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Ecology and biogeochemistry of alkenone production at Station ALOHA

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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 2800 m during 2 year-long deployments (1992–1993 and 2000–2001) 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 ($U^{K'}_{37} = 0.034\,T + 0.039$), $U^{K'}_{37}$ values in average sediment trap particles are consistent with the mean annual sea-surface temperature at Station ALOHA.
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Keywords: Haptophytes; Alkenones; Euphotic zone; Sea-surface temperature; Export production; Sedimentation; Subtropical North Pacific

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1. 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; Ohkouchi et al., 2002), 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., 1998, 2001) 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 by the depth below which photosynthesis does not occur. But, given the euphotic zone is structured thermally 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 concentrations 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 is shallowest in winter, at its greatest depth in summer and lies at the top or just above the nitracline in both seasons, 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 1-year periods (1992–1993 and 2000–2001) 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).

2. Samples and methods

2.1. 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 100 km north of the island of Oahu and has a total water depth of 4800 m.

2.2. Sediment trap samples

At Station ALOHA, settling particulate material was collected at 2800 m depth in 2 year-long time series using Parflux MK-21 sequencing sediment collectors (0.5 m^2 trap opening). In each year (June 1992–1993 and December 2000–2001), 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 5 g NaCl l^{-1}) 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.

2.3. Suspended particulate material samples

SPM samples were collected with depth throughout the euphotic zone (~0–150 m) 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 ~$1\frac{1}{2}$ h deployments of two Challenger Oceanic in situ pumps tethered together by a 20 m 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, Sea-Bird Electronics, Inc) deployed with the pump cast. SPM samples from surface seawater (~5 m) 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 (TOC) and total nitrogen concentration and isotopic analyses as well as samples for determination of 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. Chl*a* and 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 to 10 l of water for analysis of photosynthetic pigment concentration were pressure filtered (10 psi) through precombusted glass-fiber filters (25 mm dia. GF/F, 0.7 μm nominal pore size) and stored frozen at −20 °C until processing. Samples for determination of DIC isotopic composition ($\delta^{13}C_{DIC}$) were collected without aeration in 20 ml glass serum vials, preserved by addition of $HgCl_2$ (5 μl 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.

2.4. Free floating arrays

Water samples for incubation experiments were collected from the SML, the DCML and two depths between the base of the SML and the DCML using a CTD rosette equipped with 24 PVC sample bottles (10 l 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 25 l, 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 δ^{13}C of approximately +190‰ (versus 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 ~24 h 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 (135 m) was incubated at a single depth in the SML (~25 m) while on KOK-303, water collected from the DCML (120 m) was incubated at four shallower euphotic depths (40, 80, 100 and 120 m). 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 (20 ml) were taken from each carboy and processed as described above for $\delta^{13}C_{DIC}$ 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 (148 or 90 mm dia. Whatman GF/F or Gelman AE, 0.7 μm 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).

2.5. 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 (GC) approach that is calibrated using internal standards. All analyses were done using an HP6890A GC equipped with cool on-column injection and flame ionization detection. Separations were accomplished using hydrogen carrier gas, a J&W Scientific DB-1 column (60 m × 0.32 mm × 0.25 μm) and temperature programming (see Prahl et al., 1989 for further details). 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 and 90%. The precision of determinations for absolute alkenone concentration and alkenone unsaturation patterns ($U^{K'}_{37}$) was better than ±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 in ethanol to eliminate any chance of 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 δ^{13}C composition at the University of Hawaii using a MAT 252 equipped with a TraceGC and GC Combustion III interface. All compounds were chromatographically purified prior to on-line isotopic analysis using an a TraceGC equipped with cool, on-column injection, a J&W Scientific DB-1MS column (60 m × 0.32 mm × 0.25 μm) that was temperature programmed from 60 to 320 °C at 10 °C min^{-1}. Precision of replicate irmGCMS analysis of compounds with natural abundance carbon isotope composition was found in most cases to be ±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 to 80‰) by irmGCMS was typically better than ±1.5‰.

The modest ^{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 ^{13}C-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 25 mm glass fiber filters were cold-extracted ($-15\,^{\circ}C$) in polypropylene centrifuge tubes using a fixed volume of 90% acetone in water (v/v). Chromatographic separations were made using a reverse-phase column and diode array detection at 436 nm. 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 $\delta^{13}C$ 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 2000–2001 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 ± 0.2‰ for isotopic C and N analyses. In the case of the 1992–1993 sediment trap time series, the total organic 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 $\pm 10\%$.

