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Faculteit Aardwetenschappen
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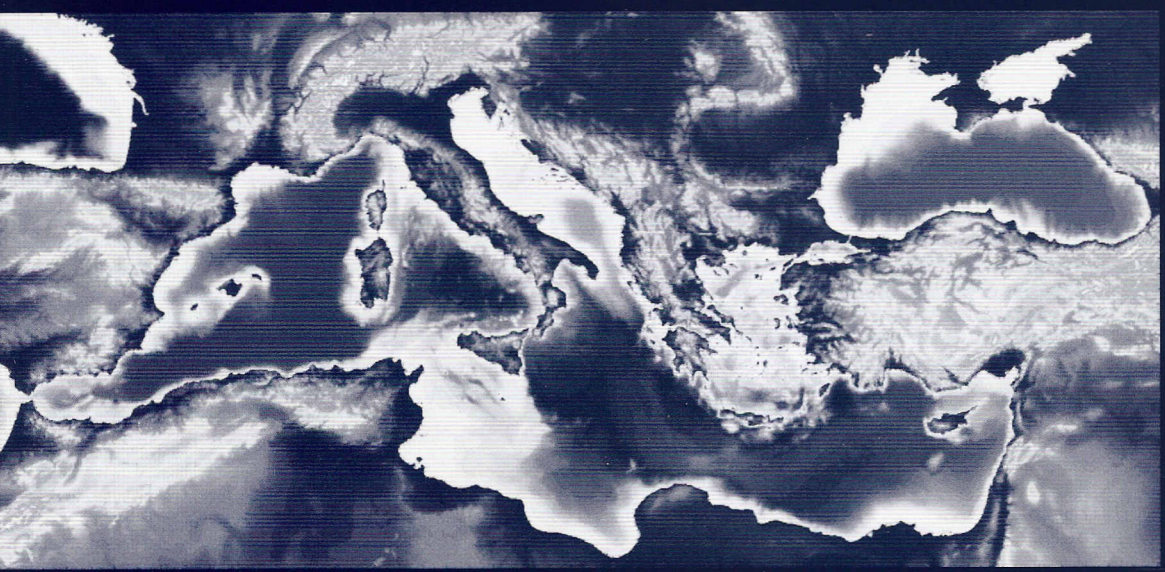

Diana Menzel

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Organic geochemical reconstruction of palaeo-environmental conditions during the deposition of Pliocene sapropels in the eastern Mediterranean Sea

Organisch geochemische reconstructie van palaeomilieu condities tijdens de afzetting van Pliocene sapropelen in de oostelijke Middellandse Zee

(met een samenvatting in het Nederlands)

Organisch geochemische Rekonstruktion friiherer Umweltbedingungen wahrend der Ablagerung von Pliocene Sapropelen im 6stlichen Mittelmeer

(mit einer Zusammenfassung in deutscher Sprache)

Proefschrift

ter verkrijging van de graad van doctor
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op gezag van de Rector Magnificus, Prof. Dr. W.H. Gispen,
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Amsterdam, The Netherlands

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Für meinen Vater

Table of contents

Chapter 1

General introduction

The modern Mediterranean Sea: climate and general circulation

The Mediterranean is a region of transition between the sub-oceanic, cool temperate zone of Europe, and the high-pressure, arid subtropics of North Africa straddling the pressure systems of the Atlantic and the monsoon climates of the Indian subcontinent and East Africa. The Mediterranean Sea is a semi-enclosed sea and an almost isolated oceanic system. The Mediterranean climate is basically characterized by hot dry summers and mild wet winters, accompanied by strong winds (Allen, 2001). Today, evaporation in the eastern basin exceeds precipitation and river-runoff. The negative water balance is compensated by the inflow of Atlantic waters through the Straits of Gibraltar. Atlantic surface waters flowing eastwards through the Mediterranean are warmed by solar radiation and the resulting evaporation causes a steady increase in salinity and density. As a result, deep water formation and therefore oxygenation in the Mediterranean takes place in the Gulf of Lion (northwestern basin) and in the Adriatic and Aegean Sea (northeastern basin). This occurs when the surface water cooling during winter results in highest densities. In the easternmost Mediterranean the high salinities combined with the winter cooling results in the formation of Levantine Intermediate Water (LIW) between (150/200 m) and 600 m, which flows westwards into the western Mediterranean and ultimately leaves through the Straits of Gibraltar (Wiist, 1961) (Fig. 1.1).

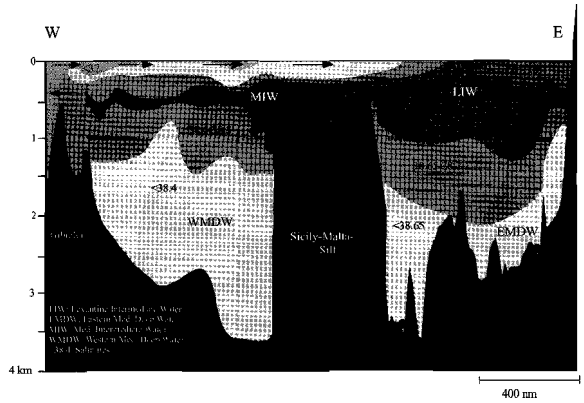

Figure 1.1 Hydrographic section through the Mediterranean Sea (after Wiist, 1961 and Vergnaud-Grazzini *et aI.,* 1977) giving the major water masses and their salinities. MIW = Mediterranean Intermediate Water, LIW = Levantine Intermediate Water, EMDW = Eastern Mediterranean Deep Water, WMDW = Western Mediterranean Deep Water.

The formation of the Eastern Mediterranean Deep Water (EMDW), originating from the Adriatic and Aegean Sea, the Western Mediterranean Deep Water (WMDW), originating

from the Gulf of Lion, and primarily Levantine Intermediate Water (UW) ensures that the basins remain well ventilated. This anti-estuarine circulation pattern makes the Mediterranean a nutrient desert: it receives nutrient-depleted surface waters and the nutrients received from rivers to the Atlantic are exported with its deeper water. This has a strong negative influence on the biological productivity in the Mediterranean resulting in deposition of sediments poor in organic matter (OM) «0.5% organic carbon) (Fig. 1.2).

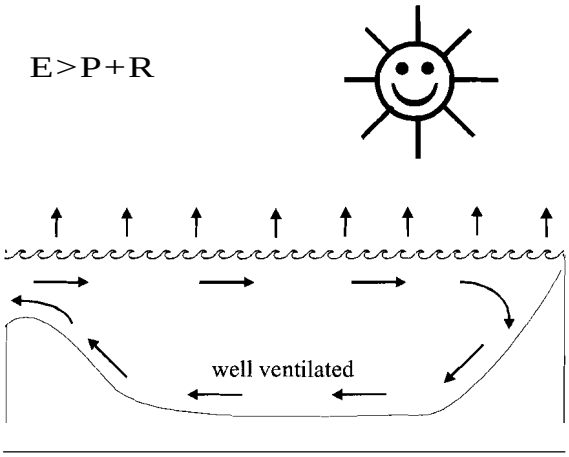

Figure 1.2 Anti-estuarine circulation pattern of the modern Mediterranean Sea. E = evaporation, P = precipitation, R = river runoff.

Climate change **in** the Mediterranean region and its impact on hydrography

Eccentricity, obliquity and precession influence the incoming solar radiation to the Earth and are thus important factors affecting global climate. Together they are responsible for 'Milankovitch cycles' (Fig. 1.3).

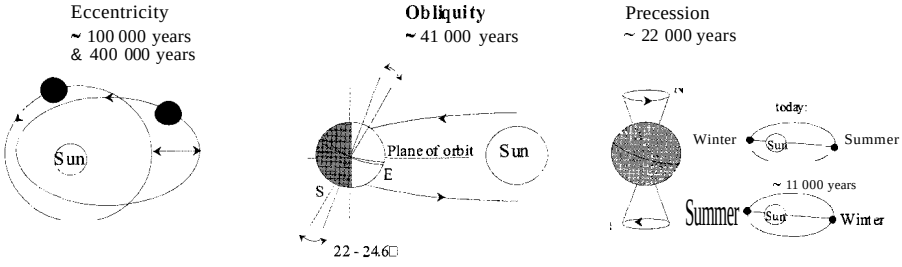

Figure 1.3 The Milankovitch cycles which influence global climate: Eccentricity, Obliquity and Precession.

The *eccentricity* cycle is caused by valiations in the shape of the earth's orbit around the sun. The shape of the Earth's orbit changes between maximum eccentricity (strong ellipse) and minimum eccentricity (near a circular orbit) with periods of 100,000 and 400,000 years. The gradual change in the angle of the Earth's rotation axis relative to the perpendicular of the plane of the Earth's orbit is called *obliquity.* This angle changes from 22.5 to 24.5 degrees and back over a period of 41,000 years. The obliquity cycle predominantly affects the distribution of sunlight between the high polar latitudes and the tropics. The *precession* cycle is related to the wobble in the Earth's rotational axis relative to the plane of the Earth's orbit around the sun. The precession cycle manifests itself in the insolation onto the Earth's surface in a period of 21,000 years governing the seasonal distribution of insolation. its impact depends on the degree of eccentricity of the orbit. Whereas in a perfect circular orbit the precession cycle would have no impact, maximum eccentricity results in maximum impact of the precession cycle. During precession minima, perihelion (i.e. when the Earth is closest to the sun) falls within Northern Hemisphere summer causing maximum insolation. Consequently, aphelion (i.e. when the Earth is farthest from the sun) falls in winter, reducing insolation. This results in the seasonal insolation contrast to be considerably higher during a precession minimum.

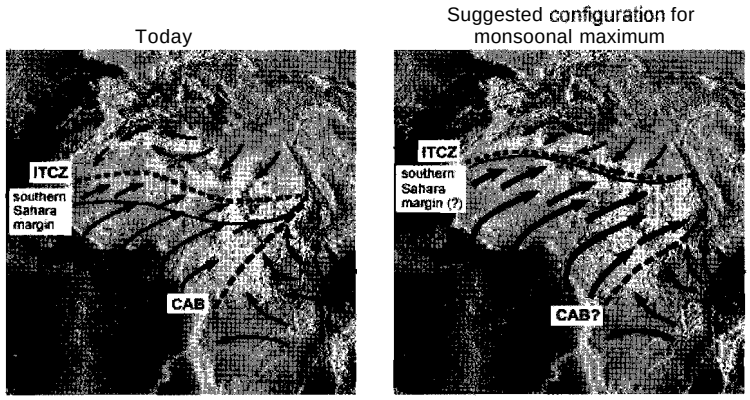

Figure 1.4 Rough location of the Intertropical Convergence Zone (ITCZ), the Congo Air Boundary (CAB), and the southern margin of the Sahara Dessert, for the present-day and in an artist's interpretation for the monsoonal maximum for the time around the boreal summer, when the sun reaches its northernmost position (www.soes.soton.ac.uk/staff/ejr/ejrhome.htm).

Higher summer insolation enhances atmospheric pressure difference between the land and sea by the large temperature contrast between the relatively cold ocean and the relatively warm continent. As a consequence, the monsoon inflow into low-latitude Africa, which has a westerly component is amplified and this results in enhanced transport of moisture from the Atlantic to the African continent. In addition, the Sahara interior heats up faster leading to a northwards shift of the Intertropical Convergence Zone (ITCZ). In addition, a periodical northwards penetration of the African summer monsoon front (northern boundary is

represented by the ITCZ) into the north and central Saharan watershed irrespective to the extent of northwards ITCZ penetration, is related to eccentricity-, obliquity- and precession controlled insolation maxima causing enhanced precipitation rates over the northern part of the African continent (Rohling *et al.,* 2002; Larrasoana *et aI.,* 2003) (Fig. 1.4). The substantially increased rainfall results in enhanced soil moisture and vegetation cover that inhibit dust production and increase the delivery of fresh water into the Mediterranean Sea. The river Nile is responsible for the largest part of the increased run-off from the North African continent affecting in particular the easternmost part of the Mediterranean Sea (e.g. Adamson *et al.,* 1980; Rossignol-Strick *et aI.,* 1982; Rossignol-Strick, 1985; Rohling, 1991, 1994). However, at many places on the North-African continent, large lakes developed and impressive fossil river systems of the northern African continent also playing an important role in freshwater discharges into the Mediterranean during times of organic carbon rich sediments (Rohling *et al.,* 2002; Larrasoana *et al., 2003).* This enhanced freshwater supply influenced the Mediterranean oceanography to a large extent. The more humid climate conditions inhibited the salinity-dependent formation of LIW in the Cyprus-Rhodes area. Under these conditions deep-water formation relied on winter cooling only. Because ofthe reduced salinity/density of newly formed deep water, this water could no longer ventilate the deeper parts of the basin (e.g. Cita *et al.,* 1977, 1982; Calvert, 1983, Mangini and Schlosser, 1983). As a result, the eastern Mediterranean became 'stagnant' downwards from 300/400 m depth. After some time bottom waters became oxygen-depleted and OM-rich sediments ("sapropels") were deposited (Fig. 1.5).

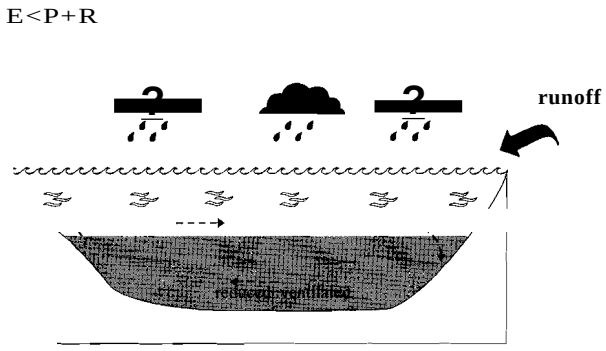

Figure 1.5 Illustration of the sluggish anti-estuarine circulation pattern during sapropel deposition in the eastern Mediterranean Basin. E = evaporation, P = precipitation, R = river runoff.

Sapropel formation

Sapropels are defined as discrete layers, greater than I cm in thickness, set in open pelagic sediment and containing greater than 2% organic carbon by weight (Kidd *et al.,* 1978). They contain more than average amounts of pyrite and benthic fauna indicative oflow bottom water oxygen concentrations (sometimes benthic fauna is completely absent) and

notably high concentrations of elements such as Sand Ba (e.g. Kullenberg, 1952; Olausson, 1961; Vergnaud-Grazzini *et aI.,* 1977). Initially, in 1947/48 sapropels were recovered in abundance mainly from the eastern Mediterranean Sea in 1947/48 (Kullenberg, 1952). These showed a prominent cyclic bedding characterized by brown to blackish coloured organic-rich sapropels from the Neogene to the Quaternary (e.g. Cita *et al.,* 1982; Vergnaud-Grazzini, 1985; Rohling, 1994). Drilling of deep-water post Messinian sedimentary sequences in the Mediterranean Sea has shown the occurrence of organic-rich sapropels and sapropel-like sediments extending from the Levantine Basin westwards into the Alboran basin (Rullkotter *et al.,* 1995, Murat, 1999). The large differences in organic carbon content of sapropels recovered in the eastern and western Mediterranean Sea can be explained by ventilation of oxygen-rich Atlantic waters via the Straits of Gibraltar into the western Mediterranean basin. In the eastern Mediterranean Sea and the Tyrrhenian Sea (easternmost part of the western Mediterranean Sea), sapropel deposition began in the early Pliocene (e.g. Olausson, 1961; Kidd *et aI.,* 1978; Emeis *et al.,* 1991). In the Alboran Sea (westernmost Mediterranean Sea) and therefore basin-wide onset of sapropel formation occurred later, in the early Pleistocene (Bouloubassi *et al.,* 1999). Very organic-rich sapropels were deposited during the middle Pliocene, and sapropel formation became less frequent with the onset of glaciations in the Northern Hemisphere (ca. 2.4 Ma). These sapropels layers range in total organic carbon content between 2-30% TOC (Emeis *et aI.,* 1996) and are intercalated by homogeneous calcareous ooze containing < 0.1 % TOC (e.g. Calvert, 1983; Calvert and Pedersen, 1992).

