Ecology and Biogeochemistry of Alkenone Production at Station ALOHA

Fredrick G. Prahl^{1*}, Brian N. Popp², David M. Karl³ and Margaret A. Sparrow¹

¹College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR

²Department of Geology & Geophysics, University of Hawaii, Honolulu, HI

³Department of Oceanography, University of Hawaii, Honolulu, HI

*corresponding author: TEL (541) 737-3969, FAX (541) 737-2064, Email
fprahl@coas.oregonstate.edu

(submitted to: Deep-Sea Research I – July 2, 2004)

Abstract

In our study of Station ALOHA, a site representative of the oligotrophic North Pacific subtropical gyre (NPSG), alkenone content and composition were examined in 1) sinking particulate materials collected in sediment traps moored at 2800m during two, year-long deployments (1992-93 and 2000-01) and 2) suspended particulate materials collected throughout the euphotic zone in two contrasting seasons (winter and summer). Each year, sediment trap records documented pronounced alkenone export events in both seasons. The winter event appears triggered by eddy diffusive mixing of nutrients into the upper water column and the release of light limitation on alkenone-producing photoautotrophs growing in the deep chlorophyll maximum layer (DCML). The summer event originates apparently from a stratified euphotic depth well above the DCML where others have now documented the occurrence of N_2 fixation-based 'new production'. Although our time series record is yet limited, it is likely that the absolute magnitude of alkenone export and its quantitative importance in winter relative to summer is an ecological feature of the NPSG which can oscillate significantly on decadal time scales in response to climatic forcing. Despite the significant seasonal and interannual differences in alkenone export noted in our study, findings suggest that paleotemperature assessments based on analysis of the alkenone unsaturation index $U^{K'}_{37}$ measured in underlying sediments would likely be unimpaired by the higher frequency temporal variability in the surface water ecology of alkenone-producers. When translated using a widely accepted calibration equation ($U^{K'}_{37} = 0.034T + 0.039$), $U^{K'}_{37}$ values in average sediment trap particles are consistent with the mean annual sea-surface temperature at Station ALOHA.

KEYWORDS: haptophytes, alkenones, euphotic zone, sea-surface temperature, export production, sedimentation, subtropical North Pacific

Introduction

C_{37-39} alkenones, most notably derived from *Emiliania huxleyi*, are observed almost universally in marine sediments of Quaternary age (BRASSELL, 1993). Throughout the world ocean, measurements of unsaturation patterns in C_{37} alkenones ($U^{K'}_{37}$) in contemporary marine sediments display a remarkably linear relationship with mean annual sea-surface temperature (maSST) (MULLER et al., 1998). This observation now provides the basis for widespread use of $U^{K'}_{37}$ for Quaternary age paleotemperature reconstruction.

Despite the strong correlation of $U^{K'}_{37}$ with maSST, the calibration is not well constrained which adds uncertainty in paleothermometric assessments based on its use. The cause for the observed variability in the $U^{K'}_{37}$ versus maSST relationship is unknown but could be explained by geological factors such as lateral redistribution of sediment (e.g. BENTHIEN and MULLER, 2000), biological factors such as interspecific and intraspecific differences in the details of biosynthesis by alkenone-producers (e.g. CONTE et al., 1998; EPSTEIN et al., 2001; EPSTEIN et al., 1998) or some combination of both. There is yet much to be learned about the sedimentology of alkenones and the biochemistry / ecology of the select set of haptophyte species which produce these unique biomarkers (see PRAHL et al., 2000) for further discussion).

Ultimately, alkenone 'export production' must originate from somewhere in the euphotic zone, defined as the depth below which photosynthesis does not occur. But, given the euphotic zone is thermally structured in most ocean environments, alkenone export need not originate from within the isothermal surface mixed layer (SML). Mounting evidence from study of locations such as the temperate NE Pacific gyre (PRAHL et al., 1993), the Mediterranean Sea (TERNOIS et al., 1997), the central Pacific (OHKOUCHI et al., 1999), the Gulf of Maine (PRAHL et al., 2001) and the Gulf of California (GONI et al., 2001) now suggests the site of export in a range of oceanic

environments is located beneath the SML within a deep chlorophyll maxima layer (DCML) at least during some periods of the year.

Station ALOHA in the oligotrophic North Pacific subtropical gyre (NPSG) is characterized by a year-round persistent DCML. At this site, 19'-hexanoyloxyfucoxanthin (19Hex), an accessory pigment found commonly but not exclusively in Haptophytes (JEFFREY et al., 1997), strongly correlates with chlorophyll *a* (Chl*a*). Given an oceanographically important subset of Haptophytes biosynthesize alkenones (BRASSELL, 1993), one could infer from the strong correlation between Chl*a* and 19Hex concentration at Station ALOHA that: 1) alkenone-producers would display highest 'standing stock' at the depth of the DCML and 2) the DCML is the depth of origin for alkenone export to the sedimentary record.

In the NPSG, the DCML occurs at its shallowest and greatest depth in winter and summer, respectively, and lies at the top or just above the nitricline, owing to the combined effect of forcing by regional meteorological and oceanographic factors (KARL et al., 2002; LETELIER et al., 2004). In view of these contrasts, we investigated the seasonality of alkenone export to sediments in the NPSG and the role of the DCML as a possible site of origin for this export production. Our study was conducted at Station ALOHA through analysis of sediment trap samples collected in time series with approximately biweekly resolution over two one-year periods (1992-93 and 2000-01) and through detailed examination of the alkenone content and composition of suspended particulate material (SPM) collected with depth in the euphotic zone in two contrasting seasons – winter and summer. In addition, we determined alkenone production rates within the euphotic zone in both winter and summer using an *in situ* experimental method modified from that pioneered by Hamanaka et al. (2000).

Samples and Methods

Sampling Site. The site for this study was the Hawaii Ocean Time-series (HOT) station ALOHA (22°45'N, 158°W; KARL and LUKAS, 1996). Station ALOHA is located 100km north of the island of Oahu and has a total water column depth of 4800m.

Sediment Trap Samples. At Station ALOHA, settling particulate material was collected at 2800m water depth in two year-long time series using Parflux MK-21 sequencing sediment collectors (0.5 m² trap opening). In each year (June 1992-93 and December 2000-01), the settling flux was sampled with 17 day time resolution. At the time of deployment, each of the 21 sampling cups on the sediment trap was filled with slightly hypersaline seawater (i.e. seawater plus 5g NaCl/L) containing 1% buffered formalin to facilitate preservation of the particulate material captured. Upon retrieval of the mooring at the end of each year, the volume of each sampling cup was split into quarters and each split was stored at 4°C until needed for chemical analysis.

Suspended Particulate Material Samples. Samples of suspended particulate material (SPM) were collected with depth throughout the euphotic zone (~0-150m) at station ALOHA on two cruises: KOK-111 (16-23 July 2001) which occurred just after HOT-128 (9-13 July 2001) and KOK-303 (17-22 February 2003) which occurred just before HOT-145 (24-28 February 2003). SPM samples for alkenone analysis were obtained using repeated ~1½hr deployments of two Challenger Oceanic *in situ* pumps tethered together by a 20m line. The actual depth of *in situ* sample collection was estimated on KOK-111 using continuous temperature data obtained using a recorder attached to each pump (HOBO StowAway TidbiT Logger) and reference to CTD profile data obtained immediately after each pump cast. On KOK-303, the depth of *in situ* sample collection was determined directly using data obtained from a temperature-pressure

recorder (SBE39) deployed with the pump cast. SPM samples from surface seawater (~ 5m) were also obtained by in-line filtration using the ship's uncontaminated seawater system.

Samples for nutrients (nitrate/nitrite, phosphate, silicate), photosynthetic pigments (chlorophylls, carotenoids), total carbon, total organic carbon and total nitrogen concentration and isotopic analyses as well as samples for determination total dissolved inorganic carbon (DIC) isotopic composition were taken from PVC water sample bottles on dedicated CTD casts. In this paper, only data from photosynthetic pigment analysis, i.e. chlorophyll a (Chla) and 19'-hexanoyloxyfucoxanthin (19Hex), are reported. The methods and data from the other analyses will be reported elsewhere. The water bottle rosette was equipped with a CTD, oxygen sensor and fluorometer. When practical, the majority of samples were collected on a single cast with multiple sample bottles closed at each depth.

SPM samples isolated from 8-10L of water for analysis of photosynthetic pigment concentration were pressure filtered (10psi) through precombusted glass-fiber filters (25mm dia. GF/F, 0.7 μ m nominal pore size) and stored frozen at -20°C until processing. Samples for determination of DIC isotopic composition ($\delta^{13}\text{C}_{\text{DIC}}$) were collected without aeration in 20mL glass serum vials, preserved by addition of HgCl_2 (5 μ L of saturated solution), sealed with a butyl rubber stopper and stored under dark, room temperature conditions for later laboratory analysis. Nutrient samples were collected in pre-cleaned polyvials directly from water bottles on the CTD rosette and stored frozen (-20°C) for later analysis in the laboratory.