3. Results

3.1. 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 1992–1993 and November 2000–2001). In both years, total particulate mass flux spanned a similar range of values (6.5–28 mg m^{-2} d^{-1} in 1992–1993; 13–28 mg m^{-2} d^{-1}in 2000–2001) 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 2000–2001 time series (~19 versus ~13 mg m^{-2} d^{-1}).

Weight percentages for two bulk chemical properties, calcium carbonate ($CaCO_3$) and TOC, were measured in each sample. $CaCO_3$ accounted for 43–63% and 22–50% of total particulate mass in samples from 1992 to 1993 and 2000 to 2001, respectively. $CaCO_3$ weight percentages in average sediment trap particulates differed quite significantly (54% versus 36%) but mean annual flux was essentially the same (6.8 versus

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

Cup number	Date open	Days open	Total mass ($mg\,m^{-2}\,d^{-1}$)	$CaCO_3$[a] (wt%)	TOC (wt%)	K37 ($ng\,m^{-2}\,d^{-1}$)	K37 ($\mu g\,g\,C^{-1}$)	$U^{K'}_{37}$
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 and 12	11/28/1992	34.8	6.5	58	30.2	230	118	0.906
13 and 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

[a]estimated in 1992–1993 time series from measurement of total calcium (Dymond and Conard, unpublished data), assuming all is present as calcium carbonate; estimated in 2000–2001 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.

7.1 $mg\,m^{-2}\,d^{-1}$) in the 2 years. TOC contribution to total particulate mass varied much more widely in 1992–1993 (5.7–36.6 wt%) than in 2000–2001 (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 1992–1993 relative to the

2000–2001 time series. However, the relative proportion of inorganic carbon to TOC in mean annual sediment trap material displayed little difference between the two time periods (0.77 versus 0.83).

C_{37} alkenone (K37, sum of tri- and di-unsaturated C_{37} ketone) flux, TOC-normalized concentration and unsaturation patterns ($U^{K'}_{37}$) were also measured in each sediment trap sample. In 1992–1993, K37 flux values varied widely ($\sim$70–1600 ng m^{-2} d^{-1}) with conspicuous peaks evident in winter ($\sim$925 ng m^{-2} d^{-1}) and in summer ($\sim$1600 ng m^{-2} d^{-1}). In 2000–2001, the seasonal pattern was qualitatively similar but the range of values was much more attenuated (70–350 ng m^{-2} d^{-1}) with the magnitude of the winter ($\sim$300 ng m^{-2} d^{-1}) and summer ($\sim$350 ng m^{-2} d^{-1}) peaks essentially equivalent. In 1992–1993, mean annual K37 flux was $\sim$2 × greater than that in 2000–2001. K37 concentration (μg g C^{-1}) spanned a similar range in both years (36–405 μg g C^{-1} in 1992–1993 versus 69–355 μg g C^{-1} in 2000–2001) and highest concentrations occurred in particulate material captured during the winter and summer periods of peak K37 flux. Mean annual K37 concentration was also quite comparable in the 2 years, i.e. $\sim$175 μg g C^{-1}.

In the 1992–1993, $U^{K'}_{37}$ values ranged from $\sim$0.80–0.94 with the lowest values occurring during the winter period of peak flux. In 2000–2001, a somewhat smaller range was observed for $U^{K'}_{37}$ ($\sim$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 2 years ($\sim$0.87).

3.2. *Chemical characterization of suspended particles in surface waters*

Tables 2 and 3 summarize for Station ALOHA the photosynthetic pigment and alkenone data acquired from analysis of depth profiles for SPM in the winter and summer euphotic zone.

In both seasons, highest Chl *a* and 19Hex concentrations (Table 2) occurred deep in the euphotic zone within a feature known as the 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 Chl *a* in both seasons, i.e. $r = +0.98$ in summer and $+0.93$ in winter. Maximum concentrations for both Chl *a* and 19Hex in the DCML were quite similar in the two seasons, i.e. $\sim$300 ng l^{-1} for Chl *a* and $\sim$60 ng l^{-1} for 19Hex.

Table 2
19Hex and Chl *a* 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^{-1})	Chl *a* (ng l^{-1})
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[a]	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**

[a]data from web archive for HOT program (http://hahana.soest.hawaii.edu/hot/hot.html).