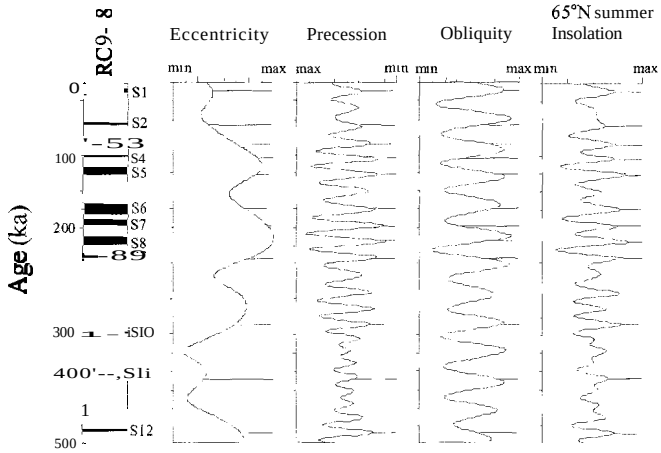

Figure 1.6 Astronomical calibration of late Pleistocene sapropels to astronomical curves of eccentricity, obliquity, precession and isolation (65°N summer insolation) (from RiIgen, 1991).

Integrated stratigraphic studies have convincingly demonstrated that these sedimentary cycles reflect astronomically induced variations in the regional Mediterranean climate. These cycles are controlled by wet-dry oscillations, whereby the sapropels are related to precession minima, i.e. insolation maxima (Fig. 1.6) (e.g. Rossignol-Strick *et al.,* 1982; Rossignol-Strick, 1985; Prell and Kutzbach, 1987; Hilgen, 1991; Rohling, 1991).

These resulted in a relatively humid part of the climate cycle as indicated by lower aeolian (Saharan) dust supply, Le. decreased Ti/Al ratios (e.g. Nijenhuis and de Lange, 2000; Wehausen and Brumsack, 2000). Further evidence is provided from changes in clay minerals, which showed cyclic variations between two groups of clay minerals. Palyorskite and kaolinite showed maximum abundance at times of aridity on the African continent, enabling them to be transported as wind-blow dust. Smectite dominates at periods of abundant rainfall, which coincides with sapropel formation (e.g. Foucault and Melieres, 2000). Enhanced fresh water input would have diluted surface waters leading to a more sluggish (but not necessarily ceased) circulation of the intermediate (Mediterranean Intermediate Water) and deeper waters associated with high amounts of nutrients into the Mediterranean Basin (Boyle and Lea, 1989).

Based on the above, two models have been proposed to explain the enrichment in organic carbon during sapropel events. In general, two types of models have been proposed or the combination of both: (1) a Stagnation Model that explains the elevated TOC levels of sapropels by enhanced OM preservation under oxygen-deficient deep-water conditions (e.g. Demaison and Moore, 1980); (2) a Productivity Model that links the occurrence of sapropels to increased marine biological production in the photic zone (e.g. Calvert, 1983, Pedersen and Calvert, 1990, Calvert and Pedersen, 1992). The Stagnation Model explains anoxic bottom conditions by increasing density stratification of the water column caused by a strong low-salinity surface layer which ultimately inhibits convective overturning and ventilation of the deeper water column (e.g. Shaw and Evans, 1984). A primary cause of sapropel deposition resulting from increase in biological productivity within the photic zone as evidenced by the recognition of OM fluxes to the sea floor in today's eastern Mediterranean Sea, would not be sufficient to produce sapropels, even if extensive water column stratification and complete bottom-water anoxia would occur (e.g. Calvert, 1983; Lourens *et at.*, 1992; van Os *et at.*, 1994). A third model combining both, increased productivity and decreased deep-water formation has also been proposed (Rohling and Gieskes, 1989, Rohling, 1991). In this model, shoaling of the pycnocline to a depth within the euphotic layer would lead to increased nutrient availability to the lower euphotic layer. This would enable the development of a deep chlorophyll maximum below the pycnocline and a concomitant increase in the downwards flux of OM from the euphotic zone (export production). According to this model, shoaling of the pycnocline could have occurred in response to an enhanced freshwater flux to the surface layer of the Mediterranean in conjunction with a continuous anti-estuarine circulation pattern.

Biomarkers as palaeoenvironmental proxies

Depending on the depositional conditions in the environment only 0.1 to 1% of biologically derived OM is preserved in most present-day oceanic settings (Calvert and Pedersen, 1992). The composition of sedimentary OM is substantially different from its precursor, biomass, because major differences exist in the rate of biodegradation between biomolecules. Bacteria mineralize polysaccharides and proteins, which represent the bulk of

biomass in most organisms, very efficiently during diagenetic processes and are thus selectively removed during transport through the water column or in top layers of sediments. As a consequence, other biochemicals, such as certain low- and high-molecular weight compounds occurring in cell walls and membranes, and aromatic biomacromolecules, such as lignin, are selectively preserved and thus enhanced in sedimentary OM (Tegelaar *et ai.*, 1989).

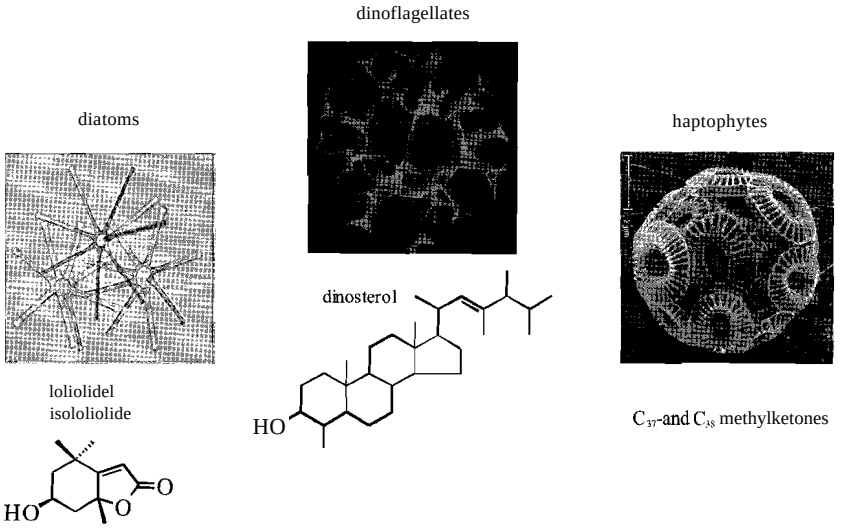

Figure 1.7 Lipid biomarker of different marine phytoplankton classes.

Organic compounds whose basic carbon skeleton suggests an unambiguous link with known, contemporary biochemicals are defined as "biological markers" or "biomarkers" although terms as "molecular fossils" are also used (Eglinton *et al.,* 1964; Eglinton and Calvin, 1967; Mackenzie, 1984) (see Fig. 1.7 for some typical examples). Molecular fossils, such as lipid biomarkers, in sediments can provide information on the presence of specific organisms and, therefore, supply valuable insights in palaeoenvironmental conditions during deposition (e.g. de Leeuw *et al.,* 1995). In Pliocene/Pleistocene sapropels of the Mediterranean Sea different lipid biomarker studies were applied to reconstruct sea surface temperature (SST). These studies used the $U_{37}^{K'}$ index of the di- and tri-unsaturated C_{37} methylketones (e.g. Emeis *et al.,* 1998; Rinna *et al.,* 2002; Bouloubassi *et al.,* 1999) derived from marine haptophyte algae (Brassell *et al.,* 1986; Volkman *et al.,* 1980; Marlowe *et al.,* 1984a; Marlowe *et al.,* I984b). Another biomarker is Isorenieratene. This is a specific aromatic carotenoid of anaerobic, photolithotrophic green sulphur bacteria requiring sulfide and light. Thus, this biomarker can indicate the shallowing of the chemocline into the photic zone (e.g. Repeta, 1993, Sinninghe Damste *et al.,* 1993a) (Fig. 1.8). The presence of diagenetic derivatives of isorenieratene in Pliocene sapropels revealed the first evidence of basin-wide water column euxinia over substantial periods in the eastern Mediterranean Sea (Passier *et al.,* 1999a). Other biomarkers, specific for marine algae, were analysed to indicate

the floral diversity during deposition of Pliocene sapropels (e.g. Bosch *et aI., 1998;* Bouloubassi *et aI.,* 1999; Rinna *et al.,* 2002). For example, haptophyte-derived long-chain methylketones and C_{28} - C_{32} alkanediols- and keto-ols, reflecting eustigmatophytes were reported (Volkman *et aI.,* 1992). Loliolides and isololiodes that are anoxic degradation products of fucoxanthin, the major carotenoid found in diatoms (Klok *et aI.,* 1984) and to some extent in dinoflagellates and haptophytes (Jeffrey and Vesk, 1997), and dinosterol, reflecting dinoflagellates, also occurr in sapropels. Changes in terrestrial input during sapropel deposition were studied using long-chain fatty acids, alcohols and alkanes (Rinna *et aI.,* 2002), biomarkers derived from higher land plant cuticular waxes (e.g. Eglinton and Hamilton, 1967).

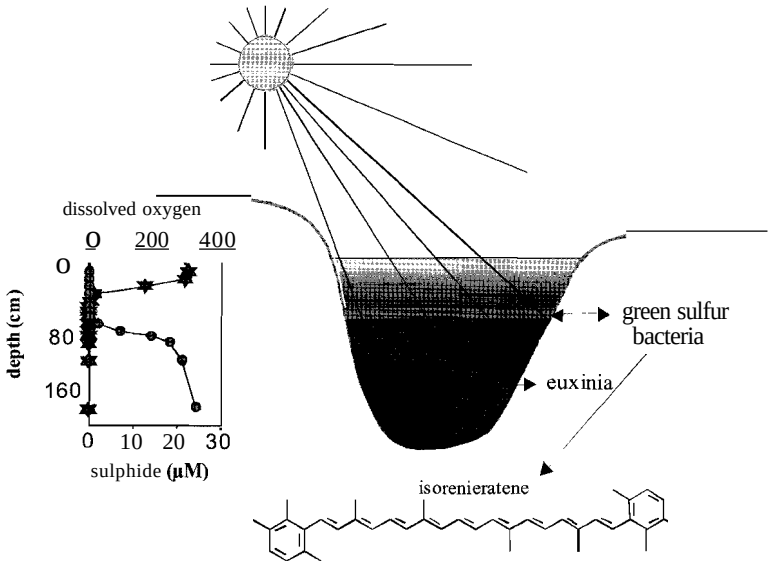

Figure 1.8 Illustration of photic zone euxinia, i.e. shoaling of the chemocline up to the photic zone of the water column.

Scope and framework of this thesis

Ocean Drilling Program (ODP) Leg 160 covered a transect of drill sites in the eastern Mediterranean Sea during March-April 1995 to investigate the tectonic and palaeoceanographic history of the area. Astronomical tuning of precession-controlled sapropel cycles recognized in the land-based sections Vrica and Singa (Italy), correspond chronically with the marine sedimentary records (Lourens *et aI.,* 1996). Sapropels recovered from three eastern Mediterranean Sea drill sites [ODP Sites 964D-lOH-l (110-103 cm), 969E-6H-6 (40-26 cm) and 967C-8H-4 (130-114 cm) (Fig. 1.9)] could unambiguously be correlated to each other and to a sapropel event of 2.943 Ma in the land-based Vrica and Singa sections (Lourens *et al.,* 1996). These late Pliocene sapropels are particularly suitable

for organic geochemical studies based on their extraordinarily high amounts of OM (C_{org}>26% TOC; Emeis *et al.,* 1996). Furthermore, OM removal from the top of a sapropel by oxic degradation, called "burning down", which can be recognized by a sharp and sudden instead of a gradual decrease in C_{org} content and has been observed in the S1 sapropel (ca. 9000-5000 BP) (Higgs *et al.,* 1994), is absent in these sapropel layers. Thus, these three time-equivalent sapropels and their corresponding homogeneous calcareous ooze (above and below the sapropels) from the late Pliocene spanning an east-west transect through the entire eastern Mediterranean basin, make an ideal section for an extensive study of the changes in organic geochemistry in relation to the palaeoenvironmental climate reconstruction during Pliocene sapropel formation. To this end, different organic geochemical tools were applied in several chapters of this thesis, including gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS), high performance liquid chromatography/mass spectrometry (HPLC/MS), isotope-ratio-monitoring gas chromatography-mass spectrometry (irm-GC/MS), Curie-point pyrolysis - GC/MS, Rock-Eval pyrolysis and palynology.

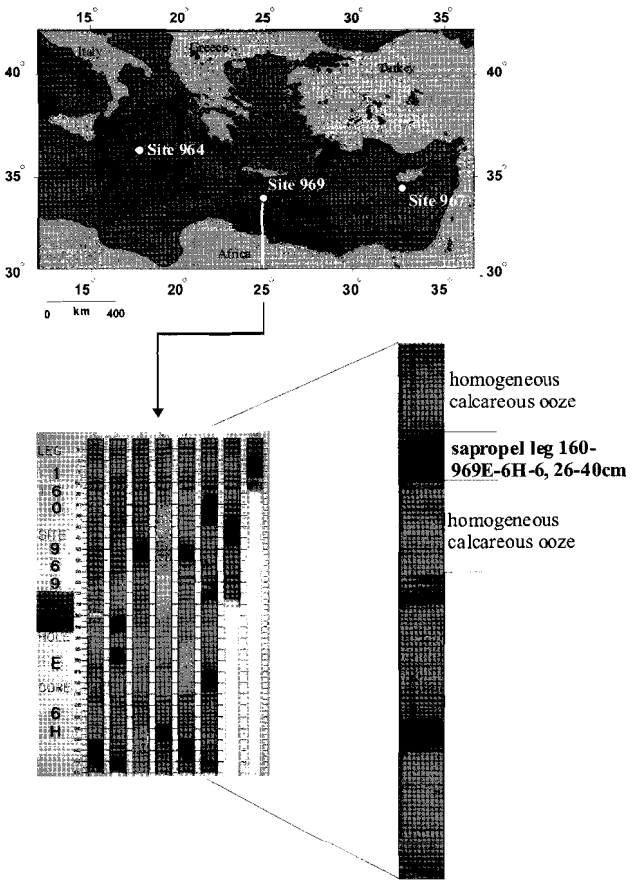

Figure 1.9 ODP location sites and sediment core ofODP Site 969, showing sapropels intercalated by homogeneous calcareous oozes.

Chapter 2 reveals that photic zone euxinia (i.e. the presence of free sulfide present in the photic zone, typically shallower than 100 m) occurred widespread over the entire eastern Mediterranean basin during Pliocene sapropel deposition. This finding is based on the presence of isorenieratene, a carotenoid biosynthesized by anaerobic, photolithotrophic-living Chlorobiaceae, in the sapropels. This carotenoid first appeared at Site 967, closest located to the river Nile and extended, with higher abundances, towards the western Site 964. This might be related to the input of fresh water and associated nutrients from the river Nile, triggering primary productivity in the Levantine basin. The subsequently enhanced export production and thus oxygen depletion in the deep water resulted in widespread anoxia. Spatial and temporal variations in isorenieratene abundances probably reflected fluctuations of the chemocline in the water column during sapropel formation. In **Chapter** 3, an "unknown purple carotenoid" is studied which is also abundant in these Pliocene sapropels. Accumulation rates of this "unknown purple carotenoid" showed a similar profile as found for isorenieratene. Two different candidates of carotenoids are considered (i) spheroidenone, a carotenoid related to various purple non-sulphur bacteria and (ii) thiothece-474, a minor carotenoid produced by the purple sulphur bacterium *Thiocystis gelatinosa.* In case thiothece-474 is the main source, the presence of this carotenoid would indicate severe water column euxinia, as purple sulphur bacteria need more light than Chlorobiaceae. **Chapter 4** shows changes in the relative distribution of the major phytoplankton classes using marine biomarkers of the haptophytes, diatoms, dinoflagellates and eustigmatophytes during Pliocene sapropel formation. At Site 967, diatoms increased in abundance relative to other phytoplankton classes at the onset of sapropel formation, indicating the enhanced supply of nutrients from the river Nile, which is in agreement with the early development of photic zone euxinia first in the most eastern Mediterranean Sea (Chapter 2). Biomarker contents differed substantially between the homogeneous intervals and sapropels, resulting partly from different redox conditions during deposition. Whereas terrestrial biomarkers, like long-chain n-alkanes and n-alcohols dominated in the homogeneous calcareous intervals, marine biomarkers were the major compounds in the sapropels. Enhanced export productivity resulted in increased export production and consequently led to oxygen depletion in the pore waters and water column. In **Chapter** 5, a newly established sea surface temperature (SST) proxy, called TEX_{86}, based on membrane lipids derived from planktonic crenarchaeota, was compared with values obtained from the most common SST proxy in palaeoceanography, the $U_{37}^{K'}$ - based on alkenones. Whereas the $U_{37}^{K'}$-based SST estimates in the homogeneous intervals and sapropels, remained almost the same, the SST estimates showed a substantial cooling of 1O-12°C during Pliocene sapropel formation. Similarities with respect to low TEX_{86}-based SST estimates were found in the contemporary euxinic Black Sea. The large drop in TEX_{86}-based SST in all sapropels is associated with the shoaling of the chemocline resulting in planktonic crenarchaeota occupying a different ecological niche. In **Chapter** 6, long-chain n-alkanes (C_{27}-C_{33}) were analysed for their stable carbon isotopic composition. Since they are derived from higher land plants, they record the effect of changes in aridity/humidity on the vegetation of the North African continent. Based on a two-end member mixing model the contribution of C4 plants to the wax lipids increased up to 20% in the sapropels at all sites. This unexpected shift from C_3 to C4 plant vegetation is

resulting from an increase in the overall plant coverage of the Mediterranean borderlands as response of the wetter climate during sapropel deposition leading to the change of formerly barren desert areas into C_4 grass-dominated savannahs. **Chapter** 7 focuses on the differences in composition of the macromolecular OM using Rock-Eval and analytical pyrolysis in combination with palynologicaly. The kerogen pyrolysates of homogeneous intervals consisted of a mixture of resistant marine and terrestrial macromolecular OM, which persisted aerobic decomposition during their deposition. The kerogen pyrolysates of the sapropels originated predominately from marine macromolecular OM with only a minor terrestrial contribution, resulting from enhanced preservation conditions leading to increased preservation of labile OM in the sapropels.

