesis of D-depleted carbon products occurs due to access to D-depleted “immediate products of photosynthesis”. One may expect that the substrates for the OPP pathway in this case (as opposed to those used on seasonal time-scales by higher plants) would be D-depleted relative to cell water (Figure 2A). Depending on the net fractionation associated with abstraction of hydrogen from this starch pool via OPP activity (e.g. Hayes 2002), greater contributions of NADPH derived from this process could lead to more D-depleted (lower α_{K37}) lipids (Figure 2A).

If this mechanism (broadly, lighter NADPH in response to increased anabolic activity) is indeed responsible, it could also explain the shift to lower α_{K37} values in nutrient-induced stationary growth phase. High metabolic losses of newly-synthesized carbon in stationary-phase plankton cells have been recognized by multiple authors (Ryther 1954; Li and Harrison 1982; Lancelot and Mathot 1985), and the high OPP pathway activity associated with high growth rates

(see above) has also been associated with stationary-phase cells (see Halsen and Jones 2014 for review). If relatively D-depleted, anabolic NADPH production is increased during stationary phase, then lipids produced under these conditions would also be expected to have relatively D-depleted isotopic compositions. Indeed, for the cultures where a full log- to stationary-phase transition has been observed (this work and the *E. huxleyi* cultures of Wolhowe et al. 2009), it appears that the decrease in α_{K37} that occurs is proportional to the corresponding percent increase in q_{K37} (Figure 2B). This linear relationship suggests that a mixing occurs between existing ‘log-phase alkenones’ and new, lighter ‘stationary-phase alkenones’. This explanation (addition of alkenones produced from a more D-depleted reductant pool) would provide mechanistic continuity for α_{K37} through batch-culture (or bloom) progression, in place of the bimodal ‘growth-phase effect’ proposed by Wolhowe et al. (2009).

This proposed mechanism is, at this time, purely speculative. Future experiments will be required to test whether the empirical correlation between α_{K37} and alkenone production is indeed mechanistically linked to the pathway of reductant formation. It would be helpful, for example, to determine whether α_{K37} correlates with the rate carbohydrate metabolism by paring δD_{K37} analysis with a carbon-labeled ‘pulse-chase’ experiment of the sort conducted by Halsey et al. (2011) to assess polysaccharide turnover rate.

3.2.2. Potential Secondary Temperature Controls

If growth rate impacts α_{K37} via changes in alkenone production rate, it is possible that the observed correlation with temperature (Figure 1D) simply reflects a tendency towards higher growth rates at higher temperatures. However, the set of experiments expressing the most distinct ‘temperature effect’, the *G. oceanica* cultures reported in Wolhowe et al. (2009; Figure 1D), show no appreciable difference in growth rate between the 21 and 25°C treatments (Table 1).

Conversely, while the *E. huxleyi* temperature treatments of Schouten et al. (2006) show a large change in growth rate between 10 and 15°C degrees (Table 1), very little change in α_{K37} is observed (Figure 1D). Additionally, variation in α_{K37} with growth temperature is still observed in stationary phase cells (Wolhowe et al. 2009; Table 1), suggesting a correlation of temperature and growth rate not (solely) responsible. Likewise, direct dependence of α_{K37} on $U_{37}^{K'}$, via changing proportions of isotopically-offset K37:3 (more D-depleted) and K37:2 (more D-enriched) (e.g. D'Andrea et al. 2007), has been ruled out as a mechanism for previously-reported correlations between α_{K37} and temperature, as A) the correlation is of the wrong sign (Wolhowe et al. 2009) and B) δD_{K37} is thought to be insensitive to changes in the relative proportions of the two compounds due to 'Rayleigh-like', fractionating consumption of K37:2 to form K37:3 (van der Meer et al. 2013). Zhang et al. (2009b) propose that multiple iso-enzymes with different temperature tolerances and fractionation factors are employed in alkenone synthesis, though this prospect has yet to be investigated.

However, any fractionating process that alters the K37:2 vs. K37:3 mass balance could lead to 'temperature effects' on α_{K37} . Differential consumption of K37:2 and K37:3, either by the cell itself or secondary consumers, is thus a potential pathway. While Pan and Sun (2011) show that consumption of alkenones by *E. huxleyi* in axenic cultures during extended periods of light starvation appears to be a first-order, non-selective process, there is also the possibility of preferential degradation of K37:3 by bacteria with consumption mechanisms that are dependent on degree of unsaturation (e.g. Rontani et al. 2008). While it is unclear to what degree any selective metabolic or bacterial processing of alkenones is already included in the empirical $U_{37}^{K'}$ temperature calibration, the latter is potentially active in many laboratory and field settings (Rontani et al. 2013). Preferential, fractionating consumption of the more-unsaturated K37:3

could drive both increases in α_{K37} and sign reversals in $\delta D_{K37:2} - \delta D_{K37:3}$ (observed by van der Meer et al. 2013 at high temperatures). This mechanism could be tested by monitoring $U_{37}^{K'}$ and δD_{K37} through the night, or extended dark periods, in both axenic and non-axenic cultures. If viable, selective bacterial consumption could explain the different responses of α_{K37} to temperature in different culture experiments (Figure 1D).

3.3 Gulf of California/ETNP Observations and Interpretation

3.3.1. Response of α_{K37} to Proposed Physiological Controls

While there is some evidence for temperature effects in the existing culture data, in general it appears that we should expect to observe lower α_{K37} values in cell populations that are A) growing at relatively high rates or B) not growing at all due to nutrient limitation. These predictions would have diametrically opposing ecological implications if used as interpretive frameworks for the examination of sedimentary data, and so we must examine α_{K37} behavior in a natural water column.