Table 3
Total C_{37} 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)

Winter samples	Depth (m)	K37 (ng l^{-1})	$U^{K'}_{37}$	CTD-T (°C)	$U^{K'}_{37}$-T (°C)	ΔT (°C)
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**		

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).

K37 concentration (Table 3) and 19Hex concentration displayed no similarity in depth distribution. This finding was not expected given that alkenone-producing haptophytes all biosynthesize 19Hex as their predominant carotenoid (Jeffrey et al., 1997), However, it is also not an enigmatic finding. It implies that alkenone-producers are minor contributors to the standing stock of 19Hex in these waters and that the ecology of alkenone-producers differs significantly from that of the major phytoplankton taxa contributing to the 19Hex distribution in Station ALOHA waters. 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 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 Chl *a* and 19Hex, the value for maximum K37 concentration differed significantly in the two seasons with the winter value ~2–3 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).

3.3. Alkenone-specific carbon isotopic determinations

The natural stable carbon isotopic composition ($\delta^{13}C$) 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, $\delta^{13}C$ values varied by only ~1‰ throughout the euphotic zone, with no recognizable depth dependence to the variation (Table 4). Mean values of $\delta^{13}C$ for K37:2 were statistically indistinguishable between summer (-26.5 ± 0.5‰) and winter (-26.9 ± 0.3‰).

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 24 h at the depth of collection with ^{13}C-labelled bicarbonate added as a tracer (see Section 2). 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 (~100 l) were also collected from the DCML in both seasons, isotopically modified as described above but incubated for 24 h at depths from 20 to 95 m 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.

4. 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°E 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 NPSG derives primarily from a euphotic depth beneath the isothermal SML. A potential subsurface site for alkenone export is the

Table 4

Total C_{37} alkenone concentrations (K37), $U^{K'}_{37}$-derived water temperature estimates ($U^{K'}_{37}$-T, see Table 3 for definition) and stable carbon isotopic ($\delta^{13}C$) composition of the di-unsaturated C_{37} alkenone (K37:2) determined from analysis of natural and ^{13}C-incubated surface waters at Station ALOHA in two different seasons: winter (February 2003) and summer (July 2001)

	Collection depth (m)	Incubation depth (m)	K37 (ng l^{-1})	$U^{K'}_{37}$-T (°C)	δ^{13}C-K37:2 (‰)	Stdev (n)	^{13}C-enrichment (‰)
Sample type/collection method							
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							
Shipboard pump	7		4.0	23.4	−26.5	±0.3 (3)	
Shipboard pump	7		2.3	24.3	−26.3	±0.0 (2)	
In situ pump	53		5.5	22.0	−27.1	±0.2 (2)	
In situ pump	63		5.5	21.4	−26.2	±0.1 (3)	
In situ pump	65		5.8	21.1	−25.8	±1.2 (3)	
In situ pump	96		3.8	24.8	−27.3	±0.2 (2)	
Average					−26.5		
Standard deviation					0.5		
Summer: in situ ^{13}C-incubations							
Free-floating array	25	25	1.6	25.6	6.5	±0.5 (2)	32.2
Free-floating array	50	50	5.6	22.6	46.7	±0.1 (2)	73.3
Free-floating array	120	120	1.2	25.7	−12.3	(1)	14.3
Summer: in situ ^{13}C-incubations (Depth shift experiments)							
Free-floating array	135	25	1.1	24.0	43.8	(1)	70.4

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. '^{13}C-enrichment' is calculated from the difference between the carbon isotopic composition of K37:2 measured in a ^{13}C-incubated sample and in the average natural sample. See text for further details.

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).

4.1. Seasonal relationship of alkenone export to surface water temperature structure

Fig. 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 1992–1993 and the 2000–2001 reconstructions. First, a cooling of surface waters and deepening of the SML occur 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 ~50–70 m 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 1992–1993 and 2000–2001 sediment trap time series (Table 1) is superimposed on its respective temperature plot (see dashed lines in Fig. 1). Both time series for K37 flux were shifted by 28 days. This shift was based on an assumed average settling rate of ~100 m per day (e.g., Deuser et al., 1990), to compensate for temporal offset caused by settling of sedimentary particles from surface waters to the depth of sediment trap collection (2800 m). 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 1992–1993 than in 2000–2001. 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 ($U^{K'}_{37} = 0.034\,T + 0.039$; Prahl et al., 1988). The estimates are plotted at appropriate times in Fig. 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.