Chapter 2

Development of photic zone euxinia in the eastern Mediterranean Basin during deposition of Pliocene sapropels

Diana Menzel ,Ellen C. Hopmans, Pim F. van Bergen, Jan W. de Leeuw and Jaap S. Sinninghe Damste

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ABSTRACT

Carotenoids were analysed in ca. l-cm thick subsamples of three laterally time-equivalent sapropels from a west-east transect of the eastern Mediterranean basin to study euxinic periods during Pliocene sapropel formation. The amount of intact isorenieratene (summed *all-trans* and *cis* isomers), ranged from non-detectable at the base and top of a sapropel up to 140 μg/g sediment in the central parts. Isorenieratene accumulation rates at the central and western site are remarkably similar and increase sharply to levels of up to 3.0 mg m^{-2} yr-] in the central part of the sapropel and then drop to low levels. This pattern indicates an expansion of euxinic conditions reaching into the photic zone, followed by deepening of the chemocline during deposition of this Pliocene sapropel. The sapropel from the easternmost site of the basin, which contains less organic carbon, shows much lower isorenieratene accumulation rates and even absence of isorenieratene in the central part of the sapropel. *Bal*Al ratios indicate enhanced palaeoproductivity during sapropel formation, supporting previously proposed models, according to which increased productivity is the driving force for the generation of euxinic conditions.

INTRODUCTION

Organic-matter-rich sediments, although atypical, are nonetheless widespread in marine environments ranging in age from the Devonian to the present day. This type of sediment was formed in a wide variety of depositional settings and under different palaeoenvironmental conditions (e.g. Arthur and Sageman, 1994). However, the precise processes that have caused the high organic matter burial are often unclear. Cores taken during Ocean Drilling Program (ODP) Leg 160 at three sites (Fig. 2.1) in the eastern Mediterranean basin revealed episodic, laminated intervals with high organic carbon contents (C_{org} >2 % TOC), so-called sapropels (Erneis *et al.*, 1996). These include Pliocene
showing variations in organic matter deposition strongly correlated to the precession cyclicity of the Earth's orbit (e.g. Rossignol-Strick, 1983, 1995; Lourens *et ai.*, 1996). Some of these sapropels have extraordinarily high amounts of organic matter (C_{org} >26 % TOC;

Emeis *et al.,* 1996). Scientific controversy remains with regard to the mechanisms and factors governing sapropel formation; the two main causes being either climate-related enhanced organic matter productivity and/or increased preservation due to oxygen depletion of the bottom waters (e.g. Canfield, 1994; Bouloubassi *et aI.,* 1999; Calvert and Fontugne, 2001). Previous geochemical studies explained the formation of sapropels by climate-induced, large-scale fluvial input by the Nile river causing water column stratification and, consequently oxygen depletion in the water column (e.g. Rossignol-Strick *et aI., 1982;* Rossignol-Strick, 1995). Nijenhuis *et al.* (1999, 2000) shed new light on the deep-water circulation pattern based on trace metal enrichments and *KJ*Al and Mg/Al ratios indicating that bottom water currents were not restricted during Pliocene sapropel formation. Primary productivity appears an essential factor based on high barium contents in the sapropels supporting an association with enhanced bioproductivity during these episodes (e.g. Wehausen and Brumsack, 1999; Nijenhuis and de Lange, 2000).

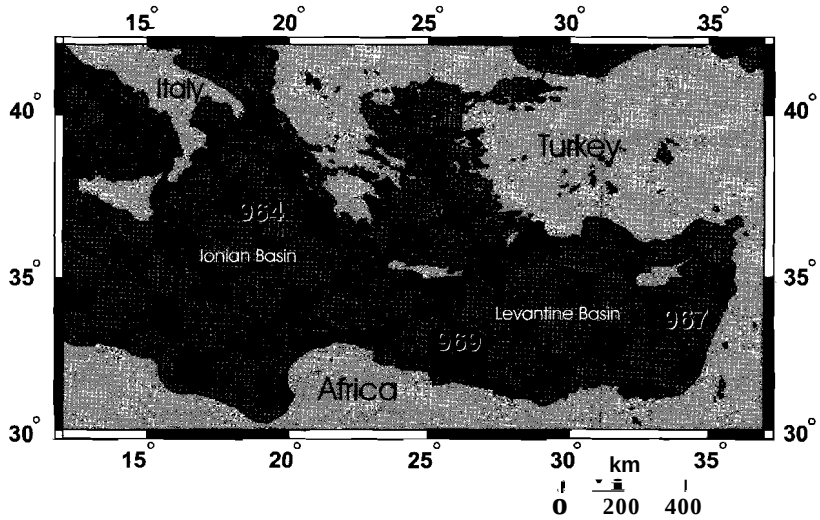

Figure 2.1 Map of the eastern Mediterranean showing the locations of the ODP drilling Sites 964, 969 and 967 (after Emeis *et ai.,* 1996).

Recent studies on individual samples of Pliocene sapropels revealed evidence of photic zone euxinia, based on the presence of isorenieratene derivatives (Bosch *et aI., 1998;* Passier *et aI.,* 1999a). Isorenieratene is the characteristic pigment biosynthesised by anaerobic, photolithotrophic Chlorobiaceae (Liaaen-Jensen, 1978). These green sulphur bacteria live at the chemocline performing anoxygenic photosynthesis requiring sulphide and light. Thus, the presence of isorenieratene in sediments indicates the past penetration of the sulphide-containing bottom water into the photic zone.

At present, the stratified, euxinic, Black Sea has a H_2S chemoc1ine at a depth of 90-150 m (Repeta *et al.,* 1989), where the obligate photoanaerobes grow under very low light conditions (e.g. <0.5 μE; Takahashi and Ichimura, 1970; Bergstein *et aI.,* 1979; Overmann *et*

al., 1992). Variations in isorenieratene contents were reported to be due to seasonal and interannual depth fluctuations of the chemocline, observed in Holocene sediments (Repeta, 1993; Sinninghe Damste *et aI.,* 1993a). Chemocline oscillations are explained by temporal changes in the balance of riverine and Mediterranean inflow to the basin, which can have a significant impact on the intensity of anoxygenic phototrophic activity (Bryantsev *et aI.,* 1988; Murray, 1991).

In this paper we report isorenieratene contents in three lateral time-equivalent Pliocene sapropels (2.943 Ma; Lourens *et aI.,* 1996) of the eastern Mediterranean basin using liquid chromatography-mass spectrometry (LC-MS) to obtain temporal and spatial insights into the development of photic zone euxinia throughout the basin. Our data indicate permanent photic zone euxinia at the central and western site during sapropel deposition. In contrast, the eastern site shows a deepening of the chemocline at time of maximum organic carbon burial probably caused by the strong increase of the discharge of fresh, less dense water of the Nile during the period of maximum humidity of the Mediterranean climate.

MATERIAL AND METHODS

Sample description

Core samples were taken during ODP Leg 160 in the eastern Mediterranean basin. Three time-equivalent sapropels (2.943 Ma; Lourens *et aI.,* 1996) were sub-sampled from cores obtained at Sites 964 (Ionian Basin), 969 (Mediterranean Ridge) and 967 (Eratosthenes Seamount; Fig. 2.1). Astronomical time scale calibrations of Mediterranean land sections have been performed on Pliocene sediments (Lourens *et aI.,* 1996). Correlation of the sapropel layers in these land sections with the distribution of sapropels in the ODP cores at Sites 964, 969 and 967 revealed unambiguously that the three investigated sapropels represent the same sapropel event (pers. commun. Lourens *et aI.,* 1997; Passier *et al.,* 1999a; Nijenhuis and de Lange, 2000). Total organic carbon (TOe) and trace metal contents were reported previously and showed distinct differences between these sapropels (Nijenhuis *et al.,* 1998).

Extraction and fractionation

Freeze-dried and ground sediment samples of 0.1 to 3 g were ultrasonically extracted with acetone (3x, 3 min) to optimise carotenoid yield. Samples were centrifuged at 3000 rpm for 5 min. The supernatants were decanted, combined and concentrated using rotary vacuum evaporation. The extract was subsequently dried under a gentle flow of nitrogen. This residue was re-dissolved in dichloromethane and applied to a silica column, where isorenieratene and other non-polar carotenoids were subsequently eluted with dichloromethane.

High performance liquid chromatography-mass spectrometry (LC-MS)

Carotenoid fractions (Fig. 2.2) were analysed on a HP 1100 series LC-MS instrument equipped with an auto-injector, photodiode array detector and mass detector. Separation was achieved on a HP XDB-C 18 column (2.1 x 120 mm, 5 maintained at 30°C, with a

gradient from methanol/water (4:1, v/v) to acetone/methanol/water (19:1:1, v/v/v) in 50 min. The flow rate was 60 ml/min. Detection was achieved by in-line UV-detection and atmospheric pressure positive ion chemical ionisation mass spectrometry (APCI-MS) of the eluent. Conditions for APCI-MS were as follows: nebuliser pressure was 60 psi, vaporiser temperature was 325°C, drying gas (Nz) was delivered at 7 l/min at 350°C, capillary voltage was -3 kV and the corona discharge was set at 6 µA. Compounds were identified based on their retention times, UV-spectra (250-750 urn) and APCI-mass spectra *(m/z 100-1000).* Isorenieratene was quantified based on UV response by comparing the peak area at 454 urn to the peak area at 454 urn of known amounts of an authentic B-carotene standard (Aldrich). Isorenieratene and B-carotene have identical UV spectra and extinction coefficients (Britton, 1995) allowing B-carotene to be used as an external standard for isorenieratene.

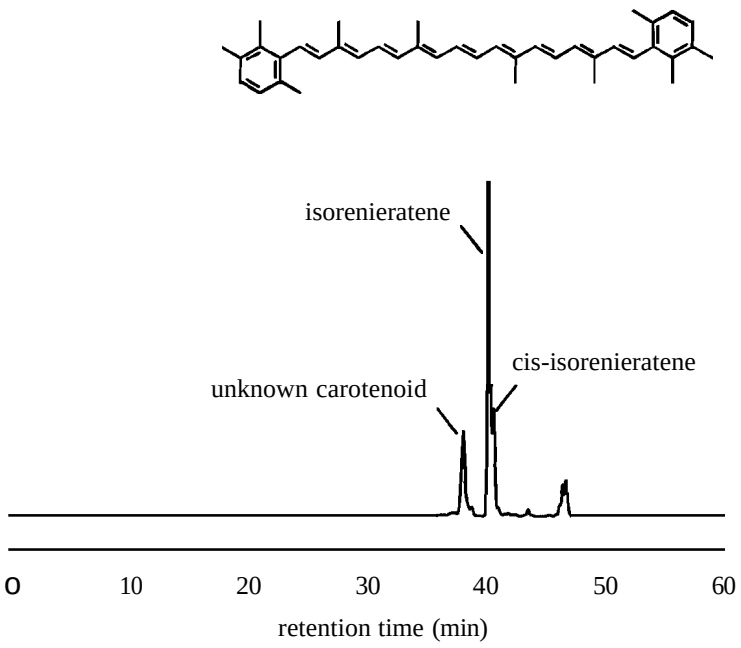

Figure 2.2 UV chromatogram at 454 nm of the isorenieratene containing fraction of 967-CH8-4, 118.5-120 em.

RESULTS

Isorenieratene contents varied within and between sapropels (Table 2.1; Fig. 2.3A-C). The isorenieratene profile of Site 964 generally followed the total organic carbon (TOC) profile through the complete sapropel with the highest content of isorenieratene (140 µg/g) being measured in the central part of the sapropel (Fig. 2.3A). Isorenieratene contents at Site 969 (Fig. 2.3B) showed a profile similar to that of Site 964. The highest content of ca. 100 was measured close to the central part of the sapropel. In contrast, Site 967 showed a

different isorenieratene content profile. Isorenieratene was absent in the sapropel centre (Fig. 2.3C) and the maximum content of 17.5 µg/g occurred at the base of the sapropel. Contents of isorenieratene measured in Holocene sediments of the world's largest stratified anoxic basin, the Black Sea ranged from non-detectable to >70 µg/gdw (Repeta, 1993).

Table 2.1 Subsamples of the ODP site, core sample and interval with TOC content and isorenieratene concentration.

ODP 964D-lOH-1 interval	TOC	Isorenieratene		ODP 969E-6H-6 interval	TOC	Isorenieratene		ODP 967C-8H-4 interval	TOC	lsorenieratene	
(em)	(%)*	(µg/g sediment)	(µg/g TOC)	(em)	(%)*	(µg/g sediment)	(µg/g TOC)	(em)	(%)*	(µg/g sediment)	(µg/g TOe)
103.5 -104	10.1	16.0	158.8	23-26	0.8	0.2	30.7	111 -114	0.2	0	5.9
104.5 -105	18.4	97.9	531.8	26-27	3.7	11.7	319.1	114 -115	1.4	0.3	19.0
105.5 -106	16.4	138.6	842.5	27-28	6.1	15.5	254.3	115 -116.5	3.3	0.6	18.1
106.5 -107	24.9	100.3	402.1	29-30	11.5	8.4	73.5	116.5 -117.5	4.1	1.3	31.1
107.5 -108	20.7	49.0	236.4	31-32	26.6	43.2	162	118.5 -120	8.9	3.4	38.6
108.5 -109	7.2	6.7	93.8	33-34	26.6	65.6	247	121.5 -122.5	10.3	0	0.02
109.5 -110	1.9	0	0	35-36	25.9	98.7	380.7	123.5 -125	15.4	0	0
				37-38	19.8	25.4	128.1	127 -128	10.3	17.5	170.7
				39-40	3.1	0.3	9.2	128 -130	4.1	4.4	108.8
				40-43	0.1	0	0	130 -134	0.2	0	11.8

* from Nijenhuis et al. (1998)

Table 2 2 Characteristics of the three lateral time-equivalent Pliocene sapropels

Core, section, interval (em)	964D-IOH-l, 103-110	969E-6H-6,26-40	967C-8H-4, 114-130
Maximum of TOC (%)	24.9	26.6	15.4
Thickness of the sapropel (em)	7	14	16
Sedimentation rate (em kyr^{-1})[a]	1[b]	2[b]	2.28[b]
Water depth (m)	3660	2201	2553
Depth	80.2	50.7	72.2

[a] assummg a constant sedImentatIOn rate
[b] assuming sapropel duration of 7000 years
[c] mbsf: metres below seafloor

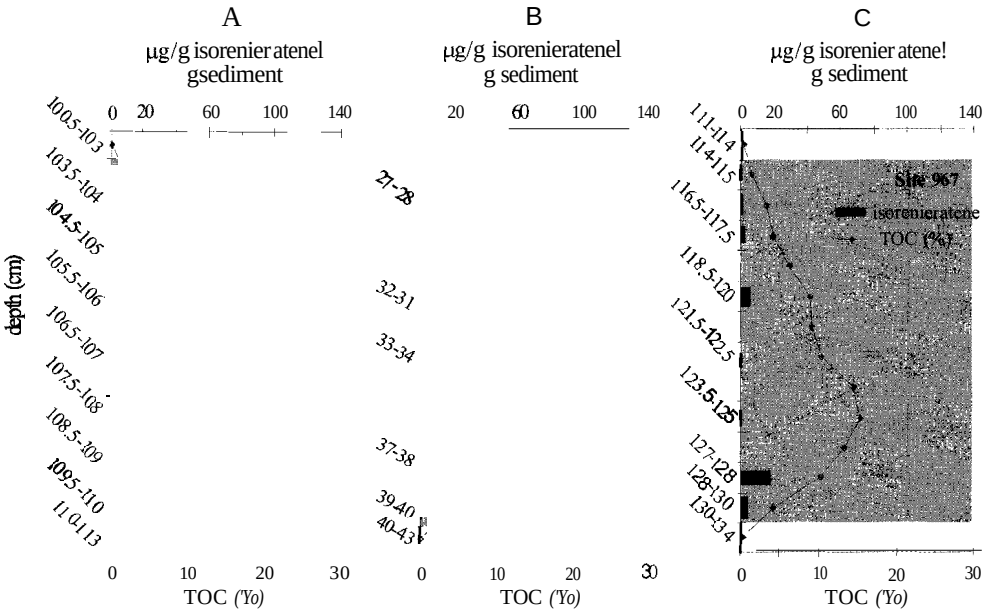

Figure 2.3 Isorenieratene contents and TOC contents measured in subsamples plotted against depth of sapropels (A) 964, (B) 969 and (C) 967. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