In the Gulf of California and ETNP, a large range in measured δD_{K37} (~58‰) and a small range in estimated δD_{H2O} (~7‰) resulted in highly variable estimates of α_{K37} (Table 2), with values at a given station generally decreasing with depth. The overall decrease with water depth is remarkably robust (Figure 3A), with the only noticeable outliers being samples from station 4-12 in the ETNP, a substantially cooler station with a surface chlorophyll maximum rather than the DCML observed at all other sites (White et al. 2013; Wolhowe et al. 2014). Can this pattern be explained in terms of the dynamics observed in culture? Variables that A) may be expected to change with depth in the GoCal and with distance north in the ETNP and B) have been previously proposed to control α_{K37} include salinity (Schouten et al. 2006), temperature

(Wolhowe et al. 2009; Zhang et al. 2009b, Figure 1D) and composition of the alkenone-producing assemblage (*E. huxleyi* vs. *G. oceanica*; Schouten et al. 2006).

Salinity both increases and decreases with depth, depending on the station (Table 2), in opposition to the consistent depth-dependence of α_{K37} . Furthermore, as per the previously-described salinity dependence of α_{K37} (Schouten et al. 2006), the narrow range of salinities sampled in the study area (~33.5 to 35.0) would be expected to shift α_{K37} values by a maximum of ~0.005 units, approximately the same as the analytical uncertainty. Thus, it appears reasonable to ascribe α_{K37} variability here to other physiological and ecological causes. The composition of the alkenone-producing assemblage varies in this setting, between ~100% *G. oceanica* and ~100% *E. huxleyi* (Table 2; Wolhowe et al. 2014), and lower α_{K37} values are observed in higher *E. huxleyi*-percentage waters. This trend, however, if species-driven, would be opposite previous reports of generally lower α_{K37} values in *G. oceanica* (e.g. Schouten et al. 2006). Temperature itself is a predictive variable for these data (Figure 3A), but even if temperature has a direct control on α_{K37} (which, as discussed above, is unclear), the apparent positive correlation (Figure 3A) opposes previously-reported temperature effects (negative correlation).

The results shown in Figure 1A suggest that a functional relationship exists between α_{K37} and q_{K37} . No correlation is observed, however, between the α_{K37} and q_{K37} values for our field samples (Figure 3B), though it is possible that any underlying trends are obfuscated by variations in species/size composition of the alkenone-producing assemblage. Wolhowe et al. (2014) reported alkenone production rate profiles (via *in situ* ^{13}C incubation) coincident with the collection of a number of these α_{K37} samples, providing another basis for comparison of our field and culture results. Estimates of the exponential growth rate, μ , can also be calculated from the ^{13}C K37:2 turnover rates ($TR_{K37:2}$) reported in Wolhowe et al. (2014). Assuming cellular K37:2

concentrations are approximately at steady state (no change in q_{K37}) over the ~24 hour incubation time, μ is given by:

$$\mu = \frac{-1.025 * \ln(1 - t * TR_{K37.2})}{t}$$

3.1

No clear relationship is observed between α_{K37} and per-cell alkenone production rate (Figure 3B) or alkenone-producer growth rate (Figure 3C). Given the highly nutrient-stratified nature of the summertime GoCal and ETNP (Wolhowe et al. 2014), it is possible that near-surface cells were in nutrient-limited stationary phase, and thus that q_{K37} was increasing with time. If true, labeled growth rate estimates would be unreliable and α_{K37} would theoretically lose any functionality with q_{K37} (i.e. Figure 1A). In this case, the growth-phase effects demonstrated in culture (Wolhowe et al. 2009; Chivall et al. 2014b) would lead us to expect low α_{K37} values near the surface, where nutrient depletion and high light should lead to nutrient-stressed physiology. Again, the trend towards lower values of α_{K37} at depth (Figure 3A) opposes this expectation.

As described above, the hypothesis of Chivall et al. (2014b) that photosynthetic rate controls δD_{K37} does not appear likely to explain the available culture data. However, all of these data are taken from light-saturated cultures. Light is another depth-dependant parameter for our field samples, and the water-column data here appears to show a dichotomy between samples collected above and below mid-day PAR values of ~100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Figure 3D). *E. huxleyi* reaches its light-saturated growth rate maximum at ~70 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Bleijswijk et al. 1994). Thus, the simplest explanation for the depth-dependant decrease in α_{K37} in our study area may be a dependence on irradiance or photosynthetic rate. If so, the implication is that there are differences in α_{K37} behavior between light-limited and nutrient-limited cells, and that the

physiological drivers discussed in Section 3.2 for nutrient-limited cells have less impact in this study area.

Is photosynthetic enrichment of cell water by NADPH formation a reasonable mechanism?

A simple mass-balance approach using literature estimates of the fractionation associated with incorporation of photosynthetic NADPH (Yakir and DeNiro 1990), average phytoplankton elemental composition (Hedges et al. 2002), and average phytoplankton cell water content suggests that improbably large amounts of hydrogen would have to be sequestered in organic matter (~4x the cell's requirements) to generate the observed shifts in δD_{K37} (up to ~50‰). Due to the large volume of water that would need to be enriched, then, it seems unlikely that the variability seen in culture or in our field samples is caused by a photosynthetic-rate-dependant D-enrichment of intracellular δD_{H2O} . However, if future work shows that isotopic communication is restricted between a smaller, sub-cellular water pool (such as that within the chloroplast) and the rest of the cytosol, then this assessment may be revised. For the time being, we must pursue another explanation for the field data consistent with the dynamics observed in culture.