Free Floating Arrays. Water samples for incubation experiments were collected from the surface mixed layer (SML), the deep chlorophyll maximum (DCML) and two depths between the base of the SML and the DCML using a CTD rosette equipped with 24 PVC sample bottles (12L each) sealed by means of non-toxic silicone or Viton o-rings. Water for incubations was

collected in darkness several hours before dawn and, to avoid light shocking the phytoplankton, kept in subdued light until used in the free-floating array. Water for each incubation depth was transferred through acid-washed and sterilized silicone tubing directly into four 25L, acid-cleaned and Milli-Q water-rinsed polycarbonate carboys. Each of the four polycarbonate carboys for a given incubation depth was then spiked with sufficient volume of a ^{13}C -labelled bicarbonate solution to yield DIC with a $\delta^{13}\text{C}$ of approximately +190‰ (vs. VPDB). The carboys were sealed and attached on-deck to a line which when deployed as an integral part of the free-floating array would tether the carboys at the nominal depth of water collection. This design was done in order to replicate *in situ* light and temperature conditions as closely as possible. The *in situ* array was fitted with a radio-frequency transmitter, deployed at predawn hours, allowed to drift freely for ~24hrs and recovered at first light the following day.

On both cruises, additional *in situ* experiments were also performed to determine the effect of light limitation on growth of alkenone-producers. During KOK-111, water collected from the DCML (135m) was incubated at a single depth in the SML (~25m) while on KOK-303, water collected from the DCML (120m) was incubated at four shallower euphotic depths (40, 80, 100 and 120m). Notably, the shift to shallower incubation depth was accompanied not only by the desired increase in light availability but, unavoidably, a small but not necessarily insignificant shift in growth temperature (~2.5°C in summer experiment; ≤1°C in winter experiment).

Upon retrieval, samples (20mL) were taken from each carboy and processed as described above for $\delta^{13}\text{C}_{\text{DIC}}$ to confirm the approximately +190‰ level of isotopic enrichment. Remaining water from all four carboys was then pressure filtered sequentially through a single precombusted glass-fiber filter (148mm or 90mm dia. Whatman GF/F or Gelman AE, 0.7µm nominal pore size) and stored frozen (-20°C) until needed for gas chromatographic analysis of alkenones using

both flame ionization detection (GC-FID) and compound specific stable isotopic detection (irmGCMS).

Analytical Techniques. At Oregon State University (OSU), alkenone fractions were isolated from sediment trap materials and SPM using established ultrasonic solvent extraction and column chromatographic methods (PRAHL et al., 1989). The alkenone content and composition of each fraction was analyzed quantitatively by a capillary gas chromatographic approach that is calibrated using internal standards. Addition of a suitable recovery standard (nonadecan-10-one) prior to extraction of all samples allowed correction for any loss of analyte during the workup procedure. Recoveries were typically between 80-90%. The precision of determinations for absolute alkenone concentration and alkenone unsaturation patterns ($U_{37}^{K'}$) was better than $\pm 10\%$ and ± 0.01 , respectively.

Alkenone fractions were subsequently saponified in preparation for compound specific carbon isotopic analysis by irmGCMS (HAYES et al., 1990). The saponification step was done to eliminate interference of a di-unsaturated C_{36} methyl ester with the primary target for isotopic analysis, the di-unsaturated C_{37} alkenone. Isotopically labeled and unlabeled alkenone samples were analyzed for $\delta^{13}C$ composition at the University of Hawaii. Precision of replicate irmGCMS analysis of compounds with natural abundance carbon isotope composition was found in most cases to be $\pm 0.3\%$ or better for individual peaks containing ≥ 5 nmoles of carbon with no indication of bias or inaccuracy in the results. Measured precision for compounds with modest ^{13}C -enrichment (δ -values ~ 50 - 80%) by irmGCMS was typically less than $\pm 1.5\%$.

The modest $\delta^{13}C$ enrichment in the DIC pool (i.e., approximately $+190\%$) provided a sufficiently unambiguous isotopic signal in the alkenones for determination of their production rate by irmGCMS. Use of irmGCMS for this purpose is markedly different from the method

employed by (HAMANAKA et al., 2000) and (SHIN et al., 2002). These workers used chemical ionization GCMS to quantify quasi-molecular ion peaks for the C_{37:2} and C_{37:3} alkenones. Their method assumes that the intrinsic intensities of the isotopically labeled and unlabeled compounds are the same. Their experiments required enrichment of the ambient DIC pool by up to 10% with 99% pure ¹³C-labeled bicarbonate. Our method requires addition of no more than 0.3% enrichment of the ambient DIC pool. Analytical uncertainty for irmGCMS is typically less than ±0.0017 atom% for labeled samples or about an order of magnitude better than the uncertainty (±0.02 atom%) reported by HAMANAKA et al. (2000) and SHIN et al. (2002).

At OSU, photosynthetic pigments (chlorophylls and carotenoids) were determined by the high performance liquid chromatographic (HPLC) method of WRIGHT et al. (1991). Briefly, SPM samples on glass fiber filters were cold-extracted (-15°C) in a polypropylene centrifuge tubes using 90% acetone in water (v/v). Chromatographic separations were made using a reverse-phase column and diode array detection at 436nm. Quantification was accomplished by comparison of integrated peak area for each pigment with response factors for corresponding authentic standards obtained commercially (Chl*a* – Sigma Chemical) or courtesy of colleagues (19Hex – R.R. Bidigare, University of Hawaii).

Values of the $\delta^{13}\text{C}$ of total inorganic carbon in water samples from the incubation experiments were determined at the University of Hawaii using a technique modified from (KROOPNICK, 1985) (for details, see LAWS et al., 1995). The concentration and isotopic analyses of total carbon (non-acidified) and organic carbon (acid-fumed – HEDGES and STERN, 1984) and nitrogen on filtered SPM and powdered sediment trap solids from the 2000-01 time series was determined using an on-line Carlo Erba CHN analyzer coupled to an isotope ratio mass spectrometer (Finnigan ConFlo II/Delta-Plus). Prior experience has indicated that the uncertainty using this

technique is better than 1-2% for measurements of C and N weight percentage and approximately $\pm 0.2\%$ for isotopic C and N analyses. In the case of the 1992-93 sediment trap time series, the total carbon (TOC) and nitrogen (TN) concentrations were determined on filtered rather than powdered sediment trap solids using a standard CHN analyzer configured with conventional thermal conductivity detection. TOC concentrations in these samples were estimated rather than directly measured. Estimates were calculated from the difference between the total carbon measurement and a measure of inorganic carbon derived from the total calcium content of these samples (Dymond and Conard, unpublished data) assuming all calcium exists as calcium carbonate. The precision of these TOC estimates is difficult to gauge but believed to be no worse than $\pm 10\%$.

Results

Chemical Characterization of Sediment Trap Samples. Table 1 summarizes all data acquired from analysis of sediment trap particulate materials collected in times series at Station ALOHA in two different year-long periods (June 1992-93 and November 2000-01). In both years, total particulate mass flux spanned a similar range of values (6.5 to 28mg/m²-d in 1992-93; 13 to 28mg/m²-d in 2000-01) with no clear seasonal pattern apparent in either case. However, values measured in summertime are arguably higher on a systematic basis than those measured at other times in the year when the data set is viewed *in toto*. Mean annual mass flux was ~50% higher in the 2000-01 time series (~19 versus ~13mg/m²-d).

Weight percentages for two bulk chemical properties, calcium carbonate (CaCO₃) and total organic carbon (TOC), were measured in each sample. CaCO₃ accounted for 43 to 63% and 22 to 50% of total particulate mass in samples from 1992-93 and 2000-01, respectively. CaCO₃ weight percentages in average sediment trap particulates differed quite significantly in the two

years (54% versus 36%) but mean annual flux was essentially the same (6.8 versus 7.1 mg/m²-d). TOC contribution to total particulate mass varied much more widely in 1992-93 (5.7-36.6 wt%) than in 2000-01 (4.2-7.4 wt%). Mean annual values for TOC in terms of weight percentage and flux were both ~2 to 3-fold higher in 1992-93 relative to the 2000-01 time series. However, the relative proportion of inorganic carbon to TOC in mean annual sediment trap material displayed little difference in the two time periods (0.77 versus 0.83).

C₃₇ alkenone (K₃₇, sum of tri- and di-unsaturated C₃₇ ketone) flux, TOC-normalized concentration and unsaturation patterns ($U^{K'}_{37}$) were also measured in each sediment trap sample. In 1992-93, K₃₇ flux values varied widely (~70 to 1600 ng/m²-d) with conspicuous peaks evident in winter (~925 ng/m²-d) and in summer (~1600 ng/m²-d). In 2000-01, the seasonal pattern was qualitatively similar but the range of values was much more attenuated (70 to 350 ng/m²-d) with the magnitude of the winter (~300 ng/m²-d) and summer (~350 ng/m²-d) peaks essentially equivalent. In 1992-93, mean annual K₃₇ flux was ~2x greater than that in 2000-01. K₃₇ concentration (μg/gC) spanned a similar range in both years (36-405 μg/gC in 1992-93 versus 69-355 μg/gC in 2000-01) and highest concentrations occurred in particulate material captured during the periods of peak K₃₇ flux in winter and summer. Mean annual K₃₇ concentration was also quite comparable in the two years, i.e. ~175 μg/gC.

