Scientific Report of project number 2177 entitled:
Identification of long-chain alkenones as paleotemperature indicators in sediment
cores from the Aegean Sea and calibration of alkenone unsaturation ratios ($U^{k'}_{37}$)
for paleotemperature estimation in Eastern Mediterranean
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Purpose of the visit

The study aimed at reconstructing the variability of past sea-surface temperature (SST) in the Aegean Sea (Eastern Mediterranean) using the alkenone unsaturation index $U^{k'}_{37}$ as paleothermometer. The important differences of the paleotemperatures that occurred during the late last Glacial to Holocene were used to explore links between local climate changes with the global climate variability. Especially the study of the Holocene S_1 sapropel is critical to advance our understanding of the large scale climate interactions during past events of climate change in the Aegean Sea. We also identified molecular biogeochemical biomarkers in suspended material and surficial sediment in order to obtain valuable information about the sources and the alteration/ diagenetic processes affecting the organic matter in the recent marine environment.

Description of the work carried out during the visit

In summary, the major steps of the work carried out in the frame of the ESF grant are:
1) training in the analysis and interpretation of alkenones as past-SST proxies
2) Analysis of alkenones in the high-resolution core HCM2/22 collected in the Eastern Mediterranean (work completed)
3) Analysis of alkenones in present-day samples (core-top and particles); (work partially completed, see details below)
4) Extension of the work on core HCM2/22: analysis of lipid biomarkers (*n*-alkanes, *n*-alkanols, sterols, diols & keto-ols) as proxies for marine productivity and terrigenous inputs (work completed)
5) First synthesis and interpretation of data. Comparison with data from other cores in the Aegean Sea. Preparation of presentations in meetings

Core HCM2/22 collected with R/V Aegaeo from the Libyan Sea, south of Crete, from a depth of 2.211m. Alkenone analyses were carried out throughout the first 58cm of the core, representing ~20,000 years BP. Detailed SST reconstructions for organic-carbon-lean sections above and below the sapropel S_1 are hampered by low and sometimes none alkenone yields, especially for the $C_{37:3}$ compound that occurs at levels often under the detection limit. SST values obtained for these sections are not considered. In the frame of my Ph.D. thesis, we have already studied two other cores from north (152SL) and southeast (NS-14) Aegean Sea. The comparison of our results will

contribute to assess differences in the paleoceanographic changes along the Aegean Sea (north to south) during the last ~20,000 years and identify how the geographical and oceanographic settings have affected the variability of S_1 formation.

The present date samples include core top sediments and marine suspended particles from 12 stations along the eastern Mediterranean, collected during a cruise (M-71/3) of R/V Meteor in January 2007. Core top sediments (12 samples) extracted and separated into three fractions at the LOCEAN research laboratories during my visit there, while suspended particles (64 samples) have been extracted and separated at HCMR, in Greece. Alkenone analysis was performed in 8 core top sediment samples. Low amounts of diunsaturated and triunsaturated C_{37} methyl ketones ($C_{37:2}$ and $C_{37:3}$, respectively) were found. Thus, no reliable evaluation of the alkenone $U^{k'}_{37}$ index was possible. Similarly, preliminary investigations on alkenones in suspended particles showed very low amounts. A reassessment of the available data from core-tops and particles is in process.

In view of the $U^{k'}_{37}$–SST data obtained for core HCM2/22, we decided to extent the initial project, considering the high importance of combining the paleoclimatic SST data with data on proxies that provide information on paleoenvironmental conditions. The use of organic biogeochemical proxies of phytoplankton assemblages and terrigenous inputs would allow us to explore links between climate changes and biogeochemical functioning for paleo-environmental reconstruction. This work involved analysis of various marine (sterols, diols and keto-ols) and terrestrial (n-alkanes, n-alkanols) biomarkers. The analysis for the various marine and terrestrial biomarkers was performed on the sediment core HCM2/22, as well as in all present date samples (core top sediments and marine suspended particles).

Description of the main results obtained

Analyses in core top sediments and suspended particles gave us preliminary results in estimation of the alkenone unsaturation index ($U^{k'}_{37}$) in order to calibrate the SST for the recent environment. We are planning to finish the evaluation of alkenones in the remaining present date samples and then we will be able to present our results and complete our research. The extension of the initial project by the study of molecular biogeochemical biomarkers as proxies for changes in marine productivity, completed during my visit in Locean and the interpretation of our results is in progress.