DISCUSSION

Sapropel chronology

Many studies have investigated the chronology of sapropels with one major question in mind: is a sapropel deposited synchronously throughout the basin or are there differences in the onset and duration of sapropel formation depending on the location within the basin (e.g. shallow vs. deep water, east vs. west; Troelstra *et al.,* 1991; Strohle and Krom, 1997; Mercone *et al.,* 2000)? Such studies are complicated by the fact that only the most recent sapropel (S1) can be dated by ^{14}C analysis. A further complication is that the S1 sapropel is often severely affected by post-depositional oxidation (e.g. de Lange *et al.,* 1989; Thomson *et al.,* 1993) resulting in almost complete removal of organic carbon from the top of the sapropel. The original thickness of the sapropel can only be assessed by high-resolution inorganic geochemical studies (i.e. BajAI and MniAI profiles; van Santvoort *et al., 1996;* Thomson *et al.,* 1999). A variety of SI sapropels from different locations and water depths investigated by radiocarbon dating revealed discrepancies in sapropel timing records ranging from depth-progressive development and vice versa to synchronous onset (e.g. Troelstra *et al.,* 1991; Fontugne *et al.,* 1994; Strohle and Krom, 1997). A recent study of the eastern Mediterranean Sea sapropel Slat different locations using accelerator mass spectrometry

radiocarbon dating in combination with Ba/Al ratios revealed an average sapropel duration of ca. 4000 years with differences between sites of approx. 700 years (i.e. by only 20 %; Mercone *et aI., 2000).*

The investigated Pliocene sapropels differ in sapropel thickness by a factor of ca. 2 (Table 2.2). Comparison of TOC and Ba/AI profiles of these sapropels indicate that post-depositional oxidation has not affected the top of the sapropels (Nijenhuis and de Lange, 2000) and is, thus, not the cause for these differences in thickness. Calculations, based on astronomical tuning and the thickness of the sapropels assuming constant sedimentation rates would result in sapropel duration of 3300 years at Site 964 and approx. 7000 years at Site 969 and 967. This difference is much higher than observed for the SI sapropel (Mercone *et aI.,* 2000) and seems unrealistic. Nijenhuis *et al.* (1999, 2000) has suggested continued bottom water circulation for the Pliocene sapropels based on trace metal enrichments and transport of Mg-rich chlorite and K-rich illite from the Adriatic and Aegean Sea into the Ionian basin. This model would be more consistent with more synchronous deposition of sapropels at the three different sites. Therefore, we feel it is more likely to attribute the difference in thickness of the sapropels to differences in sedimentation rate. These may be caused by the higher flux of detrital matter at sites closer to the Nile River and differences in preservation of carbonate (higher in the eastern sites; Nijenhuis and de Lange, 2000). Furthermore, sedimentation rates for the sapropel S1 can vary more than a factor 2 (Mercone *et aI.,* 2000) observed here for the Pliocene sapropels. To obtain temporal and spatial insights into the development of photic zone euxinia throughout the basin, it is, therefore, realistic to assume synchronous sapropel deposition at the three different sites studied.

Isorenieratene accumulation rates

Isorenieratene contents were transformed to isorenieratene accumulation rates assuming (i) a synchronous start and end of sapropel formation at the three locations, (ii) a duration of sapropel formation of approx. 7000 years (Nijenhuis and de Lange, 2000) and (iii) a constant sedimentation rate within the sapropel (Table 2.2). This transformation was undertaken to compensate for "dilution "effects and to compare these accumulation rates with rates of primary production at the three sites. The calculated accumulation rate profiles of isorenieratene at Sites 964 and 969 are similar in intensity (Fig. 2.4A). Changes in isorenieratene accumulation rates are explained by variations in the level of anoxygenic photosynthesis by green sulphur bacteria in response to the shoaling and deepening of the chemocline (Repeta, 1993; Sinninghe Damste *et al.,* 1993a). Photic zone euxinia was significantly more than in the most eastern part (Fig. 2.4A). Site 967 showed an initial increase in isorenieratene accumulation rate at the base of the sapropel to a maximum value of only 0.3 mg m,2 yr'] followed by a drop to zero at the sapropel centre. At the top of the sapropel, accumulation rates increased slightly again (Fig. 2.4A). Maximum accumulation rates for this site are, however, only 10% of those reached at Sites 964 and 969.

Based on these calculated isorenieratene accumulation rates, photic zone euxinia first appeared in the Levantine basin (Site 967) and continued to develop more strongly towards the Ionian basin (Site 964). The strongly reduced isorenieratene accumulation rates at Site 967, located closer to the mouth of the Nile, at the time of maximum isorenieratene accumulation

rates at Sites 969 and 964 is remarkable. This observation implies a deepening of the chemocline below the photic zone, most probably due to a large increase in the run-off of fresh, less dense water from the Nile. This interpretation is consistent with the fact that Site 967 also shows the highest sediment accumulation rate (Table 2.2), most probably due to fluvial delivery of mineral matter.

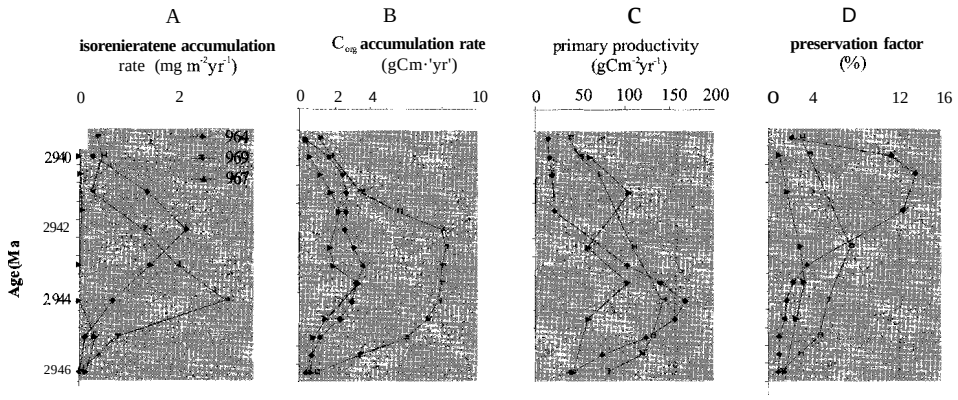

Figure 2.4 Comparison of the accumulation rates (A and B), PP (C), PF (D) among sapropel Sites 964,969 and 967.

A isorenieratene accumulation rate (AR_i):
ARi[mg m^{-2}yr-l] = x sediment rate [em kyr-l] x dry density [g *cm-3]/100.*

B C_{org} accumulation rate (AR_{Corg}):
AR_{Corg} [gCm-^{2}yr-l] = Co,g[wt%] x sediment rate [em kyr-I] x dry density [g *cm-3]/10.*

C primary productivity (PP) after Dymond *et al. (1992):*
$Ba_{(bio)}$ [µg/g] = Ba(total) - (AI x BaiAlaluminosilicate)
FbioBa [µg cm-^{2} yr-I] = (Babio_aoc)/(0.2091og(MAR)-O.213)
(Babio-acc cm-^{2} kyr-I] ~ accumulation of barium in sediments after correcting for aluminosilicate barium
MAR cm-^{2} yr -I] ~ mass accumulation rate of the sediments; after Francois *et al. (1995):*
EP [gC m-^{2} yr -I] = 1.95(FbioBa)I.41
after Eppley and Peterson, (1979):
PP [gC m-^{2} yr -I] = (new production/O.0025)II2
(EP new production)

D preservation factor (PF)
PF [%] = (AR_{Corg}/PP)*100

Because the H_2O content for the sapropel at Site 969 was not determined, an estimated dry density of 1.5 g cm-^{3}, similar to Site 964, was used for all AR calculations. Light grey shaded regions represent the sapropel. White regions represent the homogenous calcareous ooze.

Organic carbon accumulation rates, primary productivity and preservation factor

To assess if photic zone euxinia played a significant role in the burial of organic matter in the sapropels, C_{org} accumulation rates, estimated primary productivity and the estimated preservation factor were calculated from published data (Nijenhuis and de Lange, 2000) with the same assumptions as listed for the calculations of isorenieratene accumulation rates. Calculation of the C_{org} accumulation rates revealed a two-fold higher rate for the sapropel at Site 969, when compared to those at Sites 964 and 967 (Fig. *2AB).*

Barium is believed to be a good indicator for primary productivity (PP) of marine organic matter because it is removed by organic particles from the photic zone (Bishop,

1989; Dehairs *et al.,* 1987). The export production (EP) was calculated from Ba data (Nijenhuis and de Lange, 2000) using equations proposed by Francois *et al.* (1995) and Dymond *et al.* (1992) assuming a detrital *Bal*Al ratio of 0.0019 (40 % on average; van as *et al.,* 1994). Primary productivity was estimated from EP values using the equation established by Eppley and Peterson (1979). Euxinic conditions may lead to reduced formation and relatively poor preservation of barite (Dymond *et al.,* 1992; Falkner *et al.,* 1993; Francois *et al.,* 1995), which implies that the estimated PP is a minimum value.

Enhanced PP during sapropel formation is noted at Site 967 with two maxima of ca. 100 gC m^{-2} yr^{-1} (Fig. *2AC).* Sites 969 and 964 showed more or less similar estimated PP, reaching maximum values just before the central part of the sapropel of 140 gC m^{-2} yr-l and 160 gC m^{-2} yr-l, respectively (Fig. *2AC).* In the upper part of the sapropel at Site 969 estimated PP decreases slowly, ultimately reaching estimated PP values similar to those of homogenous, calcareous ooze. In contrast, estimated PP at Site 964 decreased sharply and at two third of the sapropel estimated PP levels are back to background levels.

The average PP of the present-day Mediterranean is 26 gC m^{-2} yr^{-1}. This is slightly higher than the background levels of ca. 20 gC m^{-2} yr-l in the Pliocene, because of the present excessive anthropogenic nutrient input into the Mediterranean (Bethoux, 1989). The present-day Black Sea has PP values of about 210 gC m^{-2} yr^{-1} (Karl and Knauer, 1991), similar to PP values estimated for the three time-equivalent sapropels of the Pliocene. PP values during Pliocene sapropel deposition show an approx. 5 to lO-fold increase. In addition to the input of nutrients from the Nile and other rivers (Rossignol-Strick, 1983), the substantial increase in primary production may have been caused partially by the release of labile phosphorous from deep-basin under anoxic bottom water conditions (van Cappellen and Ingall, 1994). Phosphorous regeneration has been reported in Holocene Mediterranean sapropel S1 leading to higher C: P ratios (Slomp *et al.,* 2002). An up to a factor of four higher C: P ratio has been measured for the central part of the Pliocene sapropel (2.943 Ma) at Site 969, indicating an even more significant recycling of phosphorous, caused by the enhanced C_{org} burial (Slomp *et al., 2003).*

The preservation factors (PFs, defined as the fraction of primary production preserved in the sediments) in the sapropels of Sites 967 and 969 show an average value of 2% and 4%, respectively (Fig. *2AD).* This is slightly less than the PF of 5% reported for the stratified, euxinic Black Sea (Arthur *et al.,* 1994). In the lower section of the sapropel from Site 964 there is a relatively low PF of ca. 2%. In strong contrast, the upper section shows significantly enhanced PFs of up to 13%, which is caused by the much lower estimated primary production in this section (Fig. *2AC).* It is known that barite dissolves at depth when pore water sulphate levels draw down to low levels by sulphide production (e.g. Brumsack, 1986; Torres, *et at.,* 1996; Gingele *et al.,* 1999). However, pore water analysis indicated no depletion of sulphate based on $\delta^{34}S_{pyr}$ in these sapropels. This relates to the increase of sulphate upon depth owing to the dissolution of the underlying evaporites (Emeis, *et al.,* 1996; Passier, *et al.,* 1999c). Albeit, Ba remobilisation in the upper part cannot be completely discounted. However, it is difficult to understand why this process would not have affected the other two sapropels.

With the new data it is now possible to address two major questions related to photic zone euxinia: (i) is the estimated PP during sapropel formation high enough to cause euxinic conditions in the complete water column and (ii) how did photic zone euxinia affect preservation of organic matter; in other words did it promote sapropel formation?

The first question has already been discussed thoroughly by Passier *et ai.* (1999a) based on sedimentary barium and C_{org} accumulation rate calculations, which implied increased primary productivity during sapropel formation. Trace metal budgets indicated, however, that continued circulation (as opposed to stagnant water conditions) was required to supply enough trace metals to account for the higher trace metal accumulation rates in the sapropels. Despite continued circulation, which also resulted in the delivery of oxygen to the basin, calculations indicated that the downward flux of organic matter was high enough to consume all the delivered oxygen (Passier *et aI.,* 1999a).

Our detailed isorenieratene accumulation and PP rate profiles (Fig. 2.4A,C) show no significant relationship. This is probably due to the fact that the position of the chemocline is also governed by other factors. For example, the significantly increased fresh water transport of the Nile River probably deepened the chemocline in the Levantine basin (Site 967). With respect to the second question, numerous studies have investigated whether anoxic conditions lead to increased preservation of organic matter (e.g. Demaison and Moore, 1980; Pedersen and Calvert, 1990; Arthur and Dean, 1998). Water-column anoxia often coincides with organic matter deposition on the seafloor in ancient and present-day sediments (e.g. Mediterranean Sea, Black Sea, Cariaco basin), but is not the only factor for deposition (e.g. Cowie *et ai.,* 1999). There are other factors, which also affect increased organic matter preservation (e.g. sediment accumulation rate, sources of organic matter, water depth, bacterial grazing, adsorption, geopolymerization; Canfield, 1994). Based on isorenieratene measurements in unit II sediments of the presently euxinic Black Sea, Sinninghe Damste *et ai.* (1993a) found that the top of the chemocline was within the photic zone during deposition of subunit lIb, but was below the photic zone during deposition of subunit Ila. Pyrite framboid size distributions (Wilkin *et aI.,* 1997) and $\delta^{34}S$ values of pyrite (Calvert *et ai.,* 1996) are interpreted to indicate the presence of euxinic waters during the entire period of deposition of unit II (Lyons, 1997). The content and accumulation rate of organic matter, and its degree of preservation (as evidenced by slightly lower hydrogen indices) decreased in subunit Ila (Arthur and Dean, 1998). This was interpreted to result from an erosion of the chemocline, possibly as a consequence of the cooler and drier climate during deposition of subunit IIa leading to a salinity increase of surface waters and a consequent weakened stratification. A decrease in overturn rate of nutrients and consequent lower surface-water productivity would lead to a deepening of the chemocline. These data show that there may be a tight coupling between the depth of chemocline and organic carbon accumulation in the sediment.

The isorenieratene accumulation rate and PF profiles in the sapropels show no direct correlation (Fig. 2.4A,D). Site 967 shows a maximum preservation in the central part of the sapropel, while the chemocline has deepened below the photic zone (Fig. 4A,D). Site 969 shows an approximately two-fold higher preservation factor compared to Site 967. Shoaling of the chemocline into the photic zone is clearly indicated by the large increase in the

accumulation rate of isorenieratene (Fig. 2.4A), but the increase in PF lagged slightly behind. The delay in the occurrence of photic zone euxinia at Site 964 may explain the high PF of organic matter in the upper part of the sapropel (Fig. 2AD; Table 2.2), again lagging slightly behind. However, as discussed above, the estimated factors at this site may be overestimated due to barite dissolution.