In the 1992-93, $U^{K'}_{37}$ values ranged from ~0.80-0.94 with the lowest values occurring during the period of peak flux in winter. In 2000-01, a somewhat smaller range was observed for $U^{K'}_{37}$ (~0.84-0.91) with lowest values recorded in particulate material trapped during the K₃₇ flux event in summer. Despite these seasonal differences, the value of $U^{K'}_{37}$ recorded in average sediment trap material collected was indistinguishable in the two years (~0.87).

Chemical Characterization of Suspended Particles in Surface Waters. Table 2 and 3 summarize for Station ALOHA the photosynthetic pigment and alkenone data acquired from analysis of depth profiles for suspended particulate material (SPM) in the winter and summer euphotic zone.

In both seasons, highest chlorophyll *a* (Chla) and 19'-hexanoyloxyfucoxanthin (19Hex) concentrations (Table 2) occurred deep in the euphotic zone within a feature known as the deep chlorophyll maximum layer (DCML; e.g. see LONGHURST and HARRISON, 1989). The mean depth of the position of the DCML was located in the upper thermocline and, in accordance with the summary in (KARL et al., 2002), occurred somewhat deeper beneath the sea-surface in summer than in winter. The depth profile for 19Hex concentration was highly correlated with Chla in both seasons, i.e. $r = +0.98$ in summer and $+0.93$ in winter. Maximum concentrations for both Chla and 19Hex in the DCML were quite similar in the two seasons, i.e. $\sim 300\text{ng/L}$ for Chla and $\sim 60\text{ng/L}$ for 19Hex.

K37 concentration (Table 3) and 19Hex concentration displayed no similarity in depth distribution, an unexpected finding given that alkenone-producing haptophytes all biosynthesize 19Hex as their predominant carotenoid (JEFFREY et al., 1997). In both seasons, highest K37 concentrations were measured well above the DCML but, specific seasonal differences in the K37 depth profiles were observed nonetheless. In winter, K37 concentrations were uniformly high throughout the surface mixed layer (SML) and dropped off sharply within the upper thermocline. In summer, K37 concentrations were also uniformly high in the SML but displayed a maximum in the top 20 m of the upper thermocline before dropping off sharply with further depth. In contrast to Chla and 19Hex, the value for maximum K37 concentration differed significantly in the two seasons with the winter value ~ 2 to 3 times higher than that in summer.

Values for the K37 unsaturation index $U^{K'}_{37}$ spanned a somewhat wider range in SPM collected in summer (~0.75-0.91) than in winter (~0.80-0.89). Winter values displayed a regular pattern with depth in the euphotic zone, correlating strongly with measured water temperature ($r = +0.73$). In summer, no correlation with measured water temperature was observed ($r = -0.05$), an observation warranting clear explanation given major interest in $U^{K'}_{37}$ as a paleothermometric index (EGLINTON et al., 2001) and associated publications).

Alkenone-specific Carbon Isotopic Determinations. The natural stable carbon isotopic composition ($\delta^{13}\text{C}$) of the dominant di-unsaturated C_{37} ketone (K37:2) isolated from each SPM sample collected throughout the euphotic zone in summer (July 2001) and winter (February 2003) was determined by irmGCMS. In both seasons, $\delta^{13}\text{C}$ values varied by only ~1‰ throughout the euphotic zone, with no recognizable depth dependence to the variation (Table 4). Mean values of $\delta^{13}\text{C}$ for K37:2 were statistically indistinguishable between summer ($-26.5 \pm 0.5\text{‰}$) and winter ($-26.9 \pm 0.3\text{‰}$).

Measurable uptake of ^{13}C at all depths (Table 4) was evident from isotopic analysis of K37:2 isolated from large volume water samples incubated for 24hrs at the depth of collection with ^{13}C -labelled bicarbonate added as a tracer (see Samples and Methods section). In general, the degree of ^{13}C -enrichment was greatest in samples collected nearest the sea-surface and decreased with depth. However, greatest ^{13}C -enrichment in summer was observed in the upper thermocline just below the SML where, as previously described, a conspicuous maximum in K37 concentration was evident (Table 3).

Large volumes of water (~100L) were also collected from the DCML in both seasons, isotopically modified as described above but incubated for 24hrs at depths from 20 to 95m

shallower than the depth of collection in the DCML. Isotopic analysis of K37:2 isolated from all of these samples revealed that incubation at shallower depths enhanced ^{13}C -uptake into alkenones significantly (see “Depth Shift Experiments”, Table 4). Results also show that the degree of ^{13}C -enrichment 1) increased systematically as samples from the wintertime DCML were incubated closer to the sea-surface and 2) was significantly greater than that apparent for alkenones naturally present in waters at the equivalent depth of incubation.

Discussion

OHKOUCHI et al. (1999) measured $U^{K'}_{37}$ values in surface sediments depositing along a north to south transect from 48°N to 15°S at ~175°W in the Pacific Ocean. Comparison of growth temperature estimates derived from these data with the seasonal range of sea-surface temperature (SST) measured in overlying waters provided circumstantial evidence that alkenone export in the oligotrophic North Pacific subtropical gyre (NPSG) derives primarily from a euphotic depth beneath the isothermal surface mixed layer (SML). A potential subsurface site for alkenone export is the deep chlorophyll maximum layer (DCML), a biological oceanographic feature of the NPSG present year-round and located at the base of the euphotic zone within the upper thermocline (KARL et al., 2002).

Results from our analysis of samples in a biweekly-resolved, annual sediment trap time series from two different years and water column samples collected in two different seasons at Station ALOHA (22° 45'N, 158°W), a representative site in the NPSG, corroborate Ohkouchi's inference that alkenone export originates from a depth beneath the SML. They also demonstrate that alkenone export in the NPSG occurs annually as two seasonal events, one in winter and the other in summer. The physical oceanographic dynamics of the DCML and waters above this feature could explain the winter event. But, the event in summer appears independent of the DCML and

potentially tied to the ecology of a N_2 fixation phenomenon that occurs annually in shallower, highly stratified waters of the NPSG (KARL et al., 1997; DORE et al., 2002).

Seasonal Relationship of Alkenone Export to Surface Water Temperature Structure.

Figure 1 illustrates how the depth structure of water temperature changed in the euphotic zone at Station ALOHA over the two annual periods represented by our sediment trap time series. Two seasonal features are common to both the 1992-93 and the 2000-01 reconstructions. First, a cooling of surface waters and deepening of the SML occurs consistently in late fall to early winter. This destratification phenomenon is most intense in mid-January to early February and continues until late spring. Coldest (23-24°C) water temperatures at the sea-surface are expressed in this period of the year. Notably, the depth where waters colder than 22-23°C occur is not altered much, if at all, by mixing associated with the winter destratification process. Second, thermal re-stratification of surface waters begins in late spring. Waters with a temperature of ~24°C demark the bottom of the stratified surface layer which developed. The depth of this isotherm increases through summer, reaching a maximum of ~50-70m in late summer to early fall. At this time, water temperature at the sea-surface reaches its annual maximum (~26-27°C).

The record for total C_{37} alkenone (K37) flux measured in the 1992-93 and 2000-01 sediment trap time series (Table 1) is superimposed on its respective temperature plot (see dashed lines in Figure 1). Both time series for K37 flux were shifted by 28 days. This shift was done, assuming an average settling rate of ~100 meters per day (e.g., DEUSSER et al., 1990), to compensate for temporal offset caused by settling of sedimentary particles from surface waters to the depth of sediment trap collection (2800m). As presented, data show that initiation of the seasonal K37 flux events in both years occurs at the onset of surface water destratification in winter and re-

stratification in late spring. Although the seasonal timing of these flux events is consistent in both years, the magnitude of each event was ~5 times greater in 1992-93 than in 2000-01. This scale of difference is too large to be a trapping efficiency artifact (e.g. YU et al., 2001). In view of recent biological discoveries at Station ALOHA, the difference is most likely quite real, reflecting a fundamental shift in the ecology of primary production at this NPSG site over the past decade (see (DORE et al., 2002) and later discussion).

$U^{K'}_{37}$ values recorded in sediment trap particles from each sampling period (Table 1) were converted into water temperature estimates using a commonly employed calibration equation ($U^{K'}_{37} = 0.034T + 0.039$; PRAHL et al., 1988). The estimates are plotted at appropriate times in Figure 1 to identify the euphotic depth from which alkenone export may have originated. All estimates for the late spring to early fall period plot within the euphotic zone and, at least in this regard, are environmentally realistic. Notably, predicted alkenone export depth lies within the upper thermocline just below the SML but well above the DCML which occurs at Station ALOHA typically below ~100 m throughout the year (KARL et al., 2002). In contrast, estimates for the winter to late spring period are in most cases unrealistic, being warmer than actual water temperature measured anywhere in the euphotic zone. Thus, predicted alkenone export depth during this period of the year is plotted arbitrarily at the sea-surface.

Unrealistic predictions in the winter to late spring period indicate the calibration equation used to estimate water temperature must somehow be in error. The problem is explained by either ecological factors, physiological factors or some combination of the two. Ecologically, it is now well-known that the quantitative response of $U^{K'}_{37}$ to growth temperature can differ significantly between species and even between strains of the same species of alkenone-producing haptophytes (CONTE et al., 1998). Physiologically, it is also recognized that non-thermal factors

such as nutrient and light availability can alter quite significantly the U_{37}^K value set by alkenone-producers growing under isothermal conditions (EPSTEIN et al., 2001; EPSTEIN et al., 1998; PRAHL et al., 2003).