Detailed molecular investigations have been performed on the high resolution sediment core HCM2/22, providing information on the SST fluctuations and on the supply of marine and terrestrial origin organic matter during the last 20,000 years.

We were not able to reconstruct past sea-surface temperature in core HCM2/22 during the last glacial and deglaciation periods due very low and sometimes none alkenone yields. In figure 1, we present our SST findings in HCM2/22 along with two cores (152SL and NS-14) from the southeast and north Aegean Sea respectively, that are part of our previous research. In core HCM2/22 we observe important variability in temperature over the sapropel S_1 deposition (~10 to 6 kyrs cal. BP), reflecting fluctuations in physical, biogeochemical and climatic conditions in the Eastern Mediterranean Sea during that period. At the Holocene climatic optimum, SST increases and reaches values as high as 20.3°C and 23°C indicating the onset of a warm period. Our records also shows a pronounced centennial-scale cooling that culminates around

~8.3 to 7.6 kyrs cal. BP, causing an interruption in the deposition of S_1. The temperature drop is ca. 3.3°C which falls within the 2-4°C range of winter SST shifts previously inferred in Eastern Mediterranean Sea records during the S_1 interruptions (Rohling *et al.* 2002). During the formation of the sublayer S_1b SST increases again and is characterized by slightly higher values compared to S_1a.

The *n*-alkanes and *n*-alkanols distribution patterns are characteristics of cuticular waxes of higher terrestrial plant inputs. Terrestrial biomarkers in core HCM2/22 from Libyan Sea exhibit higher concentrations and accumulation rates within the sapropelic layer (Fig. 2) suggesting increased land runoff compare to the intervals above and below its deposition (Aksu *et al.* 1995; Bouloubassi *et al.* 1998). The increased fluvial inputs related with changes in climatic conditions in which the Eastern Mediterranean became warmer and more humid into the Holocene Climatic Optimum (Rossignol-Strick 1985; Rohling 1999). S_1 interruption (~8 kyrs cal. BP) is characterized by low terrestrial inputs, indicating decrease in riverine inputs during this time, indicating cold and arid climatic conditions during that time (Casford *et al.* 2003; Gogou *et al.* 2007).