CONCLUSIONS

Based on measurements of isorenieratene, photic zone euxinia was widespread during deposition of time-equivalent sapropels at three locations in the eastern Mediterranean basin, resulting in improved organic matter preservation. The H_2S chemocline first appeared at the Levantine basin (east) and extended into the Ionian basin (west). Variations in isorenieratene content reflect fluctuations of the chemocline in the water column. The absence of isorenieratene in some parts of the sapropel at the eastern site is probably due to a deepening of the chemocline resulting from the increased delivery of fresh water from the Nile. Photic zone euxinia was most probably triggered by the large-scale input of nutrients from the Nile and other rivers leading to enhanced primary productivity and consequently high sedimentation OM fluxes, which in tum played an important role in causing anoxia and deposition of sapropels (Fig. 2.5).

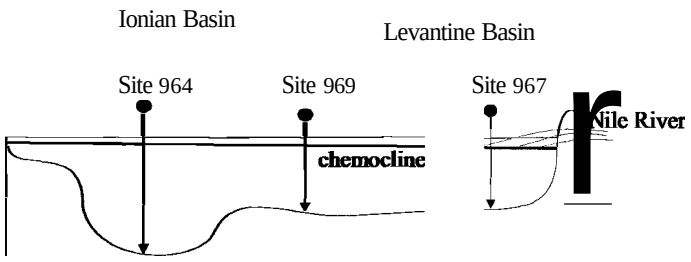

Figure 2.5 East-west gradient of the palaeochemocline based on isorenieratene data of three time-equivalent Pliocene sapropels indicating the large-scale river runoff causing the deepening of the chemocline at Site 967.

ACKNOWLEDGEMENTS

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Chapter 3

A newly discovered fossil carotenoid in Pliocene sapropels of the eastern Mediterranean Sea

Diana Menzel, Ellen C. Hopmans, Stefan Schouten and Jaap S. Sinninghe Damste

ABSTRACT

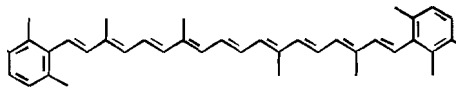
Analyses by high perfonnance liquid chromatography-mass spectrometry (LC-MS) of three time-equivalent late Pliocene sapropels (2.943 Ma) of the eastern Mediterranean Basin revealed the presence of two intact fossil carotenoids. In a previous study we reported the presence of isorenieratene, the characteristic pigment of the green sulfur bacteria, indicating that sulphide-rich bottom waters reached the photic zone for a substantial period during sapropel deposition in the entire eastern Mediterranean Basin. In addition to isorenieratene, a novel unknown carotenoid was found, which showed temporal and spatial differences in relative abundances between the three sites. Highest relative abundances of the unknown carotenoid were measured at Site 967, located closest to the Nile, and coincided with changes in isorenieratene abundances. Based on its molecular weight, mass spectrum, and its UV/VIS absorption maximum this unknown pigment reflects either (i) spheroidenone (derived from nonsulfur purple bacteria) or (ii) thiothece-474 (derived from sulfur purple bacteria). Further research will focus on the elucidation of this "unknown purple carotenoid".

INTRODUCTION

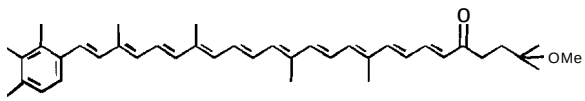
Photosynthesis is the process by which electromagnetic radiation in the fonn of photon flow is transfonned into chemical energy and is either oxygenic or anoxygenic, depending on whether there is release of oxygen. For example with green plants, algae, cyanobacteria, there is release of oxygen whilst in a number of bacterial photosynthetic processes there is not. Anoxygenic phototrophic bacteria require electron donors that are more reduced than water. This leads to extraordinary versatility, as these organisms are able to use many compounds, such as sulfide, and other reduced sulfur compounds, hydrogen and a wide range of organic molecules, and even reduced iron (Sasikala and Ramana, 1995). The photosynthetic pigments of these bacteria are bacteriochlorophylls (Bchls) *a, b,* c, *d, e* and *g,* as well as a variety of carotenoids (Eraso and Kaplan, 2001).

Based on many characteristics, including 16S rRNA and pigment analysis, the anoxygenic phototrophic bacteria have been classified into two major groups, purple and green bacteria (Sasikala and Ramana, 1995). Green bacteria are subdivided into two subgroups, the green nonsulfur bacteria and green sulfur bacteria. They contain mostly Bchl *c, d* or *e* and very little Bchl *a,* as well as carotenoids, such as chlorobactene and

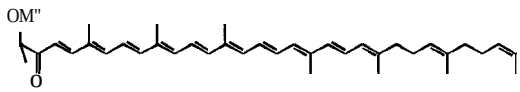
isorenieratene. Isorenieratene (I, see Fig. 3.1 for structures) is a characteristic carotenoid of the brown-coloured strain of the green sulfur bacteria, Chlorobiaceae (Liaaen-Jensen, 1978), which are obligate photoanaerobes growing under very low light conditions (e.g. <0.5 Takahashi and Ichimura, 1970; Bergstein *et al.,* 1979; Overmann *et al.,* 1992). The purple bacteria are subdivided into two subgroups, the purple sulfur bacteria, which encompass the families Chromatiaceae and Ectothiorhodospiraceae, and the purple nonsulfur bacteria with the family of the Rhodospirillaceae. This grouping is based on the ability, or inability, to use elemental sulfur as an electron donor for phototrophic carbon dioxide assimilation (Imhoff *et al.,* 1984). Purple sulfur bacteria preferentially oxidize sulfide, thiosulfate or sulfur facultatively chemoautotrophic (de Wit and van Gemerden, 1987; and Pfennig, 1986), whereas organic substrates are only poorly oxidized if at all. This is the major difference with the purple nonsulfur bacteria, of which nearly all species are facultatively chemoorganotrophic using different organic compounds as carbon source serving as respiratory substrates. The main photopigments in purple bacteria are Bchl *a* or *b,* and carotenoids, e.g. spirilloxanthin, alternative spirilloxanthins (spheroidene and spheroidenone), okenone (II) or the rhodopinal series (Eraso and Kaplan, 2001).



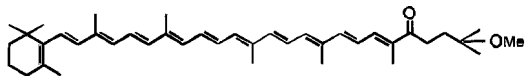
I - isorenieratene



II - okenone



III - spheroidenone



IV - thiothece-474

Figure 3.1 Structures of carotenoids discussed in the text.

Fossil photopigments in sediments can be used as chemical markers for the presence of these photosynthetic sulfur bacteria in past depositional environments (Sinninghe Damste and Koopmans, 1997). For example, Chromatiaceae have been mostly reported in different environmental settings, such as stratified lakes (Pfennig *et al.,* 1968), sewage lagoons and estuaries (Pfennig and Triiper, 1989), and shallow coastal lagoons and marine sediments

(Matheron and Baulaigue, 1972; Caumette, 1986; Pfennig, 1989). In contrast, compared Ectothiorhodospiraceaea which live under hypersaline and alkaline conditions (Triiper and Imhoff, 1981; Blankenship *et ai.,* 1995). The presence of isorenieratene and its derivatives representing Chlorobiaceae was reported in the present-day euxinic Black Sea water column (Repeta *et ai.,* 1989) and sediments (Repeta, 1993; Sinninghe Damste *et ai.,* 1993a) as well as in many older organic-rich sediments (e.g. Passier *et ai.,* 1999a; van Kaam-Peters *et ai.,* 1997; Summons and Powell, 1986; Koopmans *et ai.,* 1996)) indicating the (past) penetration of sulphide-containing bottom waters into the photic zone. Isorenieratene and its derivatives are, therefore, palaeoenvironmental indicators for "photic zone euxinia" (Repeta, 1993; Sinninghe Damste *et ai.,* 1993a). In much the same way, carotenoids of purple sulfur bacteria can be used. For example, okenone has been found in lake sediments (Hartgers *et ai., 2000;* Schaeffer *et ai.,* 1997) and in sediments of a stratified Norwegian fjord (Smittenberg *et ai.,* 2004).

The development of photic zone euxinia in the eastern Mediterranean Sea during the late Pliocene was recently studied based on the distribution of intact isorenieratene in three time-equivalent sapropels (Menzel *et ai.,* 2002). Besides isorenieratene, a newly discovered fossil carotenoid, previously also identified in the S5 sapropel of the eastern Mediterranean Sea (Hopmans *et ai.,* 2003), was also present. Here we identify this "carotenoid" as either (i) spheroidenone (III), a carotenoid related to various species of the family Rhodospirillaceae or (ii) thiothece-474 (IV), a minor carotenoid produced in *Thiocystis geiatinosa,* and discuss the palaeo-ecological implications of this finding.

MATERIAL AND METHODS

Samples

Core samples were taken during ODP Leg 160 in the eastern Mediterranean Basin. Three time-equivalent sapropels (2.943 Ma; Lourens *et ai.,* 1996) were sub-sampled from cores obtained at Sites 964 (Ionian Basin), 969 (Mediterranean Ridge) and 967, spanning an east-west transect of the eastern Mediterranean Basin (Eratosthenes Seamount; see Menzel *et ai.,* 2002). Astronomical time scale calibrations of Mediterranean land sections have been performed on Pliocene sediments (Lourens *et ai.,* 1996). Correlation of the sapropel layers in these land sections with the distribution of sapropels in the ODP cores at Sites 964, 969 and 967 revealed unambiguously that the three investigated sapropels represent the same sapropel event (e.g. Lourens *et ai.,* 1996; Passier *et ai.,* 1999a; Nijenhuis and de Lange, 2000). Total organic carbon (TOC) contents were reported previously and showed distinct differences between these sapropels.

Extraction and fractionation

Freeze-dried and ground sediment samples of 0.1 to 3 g were ultrasonically extracted with acetone (3x, 3 min) to optimise carotenoid yield. Samples were centrifuged at 3000 rpm for 5 min. The supernatants were decanted, combined and concentrated using rotary vacuum evaporation. The extract was subsequently dried under a gentle flow of nitrogen. This residue

was re-dissolved in dichloromethane and applied to a silica column, where non-polar carotenoids were subsequently eluted with dichloromethane.

High performance liquid chromatography-mass spectrometry (LC-MS)

Carotenoid fractions were analysed by LC-MS as described previously (Menzel *et ai.,* 2002). Detection was achieved by in-line UV/VIS-detection and atmospheric pressure positive ion chemical ionisation mass spectrometry (APCI-MS) of the eluent. Relative abundances of the "unknown purple carotenoid" were estimated using the UVNIS response of the peak area at 482 nm (UVNIS absorption maximum of the unknown carotenoid).

RESULTS

In addition to isorenieratene (I) (Menzel *et ai.,* 2002), the apolar carotenoid fractions of the Pliocene sapropels often contain an earlier eluting unknown carotenoid (Fig. 3.2a).

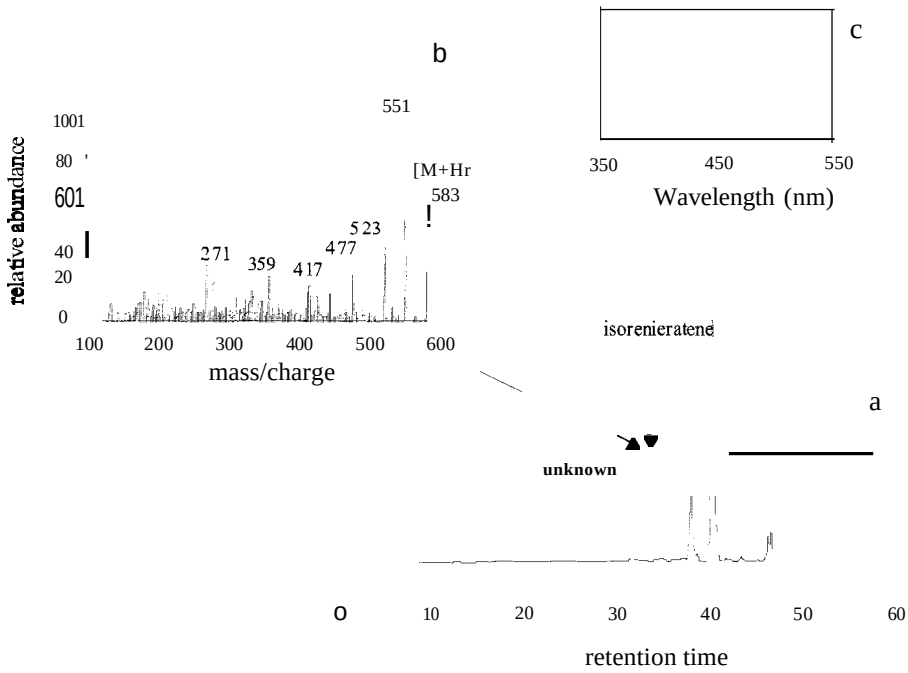


Figure 3.2 (a) UV chromatogram at 454 nm of the carotenoid fraction of Site 967-CH8-4, 118.5-120 cm showing the presence of isorenieratene and the "unknown purple carotenoid", (b) and (c) mass and UV spectrum of the "unknown purple carotenoid", respectively.

The APCI mass spectrum of this compound was dominated by an ion at *m1z 583* (representing *[M+Ht),* suggesting a molecular weight of 582 (Fig. 3.2b). Two major fragments were observed at *m1z* 551 and *m1z* 523, respectively. The first fragment, 32 Da

lower relative to the protonated molecule, may represent loss of a methoxy functionality. A second fragment at *mlz* 523, which is 18 Da lower, suggests a further loss of an alcohol, aldehyde or keton functional group as water. Its UVNIS spectrum showed an absorption band with a maximum at 482 nm (Fig. 3.2c). Comparison with literature data indicated that this carotenoid is either (i) spheroidenone (III) or (ii) thiothece-474 (IV) as these have molecular weights of 582 Da, possess functional groups that could lead to the observed fragments in the APCI mass spectrum, and similar absorption maxima (Pfennig *et al., 1968),* (Andrews and Liaaen-Jensen, 1972), (Staub, 1987). Unfortunately, authentic standards of these components were not available to check if relative retention time data could be used to discriminate between these two possibilities. Small amounts of this carotenoid were isolated by repetitive preparative HPLC to further elucidate its structure, but 1H NMR studies of the isolate indicated that the carotenoid had become oxidized during the isolation process. Therefore, we are at present not able to further identify this carotenoid.

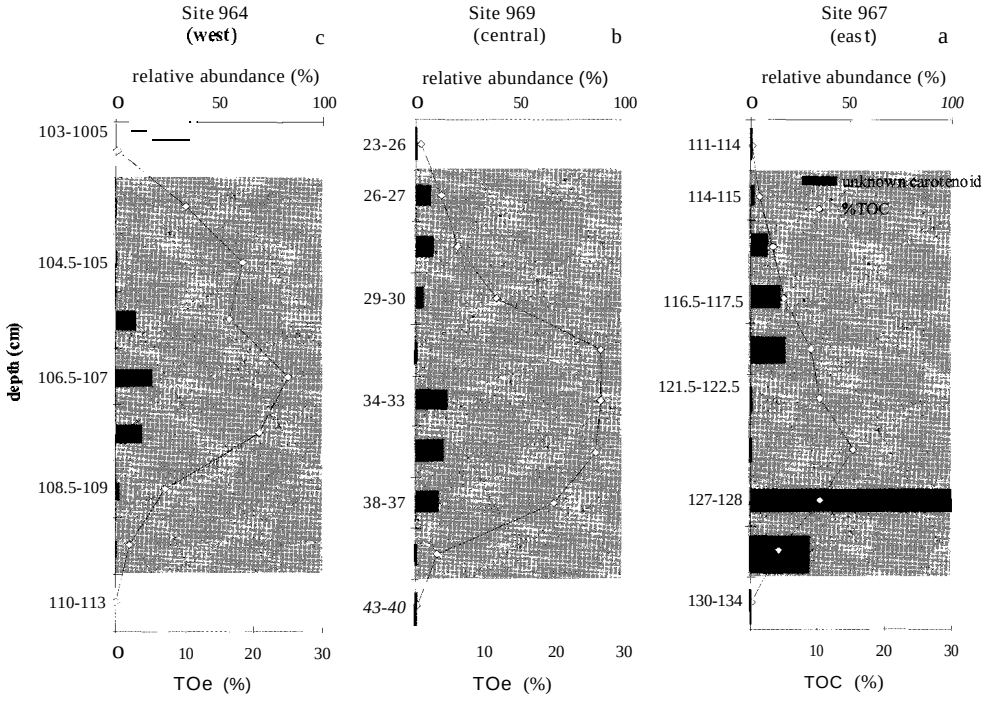


Figure 3.3 Relative abundances of the "unknown purple carotenoid" in sub-samples plotted against depth of sapropels (a) Site 967, (b) Site 969 and (c) Site 964 and total organic carbon (TOC) (*data from Nijenhuis *et al.,* 1998). Light grey shaded regions represent the sapropel and white regions represent the homogeneous calcareous ooze.