CORTES et al. (2001) studied the coccolithophorid ecology at Station ALOHA. *E. huxleyi* and *Geophyrocapsa ericsonii* were reported as co-occurring, major contributors to the overall coccolithophorid community. The former species and the latter genus are well-recognized alkenone-producers (CONTE et al., 1998). Three morphotypes of *E. huxleyi* were also identified. Two co-occurred in time and space while the third was present only in waters characterized by phosphate depletion. Thus, ecological and physiological grounds exist to question the accuracy of water temperature estimates and hence, the absolute veracity of alkenone export depth predictions derived exclusively from the sediment trap data (Figure 1).

Seasonal Relationship of Alkenone Export to Surface Water Dissolved Oxygen Content.

Figure 2 illustrates how excess dissolved oxygen (excDO) concentration varied with depth in the euphotic zone at Station ALOHA over the year-long period represented by our 2000-01 sediment trap time series. For a water sample, excDO is defined by the difference between its measured dissolved oxygen (DO) concentration and the value for DO saturation predicted from its measured temperature and salinity (see algorithm in Appendix D of PILSON, 1998). This calculated property provides a quantitative measure of photosynthetic relative to respiratory biological activity where positive values depict net community photosynthesis and negative values depict net community respiration.

Two important features warrant highlighting in Figure 2. First, the depth where excDO equals zero (i.e. photosynthesis balances respiration) is ~100m throughout the year. This depth approximates that of the DCML in winter but, in other seasons, is ~10m shallower than that of

the DCML (see hashed line). Second, a zone of net photosynthetic activity is readily apparent in the late spring to early fall period when surface waters are most highly stratified. At this time, excDO reaches a subsurface maximum of +10 to 15 μ M.

Alkenone export depth predictions derived from $U^{K'}_{37}$ measurements for the 2000-01 sediment trap time series (Figure 1) are overlaid in Figure 2 (see filled circles). This comparison shows the trend for predicted alkenone export depth tracks quite well that of the conspicuous excDO feature. The same finding results if the 1992-93 dataset is subjected to similar analysis (illustration not shown). This interannual concurrence provides further evidence that alkenone export at Station ALOHA could originate in the summer to fall period from highly stratified, nitrate-depleted waters in the euphotic zone located well above the DCML.

Primary production in surface waters of the NPSG is nitrogen-limited and controlled by two significant sources of new nitrogen: 1) physically-controlled upward flux of nitrate from deep water and 2) biological fixation of nitrogen (N_2) gas in near-surface waters (KARL et al., 2002). At Station ALOHA, DORE et al. (2002) investigated the relative seasonal importance and the long-term annual trend for these two primary production phenomena using continuous time series measurements of particulate nitrogen (PN) flux and nitrogen stable isotopic ($\delta^{15}N$) composition spanning a ten-year period. Results showed that export production from N_2 fixation is greatest when surface waters are most highly stratified (June to August), while more traditional, nitrate-supported export production maximizes in winter (February to March) when surface mixing penetrates deepest. They also revealed that the contribution of N_2 fixation to total export production has increased significantly over the past decade.

Notably, the alkenone export events documented by our 2800m sediment trap time series (Figure 1) are synchronized with the seasonal pattern for total export production just described.

Although the correlation does not prove cause-effect, this concurrence provides reason to believe that alkenone export at Station ALOHA and probably throughout the NPSG is controlled by two fundamentally different processes during the year. In winter, episodic mixing and physical entrainment of nitrate and phosphate from deep waters into surface waters controls alkenone export. On the other hand, alkenone export is an ecological byproduct of the N₂-fixation phenomenon in summer. At this time of year, N₂-fixing organisms provide the source of new nitrogen to a highly stratified system that would otherwise be phosphate-replete (KARL et al., 1997) but nitrate-depleted. Nitrogen required by the alkenone-producers, organisms incapable of N₂-fixation, is made available subsequently by *in situ* trophic recycling of the N₂-fixing phytoplankton blooms (HOOD et al., 2000 and references therein).

Over the 1990s, various climatic indices for the Pacific Ocean indicate that meteorologically-derived mixing energy declined at Station ALOHA. Consequently, the thermal destratification process in winter has not been sufficiently intense to mix deeply into the nitricline, thereby reducing vertical transport of nitrate into the euphotic zone (KARL et al., 2002). DORE et al. (2002) speculate that such climatic ‘winding down’ over the past decade is a root cause for the apparent shift in the contribution of N₂ fixation to total export production at Station ALOHA. Deep mixing in winter is ultimately the route of supply for phosphate required for primary production in surface waters throughout the year. Clearly, a decline in winter mixing would have had an immediate, direct impact on primary production in wintertime. But, it also could impact primary production in summertime since N₂ fixation is ultimately limited by phosphate availability in the stratified surface waters and, in the absence of a biological transport mechanisms involving vertical migration (e.g., VILLAREAL et al., 1999), the magnitude of this nutrient’s physical replenishment in winter. Given this scenario is valid, it is possible that the

significant decline in both the seasonal and annual average alkenone export flux measured in the 2000-01 relative to 1992-93 sediment trap time series is environmentally quite real (Figure 1). It reflects a climatic shift in oceanographic conditions at Station ALOHA and, like the N₂-fixation phenomenon, is prone to variability in its magnitude depending upon long-term trends in meteorologically dependent ocean mixing conditions in the NPSG.

Alkenone Production and Unsaturation Patterns in the Euphotic Zone. Detailed depth profiles of K37 concentration and $U^{K'}_{37}$ through the euphotic zone were determined for both the summer (July 2001) and winter (February 2003) season (Figure 2). This exercise was done in an effort to validate the major inferences about alkenone biogeochemistry at Station ALOHA made from analysis of the sediment trap time series records. In many regards, our water column findings corroborate the salient sediment trap interpretations. But, there are some clearly identifiable discrepancies of biogeochemical significance which warrant explanation.

In both seasons, K37 concentrations were highest in waters well above the depth of the DCML (~120 m, Figure 3A and B). Maximum values in summer were also not found within the SML but rather in the excDO zone located just beneath this layer. At face value, these aspects of our water column work fully support the sediment trap inference that alkenone export does not originate from the DCML but rather from shallower in the euphotic zone.

Using the method previously described, $U^{K'}_{37}$ -derived temperature estimates were made with depth in each water column (Table 3). Comparison of CTD temperatures characteristic of the SML in wintertime (Figure 3C) and the excDO feature in summertime (Figure 3D) with temperature estimates for cells actually growing at predicted alkenone export depths in these time periods (see filled circles, Figure 1) reveals noteworthy features. $U^{K'}_{37}$ -derived temperatures over-estimate *in situ* wintertime SML temperatures by 1-2°C. This finding is

consistent with the inference from the alkenone signal recorded in sediment trap materials collected in this season (see previous discussion). However, $U^{K'}_{37}$ -derived temperature estimates in the summertime excDO feature significantly under-estimate *in situ* temperatures (i.e., they are significantly ‘colder’ than the CTD measurement). This finding is enigmatic if alkenone export does derive in summertime from the depth of the excDO feature and growth temperature provided the only control on values set by $U^{K'}_{37}$. An examination of other results from our water column work is required before providing a physiological explanation for the cause of this enigma in later discussion.

In winter, the seemingly uniform $\sim 1.4^\circ\text{C}$ over-estimate (Table 3) of *in situ* temperatures throughout the euphotic zone (Figure 3B) is explained perhaps most simply by ecology. The average alkenone-producing community extant at Station ALOHA throughout the deeply mixed, wintertime euphotic zone has a $U^{K'}_{37}$ response to growth temperature like that for the *E. huxleyi* strain calibrated in batch culture (0.034 unit change per $^\circ\text{C}$) but with a somewhat different linear offset (e.g., 0.085 versus 0.039). Such a calibration difference is consistent with reports in the literature (e.g. CONTE et al., 1998).

Observations in summer cannot be accounted so simply, however. A significant difference between estimated and measured water temperature is again apparent but, in this case, the offset is not uniform with depth (Figure 3D). In the SML, $U^{K'}_{37}$ -derived temperature estimates are either equivalent to or up to 2°C less than *in situ* temperatures. In the excDO feature in the thermocline just below the SML, $U^{K'}_{37}$ -derived temperatures under-estimate *in situ* temperatures (i.e., they are $\sim 2\text{--}3^\circ\text{C}$ too ‘cold’). Whereas in the DCML, $U^{K'}_{37}$ -derived temperature over-estimate *in situ* temperatures (i.e., they are $\sim 2\text{--}3^\circ\text{C}$ too ‘warm’). To explain this summer pattern on purely ecologically grounds, the presence of alkenone-producers with not only different

$U^{K'}_{37}$ -T calibrations but also significantly different depth distributions in the euphotic zone is required.

The community of coccolithophores at Station ALOHA is known to be diverse and its species composition is not uniformly distributed with depth and time (CORTES et al., 2001).