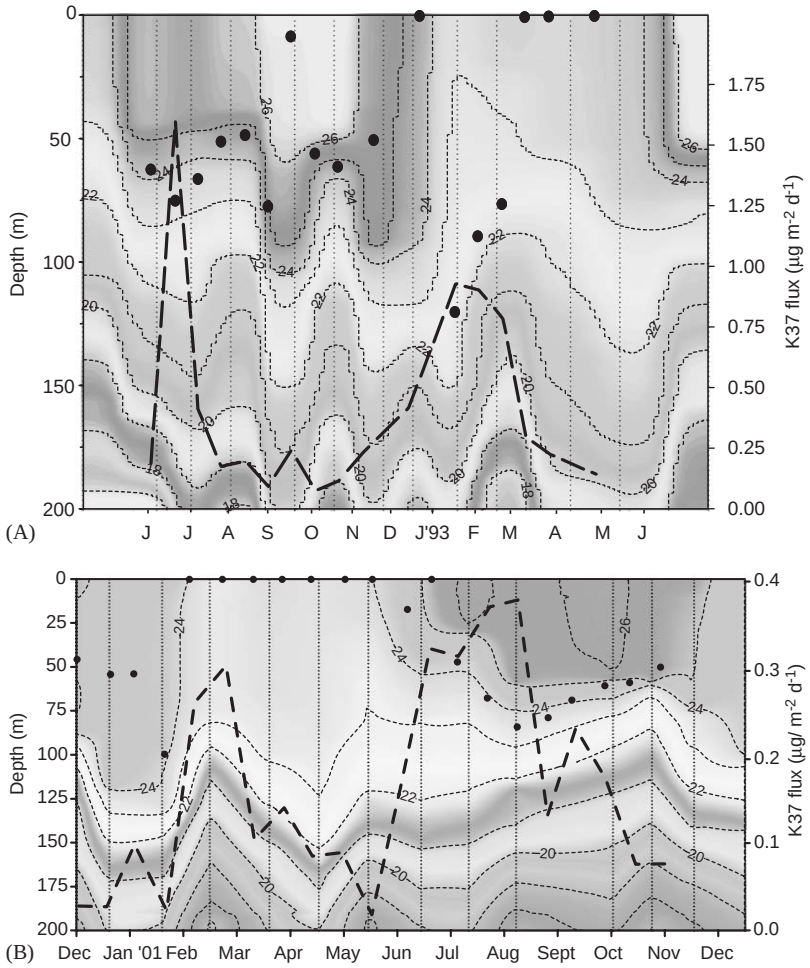


Fig. 1. Contour plots for temperature measured with depth in the euphotic zone at Station ALOHA over two annual periods: (A) June 1992–May 1993 and (B) December 2000–November 2001. The illustrated temperature reconstructions were accomplished using monthly CTD data available in the WWW archive for the 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 2800 m assuming average particle settling velocity of 100 m d^{-1} (e.g., Deuser et al., 1990).

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 $U^{K'}_{37}$ value set by alkenone-producers growing under isothermal conditions (Epstein et al., 1998, 2001; Versteegh et al., 2001; 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 coccolithorphorid 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 (Fig. 1).

4.2. Seasonal relationship of alkenone export to surface water dissolved oxygen content

Fig. 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 2000–2001 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 Fig. 2. First, the depth where excDO equals zero (i.e. photosynthesis balances respiration) is ~100 m throughout the year. This depth approximates that of the DCML in winter but, in other seasons, is ~10 m 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 +10–15 μM.

Alkenone export depth predictions derived from $U^{K'}_{37}$ measurements for the 2000–2001 sediment trap time series (Fig. 1) are overlaid in Fig. 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 1992–1993 data set is subjected to similar analysis (illustration not shown). This interannual concurrence provides further evidence that alkenone export at Station