The peak area of the unknown carotenoid was in few sub-samples of the sapropels as large as the peak area of isorenieratene. Thus, the unknown carotenoid is an important fossil bacterial pigment in addition to isorenieratene preserved in the late Pliocene sapropels. Abundances of this carotenoid varied within and between the three sapropels (Fig. 3.3). In the

homogeneous, calcareous intervals below and above the sapropels it was absent at all three sites. In the lower part of sapropel at Site 967 (closest to the mouth of the Nile), the highest relative abundances of the unknown carotenoid ranged between 30 to 100 % (Fig. 3.3a). This carotenoid was absent in the central part of the sapropel parting the top of the sapropel its relative abundance declined upwards to about 15%. Site 969 (central eastern Mediterranean) showed at the base of the sapropel low relative abundances of about 10% with increasing values towards the central part of the sapropel of about 15% and remained unchanged in relative abundances in the top of the sapropel of about 7% (Fig. 3.3b). At Site 964 (western eastern Mediterranean), the carotenoid only occurs in relatively abundances (ca. 15%) in the central part of the sapropel (Fig. 3.3c).

DISCUSSION

Possible biological origin for the unknown carotenoid

Spheroidenone (III) and thiothece-474 (IV) are proposed as the two candidates for the structure of the "unknown purple carotenoid". Spheroidenone has been described as end product of the carotenoid biosynthesis pathway of various species of the family Rhodospirillaceae (purple nonsulfur bacteria). Thiothece-474 is a minor carotenoid (ca. 1%) of the species *Thiocystis gelatinosa,* family belonging to the Chromatiaceae (purple sulfur bacteria) (Andrews and Liaaen-Jensen, 1972). *Thiocystis geIatinosa* is generally characterised by okenone as the major pigment (e.g. Pfennig *et aI.,* 1968). Thus, the carotenoid represents the fossil remains of a purple photosynthetic bacteria, either a purple sulfur bacteria or a purple nonsulfur bacteria. Based on the abundance of spheroidenone among photosynthetic bacteria, compared to thiothece-474 and the absence of okenone, spheroidenone seems the most likely candidate for the carotenoid found in the late Pliocene sapropels. However, work to confirm the structure identification is ongoing.

Fossil photopigments of anaerobic, green and purple photosynthetic bacteria are common components of meromictic lakes (Lami *et aI.,* 2000) and have been used to reconstruct the palaeo-environmental history of different lakes (e.g. Brown *et aI., 1984;* Ryves *et aI.,* 1996; Lami *et aI.,* 2000). Spheroidenone was used as indicator of the development of transparency in meromictic lakes and therefore, its trophic state (e.g. Brown *et aI.,* 1984; Ryves *et aI.,* 1996; Lami *et aI.,* 2000). For instance, changes in palaeoenvironmental conditions during the Holocene of the small, shallow, eutrophic Lake Candia (N. Italy) revealed enhanced accumulation rates of algal and anaerobic photosynthetic bacterial pigments indicating more intense microbial mineralisation and consequently oxygen depletion (Ryves *et aI.,* 1996; Lami *et aI., 2000).*

Palaeo-ecological implications

With respect to the light intensity, green bacteria require in general less light, based on the chlorosomes as light harvesting units, which are unique among all phototrophic organisms in that they require only about one-fourth of the light intensity of the purple bacteria in order to grow at comparable growth rates (Biebl and Pfenning, 1978). Therefore,

the green sulfur bacteria populations regularly occur below the layers of all other phototrophs, where light penetration is severely hampered (Blankenship *et aI., 1995;* Pfenning, 1978) and are closest to the sulfide zone (Fig. 3.4).

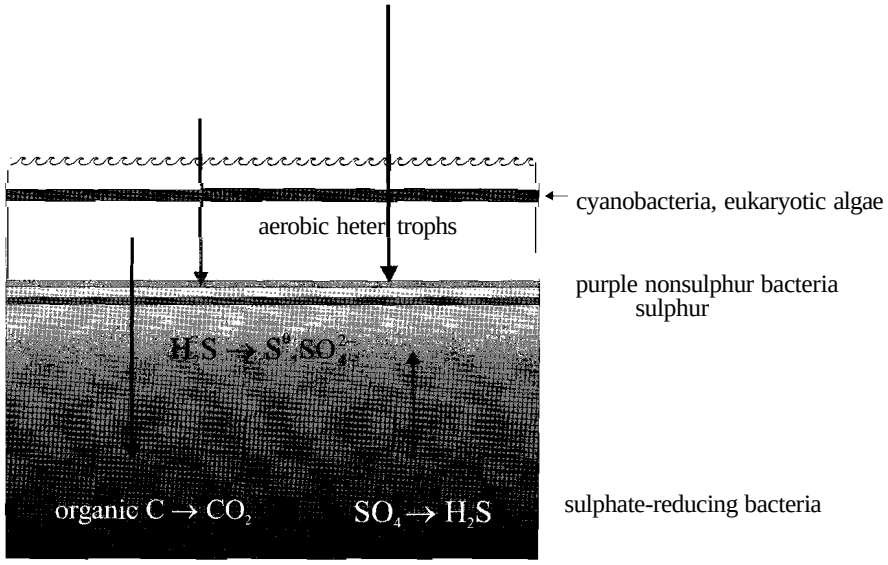


Figure 3.4 mustration of a water ecosystem involving oxygenic and anoxygenic photosynthesis in the upper water column and sulphate reduction activities near the bottom of the water column.

In previous studies of the eastern Mediterranean Sea, the presence of isorenieratene revealed sulfide-rich water up into the photic zone in the entire basin during Pliocene sapropel deposition (Passier *et al.,* 1999a). Sulfide was formed as an end product of the dissimilatory sulphate reduction of sulphate-reducing bacteria due to anoxic degradation of organic matter (e.g. Passier and de Lange, 1998), which have been caused by enhanced primary productivity leading to high sedimentation rates of OM and consequently oxygen depletion in the pore waters and water column up to the photic zone (e.g. Nijenhuis and de Lange, 2000; Passier *et aI.,* 1999a; Menzel *et al.,* 2003). Variation of isorenieratene abundances at the three different sites reflected fluctuation of the chemocline in the water column during sapropel deposition (Menzel *et aI.,* 2002). The HzS chemocline first appeared at the Levantine basin (east) and extended with higher abundances into the Ionian Basin (west). Abundances of the "carotenoid likely derived from Rhodospirilaceae" showed highest relative at Site 967, located closest to the Nile and decreased towards the Ionian basin (Site 964). These bacteria were more abundant at Site 967, compared with Sites 969 and 964 (Fig. 4.5). Rhodopirillaceae are facultative anaerobes possessing the adaptive capacity to grow anaerobically in the light (photosynthetically) and aerobically in darkness by oxidative phosphorylation using organic compounds as electron donor (Saunders, 1978). It is the great ability of this group to practice photoheterotrophy using a wide range of carbon sources, that likely accounts for their competitive success in nature (Brock, 2000) explaining their true

omnipresence (Eraso and Kaplan, 2001). Based on the wide range of physiological adaptations of these Rhodospirillaceae, the palaeo-ecological implication of the presence of these bacteria is uncertain but probably related to the temporal occurrence of shallow photic zone anoxia (i.e. even more shallow than indicated by the presence of the Chlorobiaceae).

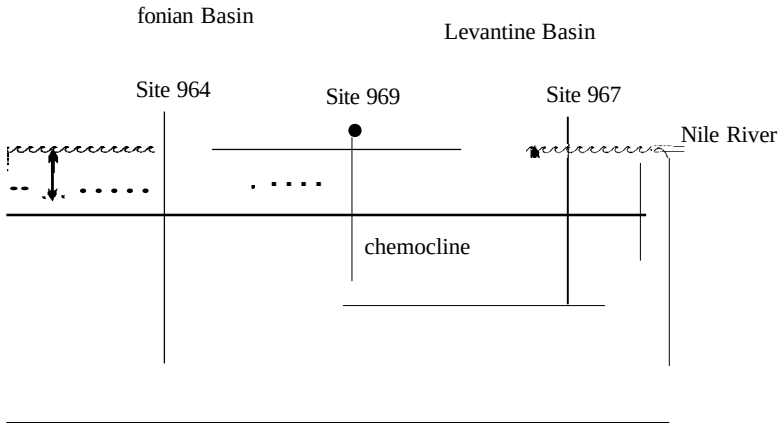


Figure 3.5 Illustration of the east-west gradient of the palaeochemocline based on isorenieratene (see Menzel *et al.*, 2002) and "unknown purple carotenoid" (dotted line) data of the three time-equivalent Pliocene sapropels.abundances.

CONCLUSIONS

A novel sedimentary carotenoid was tentatively identified in sapropel sediments. It is likely derived from photosynthetic purple nonsulfur bacteria, i.e. Rhodospirillaceae. Based on the wide range of physiological adaptations of these Rhodospirillaceae, the palaeo-ecological implication of the presence of these bacteria is uncertain but probably related to the temporal occurrence of shallow photic zone anoxia, (i.e. even more shallow than indicated by the presence of pigments of the Chlorobiaceae).

ACKNOWLEDGEMENTS

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Chapter 4

Reconstruction of changes in export productivity during Pliocene sapropel deposition: a biomarker approach

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ABSTRACT

Biomarkers (alkenones, loliolide/isololiolide, dinosterol and C_{30} 1,15-diol/keto-ol) representative of four major classes of marine primary producers (haptophytes, diatoms, dinoflagellates, eustigmatophytes, respectively) were studied for changes in phytoplankton export production before, during and after deposition of a Pliocene, TOC-rich (up to 27%) sapropel (2.943 Ma) from three sites of an east-west transect spanning the entire eastern Mediterranean basin. Biomarker contents showed substantial differences between non-sapropelic and sapropelic sediments. In non-saprope1ic sediments, small contents are observed, reflecting extensive oxic degradation in oxic pore waters during deposition. In the sapropels, biomarker accumulation rates (ARs) showed a substantial increase from the base to the centre, likely resulting from increased biological productivity since bottom waters were continuously anoxic and preservation conditions are similar. The diatom biomarker, loliolide/isololiode became distinctly more abundant in the sapropels indicating that the eastern Mediterranean basin was enriched in nutrients. This caused enhanced export production by the major phytoplankton classes leading to periods of sapropel deposition. Alkenone-based SST reconstructions indicated that this change in phytoplankton composition was not influenced by changes in sea surface temperature, but was mainly nutrient-controlled. Compound-specific $\delta^{13}C$ measurements did not provide additional evidence for substantially increased primary production rates: $\delta^{13}C$ values of $C_{37:Z}$ alkenones and lolioloide/isololiolide showed a slight negative shift instead of the anticipated positive shift. This was probably caused by the enrichment of dissolved inorganic carbon in the upper water column, caused by enhanced recycling of respired COz due to the shallow chemocline.

INTRODUCTION

Since the discovery of sapropels, i.e. sediments rich in organic matter, in the Mediterranean seabed there has been extensive research on their origin, but the exact mechanism of their formation is still not well understood. Sapropels are characterized by carbonate-poor and organic-rich (up to 30% TOC) material interspersed between organic-poor «0.3% TOC) nannofossil and foraminiferal marl ooze (Calvert *et ai.,* 1992). Benthic

foraminifera are generally rare in sapropels, or even absent, due to suboxic/anoxic conditions of the bottom waters (e.g. Lourens *et al.,* 1996). Sapropels occur in regular intervals in the eastern Mediterranean basin (Emeis *et al.,* 1996) showing a mainly precession-controlled correlation (Lourens *et al.,* 1996). Minima of the Earth's cycle precession are characterized by the intensification of the African monsoon leading to increased precipitation over the Mediterranean region as a whole and consequently an increase in river discharges and nutrients (e.g. Rossignol-Strick, 1985; Rohling, 1994). These river-borne nutrients most probably led to enhanced biological productivity causing increased export production of organic matter (OM) to the seafloor. Evidence for enhanced productivity is derived from substantial barite enrichments in sapropels (Wehausen and Brurnsack, 1999; Passier *et al.,* 1999c; Nijenhuis and de Lange, 2000; Rinna *et al.,* 2002). As a consequence of increased export production, oxygen depletion and bottom water anoxia occurred, which in tum resulted in increased preservation of settling OM. Sapropel formation is thus both a consequence of increased productivity and improved preservation of OM.

In contrast to Pleistocene sapropels, which generally have total organic carbon (TOC) contents of less than 10% and often below 3%, Pliocene sapropels are characterised by extreme enrichments in Toe (up to 30%; Nijenhuis *et at.,* 1998; Passier *et al.,* 1999a; Rinna *et al.,* 2002). This seems to point to an even larger change in climatic and environmental conditions during the "sapropel mode" of the Mediterranean. Passier *et at.* (l999a) described a model for Pliocene sapropel deposition under continued deep-water circulation, based on trace metal data and organic geochemical evidence for photic zone euxinia (i.e. the presence of sulphide in the photic zone). Detailed studies of isorenieratene, a biomarker for photic zone euxinia (Repeta, 1993; Sinninghe Damste *et al.,* 1993a), revealed an early development of euxinia in the Levantine basin most probably caused by the large-scale nutrient input from the Nile that subsequently spread towards the Ionian basin (Menzel *et al., 2002).*

The dominance of siliceous or organic-walled plankton over calcareous species at times of sapropel formation suggests that the phytoplankton composition of the Mediterranean Sea might have varied substantially (van Os *et al.,* 1994; Nijenhuis *et al.,* 1996). Studies on late Quaternary Mediterranean sapropels reported enhanced diatom production and inferred their contribution to the organic-rich nature of the sapropels (Kemp *et al.,* 1999). However, to date little information is known about the composition and distribution of the major primary producers during sapropel formation, especially in cases where siliceous and calcareous skeletons have dissolved, as is often the case in sapropels with a high Toe content. In such cases, lipid biomarkers can provide valuable insights into the changes in phytoplankton composition.

In the current paper four biomarkers, characteristic of different phytoplankton taxa, occurring in three time-equivalent Pliocene sapropels (2.943 Ma; Lourens *et at.,* 1996) from an east-west transect spanning the entire eastern Mediterranean basin were used to reconstruct changes in phytoplankton export production before, during and after sapropel formation. The extraordinarily high organic matter content (up to 27% TOC), in these Pliocene sapropels was predominately of marine origin and terrestrial OM contribution was insignificant (Bosch *et at.,* 1998). Results of this study show that loliolide/isololiode, a biomarker of diatoms previously reported from sediments underlying highly productive

surface waters (e.g. ten Haven *et ai.,* 1987b; Repeta, 1989), become distinctly more abundant in the sapropels, compared with biomarkers of haptophytes, eustigmatophytes and dinoflagellates. In addition, measurements of $C_{37:2}$ alkenones and loliolide/isololiolide were used as a tool to assess changes in biological productivity during sapropel formation since during extensive periods of enhanced productivity, the isotopic fractionation (ε_p) between DIC and phytoplanktonic biomass can be reduced (Laws *et ai., 1995).*

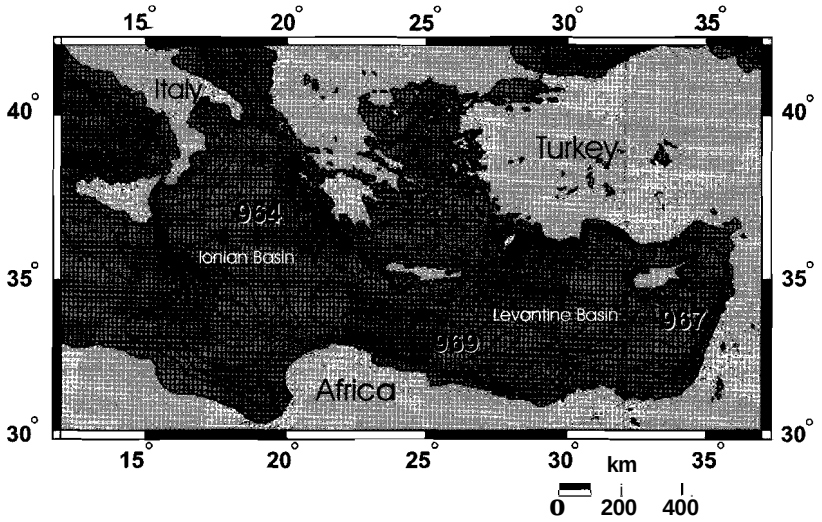

Figure 4.1 Location of the ODP drilling sites.