Furthermore, each member of this haptophyte (aka prymnesiophyte, see GREEN and LEADBETTER, 1994) community is a potential alkenone-producer (BRASSELL, 1993). Of the species reported by CORTES et al. (2001), however, only *E. huxleyi* and perhaps *Gephyrocapsa ericsonii* are now known to produce alkenones (BRASSELL, 1993; CONTE et al., 1998).

Nonetheless, given that the community at Station ALOHA is comprised of more than just one alkenone-producing species, it is certainly possible that an assemblage of such species characterized by different $U^{K'}_{37}$ -T responses (PRAHL et al., 2000) are segregated in the highly stratified water column in the depth range spanning the excDO zone and the DCML.

Although this more elaborate ecological explanation is viable, it cannot account for why water temperature estimates within the isothermal SML (Figure 3D) display so much variability. For this reason, a physiological cause is posed as an alternative, more suitable explanation for the contrasting relationship between predicted and measured temperature with depth in Station ALOHA's highly stratified, summertime euphotic zone. Recent laboratory work with *E. huxleyi* in batch culture reveals that the $U^{K'}_{37}$ value set by isothermally grown cells can be affected significantly by factors such as nutrient and light availability (EPSTEIN et al., 2001; EPSTEIN et al., 1998; PRAHL et al., 2003). In the strain of *E. huxleyi* which best matches the empirical $U^{K'}_{37}$ -T field calibration of MULLER et al. (1998), the impact of nutrient depletion and darkness on cells grown otherwise isothermally is to decrease and increase, respectively, the $U^{K'}_{37}$ value by an amount equivalent to a 2-3°C change in growth temperature (PRAHL et al., 2003). Assuming

these experimental findings for a laboratory culture apply to natural populations, it is unnecessary to invoke significantly different alkenone-producing communities with depth in the summertime water column at Station ALOHA. The under-estimate of CTD temperature within the excDO feature could reflect the communities' physiological response to the highly limited availability of fixed inorganic nitrogen at this depth (KARL et al., 2002). The over-estimate at the DCML could reflect the same communities' physiological response to the lack of light in an environment situated in close proximity with an abundant supply of fixed inorganic nitrogen, i.e. the top of the nitricline (LETELIER et al., 2004).

Alkenone Productivity in the Euphotic Zone. Results from our *in situ* ^{13}C -incubation experiments show that alkenone production rates are depth dependent (Figure 4). Despite the low resolution of our experimental data, the depth pattern for ^{13}C -uptake into alkenones displays remarkable similarity to that for K37 concentration. As for K37 concentration (Figure 3A and C), the depth of greatest ^{13}C -uptake in summertime is within the zone of excDO (Figure 4B). These findings show that alkenone-producers at Station ALOHA are most productive at the depth of their biomass maximum. They also support the inference drawn from our sediment trap analysis (Figure 1 and 2) that the depth of alkenone export in winter and summer is the SML and the subsurface excDO zone, respectively. Although enticing, this inference is nonetheless not proven by the apparent consistency of the two datasets. Alkenone measurements in the euphotic zone gauge 'standing stock' and 'productivity' while those for sediment trap materials gauge 'export production'. These biological properties, being conceptually quite different, are not equivalent (PARSONS et al., 1984). 'Export production' represents the small, residual fraction of alkenone 'standing stock' produced in surface waters that survives trophic metabolism in the

water column, is transported via a sedimentation mechanism to the abyssal sea and ultimately becomes preserved within the sediment record.

In both seasons, our experimental water column work also revealed that a shift to shallower incubation depth resulted in enhanced ^{13}C -uptake into alkenones (Table 4). Relief of light limitation on the growth of alkenone-producers residing in the nutrient-replete DCML (LETÉLIER et al., 2004) provides an obvious explanation for this response. If correct, then wintertime alkenone export events at Station ALOHA (Figure 1) may be tied ecologically to the thermally-driven mixing dynamics of waters in the euphotic zone nearest the sea-surface and not only the seasonal shoaling behavior of the DCML (KARL et al., 2002) but also mechanistically to the much shorter term, physical oceanographic dynamics of the DCML. Although the DCML persists at a specific potential density surface, high temporal resolution CTD profiling shows its depth below the sea-surface is not constant on the timescale of hours to days (KARL et al., 2002). It varies by up to 50m as a consequence of inertial period and semi-diurnal tidal oscillations; and the timing of this motion is asynchronous with the daily light cycle. Given results from our depth shift experiments, this physical oceanographic condition could significantly impact integrated alkenone production within the DCML on a day-to-day basis and potentially play an influential role in what controls the magnitude of wintertime alkenone export.

In earlier discussion, the SML was assigned as the depth from which alkenone export originates throughout the wintertime period based on cursory examination of sediment trap time series results (Figure 1). It is now worth reconsidering the DCML as the depth from which alkenone export originates in wintertime. At this time, the DCML lies within the top of the nitricline, is therefore nutrient replete and, unlike the nitrate-depleted SML (KARL et al., 2002), thereby able to sustain appreciable alkenone productivity and subsequently export production if sufficient

light were made available. Notably, in both sediment trap series (Figure 1), $U^{K'}_{37}$ -based alkenone export depth predictions approach that of the DCML at the onset of thermal destratification in winter. This feature of the dataset is most conspicuous in the 1992-93, a period when climatic indices suggest meteorologically-derived mixing energy in winter was appreciably greater than in 2000-01 (DORE et al., 2002).

Sedimentation Effect on Alkenone Unsaturation Patterns. If alkenone export does originate from the excDO feature in summer, as suggested from our sediment trap analysis (Figures 1 and 2), this fact is not clearly encoded in $U^{K'}_{37}$ data for the water column. An analytically significant mis-match exists between $U^{K'}_{37}$ measured in cells at the depth of the excDO feature (0.75-0.80, Table 3) and sediment trap materials collected concurrently (0.88-0.89, Table 1). In principle, the occurrence of a diagenetic process such as selective degradation of the tri-unsaturated relative to the di-unsaturated C_{37} alkenone during sedimentation would yield a $U^{K'}_{37}$ shift in the proper direction. However, most lines of geochemical evidence now suggest that once set by the alkenone producer, a $U^{K'}_{37}$ value is relatively insensitive to alteration post-mortem (EGLINTON et al., 2001 and associated articles). Therefore, it seems unlikely that the discrepancy is an artifact produced diagenetically during the sedimentary process.

However, a process occurring during sedimentation could still be responsible for the observed shift in $U^{K'}_{37}$. A physiological explanation can be invoked if alkenone-producing cells were not dead when packaged into settling particles in summertime but rather remained biochemically viable by some means. Laboratory batch culture experiments with a single *E. huxleyi* strain (PRAHL et al., 2003) have shown that cells shifted under isothermal conditions into continuous, prolonged darkness catabolize alkenones on a compound specific basis. Over a 5-day period, the value of $U^{K'}_{37}$ can shift by as much as +0.11 units. Notably, this shift is the same magnitude as

the discrepancy warranting explanation. Given this physiological possibility, the credibility of the explanation now suggested would then depend on identifying how cells packaged within sedimentary particles could remain biochemically viable and thereby be able to re-adjust their $U^{K'}_{37}$ while settling.

SCHAREK et al. (1999) collected settling particulate material just below the euphotic zone at Station ALOHA using free-floating sediment traps. Aggregates of endosymbiont-containing diatom species potentially capable of N_2 fixation were documented as major contributors to the particulate material collected in summer during the period when maximum alkenone export occurs. Our physiological explanation for the mis-matched $U^{K'}_{37}$ values would be supported if these aggregates originated from the depth of the excDO feature and inadvertently incorporated viable alkenone-producing cells in the process of their formation and settling. Unfortunately, there is not sufficient information now available about particle aggregation and sedimentation processes at Station ALOHA to fully advance this explanation. Nonetheless, it seems credible in view of recent experimental results from study of laboratory cultures and warrants further investigation.

Despite all of the apparent complexities in alkenone biogeochemistry that our study has identified, the average $U^{K'}_{37}$ value transmitted to the paleo-record at Station ALOHA provides a reasonable estimate of mean annual sea-surface temperature (maSST). In this regard, our results fully comply with the paleoceanographic interpretation that MULLER et al. (1998) have attached to $U^{K'}_{37}$ values preserved in sediments through most of the global ocean. In our work, maSST was calculated for the time periods represented by our 1992-93 and 2000-01 sediment trap time series by averaging data from monthly CTD casts. Comparison shows maSST was only slightly colder ($\sim 1^\circ\text{C}$) in 1992-93 (24.4 versus 23.2 $^\circ\text{C}$) and essentially the same in 2000-01 (24.7 versus

24.9°C) as temperature estimates derived from the flux-weighted average $U^{K'}_{37}$ in particulate materials from respective sediment trap time series.

Conclusions

Although initiation of the seasonal K37 flux events occurs at the onset of surface water destratification in winter and re-stratification in late spring, the magnitude of each event was ~5x greater in 1992-93 than in 2000-01. This difference could reflect a fundamental shift in the ecology of primary production at this NPSG site over the past decade or a so-called “regime shift” (HARE and MANTUA, 2000). It has been suggested that bottom-up effects from either (a) enhanced nitrogen fixation (e.g., (KARL et al., 1997; DORE et al., 2002) or (b) increased deep-water nitrate entrainment may have forced the 1997 regime shift. (SHERIDAN and LANDRY, 2004) recently documented a significant increase in mesozooplankton biomass at Station ALOHA. This increase occurred in 1997 in conjunction with major changes in water column stability and marine phytoplankton community composition. Although it is certain that new nitrogen inputs must have instigated the central North Pacific ‘regime shift’, the mode of bottom-up forcing is unknown.