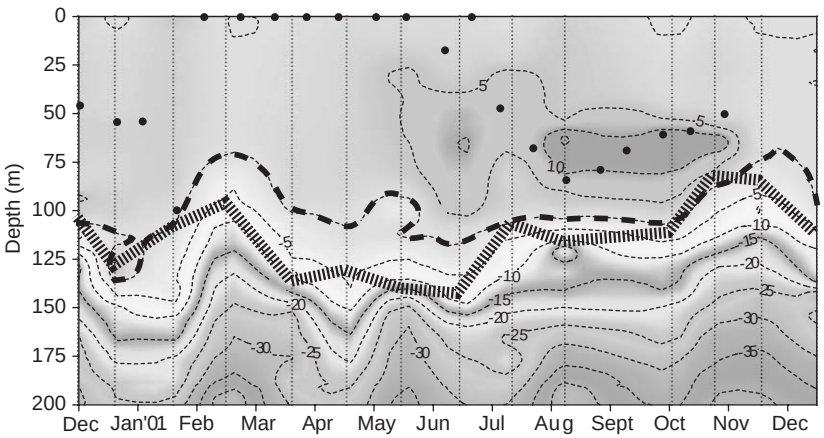


Fig. 2. Contour plot for excDO concentration predicted with depth at Station ALOHA over the annual time period from December 2000 to November 2001. excDO in a water sample is calculated from the difference between its measured DO concentration and the value for DO saturation predicted from its measured 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 were obtained from the 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 Fig. 1).

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 stable isotopic ($\delta^{15}N$) composition spanning a 10-year period. Results showed that export production from N_2 fixation is greatest when surface waters are most highly stratified (June–August), while more traditional, nitrate-supported export production maximizes in winter (February–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 2800 m sediment trap time series (Fig. 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 N_2-fixation phenomenon in summer. At this time of year, N_2-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 N_2-fixation, is made available subsequently by in situ trophic recycling of the N_2-fixing phytoplankton blooms (Hood et al., 2000; Montoya et al., 2004).

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 nitracline, 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 N_2 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 N_2 fixation is ultimately limited by phosphate availability in the stratified surface waters and, in the absence of a biological transport mechanism 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 2000–2001 relative to 1992–1993 sediment trap time series is environmentally quite real (Fig. 1). It reflects a climatic shift in oceanographic conditions at Station ALOHA and, like the N_2-fixation phenomenon, is prone to variability in its magnitude depending upon long-term trends in meteorologically dependent ocean mixing conditions in the NPSG.

4.3. *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 (Fig. 3). 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, Fig. 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 (Fig. 3C) and the excDO feature in summertime (Fig. 3D) with temperature estimates for cells actually growing at predicted alkenone export depths in these time periods (see filled circles, Fig. 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-estimated 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 ~1.4 °C overestimate (Table 3) of in situ temperatures throughout the euphotic zone (Fig. 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 °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).

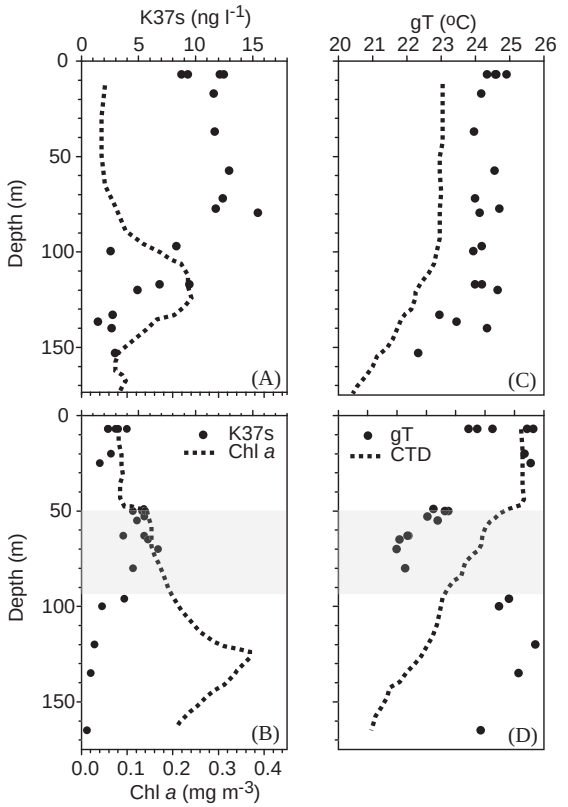


Fig. 3. Profiles for K37 concentration and $U^{K'}_{37}$ measured with depth throughout the euphotic zone at Station ALOHA in winter (February 2003) and summer (July 2001). For reference purposes, plots for K37 concentration in (A) winter and (B) summer have been annotated with the profile for Chl *a* concentration measured with 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 excDO concentration (see definition in legend for Fig. 2) were observed.