3.3.2. Alternate Assessment of α_{K37} Relationship to Growth Rate

On the whole, the field data do not appear to meet our culture-driven expectations of an α_{K37} response (causative or otherwise) to q_{K37} or alkenone production rate. There are potential pitfalls, however, in comparing alkenone abundance profiles collected by Niskin bottle, cell abundance profiles collected on different casts, rate estimates from 24-hour, free-floating fixed-depth incubations, and δD_{K37} measurements from multi-hour *in situ* pump deployments, all collected at different times across ~36 hours of station occupation and with low depth-resolution (White et al. 2013; Wolhowe et al. 2014). If coccolithophore growth at these stations is balanced by grazing losses, alkenone labeling rates (~0.1 to 0.4 d⁻¹ at the depths of our isotopic samples;

Wolhowe et al. 2014) imply the alkenone pool turns over on a time scale of 2.5-10 days. Thus it may be problematic to compare 24-hour production rate measurements, made at fixed water depths, to isotopic information from a time-integrating alkenone standing stock that potentially represents both a variable depth habitat and contributions from sinking, detrital material.

There is, however, a way to potentially assess growth rate on time and depth scales more directly applicable to those represented by the δD of the K37 standing stock. An unexpected positive relationship is observed between α_{K37} and another potential physiological indicator, ε_p ($R^2 = 0.92$; Figure 4A). This parameter, an estimate of the photosynthetic ^{13}C fractionation between aqueous CO_2 and phytoplankton biomass, is defined for coccolithophores via $\delta^{13}C_{K37:2}$ measurements as:

$$\varepsilon_p = \frac{\delta^{13}C_{CO_2} + 1}{\delta^{13}C_{K37:2} + \varepsilon_{cell-K37:2} + 1} - 1 \quad 3.2$$

where $\varepsilon_{cell-K37:2}$ is an average offset between K37:2 alkenones and bulk cell carbon (4.2‰) proposed by Popp et al. (1998b). Natural-abundance $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{K37:2}$ (Appendix 1) were determined in the course of Wolhowe et al.'s (2014) production rate experiments, allowing us to calculate ε_p (Table 2). Alkenone $\delta^{13}C$ is the subject of a considerable volume of literature concerning a relationship observed between ε_p and $\mu/[CO_2]$ (see Pagani 2014 for review). While the behavior of ε_p has been shown to be increasingly complicated in recent years, for certain species, including *E. huxleyi*, ε_p appears to have linear functionality with $(\mu/[CO_2])(\text{volume/surface area})$ of the form:

$$\varepsilon_p = a \frac{\mu}{[CO_2]} \frac{V}{SA} + b \quad 3.3$$

where a and b are the constants -182 and 25.3, respectively (when ε_p is expressed in per mil; Popp et al. 1998a). Linearity appears to be maintained within the limited range of

$(\mu/[CO_2])(V/SA) = 0$ to $\sim 0.08 \text{ kg } \mu\text{m } \mu\text{mol}^{-1} \text{ d}^{-1}$ (Popp et al. 1998a). At the depths associated with our δD_{K37} measurements, $[CO_2]$ values (Appendix 1) and 24-hour growth rate estimates (Table 2) range from ~ 10 to $16 \text{ } \mu\text{mol kg}^{-1}$ and ~ 0 to 0.56 d^{-1} , respectively. Assuming spherical coccolithophore cells of $\sim 5 \text{ } \mu\text{m}$ diameter (see Appendix 1), $(\mu/[CO_2])(V/SA)$ estimates fall in the range of 0 to $\sim 0.04 \text{ kg } \mu\text{m } \mu\text{mol}^{-1} \text{ d}^{-1}$, a range where ϵ_p is well-described by this model. While we cannot assess the time-scales upon which $\delta^{13}C_{CO_2}$ and $[CO_2]$ vary here, $\delta^{13}C_{K37}$ and δD_{K37} are measured on the same analyte pool, and thus we should be able to use ϵ_p and $[CO_2]$ measurements to estimate growth rates corresponding more precisely to the time period and depth range integrated by our δD_{K37} data.

When Equation 3.3 is solved for μ , estimates range from ~ 0.4 to 1.2 (Table 2). Higher growth rates are estimated for deeper (closer to the nitracline) samples; subsurface maxima in estimated q_{K37} (Table 2) near these depths may thus be consistent with the hypothesis of higher per-cell alkenone production at higher growth rates. Additionally, there is a clear inverse correlation between these rate estimates and α_{K37} ($R^2 = 0.78$; Figure 4B), similar to what has previously been observed in culture. This relationship in Figure 4B is data-sparse due to incomplete overlap of carbon-isotopic, hydrogen-isotopic, and CO_2 system measurements. However, cell abundance appears to be a rough indicator of growth rate here, as well; while necessarily weak because of the unquantified influence of grazing on phytoplankton standing stock, there is a broad association of higher ϵ_p -estimated growth rates with higher cell abundances (Figure 4B color axis). If higher cell abundances are generally indicative of higher time-integrated growth rates, the inverse correlation observed between α_{K37} and cell abundance (Figure 4C) lends support to the relationship between α_{K37} and μ indicated in Figure 4B.