The euphotic depth from which alkenone export originates, as inferred from sediment traps for the late spring to early fall period, lies within the upper thermocline just below the SML but well above the DCML. Depth estimates for alkenone export for the winter to late spring period are in most cases unrealistic, being warmer than actual water temperature measured anywhere in the euphotic zone. The cause for the unrealistic predictions in the winter to late spring period may be due to ecological differences in the alkenone-producer community but remain a quandary.

Predictions of alkenone export depth derived from $U^{K'}_{37}$ measurements in the sediment trap time series track quite well that of conspicuous, subsurface excDO features throughout summer.

Alkenone export events documented by our 2800m sediment trap time series are synchronized with the seasonal pattern for total export production and appear controlled by two fundamentally different processes during the year: export production from N_2 fixation which is greatest when surface waters are most highly stratified (June to August), and more traditional, nitrate-supported export production which maximizes in winter (February to March) when surface mixing penetrates deepest.

Alkenone concentration and production maximum coincide throughout the year. The maximum is found in the SML or just below the SML in the upper thermocline. Concentration and production are consistently lowest in the DCML. Growth of alkenone-producers in the DCML appears to be light-limited.

$U^{K'}_{37}$ -derived temperatures over-estimate *in situ* wintertime temperatures in the SML by 1-2°C and is consistent with the inference from the alkenone signal recorded in sediment trap materials collected in this season. However, $U^{K'}_{37}$ -derived temperature estimates in the summertime excDO feature significantly under-estimate *in situ* temperatures whereas in the DCML, $U^{K'}_{37}$ -derived temperature overestimate *in situ* temperatures. These observations require physiological explanations. The under-estimate of CTD temperature within the excDO feature could reflect the communities' physiological response to the highly limited availability of fixed inorganic nitrogen at this depth. The over-estimate at the DCML could reflect the same communities' physiological response to light-limited growth.

Despite all of the apparent environmental complexities in alkenone biogeochemistry that our study has identified, the average $U^{K'}_{37}$ value transmitted to the paleo-record at Station ALOHA provides a reasonable estimate of mean annual sea-surface temperature (maSST). Temperature estimates derived from the flux-weighted average $U^{K'}_{37}$ in particulate materials from respective

sediment trap time series are only slightly warmer than maSST in 1992-1993 and essentially the same in 2000-2001.

Acknowledgements. We wish to thank: the JGOFS HOT Program for access to ancillary oceanographic data from Station ALOHA; Bobbi Conard and the late Dr. Jack Dymond (OSU) for sharing their unpublished total calcium data for the 1992-93 sediment trap time series from ALOHA; Dave Gravitt, the captain and crew of the R/V Ka'Imikai O' Kanaloa for facilitating all of our field work at Station ALOHA; and the National Science Foundation for all research support (OCE-9986306, -0094329 and -0049272).

References

- Benthien A. and Muller P. J. (2000) Anomalous low alkenone temperatures caused by lateral particle and sediment transport in the Malvinas Current region, western Argentine Basin. *Deep-sea Research I* **47**, 2369-2393.
- Brassell S. C. (1993) Applications of biomarkers for delineating marine paleoclimatic fluctuations during the Pleistocene. In *Organic Geochemistry: Principles and Applications* (ed. M. H. Engel and S. A. Macko), pp. 699-738. Plenum Press.
- Conte M. H., Thompson A., Lesley D., and Harris R. (1998) Genetic and physiological influences on the alkenone/alkenoate versus growth temperature relationship in *Emiliana huxleyi* and *Gephyrocapsa oceanica*. *Geochimica et Cosmochimica Acta* **62**, 51-68.
- Cortes M. Y., Bollmann J., and Thierstein H. R. (2001) Coccolithophore ecology at the HOT station ALOHA, Hawaii. *Deep-sea Research II* **48**, 1957-1981.
- Deusser W. G., Muller-Karger F. E., Evans R. H., Brown O. B., Esaias W. E., and Feldman G. C. (1990) Surface-ocean color and deep-ocean carbon flux: how close a connection? *Deep-sea Research I* **37**, 1331-1343.

- Dore J. E., Brum J. R., Tupas L. M., and Karl D. M. (2002) Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. *Limnology and Oceanography* **47**, 1595-1607.
- Eglinton T. I., Conte M. H., Eglinton G., and Hayes J. M. (2001) Proceedings of a workshop on alkenone-based paleoceanographic indicators. *Geochemistry, Geophysics and Geosystems* **2**, 2000GC000122.
- Epstein B. L., d'Hondt S., and Hargraves P. E. (2001) The possible metabolic role of C₃₇ alkenones in *Emiliana huxleyi*. *Organic Geochemistry* **32**, 867-875.
- Epstein B. L., d'Hondt S., Quinn J. G., Zhang J., and Hargraves P. E. (1998) An effect of dissolved nutrient concentrations on alkenone-based temperature estimates. *Paleoceanography* **13**, 122-126.
- Goni M. A., Hartz D. M., Thunell R. C., and Tappa E. (2001) Oceanographic considerations for the application of the alkenone-based paleotemperature U^{K'}₃₇ index in the Gulf of California. *Geochimica et Cosmochimica Acta* **65**, 545-557.
- Green J. C. and Leadbetter B. S. C. (1994) *The Haptophyte Algae*. Claredon Press.
- Hamanaka J., Sawada K., and Tanoue E. (2000) Production rates of C₃₇ alkenones determined by ¹³C-labeling technique in the euphotic zone of Sagami Bay, Japan. *Organic Geochemistry* **31**, 1095-1102.
- Hare S. R. and Mantua N. J. (2000) Empirical evidence for North Pacific regime shifts in 1977 and 1989. *Progress in Oceanography* **47**, 103-145.
- Hayes J. M., Freeman K. H., Popp B. N., and Hoham C. H. (1990) Compound-specific isotopic analyses: a novel tool for reconstruction of ancient biogeochemical processes. *Organic Geochemistry* **16**, 1115-1128.

- Hedges J. I. and Stern J. H. (1984) Carbon and nitrogen determinations of carbonate-containing solids. *Limnology and Oceanography* **29**(3), 663-666.
- Hood R. R., Michaels A. F., and Capone D. G. (2000) Answers sought to the enigma of marine nitrogen fixation. *EOS, Transactions, American Geophysical Union* **81**(13), 138-139.
- Jeffrey S. W., Mantoura R. F. C., and Wright S. W. (1997) *Phytoplankton pigments in oceanography*. UNESCO Publishing.
- Karl D., Letelier R., Tupas L., Dore J., Christian J., and Hebel D. (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical Pacific Ocean. *Nature* **388**, 533-538.
- Karl D. M., Bidigare R. R., and Letelier R. M. (2002) Chapter 9: Sustained and aperiodic variability in organic matter production and phototrophic microbial community structure in the North Pacific subtropical gyre. In *Phytoplankton Productivity: Carbon Assimilation in Marine and Freshwater Ecosystems* (ed. P. J. I. Williams, D. N. Thomas, and C. S. Reynolds), pp. 222-264. Blackwell Science Ltd.
- Karl D. M. and Lukas R. (1996) The Hawaii Ocean Time-series (HOT) program: background, rationale and field implementation. *Deep-sea Research II* **43**, 129-156.
- Kroopnick P. (1985) The distribution of ^{13}C in ΣCO_2 in the world oceans. *Deep-sea Research I* **32**, 57-84.
- Laws E. A., Popp B. N., Bidigare R. P., Kennicutt M. C., and Macko S. A. (1995) Dependence of phytoplankton carbon isotopic composition on growth rate and $[\text{CO}_2]_{\text{aq}}$: theoretical considerations and experimental results. *Geochimica et Cosmochimica Acta* **59**, 1131-1138.
- Letelier R. M., Karl D. M., Abbott M. R., and Bidigare R. R. (2004) Light driven seasonal patterns of chlorophyll and nitrate in the lower euphotic zone of the North Pacific Subtropical Gyre. *Limnology and Oceanography* **49**(2), 508-519.