MATERIAL AND METHODS

Samples

Core samples were taken during ODP Leg 160 in the eastern Mediterranean basin (Erneis *et ai.,* 1996). Site 967C-8H-4, 111-134 cm (Eratosthenes Seamount) and Site 969E-6H-6, 23-43 cm (Mediterranean Ridge) are located in the Levantine basin. Site 964D-lOH-l, 100.5 -113 cm is located in the Ionian basin (Fig. 4.1). The detailed chronology for these cores obtained by astronomical tuning enabled us to select three laterally equivalent sapropels with an age of 2.943 Ma (see Lourens *et ai.,* 1996 for details). Each sapropel was sub-sampled in 0.5, 1 and 1.5 cm slices above, through and below the sapropel. Total organic carbon (TOC) was reported previously and showed distinct differences between these sapropels (Nijenhuis *et ai., 1998).*

Extraction and total lipid fraction

Ground, freeze-dried sediment samples (0.1 to 3 g) were Soxhlet extracted with dichloromethane/methanol (1:1, v/v) mixture for 24 h. The extracts were concentrated with a rotary evaporator at 30°C. Totallipid fractions (TLF) were obtained using a Pasteur capillary

pipette (150 mm), packed with silica gel and eluted with ethyl acetate to remove very polar material and silylated using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA); (1 h; 80°C). 10-Nonadecanone was used as an internal standard. The TLF was dried under a gentle stream of nitrogen and analysed using gas chromatography (GC) and GC-mass spectrometry (GC-MS). Quantitation was perfonned by integration of relevant peak areas of biomarkers of interest and the internal standard. C_{30} 1,15-diol and keto-ol, co-eluting with their isomers (C30 1,14-1,12-diols) were quantified by integration of individual peak areas in mass chromatograms using characteristic *m/z* values.

Calculations of accumulation rates were perfonned using absolute biomarker amounts (µg g-l dry weight sediment), assuming (i) a synchronous start and end of sapropel fonnation at the three locations, (ii) a duration of sapropel fonnation of approximately 7000 years (Nijenhuis and de Lange, 2000) and (iii) a constant sedimentation rate during sapropel fonnation (Menzel *et ai.*, 2002). Accumulation rates in the homogeneous intervals were not calculated, because sedimentation rates are expected to be substantially different during times of sapropel and non-sapropel fonnation.

Fractionation of compound classes

An aliquot of the total extract (1.5 to 3 mg) was fractionated using a Pasteur capillary pipette (150 mm length) packed with (activated for 2.5 h at l20°C). The apolar fraction (Fl) was eluted using a mixture of hexane/dichloromethane (9:1, v/v; 4 ml). Subsequently, the ketone fraction (F2) was eluted using hexane/dichloromethane (1:1, v/v; 4 ml). Finally, the polar fraction was obtained using dichloromethane/methanol (1:1, v/v; 4 ml). Lipids in the polar fraction were derivatized using BSTFA (30 min at 60°C) with a known isotope value of - 49.29 %0 and corrected accordingly to obtain $\delta^{13}C$ values. All fractions were dried under a flow of nitrogen and analysed by GC, GC-MS and isotope-ratio monitoring (inn)-GC-MS.

Gas **chromatography,** GC-mass **spectrometry and irm-GC-MS**

GC was perfonned on a Hewlett-Packard 6890 series gas chromatograph equipped with a fused silica capillary column (50 m x 0.32 mm or 25 m x 0.32 mm) coated with CP-Sil 5 (film thickness 0.12 µm). Helium was used as a carrier gas. The samples were injected on-column at 70°C and the oven was subsequently programmed to 320°C at 4°C/min, where it remained isothennal for 20 min. Compounds were detected using a flame ionisation detector (FID).

GC-MS analyses were perfonned using a Hewlett-Packard 5890 series II gas chromatograph connected to a Fisions instruments VG platfonn II mass spectrometer operated at 70 eV, with a mass range *m/z* 50-650 and cycle time of 0.65 s (resolution 1000). The capillary column and temperature programme were as described for the GC analyses.

Isotope-ratio-monitoring gas chromatography-mass spectrometry (inn-GC-MS) was perfonned using a Delta-plus XL-inn-GC-MS system (cf. Schouten *et ai.*, 1998), equipped with an on-column injector and fitted with a 25 m x 0.32 mm fused silica capillary column coated with CP-SiI5 (film thickness 0.12 Helium was used as carrier gas and the oven was programmed from 70 to 130°e at 20°C/min., followed by an increase of 4°C/min. to

320°C (20 min.). Isotopic values were calculated by integrating the mass 44, 45 and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and those of CO_2-spikes produced by admitting CO_2 with a known l3C-content at regular intervals into the mass spectrometer. Two analyses were carried out for each sample and the results were averaged to obtain a mean value. $\delta^{13}C$ values have been corrected for carbon added during derivatization and have typically an error of <0.5%0.

RESULTS

Phytoplankton biomarkers

The TLF of the extracts of the homogeneous intervals (TOC up to 0.2%) below and above the sapropels are dominated by n-alkanes and n-a1cohols with relatively small amounts of fatty acids, sterols and long-chain unsaturated ketones (Fig. 4.2A).

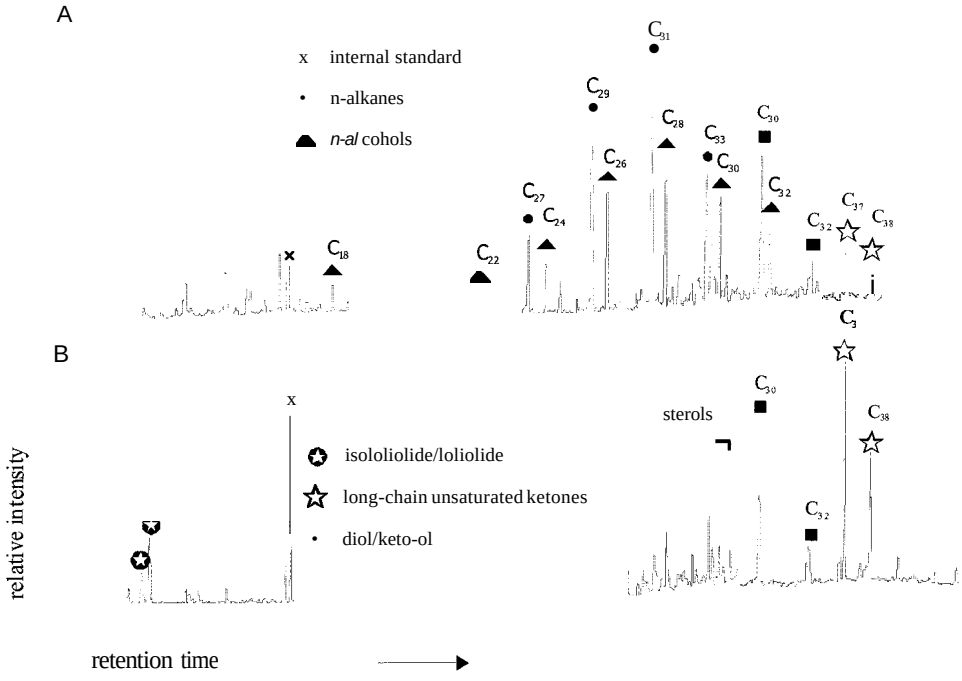

Figure 4.2 Gas chromatograms of the total lipid fractions of (A) the homogeneous interval before sapropel fOlmation (C_{org} up to 0.12%), and (B) the sapropel (C_{org} up to 30%) of Site 969E-6H-6.

The substantially higher contribution of land plant to these TLFs is indicated by the odd-over-even predominance of the long-chain (C23-C_{33}) n-alkanes and even-over-odd carbon number predominance of the dominant C_24-C_{30} n-alcohols. In contrast, the TLF of the sapropels (TOC up to 30%) are dominated by biomarkers derived from phytoplankton, either directly biosynthesized lipids (e.g. long-chain alkenones, dinosterol, long-chain diols) or compounds formed from lipids or carotenoids by early diagenetic reactions (e.g. loliolide/isololiolide,

long-chain keto-ols, Fig. 4.2B). The relative contribution of terrestrial lipids to the TLF of the sapropels was insignificant and is probably caused by the overprinting marine biomarkers (Fig. 4.2B). Four biomarkers, each characteristic for a specific phytoplankton class, were used to reconstruct changes in phytoplankton composition during sapropel formation (cf. ten Haven *et aI.,* 1987a).

Table 4.1 Biomarker contents and accumulation rates (AR) of the homogeneous intervals and sapropels.

Compound	Sapropel position	ODP 964D-10-1	AR (mgm^{-2} yr-I)	ODP 969E-6H-6	AR (mgm^{-2} yr-!)	OOP 967C-8H-4	AR (mgm^{-2} yr-l)
Loliolidel isololiolide (µg/g OC)	above	0		60		0	
	within (n=8)	100-730	0.03-2.0	35-315	0.03-2.5	30-840	0.02-1.7
	below	0		0		0	
Long-chain alkenones (µg/g OC)	above	305		255		120	
	within (n=8)	170-1180	0.1-4.7	90-630	0.08-2.6	160-1485	0.09-1.9
	below	80		115		320	
Dinosterol (µg/g OC)	above	0		70		0	
	within (n=8)	20-280	0.01-0.65	5-90	0.005-0.5	60-400	0.06-1
	below	45		0		38	
C_{30} 1,15-diol and keto-oj (µg/g OC)	above	325		105		170	
	within (n=8)	100-1020	0.07-2.3	55-310	0.05-1.93	130-1030	0.05-1.4
	below	53		57		280	

Loliolidelisololiolide is derived on a mole to mole basis from anoxic degradation of fucoxanthin (Repeta, 1989), the major carotenoid in diatoms, but also in dinoflagellates and haptophytes (Klok *et al.,* 1984; Jeffrey and Vesk, 1997). Diatom blooms are characteristic of highly productive areas, like upwelling regions where cold and nutrient-rich waters reaches the euphotic zone. Loliolide/isololiolide was absent in the homogeneous intervals below the sapropels at all three sites. The Site 967 and 964 sapropels showed the highest mean contents of ~380 µg/g *DC* and lower contents of 215 *DC* were measured a Site 969 (Table 4.1, Fig. 4.3). Above the sapropel, at Site 969 loliolide/isololiolide content of 60 *DC* was measured (Fig. 4.3). Accumulations rates (ARs) in the sapropel ranged from 0.02-2.5 mg.m-2.yr-1 (Table 4.1, Fig. 4.4B).

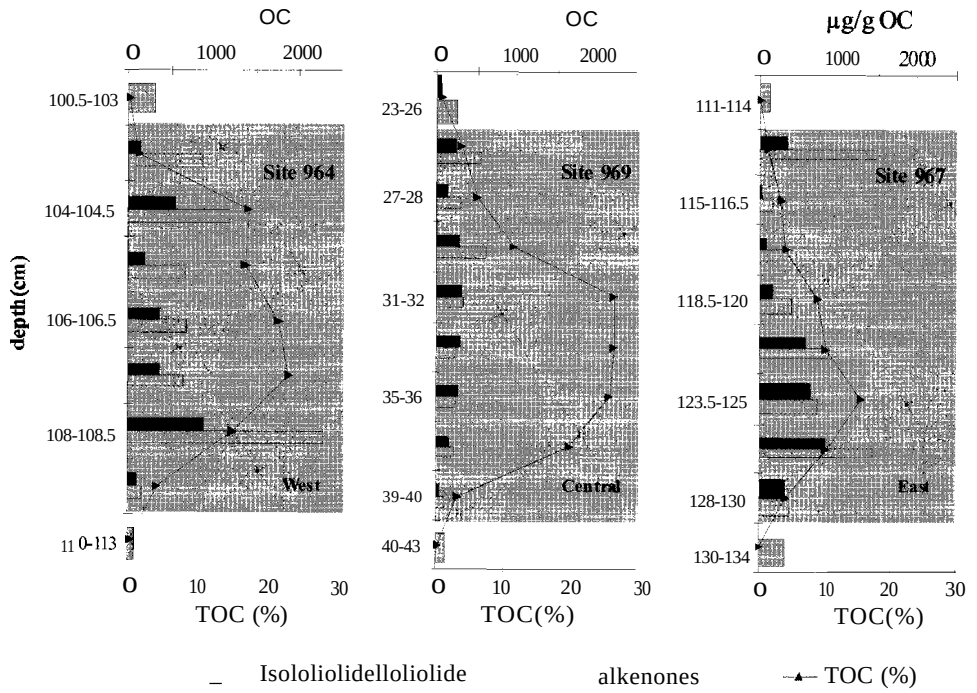

Figure 4.3 Loliolide/isololiolide and alkenone contents (nonnalized to TOC) and TOC contents plotted against depth of the sapropels. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

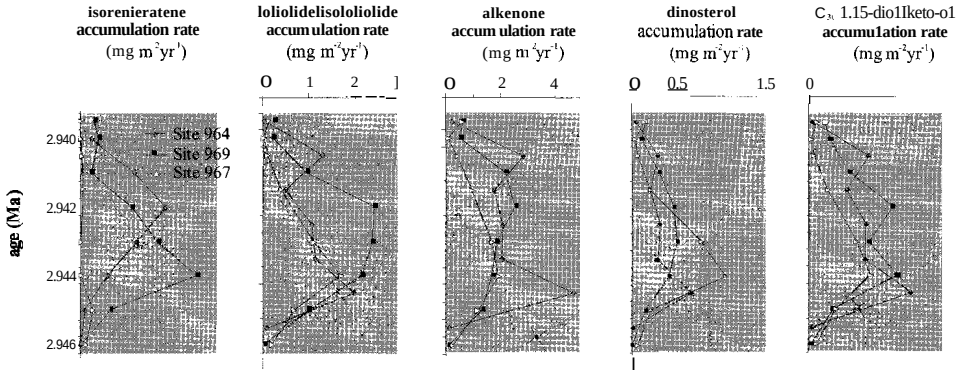

Figure 4.4 Accumulation rates of isorenieratene (A), loliolide/isololiolide (B), alkenone (C), dinosterol (D), and C_{30} 1,IS-diol/keto-ol (E) at Sites 967, 969 and 964. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

Long-chain alkenones are exclusively biosynthesised by some haptophyte algae (Volkman *et aI.,* 1980; Marlowe *et aI.,* 1984a), which are found in the phytoplankton living in low to moderate productive areas, such as the present-day Mediterranean Sea (Dugdale and Wilkerson, 1988). Mean contents of long-chain alkenones in the homogeneous intervals,

below the sapropels, ranged from 80 to 320 oe decreasing towards the western Eastern Mediterranean basin (Table 4.1, Fig. 4.3). In the sapropels, mean contents ranged from 320 to 930 oe. In the homogeneous interval above the sapropel, mean contents were lower ranging from 120 to 305 µg/g Oc. The AR in the sapropels showed an east to west trend with values between 0.08 to 4.7 mg.m'2.yr'l (Table 4.1, Fig. 4.4C). The distribution of C37:2 and em alkenones (as expressed in the $U_{37}^{k'}$ = [37:2]/{[37:2]+[37:3]}), which is correlated with sea surface temperature (SST; Brassell *et al.,* 1986), was used for SST reconstruction using the temperature calibration established for the north-western Mediterranean Sea by Ternois *et al.* (1997). Inferred SSTs ranged from 24°C to 27°e (Fig. 4.5).

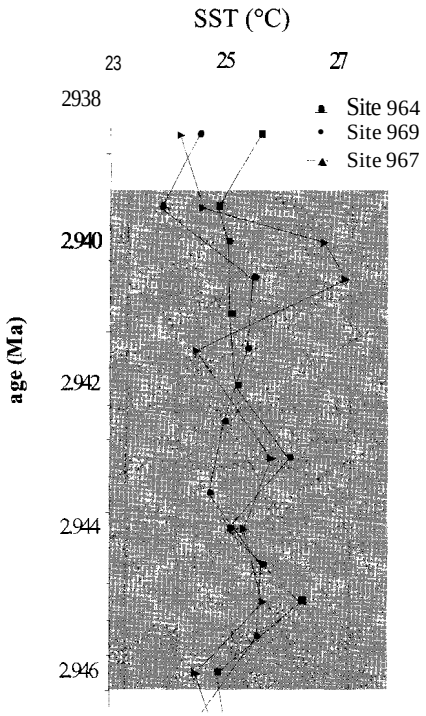

Figure 4.5 SST (0C) reconstruction based on the $U_{37}^{k'}$-SST correlation reported by Ternois *et at., 1997* for the northwestern Mediterranean Sea. Light grey shaded regions represent the sapropels. White regions represent the homogenous calcareous ooze.