The data presented here are sparse and from a single oceanographic setting; study of samples from other settings and production regimes (such as developing coccolithophore blooms,

where growth status can be more objectively assessed) would be beneficial. Specifically, it would be informative to determine whether culture-predicted inverse correlations between α_{K37} and q_{K37} can be observed in cooler, better mixed settings like station 4-12 where the species assemblage is less variable.

4. SUMMARY AND CONCLUSIONS:

The trends observed in culture and suspended material have indicated what physiological information environmental δD_{K37} may convey, but not without complication. To summarize the culture component of this study:

- 1) In nutrient-limited batch cultures with low narrow salinity ranges, α_{K37} appears to correlate negatively with alkenone production rate (Figure 1B) and, to a lesser degree, per-cell alkenone quota (Figure 1A), more strongly than the previously-identified factors of growth rate or temperature.
- 2) As a possible explanation for these data, we have hypothesized that ‘production rate/cell quota effects’ on α_{K37} may be manifestations of the increased use of anabolic NADPH sources under increased lipid synthetic demand. This mechanism would explain the observed, but inconsistent, response of α_{K37} to growth rate. While there is evidence that the temperature effects observed in culture are not related to temperature-driven growth rate changes, a satisfying alternate explanation is still lacking.

Field samples, limited by both nutrients and light from the Gulf of California and ETNP in summer, were analyzed to determine whether the dynamics observed in culture are expressed in the natural environment. To summarize the investigation of the field samples:

- 1) α_{K37} was observed to decrease with depth, resulting in correlations with light availability (positive, Figure 3D), alkenone-producing cell abundance (negative, Figure 4C) and,

unexpectedly, the carbon isotopic fractionation parameter ϵ_p (positive, Figure 4A).

Anticipated correlations with alkenone cell quota, temperature, and growth rate estimated from *in situ* ^{13}C incubations were not observed.

- 2) We have proposed two possible explanations for the observed α_{K37} field data. First, there is the possibility that changes in light-availability with depth control α_{K37} , and that isotopic contrasts between nutrient-limited (surface) and light-limited (at depth) cells are more significant in this setting than the impacts of alkenone production rate or temperature observed in culture. Alternately, we suggest that our ϵ_p -derived estimates of growth rate are more directly comparable to the time- and depth-integrated δD_{K37} signal of the alkenone standing stock than are 24-hour, fixed-depth production rate measurements. If true, the ϵ_p results imply that the depth-dependant decrease in α_{K37} is related to increased growth rates in proximity to the nitracline, consistent with the α_{K37}/μ dynamics that are hypothesized to explain the culture data.

In the open ocean, salinity effects should be minimal and the isotopic composition of water relatively constrained. If the physiology of alkenone-producers exerts a control on α_{K37} such that its values provide information about either light availability or growth rate in these settings, then δD_{K37} may prove a powerful modern ecological and paleoceanographic tool. If light controls α_{K37} in the field, then paleoceanographic assessment of α_{K37} may provide information about changes in surface vs. DCML production of coccolithophores – information useful both in reconstructions of temperature (via $U_{37}^{K'}$) and of the marine carbon cycle. If α_{K37} is predictive of alkenone-producer growth rate, then estimates of its value may allow us to overcome the major hurdle in alkenone-based paleo- $p\text{CO}_2$ reconstructions (the inability to constrain μ) in addition to providing

634 a coccolithophore-specific productivity proxy. Further work comparing α_{K37} to alkenone-
635 producing coccolithophore growth rate in the field may revitalize a paleoceanographic technique
636 that has appeared unreliable (e.g. Pagani 2014) when constrained only by global scale, correlative
637 estimates of growth rate (i.e. geochemical macronutrient proxies).

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CAPTIONS:

Figure 1. Compiled experimental culture results for oceanic alkenone producers. In **A**, α_{K37} measured in culture (Table 1) is plotted as a function of the alkenone cell abundance q_{K37} (q_{K37} not available for Schouten et al. or M'boule et al. data); black symbols represent log-phase samples and grey symbols represent stationary-phase samples or, in the case of the Wolhowe et al. 2009 *G. oceanica* data, late-log 'transition phase'. α_{K37} is plotted versus alkenone production rate (log-phase samples only) in **B**, versus exponential growth rate μ in **C**, and versus growth temperature in **D**. Note that only data from the salinity range of ~29-32 are displayed from Schouten et al. (2006) and M'boule et al. (2014) in **C** and **D**. The relationship with growth temperature is shown only for log-phase samples in **D** for simplicity – a similar, negatively offset pattern is observed for stationary phase samples (Table 1).

Figure 2. Schematic (**A**) of the changes in biochemical partitioning, all occurring within the chloroplast, hypothesized as a possible explanation for the dependence of α_{K37} on alkenone content and production rate in Section 3.2.1. Arrow weight, box size, and vertical position represent flux, pool size, and δD , respectively (not to scale). Under low lipid demand (upper panel), the majority of the reductant used in lipid synthesis is derived directly from photosynthesis, with only minor contributions from OPP activity. At higher growth rates/lipid demands (lower panel), rapid turnover of the primary NADP(H) pool is achieved by shunting reductant into labile, short-lived carbohydrates. The additional NADPH required for the increased lipid synthesis is then derived from OPP processing of this carbohydrate pool. If this NADPH is D-depleted relative to primary, photosynthetic NADPH, the resulting lipid pool will be increasingly D-depleted. Relative depletion will depend on both on the D-depletion the carbohydrate substrate and the net fractionation inherent in the OPP process (see Section 3.2.1).