- Longhurst A. R. and Harrison W. G. (1989) The biological pump: Profiles of plankton production and consumption in the upper ocean. *Progress in Oceanography* **22**, 47-123.
- Muller P. J., Kirst G., Ruhland G., von Storch I., and Rosell-Mele A. (1998) Calibration of the alkenone paleotemperature index $U_{37}^{K'}$ based on core-tops from the eastern South Atlantic and the global ocean (60°N-60°S). *Geochimica et Cosmochimica Acta* **62**, 1757-1772.
- Ohkouchi N., Kawamura K., Kawahata H., and Okada H. (1999) Depth ranges of alkenone production in the central Pacific Ocean. *Global Biogeochemical Cycles* **13**, 695-704.
- Parsons T. R., Takahashi M., and Hargrave B. (1984) *Biological Oceanographic Processes*. Pergamon Press.
- Pilson M. E. Q. (1998) *An Introduction to the Chemistry of the Sea*. Prentice Hall.
- Prahl F. G., Collier R. B., Dymond J., Lyle M., and Sparrow M. A. (1993) A biomarker perspective on prymnesiophyte productivity in the northeast Pacific Ocean. *Deep-sea Research I* **40**, 2061-2076.
- Prahl F. G., Herbert T., Brassell S. C., Ohkouchi N., Pagani M., Repeta D., Rosell-Mele A., and Sikes E. (2000) Status of alkenone paleothermometer calibration: report from Working Group 3. *Geochemistry, Geophysics and Geosystems* **1**, 1-13.
- Prahl F. G., Muehlhausen L. A., and Lyle M. (1989) An organic geochemical assessment of oceanographic conditions at MANOP Site C over the past 26,000 years. *Paleoceanography* **4**(5), 495-510.
- Prahl F. G., Muehlhausen L. A., and Zahnle D. L. (1988) Further evaluation of long-chain alkenones as indicators of paleoceanographic conditions. *Geochimica et Cosmochimica Acta* **52**, 2303-2310.

- Prahl F. G., Pilskaln C. H., and Sparrow M. A. (2001) Seasonal record for alkenones in sedimentary particles from the Gulf of Maine. *Deep-sea Research I* **48**, 515-528.
- Prahl F. G., Wolfe G. V., and Sparrow M. A. (2003) Physiological impacts on alkenone paleothermometry. *Paleoceanography* **18**, 1025, 2002PA000803.
- Scharek R., Latasa M., Karl D. M., and Bidigare R. R. (1999) Temporal variations in diatom abundance and downward vertical flux in the oligotrophic North Pacific gyre. *Deep-sea Research I* **46**, 1051-1075.
- Sheridan C. C. and Landry M. R. (2004) A 9-year increasing trend in mesozooplankton biomass at the Hawaii Ocean Time-series Station ALOHA. *Journal of Marine Science* **61**, 457-463.
- Shin K.-H., Tanaka N., Harada N., and Marty J.-C. (2002) Production and turnover rates of C₃₇ alkenones in the eastern Bering Sea: implication for the mechanism of a long duration of *Emiliana huxleyi* bloom. *Progress in Oceanography* **55**, 113-129.
- Ternois Y., Sicre M.-A., Boireau A., Conte M. H., and Eglinton G. (1997) Evaluation of long-chain alkenones as paleo-temperature indicators in the Mediterranean Sea. *Deep-sea Research I* **44**, 271-286.
- Villareal T. A., Pilskaln C., Brzezinski M., Lipschultz F., Dennett M., and Gardner G. B. (1999) Upward transport of oceanic nitrate by migrating diatom mats. *Nature* **397**, 423-425.
- Wright S. W., Jeffrey S. W., Mantoura R. F. C., Llewellyn C. A., Bjornland T., Repeta D., and Welschmeyer N. (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Marine Ecology Progress Series* **77**, 183-196.
- Yu E.-F., Francois R., Bacon M. P., Honjo S., Fleer A. P., Manganini S. J., van der Loeff M. M. R., and Ittekkot V. (2001) Trapping efficiency of bottom-tethered sediment traps estimated from the intercepted fluxes of ²³⁰Th and ²³¹Pa. *Deep-sea Research II* **48**, 865-889.

Figure 1. Contour plots for temperature measured with depth in the euphotic zone at Station ALOHA over two annual periods: A) June 1992 to May 1993 and B) December 2000 to November 2001. The illustrated temperature reconstructions were accomplished using monthly CTD data available in the WWW archive for the JGOFS Hawaii Ocean Time Series (HOT) program (<http://hahana.soest.hawaii.edu/hot/hot.html>). In both plots, time series data for K37 flux (dashed lines) and $U^{K_{37}}$ -predicted alkenone export depth (filled circles, see text for details) are depicted. Both time series have been adjusted by 28 days to account for particle settling time between surface waters and collection depth at 2800m assuming average particle settling velocity of 100m/d (e.g., DEUSSER et al., 1990).

Figure 2

Figure 2. Contour plot for excess dissolved oxygen (excDO) concentration predicted with depth at Station ALOHA over the annual time period from December 2000 to November 2001. excDO in a water sample is calculated from the difference between its measured dissolved oxygen (DO) concentration and the value for DO saturation predicted from its measured temperature and salinity (see algorithm in Appendix D of PILSON, 1998). Approximately monthly resolved CTD data for DO, T and S used to make the illustrated excDO reconstruction was obtained from WWW archive for the JGOFS HOT program (<http://hahana.soest.hawaii.edu/hot/hot.html>). The hashed line depicts the depth of the DCML determined using fluorometric data obtained in each CTD cast. Filled circles depict $U^{K'}_{37}$ -based predictions for alkenone export depth (see legend for Figure 1).

Figure 3

Figure 3. Profiles for K37 concentration and $U^{K'_{37}}$ measured with depth throughout the euphotic zone at Station ALOHA in winter (February 2003) and summer (July 2001). For reference purposes, plots for K37 concentration in A) winter and B) summer have been annotated with the profile for chlorophyll a concentration measured using an *in situ* fluorometer attached to the CTD – water sampling package. Plots for $U^{K'_{37}}$ -derived water temperature estimates (see text for details) in C) winter and D) summer have been annotated with the profile for CTD temperature. The highlighted part of both summer plots (B and D) identifies the depth range over which significant positive values of excess dissolved oxygen (excDO) concentration (see definition in legend for Figure 2) were observed.

Figure 4

Figure 4. Profiles illustrating depth dependence for ^{13}C -enrichment in the di-unsaturated C_{37} alkenone (K37:2) as determined by *in situ* incubation experiments. Grey filled circles show the effect on ^{13}C -uptake of incubating nutrient-replete samples from the DCML at shallower depths.

Table 1. Times series data for fluxes and compositional properties measured in sediment trap materials collected at 2800 m water depth at Station ALOHA in two different one-year periods: June 1992 to 1993 and December 2000 to 2001.

Cup Number	Date Open	Days Open	Total Mass mg/m ² -d	CaCO ₃ ¹ wt%	TOC wt%	K37 ng/m ² -d	K37 ug/gC	U ^K ₃₇ n.a.
1	6/7/1992	17.4	11.2	50%	10.1%	n.a.	n.a.	n.a.
2	6/24/1992	17.4	28.3	63%	9.8%	187	67	0.862
3	7/12/1992	17.4	26.2	43%	23.7%	1597	257	0.833
4	7/29/1992	17.4	16.9	44%	18.5%	410	131	0.843
5	8/15/1992	17.4	12.5	53%	20.7%	173	67	0.869
6	9/2/1992	17.4	11.2	58%	36.6%	195	48	0.875
7	9/19/1992	17.4	28.3	55%	5.7%	91	56	0.882
8	10/6/1992	17.4	12.4	55%	13.7%	241	142	0.938
9	10/24/1992	17.4	13.6	56%	15.2%	74	36	0.906
10	11/10/1992	17.4	8.3	57%	34.1%	114	40	0.873
11 & 12	11/28/1992	34.8	6.5	58%	30.2%	230	118	0.906
13 & 14	1/1/1993	34.8	7.3	57%	21.5%	416	267	0.924
15	2/5/1993	17.4	8.5	58%	26.8%	927	405	0.802
16	2/22/1993	17.4	10.2	58%	31.8%	900	278	0.797
17	3/12/1993	17.4	12.1	56%	21.4%	784	304	0.803
18	3/29/1993	17.4	15.6	51%	7.3%	294	258	0.870
19	4/16/1993	17.4	11.7	52%	13.6%	223	140	0.887
20	5/3/1993	17.4	11.1	55%	18.3%	n.a.	n.a.	n.a.
21	5/20/1993	17.4	9.6	60%	9.6%	141	153	0.882
Annual Average			13.1	54%	17.8%	402	173	0.868
1	12/15/2000	16	12.8	n.a.	4.7%	77	128	0.875
2	12/31/2000	17	14.4	32%	4.6%	76	116	0.865
3	1/17/2001	17	18.4	35%	4.2%	133	171	0.880
4	2/3/2001	16	15.7	39%	5.2%	72	90	0.858
5	2/19/2001	17	19.8	23%	4.4%	261	301	0.886
6	3/8/2001	17	19.2	47%	5.2%	295	296	0.892

7	3/25/2001	16	17.7	31%	4.7%	137	166	0.879
8	4/10/2001	17	15.8	50%	5.6%	166	190	0.919
9	4/27/2001	17	15.9	35%	5.1%	122	149	0.912
10	5/14/2001	16	17.1	45%	6.0%	125	123	0.900
11	5/30/2001	17	14.9	32%	5.6%	69	82	0.896
12	6/16/2001	17	24.6	35%	5.6%	183	133	0.872
13	7/3/2001	16	19.3	40%	5.9%	311	273	0.905
14	7/19/2001	17	23.2	43%	6.0%	304	217	0.861
15	8/5/2001	17	27.7	41%	7.4%	349	171	0.847
16	8/22/2001	16	27.8	36%	6.7%	355	191	0.839
17	9/7/2001	17	24.0	22%	5.3%	159	126	0.845
18	9/24/2001	17	18.3	46%	6.0%	238	217	0.855
19	10/11/2001	16	15.4	38%	6.2%	192	200	0.874
20	10/27/2001	17	15.1	34%	5.6%	115	135	0.874
21	11/13/2001	17	16.5	34%	5.5%	115	126	0.896
Annual								
Average			18.8	36%	5.6%	184	175	0.878

¹estimated in 1992-93 time series from measurement of total calcium (Dymond and Conard, unpublished data), assuming all is present as calcium carbonate; estimated in 2000-01 time series from total inorganic carbon value determined from difference between total carbon and total organic carbon measurements, assuming all is present as calcium carbonate

Table 2. 19'-hexanoyloxyfucoxanthin (19Hex) and chlorophyll a (Chla) concentrations measured with depth in surface waters at Station ALOHA in two different seasons: winter (February, 2003) and summer (July, 2001).