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 (Fig. 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., ~2–3 °C too 'cold'). Whereas in the DCML, $U^{K'}_{37}$-derived temperature over-estimate in situ temperatures (i.e., ~2–3 °C too 'warm'). To explain this summer pattern on purely ecological 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 consists 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 (Fig. 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 a nutrient and light availability (Epstein et al., 1998, 2001; 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 nitracline (Letelier et al., 2004).

4.4. Alkenone productivity in the euphotic zone

Results from our in situ ^{13}C-incubation experiments show that alkenone production rates are depth dependent (Fig. 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 (Fig. 3A and C), the depth of greatest ^{13}C-uptake in summertime is within the zone of excDO (Fig. 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 (Figs. 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 data sets. 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'

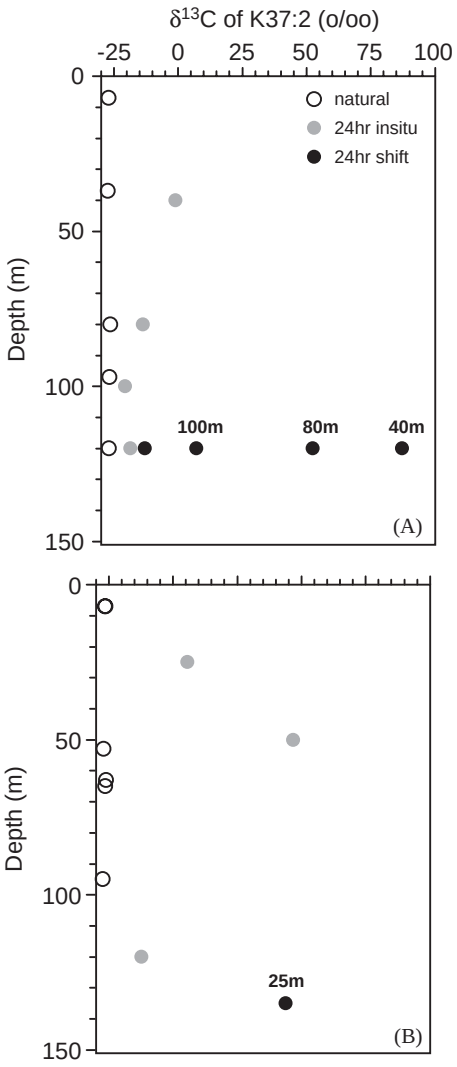


Fig. 4. Profiles illustrating depth dependence for ^{13}C-enrichment in the di-unsaturated C_{37} alkenone (K37:2) as determined by in situ incubation experiments. Grey filled circles show the effect on ^{13}C-uptake of incubating nutrient-replete samples from the DCML at shallower depths.

produced in surface waters that survives trophic metabolism in the water column, is transported via a sedimentation mechanism to the abyssal sea and ultimately may become 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 (Letelier et al., 2004) provides an obvious explanation for this response. If correct, then wintertime alkenone export events at Station ALOHA (Fig. 1) may be an ecological response to the thermally driven mixing dynamics of waters in the euphotic zone nearest the sea-surface. The response is tied not only to the seasonal shoaling behavior of the DCML (Karl et al., 2002) but also to the vertical motion of the DCML on much shorter, tidal time scales. 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 50 m 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 winter period based on cursory examination of sediment trap time series results (Fig. 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 nitracline, 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 (Fig. 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 data set is most conspicuous in 1992–1993, a period when climatic indices suggest meteorologically derived mixing energy in winter was appreciably greater than in 2000–2001 (Dore et al., 2002).

4.5. Sedimentation effect on alkenone unsaturation patterns

If alkenone export does originate from the excDO feature in summer, as suggested from our sediment trap analysis (Figs. 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 post-mortem alteration (Eglinton et al., 2001). 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 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 1992–1993 and 2000–2001 sediment trap time series by averaging data from monthly CTD casts. Comparison shows maSST was only slightly colder ($\sim$1 °C) in 1992–1993 (24.4 versus 23.2 °C) and essentially the same in 2000–2001 (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.

5. 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 $\sim 5\times$ greater in 1992–1993 than in 2000–2001. 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 2800 m 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–August), and more traditional, nitrate-supported export production which maximizes in winter (February–March) when surface mixing penetrates deepest.

Alkenone concentrations and production maxima 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 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.

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