The linear response of the decrease in α_{K37} to the increase in q_{K37} , observed for *E. huxleyi* when moving from log-phase to stationary-phase, is shown in **B**. This relationship suggests that the ‘growth phase effect’ is mechanistically connected to increased lipid content as discussed in Section 3.2.1. Note that the Wolhowe et al. (2009) data are from log- and stationary-phase sampling points of the same batch culture; values from this work are determined by comparing log- and stationary-phase samples from parallel cultures with different nutrient conditions (see Section 2.1).

Figure 3 Alkenone isotopic composition (α_{K37}) relative to estimated δD_{H_2O} (Section 2.4.3) from SPM filters collected in the Gulf of California and ETNP (Table 2). Data plotted as a function of **A**) collection depth and temperature; **B**) estimated per-cell K37 abundance (q_{K37}) and per-cell K37 production rate (where available); **C**) estimated alkenone-producer growth rate (where available); and **D**) mid-day spherical PAR. Per-cell alkenone production rate and q_{K37} profiles (from Wolhowe et al. 2014) were linearly interpolated to the depths of the δD_{K37} filter samples. Growth rates, estimated from K37:2 turnover in labeled incubations, are calculated via Equation 3.1 using turnover rate data from Wolhowe et al. (2014). Uncolored symbols in plot **B** are data from all stations at which rate data is not available (4-2b, 4-10, 4-11); the 57m sample from station 4-10 is also absent from this plot as q_{K37} data was not available. The data from station 4-12 is indicated in **A** as per the discussion in Section 3.3.1.

Figure 4 α_{K37} from the Gulf of California and ETNP stations (Table 2) compared to **A**) carbon isotopic fractionation (ϵ_p , Equation 3.2) and depth; **B**) growth rate (μ), estimated from ϵ_p , via Equation 3.3 (see Section 3.3.2 and Appendix 1); and **C**) and alkenone-producer cell abundance. The dependant variable of **B** is shown as the color axis in **C**, and vice versa. Uncolored symbols

in plot **C** are data from all stations at which rate estimates are not available (4-2b, 4-10, 4-11).
The annotations in **A** indicate the consistent decrease in ϵ_p with depth in the GoCal and the offset decrease with depth at 4-12 in the ETNP.

Table 1. Growth parameters, quantitative alkenone parameters, and alkenone isotopic composition from the culture experiments described in Section 2.1. Chemical and isotopic analyses are described in Sections 2.3 and 2.4. Data from Wolhowe et al. (2009) is provided along with previously unreported carbon and uncertainty data. Note that Wolhowe et al. (2009) log- and stationary-phase data are different sampling points of the same cultures; new log- and stationary-phase samples described here are separate treatments as described in Section 2.1.

Table 2. Ecological and isotopic field data from the Gulf of California and ETNP. In addition to the alkenone chemical and isotopic (δD and ϵ_p) data reported here, alkenone-producer species composition and abundance, temperature, light levels, estimated per-cell alkenone content, and estimated growth rate are provided (all linearly interpolated from the results of Wolhowe et al. 2014). ϵ_p and ϵ_p -estimated growth rate are calculated as described in Section 3.3.2.

Table 1

Table 1. Chemical and Isotopic Composition of Nutrient-Replete Exponential and Nutrient-Depleted Stationary Cultures.