	Depth (m)	19Hex (ng/L)	Chla (ng/L)
Winter	20	12	115
	40	6	101
	40	12	120
	60	14	120
	80	15	150
	80	16	133
	100	25	227
	100	36	259
	100	41	282
	100	23	179
	120	54	302
	120	49	252
	140	31	159
	maximum	54	302
	minimum	6	101
	correlation		0.93
Summer ¹	4	11	54
	24	11	51
	44	23	103
	60	30	133
	74	44	199
	84	43	211
	99	51	250
	114	59	280
	125	66	312
	134	69	286
	150	49	214
	175	29	89
	maximum	69	312
	minimum	11	51
	correlation		0.98

¹data from web archive for HOT program
(<http://hahana.soest.hawaii.edu/hot/hot.html>)

Table 3. Total C₃₇ alkenone concentrations (K₃₇) and unsaturation patterns (U^{K'}₃₇) determined from analysis of surface waters at Station ALOHA in two different seasons: winter (February 2003) and summer (July 2001). ΔT is defined by the difference between measured (CTD-T) and estimated (U^{K'}₃₇-T) temperature, where U^{K'}₃₇-T is calculated from U^{K'}₃₇ using the calibration equation of (PRAHL et al., 1988).

	Depth	K ₃₇	U ^{K'} ₃₇	CTD-T	U ^{K'} ₃₇ -T	ΔT
	(m)	(ng/L)		(°C)	(°C)	(°C)
Winter Samples						
Shipboard Pump	7	9.3	0.886	23.1	24.9	1.8
Shipboard Pump	7	8.8	0.876	23.1	24.6	1.5
Shipboard Pump	7	12.1	0.875	23.1	24.6	1.5
Shipboard Pump	7	12.5	0.866	23.1	24.3	1.2
In Situ Pump	17	11.6	0.861	23.1	24.2	1.1
In Situ Pump	37	11.7	0.854	23.0	24.0	1.0
In Situ Pump	57	12.9	0.874	23.2	24.6	1.4
In Situ Pump	72	12.4	0.855	23.0	24.0	1.0
In Situ Pump	77	11.8	0.879	23.0	24.7	1.7
In Situ Pump	80	15.5	0.859	23.1	24.1	1.1
In Situ Pump	97	8.3	0.861	23.0	24.2	1.2
In Situ Pump	100	2.5	0.853	23.0	23.9	1.0
In Situ Pump	117	6.8	0.855	22.7	24.0	1.3
In Situ Pump	117	9.5	0.862	22.4	24.2	1.8
In Situ Pump	120	4.9	0.877	22.7	24.6	1.9
In Situ Pump	133	2.7	0.819	22.2	22.9	0.7
In Situ Pump	137	1.4	0.836	21.5	23.5	1.9
In Situ Pump	140	2.6	0.867	22.2	24.3	2.2
In Situ Pump	153	2.9	0.798	21.6	22.3	0.7
maximum		15.5	0.886	23.2	24.9	2.2
minimum		1.4	0.798	21.5	22.3	0.7
average						1.4
Standard deviation						0.4
correlation				0.74		
Summer Samples						
Shipboard Pump	7	3.0	0.911	25.3	25.6	0.3
Shipboard Pump	7	3.2	0.846	25.3	23.7	-1.6
Shipboard Pump	7	2.4	0.904	25.3	25.4	0.1
Shipboard Pump	7	4.0	0.836	25.3	23.4	-1.9
Shipboard Pump	7	2.3	0.864	25.3	24.3	-1.0
In Situ Pump	20	2.6	0.901	25.3	25.3	0.0
CTD Rosette	25	1.6	0.911	25.3	25.6	0.3
In Situ Pump	49	5.5	0.795	24.8	22.2	-2.5
In Situ Pump	50	5.3	0.809	24.7	22.6	-2.0
In Situ Pump	50	4.5	0.812	24.7	22.7	-1.9
CTD Rosette	50	5.6	0.808	24.7	22.6	-2.0
In Situ Pump	53	5.5	0.788	24.4	22.0	-2.3

In Situ Pump	55	4.9	0.800	24.2	22.4	-1.8
In Situ Pump	63	5.5	0.766	23.9	21.4	-2.5
In Situ Pump	65	5.8	0.756	23.9	21.1	-2.8
In Situ Pump	70	6.7	0.753	23.8	21.0	-2.8
CTD Rosette	80	4.5	0.762	23.2	21.3	-2.0
In Situ Pump	96	3.8	0.883	22.6	24.8	2.3
CTD Rosette	100	1.8	0.871	22.5	24.5	1.9
CTD Rosette	120	1.2	0.913	22.0	25.7	3.7
CTD Rosette	135	0.8	0.894	21.3	25.1	3.8
CTD Rosette	165	0.5	0.850	20.2	23.9	3.7
maximum		6.7	0.913	25.3	25.7	3.8
minimum		0.5	0.753	20.2	21.0	-2.8
average						-0.5
standard deviation						2.2
correlation				-0.05		

Table 4. Total C₃₇ alkenone concentrations (K37), U^{K'}_{37-T}-derived water temperature estimates (U^{K'}_{37-T}, see Table 3 for definition) and stable carbon isotopic (δ¹³C) composition of the di-unsaturated C₃₇ alkenone (K37:2) determined from analysis of natural and ¹³C-incubated surface waters at Station ALOHA in two different seasons: winter (February 2003) and summer (July 2001). Stdev (n) represents the standard deviation of the d13C measurement on K37:2 and the number of injections for the sample (n) on the irmGCMS. 'δ¹³C-enrichment' is calculated from the difference between the carbon isotopic composition of K37:2 measured in a ¹³C-incubated sample and in the average natural sample. See text for further details.

Sample Type / Collection Method		Collection Depth (m)	Incubation Depth (m)	K37 (ng/L)	U ^{K'} _{37-T} (°C)	δ ¹³ C-K37:2 (o/oo)	Stdev (n)	¹³ C-enrichment (o/oo)
Winter: Natural								
Shipboard Pump		7		9.3	24.9	-27.0	(1)	
In Situ Pump		37		11.7	24.0	-27.3	±0.3 (4)	
In Situ Pump		80		15.5	24.1	-26.4	±0.1 (2)	
In Situ Pump		97		8.3	24.2	-26.7	±0.4 (6)	
In Situ Pump		120		4.9	24.6	-26.9	±0.4 (4)	
Average						-26.9		
Standard Deviation						0.3		
Winter: In situ ¹³ C-Incubations								
Free-floating Array		40	40	9.0	25.3	-1.0	±0.1 (2)	25.9
Free-floating Array		80	80	8.8	24.6	-13.8	±0.2 (3)	13.1
Free-floating Array		100	100	4.0	24.6	-20.7	±0.3 (2)	6.2
Free-floating Array		120	120	2.7	25.2	-18.6	±0.4 (2)	8.3
Winter: In Situ ¹³ C-Incubations (Depth Shift Experiments)								
Free-floating Array		120	40	10.3	24.3	87.1	±0.4 (3)	114.0
Free-floating Array		120	80	5.5	24.8	52.5	±0.4 (2)	79.3
Free-floating Array		120	100	1.9	23.2	7.0	±0.2 (2)	33.9
Free-floating Array		120	120	2.2	23.9	-12.9	(1)	13.9

Summer: Natural						
<i>Shipboard Pump</i>	7	4.0	23.4	-26.5	±0.3 (3)	
<i>Shipboard Pump</i>	7	2.3	24.3	-26.3	±0.0 (2)	
<i>In Situ Pump</i>	53	5.5	22.0	-27.1	±0.2 (2)	
<i>In Situ Pump</i>	63	5.5	21.4	-26.2	±0.1 (3)	
<i>In Situ Pump</i>	65	5.8	21.1	-25.8	±1.2 (3)	
<i>In Situ Pump</i>	96	3.8	24.8	-27.3	±0.2 (2)	
Average				-26.5		
Standard Deviation				0.5		
Summer: In Situ ¹³C-Incubations						
<i>Free-floating Array</i>	25	25	1.6	25.6	±0.5 (2)	32.2
<i>Free-floating Array</i>	50	50	5.6	22.6	±0.1 (2)	73.3
<i>Free-floating Array</i>	120	120	1.2	25.7	(1)	14.3
Summer: In Situ ¹³C-Incubations (Depth Shift Experiments)						
<i>Free-floating Array</i>	135	25	1.1	24.0	(1)	70.4