Marine sterols that we identified are major constituents of several marine zooplankton and phytoplankton (diatoms and dinoflagellates) groups (Volkman *et al.* 1999; Bouloubassi *et al.* 1999). The vertical distributions of the marine sterols exhibit low concentrations before the S_1 deposition, reflecting low productivity/ high diagenesis of biomarkers under well oxygenated conditions (Fig. 3). Within S_1 significantly larger proportions of these compounds, with maxima at the S_1a period, indicating higher productivity and/or preservation of organic matter, typical of a productive marine system (Bouloubassi *et al.* 1998; Menzel *et al.* 2003; Gogou *et al.* 2007). An important decline in the abundances of all marine biomarkers recorded in the cool interval of S_1 interruption compared to the sapropelic layers that can be related either to fluctuation in the supply of organic matter in our sampling site and/or lower preservation of organic material due to bursts of ventilation to greater depths at this time.
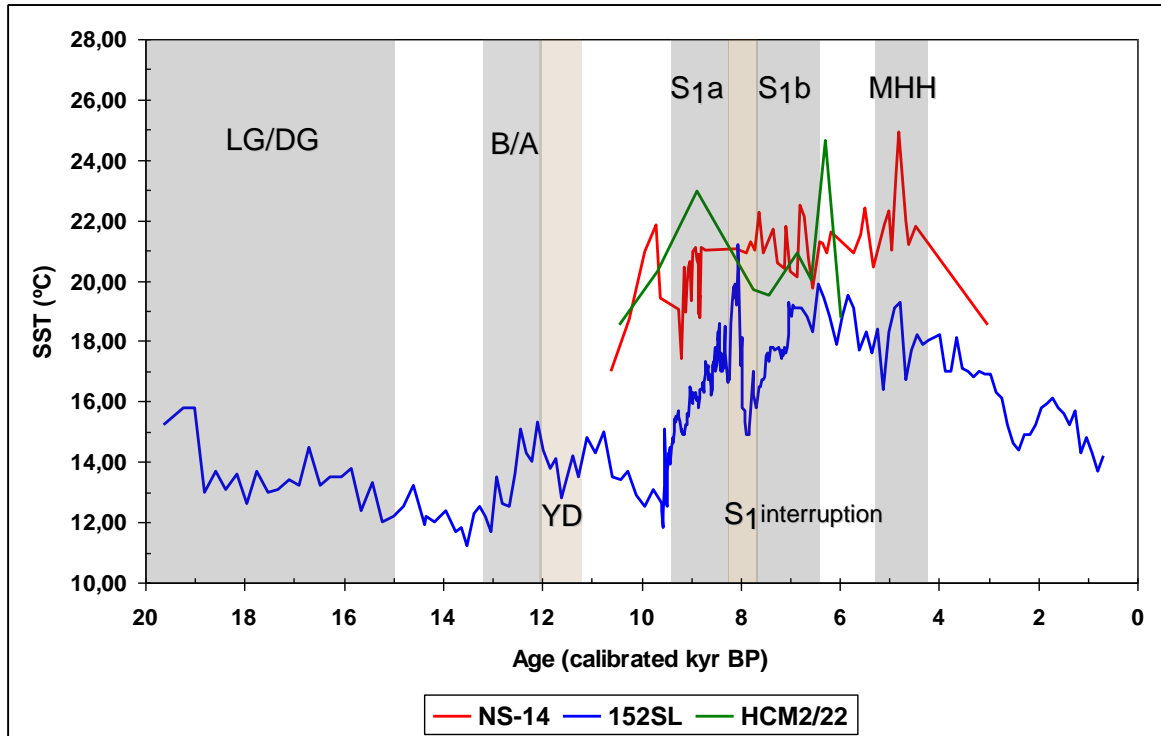
Fig. 1 Alkenone-derived sea surface paleotemperature record in core HCM2/22 (green line), compare with SST record in cores NS-14 and 152SL (red and blue line respectively) from the southeast and north Aegean Sea respectively.
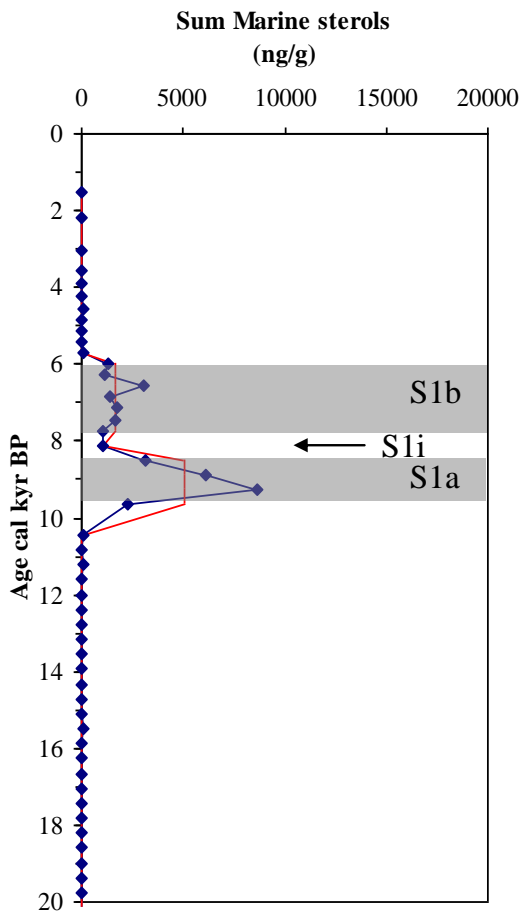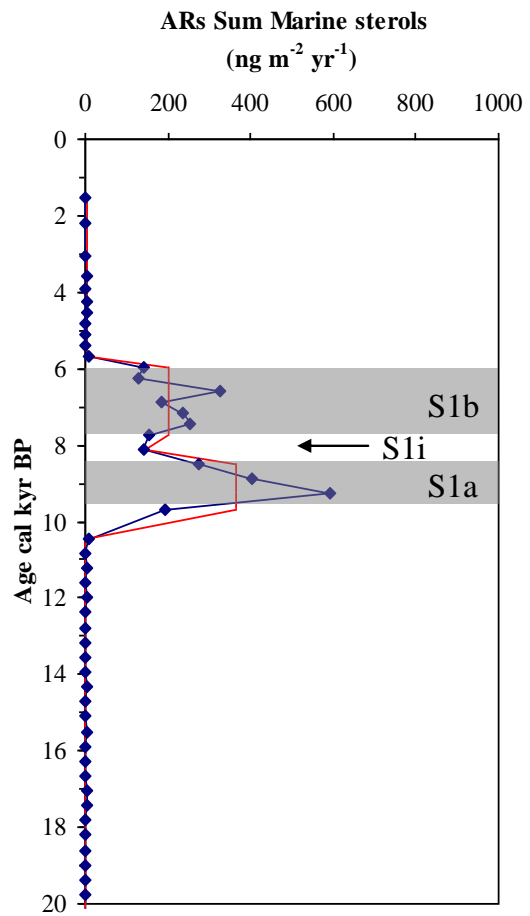
HCM 2/22