<i>E. huxleyi</i> Strain	Treatment ^a	Temperature (°C)	Salinity	PAR (μmol quanta m ⁻² s ⁻¹)	Day Length (h)	Growth Rate (d ⁻¹)	Standard Error ^b	U ^{K₃₇} ₃₇	q _{K37} (pg K37 cell ⁻¹)	± ^c	K37 Production Rate (pg K37 cell ⁻¹ d ⁻¹)	±	Organic Carbon (pg OC cell ⁻¹)	± ^d	OC Production Rate (pg OC cell ⁻¹ d ⁻¹)	±	K37:OC (pg K37 pg OC ⁻¹)	±	δD _{K37} (‰ vs. SMOW)	σ	δD _{H2O} (‰ vs. SMOW)	σ	α _{K37}	±
<i>Wolhowe et al. 2009 E. huxleyi (Non-Calciifying)</i> ^e																								
CCMP 1742		15	32	70	12	0.58	0.02	0.409	0.44	0.05	0.22	0.03	8.1	0.4	4.7	0.3	0.054	0.007	-186.4	0.9	-8.7	1.8	0.821	0.002
CCMP 1742		15	32	70	12	0.56	0.02	0.413	0.46	0.05	0.21	0.03	8.1	0.4	4.5	0.3	0.057	0.007	-191.0	0.1	-9.2	2.7	0.817	0.002
CCMP 1742		15	32	70	12	Stationary		0.304	1.32	0.16			11.7	0.6			0.112	0.015	-215.3	3.5	-7.7	3.4	0.791	0.004
CCMP 1742		15	32	70	12	Stationary		0.302	1.37	0.17			13.3	0.7			0.103	0.014	-212.9	2.1	-7.6	0.8	0.793	0.002
<i>Wolhowe et al. 2009 G. oceanica (Calciifying)</i> ^e																								
PZ3-1		17	32	150	14	0.62	0.04	0.437	0.60	0.08	0.37	0.06	19.6	1.0	12.1	1.0	0.031	0.005	-190.9	1.3	0	2	0.809	0.002
PZ3-1		21	32	150	14	0.92	0.04	0.744	0.82	0.12	0.75	0.11	26.7	1.3	24.5	1.7	0.031	0.005	-204.6	0.2	0	2	0.795	0.002
PZ3-1		25	32	150	14	0.93	0.05	0.985	1.98	0.28	1.84	0.28	53.4	2.7	49.6	3.5	0.037	0.006	-229.9	2.0	0	2	0.770	0.003
PZ3-1		17	32	150	14	Stationary		0.384	0.78	0.11			23.7	1.2			0.033	0.005	-213.2	7.6	0	2	0.787	0.008
PZ3-1		21	32	150	14	Stationary		0.683	1.17	0.17			16.6	0.8			0.070	0.011	-235.5	0.3	0	2	0.765	0.002
PZ3-1		25	32	150	14	Stationary		0.976	1.08	0.15			17.8	0.9			0.061	0.009	-262.6	0.5	0	2	0.737	0.002
<i>Mediterranean Strains (Calciifying)</i>																								
RCC1812	P-Replete	20	29.6	400	16	1.14	0.02	0.642	0.99	0.14	1.12	0.16	12.0	0.6	13.6	0.7	0.083	0.012	-258.2	2.0	-32.0	0.2	0.766	0.002
RCC1812	P-Replete	20	29.6	400	16	1.12	0.02	0.643	1.19	0.17	1.33	0.19	11.2	0.6	12.5	0.7	0.106	0.016	-254.0	0.7	-32.8	0.1	0.771	0.001
RCC1813	P-Replete	20	29.6	400	16	1.05	0.02	0.606	0.84	0.12	0.89	0.13	8.6	0.4	9.0	0.5	0.098	0.015	-230.3	0.9	-31.1	0.1	0.794	0.001
RCC1813	P-Replete	20	29.6	400	16	1.05	0.02	0.611	1.05	0.15	1.09	0.16	8.6	0.4	9.0	0.5	0.122	0.018	-229.4	3.0	-33.1	0.1	0.797	0.003
RCC1817	P-Replete	20	29.6	400	16	1.03	0.03	0.664	0.89	0.13	0.92	0.13	8.5	0.4	8.8	0.5	0.104	0.016	-239.7	1.3	-31.8	0.1	0.785	0.001
RCC1817	P-Replete	20	29.6	400	16	1.06	0.03	0.673	1.00	0.14	1.07	0.15	8.4	0.4	8.9	0.5	0.120	0.018	-246.0	0.1	-32.2	0.1	0.779	0.000
RCC1827	P-Replete	20	30.3	400	16	1.01	0.03	0.758	0.94	0.13	0.95	0.14	8.1	0.4	8.2	0.5	0.116	0.017	-260.6	0.8	-31.6	0.2	0.764	0.001
RCC1827	P-Replete	20	29.6	400	16	0.99	0.02	0.760	0.97	0.14	0.96	0.14	8.0	0.4	7.9	0.4	0.121	0.018	-262.5	1.7	-30.5	0.1	0.761	0.002
RCC1830	P-Replete	20	29.6	400	16	1.22	0.02	0.607	1.01	0.14	1.24	0.18	10.2	0.5	12.5	0.7	0.100	0.015	-256.6	3.2	-31.1	0.2	0.767	0.003
RCC1830	P-Replete	20	29.6	400	16	1.21	0.03	0.618	1.10	0.16	1.33	0.19	10.8	0.5	13.0	0.7	0.102	0.015	-258.7	3.2	-29.4	0.1	0.764	0.003
RCC1833	P-Replete	20	30.3	400	16	1.02	0.02	0.572	0.77	0.11	0.78	0.11	7.8	0.4	7.9	0.4	0.099	0.015	-231.9	2.1	-31.5	0.1	0.793	0.002
RCC1833	P-Replete	20	29.6	400	16	1.02	0.02	0.557	0.80	0.11	0.81	0.12	8.1	0.4	8.2	0.4	0.099	0.015	-229.9	0.3	-30.4	0.1	0.794	0.000
RCC1812	P-Depleted	20	29.6	400	16	Stationary		0.659	3.08	0.44			33.7	1.7			0.091	0.014	-281.3	2.4	-32.8	0.1	0.743	0.002
RCC1812	P-Depleted	20	29.6	400	16	Stationary		0.649	4.21	0.60			35.8	1.8			0.118	0.018	-282.1	3.4	-32.7	0.2	0.742	0.004
RCC1813	P-Depleted	20	29.6	400	16	Stationary		0.639	3.88	0.55			22.6	1.1			0.172	0.026	-253.6	2.4	-32.0	0.1	0.771	0.002
RCC1813	P-Depleted	20	29.6	400	16	Stationary		0.633	5.20	0.74			24.5	1.2			0.212	0.032	-252.3	1.1	-32.3	0.1	0.773	0.001
RCC1817	P-Depleted	20	28.8	400	16	Stationary		0.668	4.09	0.58			31.3	1.6			0.131	0.020	-256.7	4.2	-30.8	0.1	0.767	0.004
RCC1817	P-Depleted	20	29.6	400	16	Stationary		0.671	3.87	0.55			29.4	1.5			0.131	0.020	-260.7	2.0	-30.9	0.1	0.763	0.002
RCC1827	P-Depleted	20	29.6	400	16	Stationary		0.792	4.52	0.64			24.8	1.2			0.182	0.027	-282.1	2.1	-31.4	0.1	0.741	0.002
RCC1827	P-Depleted	20	30.3	400	16	Stationary		0.786	3.99	0.56			24.2	1.2			0.165	0.025	-282.3	0.4	-29.4	0.1	0.739	0.000
RCC1830	P-Depleted	20	29.6	400	16	Stationary		0.676	3.67	0.52			34.2	1.7			0.107	0.016	-284.6	0.2	-30.6	0.2	0.738	0.000
RCC1830	P-Depleted	20	30.3	400	16	Stationary		0.690	3.53	0.50			31.8	1.6			0.111	0.017	-283.9	2.6	-29.3	0.1	0.738	0.003
RCC1833	P-Depleted	20	30.3	400	16	Stationary		0.528	2.74	0.39			20.0	1.0			0.136	0.020	-254.7	1.7	-32.0	0.1	0.770	0.002
RCC1833	P-Depleted	20	30.3	400	16	Stationary		0.526	3.13	0.44			24.5	1.2			0.128	0.019	-260.2	2.3	-29.9	0.1	0.763	0.002
<i>Norwegian Strain (Calciifying)</i>																								
PML B92/11	N-Replete	15	31.1	400	16	1.32	0.02	0.455	0.60	0.09	0.80	0.11	8.2	0.4	10.8	0.6	0.074	0.011	-227.6	2.0	-38.7	0.1	0.803	0.002
PML B92/11	N-Replete	15	31.1	400	16	1.32	0.02	0.459	0.63	0.09	0.83	0.12	8.4	0.4	11.1	0.6	0.073	0.011	-230.7	2.0	-36.7	0.1	0.799	0.002
PML B92/11	N-Depleted	15	31.1	400	16	Stationary		0.600	1.17	0.17			16.2	0.8			0.073	0.011	-231.4	1.0	-39.1	0.1	0.800	0.001
PML B92/11	N-Depleted	15	30.3	400	16	Stationary		0.607	1.35	0.19			15.9	0.8			0.085	0.013	-235.2	0.1	-37.1	0.1	0.794	0.000
PML B92/11	P-Replete	15	30.3	400	16	1.13	0.04	0.495	0.32	0.05	0.36	0.05	8.2	0.4	9.2	0.6	0.039	0.006	-203.4	6.4	-19.2	0.1	0.812	0.007
PML B92/11	P-Replete	15	31.1	400	16	1.14	0.04	0.494	0.28	0.04	0.32	0.05	8.6	0.4	9.8	0.6	0.033	0.005	-208.9	0.6	-18.4	0.1	0.806	0.001
PML B92/11	P-Depleted	15	31.1	400	16	Stationary		0.544	3.26	0.46			40.2	2.0			0.082	0.012	-256.3	0.6	-19.5	0.1	0.759	0.001
PML B92/11	P-Depleted	15	31.1	400	16	Stationary		0.538	2.46	0.35			39.5	2.0			0.063	0.009	-263.0	3.7	-16.8	0.1	0.750	0.004