HCM 2/22

**Ter *n*-alkanes
(ng/g)**

**ARs Ter n-alkanes
(ng m^{-2} yr^{-1})**
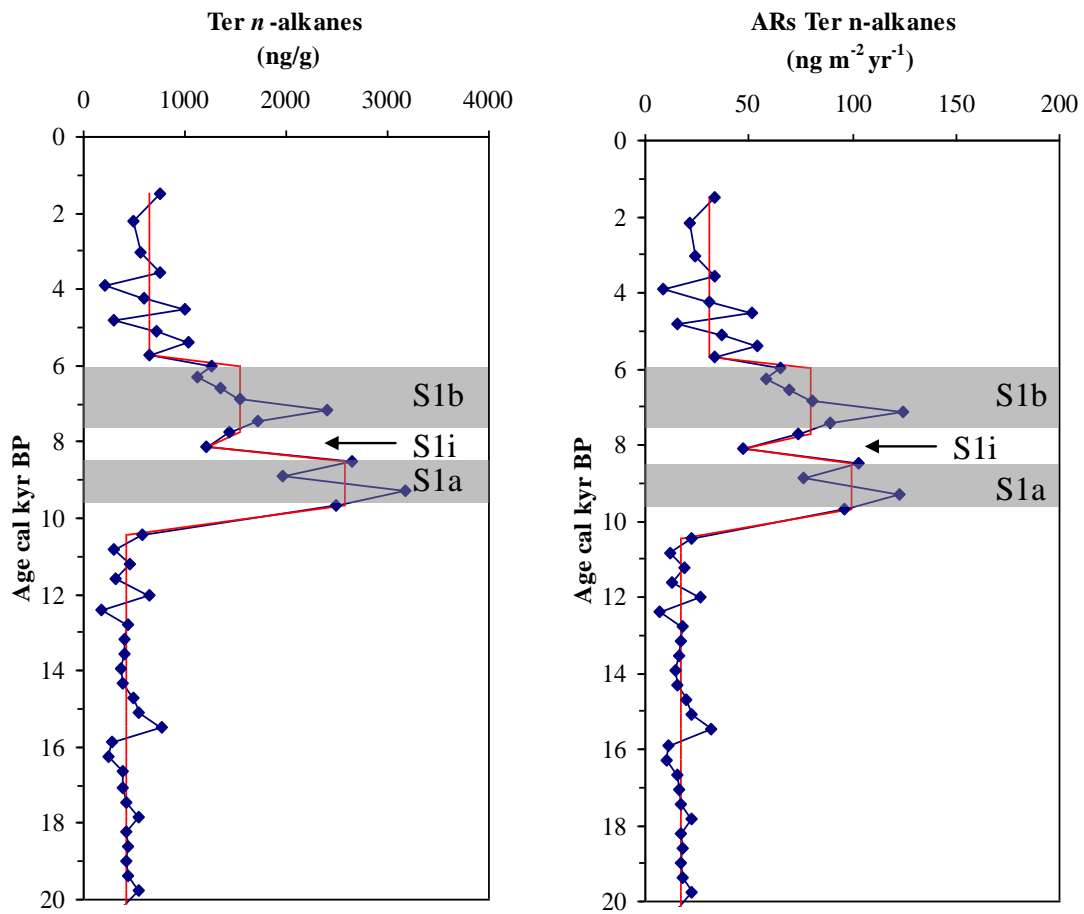
Fig. 2 Vertical distribution of concentration (ng/g) and Accumulation rates (ng m^{-2} yr^{-1}) of terrigenous *n*-alkanes the last 20,000 years in core HCM2/22

HCM 2/22

HCM 2/22

Sum Marine sterols
(ng/g)

ARs Sum Marine sterols
(ng m^{-2} yr^{-1})

Fig. 3 Vertical distribution of concentration (ng/g) and Accumulation rates (ng m^{-2} yr^{-1}) of marine-derived sterols the last 20,000 years in core HCM2/22

On-going collaboration with host institution

The collaboration between the host institute LOCEAN in Paris and the institute of Oceanography of the Hellenic Center of Marine Research (HCMR) is continuing. The main focus is on the interpretation and synthesis of the data, which form part of my PhD Thesis and on the writing of scientific papers.