^a 'Replete' treatments are stoichiometrically limited by the indicated nutrient (see Section 2.1).

^b Standard error of the slope of the linear regression of ln(cells mL⁻¹) vs. time.

^c Propagated from assumed 10% uncertainty in alkenone quantification and the number of cells sampled.

^d Assumed 5% uncertainty based on typical standard deviation of replicate measurements.

^e Alkenone abundance and isotopic data from Wolhowe et al. 2009, reproduced here for convenience with previously unreported uncertainty estimates and organic carbon quantification.

Table 2. Isotopic Composition and Production Rate of Alkenones in Gulf of California/ETNP SPM

Station ^a	Lat	Lon	Occupation Date	Depth	U _{K37} ^K	δD _{K37}	σ	S	Modeled δD _{H2O}	α _{K37}	± ^b	Temperature	Noon PAR ^c	<i>E. huxleyi</i> + Gephyrocapsa ^{c,d}	±	Fraction <i>E.huxleyi</i> ^e	<i>q</i> _{K37} ^c	±	Per-Cell PR _{K37} ^c	±	Incubation Esitimated μ	± ^e	ε _p ^f	ε _p Esitimated μ ^f	± ^f
	(°N)	(°W)		(m)	(‰ vs. SMOW)			(‰ vs. SMOW)				(°C)	(μmol quanta m ⁻² s ⁻¹)	(cs L ⁻¹ x 10 ³)		(of <i>E.hux</i> + Gephyrocapsa)	(pg K37 cell ⁻¹)		(pg K37 cell ⁻¹ d ⁻¹)		(d ⁻¹)		(‰ vs.PDB ± 0.15)	(d ⁻¹)	
4-1	26.5	111.5	July 11th-13th 2008	30	0.981	-199.7	5.0	34.8	12.3	0.791	0.007	22.6	107	44	5	0.99	0.4	0.1	0.39	0.06	0.56	0.02	16.2	0.75	0.17
4-1				50	0.876	-216.5	1.6	34.7	12.0	0.774	0.006	19.0	18	35	5	0.73	0.15	0.03	0.07	0.01	0.12	0.01			
4-2a	24.5	109.0	July 14th-16th 2008	10	0.973	-192.7	1.0	35.0	13.4	0.797	0.006	29.0	1400	44	6	0.19	0.8	0.1	0.16	0.03	0.24	0.01	16.8	0.53	0.14
4-2a				20	0.968	-192.9	4.6	35.0	13.3	0.796	0.007	28.5	581	43	6	0.21	0.8	0.1	0.15	0.02	0.24	0.01	16.7	0.54	0.14
4-2a				30	0.978	-192.8	2.6	35.1	13.6	0.796	0.006	27.8	239	47	7	0.34	0.4	0.1	0.07	0.01	0.26	0.01	16.2	0.62	0.16
4-2a				40	0.979	-202.8	5.0	35.0	13.4	0.787	0.007	26.6	114	60	8	0.53	0.27	0.05	0.022	0.004	0.38	0.01	15.9	0.75	0.18
4-2a				50	0.970	-201.2	5.0	35.0	13.4	0.788	0.007	24.0	63	22	4	0.70	0.6	0.1	0.04	0.01	0.28	0.01	14.9	0.92	0.22
4-2b	24.5	109.0	July 22nd-24th 2008	10	0.992	-191.9	1.1	34.3	10.4	0.800	0.006	29.6	684	16	3	0.08	1.4	0.3							
4-2b				20	0.992	-195.6	3.3	34.4	10.6	0.796	0.006	29.0	450	13	2	0.16	1.0	0.2							
4-2b				45	0.992	-200.9	5.0	34.8	12.5	0.789	0.007	27.1	64	28	4	0.45	0.4	0.1							
4-8	20.5	106.5	July 18th-21st 2008	10	0.947	-194.0	0.6	34.2	9.7	0.798	0.006	29.2	415	27	3	0.02	1.0	0.2	0.20	0.03	0.21	0.01	17.1	0.43	0.12
4-8				20	0.967	-190.6	5.1	34.4	10.7	0.801	0.008	28.5	181	32	5	0.07	0.9	0.2	0.17	0.03	0.21	0.01	17.0	0.48	0.13
4-8				57	0.956	-241.9	5.0	34.9	12.8	0.749	0.007	25.8	10	47	6	0.50			0 ^g		0 ^g	0 ^g			
4-10	24.7	113.3	July 28th-31st 2008	10	0.907	-187.5	5.0	33.9	8.3	0.806	0.008	23.6	923	7	1	0.68	0.36	0.06							
4-10				25	0.650	-209.4	8.8	33.7	7.5	0.785	0.010	18.8	396	22	3	0.77	1.2	0.2							
4-10				52	0.500	-224.6	6.1	33.7	7.5	0.770	0.008	14.5	37	56	7	0.86	0.40	0.06							
4-10				57	0.483	-208.9	5.0	33.7	7.6	0.785	0.007	14.1	23	85	9	0.90	0.32	0.04							
4-11	27.5	117.5	Aug. 1st-3rd 2008	20	0.689	-191.6	5.0	33.6	7.1	0.803	0.008	20.8	232	17	2	0.73	1.7	0.3							
4-11				50	0.401	-220.6	0.4	33.5	6.4	0.774	0.005	14.8	15	78	11	0.78	0.43	0.07							
4-12	32.5	120.5	Aug. 5th-7th 2008	10	0.533	-237.2	0.2	33.6	7.2	0.757	0.005	16.9	344	62	7	0.90	1.0	0.1	0.35	0.06	0.48	0.03	13.5	0.92	0.21
4-12				25	0.538	-245.0	3.1	33.6	7.2	0.750	0.006	15.7	23	68	9	0.91	0.52	0.08	0.006	0.004	0.01	0.01	13.0	1.15	0.26

^a Stations detailed in Wolhowe et al. (2014) and White et al. (2013).

^b Propagated from standard deviation of replicate alkenone isotopic measurements and assumed 3.7‰ uncertainty in estimated water values (see Section 2.4.3).

^c Mean density/property profiles of Wolhowe et al. (2014) interpolated to the density at time and depth of LVF collection.

^d Gephyrocapsa counts include species *muellerae*, *ericsonii*, and *ornata*, but were >90% *G. oceanica* (Wolhowe et al. 2014).

^e Propagated from TR_{K37.2} and incubation time uncertainties reported in Wolhowe et al.(2014).

^f See Appendix A for data used in calculation.

^g The isopycnal where this LVF was collected was ~8 m below the isopycnal corresponding to the deepest incubation depth (mean density/production rate profile of Wolhowe et al. 2014). Rate extrapolates to 0 between these two depths.

Table A1. Values used in Calculation of μ via Eq. 3.3

Station	Depth	$\delta^{13}\text{C}_{\text{K37:2}}$	$\delta^{13}\text{C}_{\text{DIC}}$	$\delta^{13}\text{C}_{\text{CO2}}$	$[\text{CO}_{2(\text{aq})}]$	Estimated SA:V
	(m)	(‰ vs. PDB)	(‰ vs. PDB)	(‰ vs. PDB)	($\mu\text{mol kg}^{-1}$)	(m^{-1})
4-1	30	-27.12	1.24	-7.14	12.5	1.20
4-2a	10	-27.41	1.31	-6.83	11.4	1.00
4-2a	20	-27.41	1.30	-6.85	11.5	1.00
4-2a	30	-26.93	1.28	-6.95	11.9	1.04
4-2a	40	-27.22	0.92	-7.45	13.4	1.08
4-2a	50	-26.44	0.84	-7.68	14.2	1.13
4-8	10	-27.60	1.43	-6.71	9.9	0.96
4-8	20	-27.56	1.38	-6.78	10.9	0.97
4-8	30	-26.24	1.14	-7.11	11.7	1.04
4-12	10	-25.11	1.50	-7.66	12.2	1.17
4-12	25	-25.07	1.34	-8.13	14.4	1.18

Figure 1

Figure 2

Figure 3

Figure 4