Presentations of the data – projected publications

We have presented our findings in core HCM2/22 along with the already available data in two cores (152SL and NS-14) from the Aegean Sea in the following meetings:

1. KATSOURAS G., GOGOU A., BOULOUBASSI I., EMEIS K.-C., TRIANTAPHYLLOU M.V., LYKOUSIS V., 2009. A biomarker study of high resolution sedimentary records in the eastern Mediterranean Sea since the last glacial maximum. Oral presentation in the EGU General Assembly 2009. Vienna, Austria, 19-24 April. Geophysical Research Abstracts, Vol. 11, EGU-2009-10852-3

2. KATSOURAS G., GOGOU A., BOULOUBASSI I., EMEIS K.-C., TRIANTAPHYLLOU M.V., SYMSARIS E., LYKOUSIS V., 2009. Paleoceanographic study of climate variability in the north-eastern Mediterranean during the last 20 kyrs: a biogeochemical and stable isotopes approach. 9o Hellenic Symposium in Oceanography & Fishery, Patra, Greece, 13-16 May, Program and Abstracts, page 79.

A paper dealing with the climate sensitivity of eastern Mediterranean using paleotemperatures and biogeochemical proxies will be prepared after the data synthesis and interpretation are completed. A second one about the SST alkenone-derived calibration and the productivity patterns in recent environment of eastern Mediterranean will be also prepared in the near future.

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ANNEXE

Analytical Protocol

Lipids biomarkers were extracted with a mixture of dichlomethane/methanol. The extracts were separated into different compound classes by column chromatography using silica gel that had been activated for 1 hour at 150°C. We used the following solvent systems to isolate different compound classes: (1) n-hexane (aliphatic hydrocarbons), (2) dichlomethane/n-hexane, 4:6 (carbonyl compounds such as alkenones), (3) ethylacetate/ n-hexane, 5:10 (alcohols and sterols).

Alkenones were identified and quantified by gas chromatography using flame ionization detection (GC-FID), whereas aliphatic hydrocarbons, alcohols and sterols were identified and quantified by gas chromatography coupled to mass spectrometry (GC-MS). The sterols/alcohols were silylated using BSTFA prior to analysis. The GC-FID analyses were performed on a 6890 Hewlett Packard gas chromatograph equipped with a Saphir-SRA data system, an on-column injector, and a fused silica capillary column (CPSil-5, 50m×0.32 mm×0.45 mm). Helium was used as the carrier gas with a backpressure of 1atm. GC-MS analyses were carried out on a 5973N Hewlett Packard Mass Selective Detector with the appropriate data system. A 6890 Hewlett Packard gas chromatograph equipped with an on-column injector was directly coupled with the fused silica capillary column (DB-5 MS, 30m×0.249 mm×0.25 mm) to the ion source. The temperature programs for different compounds class were as follows: (a) for n-alkanes, from 60 (2 min) to 80 °C at 25 °C/min, from 80 to 300 at 5 °C/min and 300 °C (15min), (b) for the long chain alkenones, from 50 °C (1min) to 140 °C at 30 °C/min, from 140 to 280 at 15 °C/min, from 280 to 310 at 0,5 °C/min and 310 °C (80min), (c) for n-alkanols and sterols, from 60 (2 min) to 80 °C at 25 °C/min, from 80 to 300 at 3 °C/min and 300 °C (30min). The individual lipid components were identified by a combination of comparison of GC-retention times to authentic standards and comparison of their mass spectral data to those in the literature. Quantification was based on the GC-FID and GC-MS response and achieved by comparison of peak areas with those of known quantities of standards (*n*-$C_{24}D_{50}$ for *n*-alkanes, *n*-$C_{21}H_{43}OH$ for *n*-alkanols, 5a-androstanol-3H-ol for sterols and *n*-$C_{36}H_{74}$ for long chain alkenones).

Estimates of past sea-surface temperature (SST) were made using the unsaturation ratios of alkenones ($U^{k'}_{37}$). The alkenone SST proxy expressed in terms of the relative proportions of diunsaturated and triunsaturated C_{37} methyl ketones ($C_{37:2}$ and $C_{37:3}$, respectively):

$$U^{k'}_{37} = [C_{37:2}]/[C_{37:2} + C_{37:3}]$$

The $U^{k'}_{37}$ index was convert into SST using the global calibration given by Müller *et al.* 1998:

$$SST (°C) = (U^{k'}_{37} - 0.0044) / 0.033$$