Dinosterol is produced by many dinoflagellates (Boon *et al.,* 1979), although exceptional occurrences have been observed in diatoms (Volkman *et al.,* 1993). Dinosterol mean contents in the homogeneous intervals below the sapropels ranged from 0 to 40 oe (Table 4.1, Fig. 4.6). In the sapropels, the highest mean dinosterol contents revealed a range from 60 to 120 OC (Fig. 4.6). ARs in the sapropels at all three sites ranged from 0.005 to 1 mg.m·2.yr,1 (Table 4.1, Fig. 4.4D).

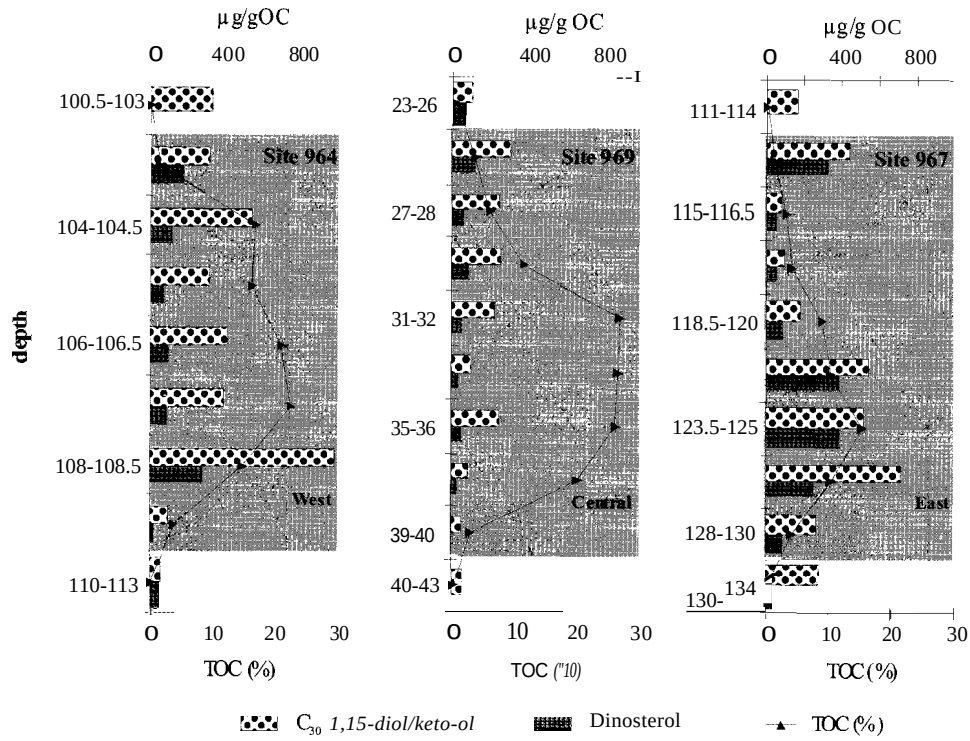

Figure 4.6 Dinosterol and C_{30} 1,15-diollketo-ol contents (normalized to TOC) and TOC contents plotted against depth of the sapropels. Light grey shaded regions represent the sapropel. White regions represent the homogenous calcareous ooze.

C30 1,15-diol and keto-ol were quantified to estimate the abundance of eustigmatophyte algae. C_{30} I,l5-diol is biosynthesised by eustigmatophytes (Volkman *et ai.,* 1992; Volkman *et ai.,* 1999; Gelin *et ai.,* 1999). C_{30} 1,15-keto-o1 is probably an oxidation product (Ferreira *et ai.,* 2001) of the C_{30} 1,15-diol formed during settling of particles in the water column or in the sediment. Direct evidence for the formation through oxidation was obtained through the increase of the keto-ol relative to the diol at the post-depositional oxidation front in the Mediterranean Sl sapropel (Ferreira *et ai.,* 2001). In the homogeneous intervals, below the sapropels the mean summed diollketo-ol contents ranged from 50 to 280 µg/g OC (Table 1, Fig. 4.6). In the sapropels mean contents ranged from 190 to 440 OC. The homogeneous intervals above the sapropels showed mean contents from 105 to 325 µg/g Oc. ARs in the sapropels showed an increase from the east to the west ranging from 0.05 to 2.3 mg.m^{-2}.yr^{-1} (Fig. 4.4E).

Compound-specific $\delta^{13}C$ measurements

The $\delta^{13}C$ values of the $C_{37:2}$ alkenones at Site 969 below the sapropel and at Site 964 below and above the sapropel were not measured, because their content was too low. C37:2 a1kenones in the sapropel showed slightly depleted $\delta^{13}C$ values towards the sapropel centre,

compared with the homogeneous intervals at all three sites (Fig. 4.7A-C). Site 967 showed mean values of -25.2%0 in the homogeneous calcareous ooze and values of -25.7%0 in the sapropel (Fig. 4.7C). Site 969 in the central part of the eastern Mediterranean Sea, showed a general depletion of ca. 1%0 in $\delta^{13}C$ compared with Sites 976 and 964 (Fig. 4.7B). Above the sapropel the $\delta^{13}C$ value was -26.8%0, whereas the mean $\delta^{13}C$ value in the sapropel was -26.4%0 (Fig. 4.7B). The average $\delta^{13}C$ value of -25.4%0 in the sapropel at Site 964 is similar to Site 967 (Fig. 4.7A). $\delta^{13}C$ measurements of loliolide/isololiolide were only performed in the sapropel of Site 969. Like the $\delta^{13}C$ values of $e_{37:2}$ alkenones, loliolide/isololiolide showed slight depletion in $\delta^{13}C$ through the sapropel. The average $\delta^{13}C$ value was -25.7%0 (Fig. 4.7B).

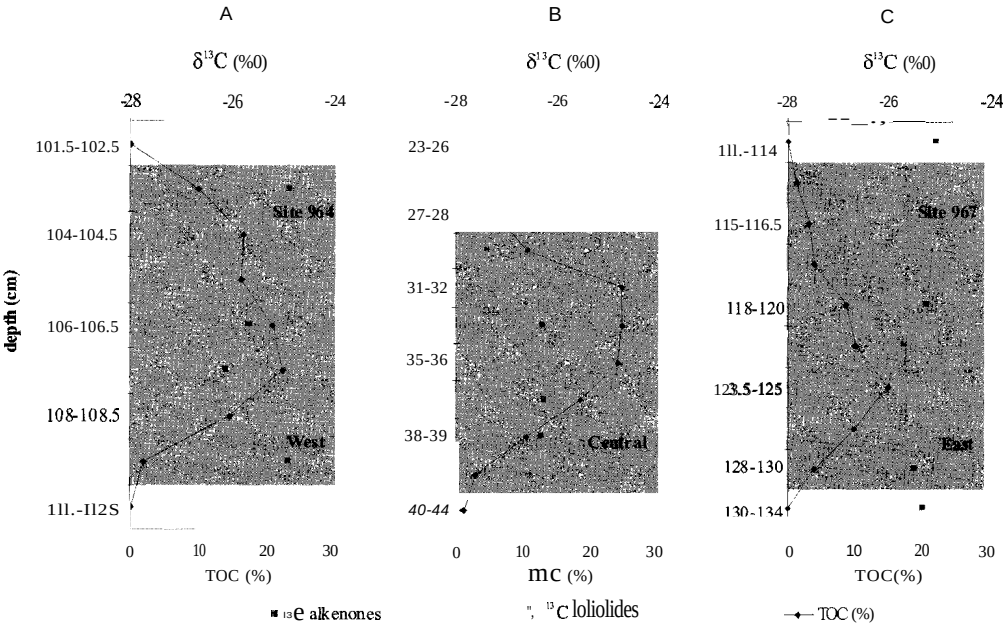

Figure 4.7 $\delta^{13}C$ measurements of alkenones at sapropel Sites 964 (A), 969 (B) and 967 (C) and loliolide/isololiolide at Site 969. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

DISCUSSION

Changes in palaeoexport productivity

A primary signal of enhanced export productivity in these Pliocene sapropels has been shown by the enrichment of barium/aluminium data (Nijenhuis and de Lange, 2000), a common palaeoproxy used in the marine environment (e.g. Dehairs et aI, 1987). The resulting high OM fluxes consequently caused a higher oxygen demand leading to anoxia and in tum played an important role in preservation of OM (Passier *et ai.,* 1999a). Evidence of phosphate regeneration in the most recent sapropel S1 has been reported by enhanced Corg/Porg ratios by Slomp *et al.* (2002) showing the enhanced release of phosphate from organic matter in the

sediments under anoxic pore water conditions. In Pliocene sapropels at Site 969, the Corg/Porg ratios were even a factor of four higher than those of the S1 sapropel (Slomp *et al., 2003.),* indicating that phosphate regeneration may have played an important role in sustaining elevated export productivity in the euxinic basin.

At all three sites phytoplankton biomarker contents, normalized to TOC, were substantially higher in contents in the sapropel than in the homogeneous intervals below and above the sapropel (Table 1). These differences between homogeneous intervals and sapropels are predominantly caused by different redox conditions in the sediment and water column before, during and after sapropel deposition. This was recently demonstrated by a biomarker study of Arabian Sea sediments deposited in and below the oxygen minimum zone (Sinninghe Damste *et aI.,* 2002). During the formation of these sapropels the water column was euxinic up into the photic zone as evidenced by the presence of isorenieratene, a pigment derived from anoxygenic photosynthetic sulphur bacteria (Fig. 4.4A; cf. Passier *et aI.,* 1999a; Menzel *et aI.,* 2002). Consequently, sedimentary pore waters were anoxic during sapropel deposition, resulting in significantly less oxic degradation of organic matter and biomarkers. The largest contents of the four phytoplanktonic biomarkers within the sapropel are observed at Sites 967 and 964 (Fig. 4.3 and 4.6). At Site 969, OC-normalized biomarker contents were generally lower. This may have been caused by a lower export flux of biomarkers or a higher export flux of TOC. During deposition of the homogeneous intervals below and above the sapropel, the pore waters were probably oxic and the organic matter (including the biomarkers) was severely affected by oxic degradation processes. It has been shown that biomarkers are more susceptible to oxic degradation than TOC (Hoefs *et aI.,* 2002; Sinninghe Damste *et aI.,* 2002c), which probably explains the decrease in biomarker content (normalized to OC) outside the sapropel albeit that this may also partly be due to a larger contribution to TOC of terrestrial TOC. The significant difference in redox conditions during deposition has also implications for the *relative* biomarker distribution since sterols like dinosterol are degraded to a greater extent than other marine biomarkers such as alkenones and long-chain diols/keto-ols (Hoefs *et aI.,* 2002; Sinninghe Damste *et al.,* 2002). During oxic depositional conditions, formation of loliolide from fucoxanthin is highly unlikely since it is an anoxic degradation product (Repeta, 1989) and carotenoids are very sensitive to oxic degradation. These considerations indicate that there are severe complications to compare biomarker contents from within the sapropel with those from the homogenous intervals (Fig. 4.3 and 4.6). However, because sedimentary pore waters were anoxic during sapropel formation (Menzel *et aI.,* 2002), biomarker contents within the sapropels can be compared directly. In fact, they were transformed to ARs to remove potential "dilution" effects of the inorganic matrix and to obtain further insights concerning the differences in export production between these three different sites studied (Fig. 4.4A-E).

Phytoplanktonic biomarkers generally showed a strong increase in ARs towards the centre of the sapropel, reflecting an increase in export production to the seafloor caused by increased productivity (Fig. 4.4B-E), and a subsequent decrease. These biomarker AR profiles generally correspond well with the TOC profile at a particular site; e.g. the maximum ARs of biomarkers at Site 967 is reached at one third of the sapropel (Fig. 4.4B-E), just as observed for the TOC profile (Fig. 4.3). In general, highest ARs were observed at Site 969, in

good agreement with the high TOC values for this site. Enhanced export productivity is noted for all four plankton taxa but the increase from the base of the sapropel to the centre is most evident for loliolide/isololiolide, the biomarker for diatoms (Fig. 4.4). Diatoms are the most important phytoplankton species in spring blooms and upwelling events, if silicate is delivered to the photic zone (Nybakken, 1993; Abrantes and Moita, 1999) and thus indicate nutrient-rich conditions. Consequently, the rapid increase in loliolide/isololiolide ARs reveals a rapid change to nutrient-rich conditions, probably caused by the increased nutrient delivery from the Nile and surrounding rivers as a consequence of the increased monsoonal activity (e.g. Rossignol-Strick, 1985). Dinosterol and C30 1,15-diollketo-ol ARs showed successive increase in phytoplankton growth, with highest ARs at the middle part of the sapropel. An increase in the ARs of alkenones in the upper part of the sapropel and succeeding decrease of loliolide/isololiolide ARs indicates most probably a tendency towards nutrient-poorer conditions.

Sapropels at sites 967 and 964 show alkenone ARs corresponding directly to their TOC profile (Figs. 4.3 and 4.4C). It is worthy of note that Site 964 showed highest ARs of alkenones implying that growth conditions favoured haptophytes, which proliferate at lower nutrient conditions (Table I, Fig. 4.4C, Fig. 4.8). Lower nutrient conditions favour smaller photosynthetic organisms, like haptophytes and very small flagellates that have a proportionately greater surface area to adsorb the nutrients, but a lower relative need (Nybakken, 1993).

Reconstruction of phytoplankton composition during sapropel deposition

Although ARs of biomarkers provided direct insights into changes in export production of the different phytoplankton species, changes in the phytoplankton composition are somewhat difficult to assess from these data. To overcome these problems, relative abundances of biomarker were used to reconstruct phytoplankton patterns during sapropel fonnation. Based on biomarker contents the relative distribution of the four main phytoplankton classes during sapropel fonnation was detennined (Fig. 4.8). To some degree this is a biased view of export productivity, because biomarker contents may vary depending upon the type of algae and even within one class of algae (e.g. Brassell, 1993).

Considering the diatom population at Site 967, the contribution of diatoms is highest in the lower part of the sapropel and decreases through the sapropel. This probably reflects the nutrient supply at this site derived from the Nile River. The sapropel at Site 969 showed a successive increase in diatom populations reaching highest abundances in the middle part of the sapropel at the maximum of TOC content. This probably indicates that the high productivity of diatoms in the central part of the eastern Mediterranean basin (Fig. 4.4B) was for a substantial part responsible for the significantly increased TOC flux. At Site 964, the diatom population appears with sapropel onset and remained constant during sapropel fonnation.

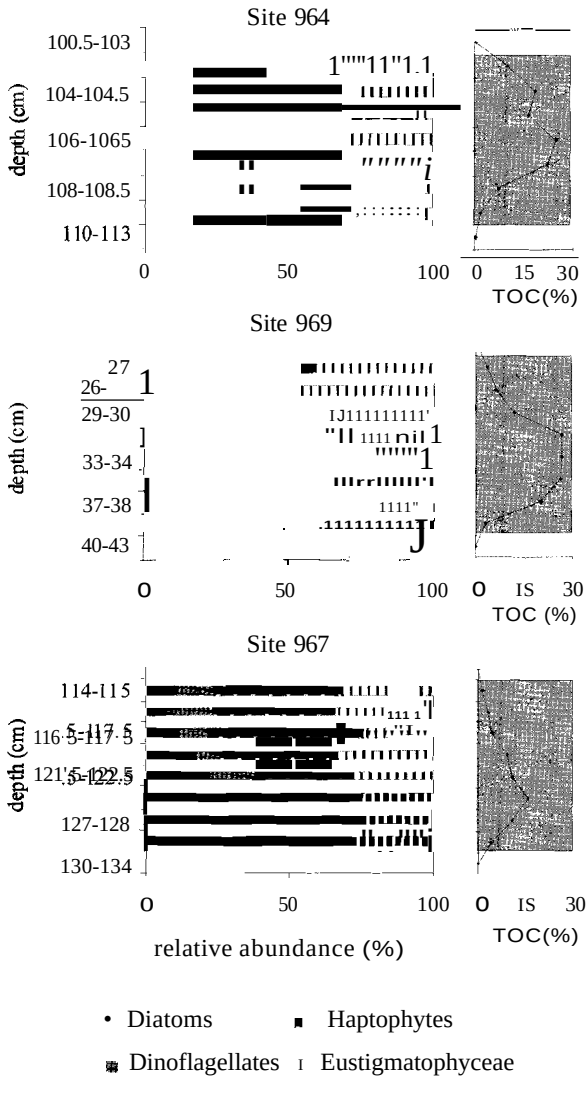

Figure 4.8 Relative distribution of four phytoplankton classes at sapropel Sites 967, 969 and 964 and their TOe profile,

Generally, the relative abundances of loliolide/isololiode in the sapropels correspond roughly with their TOe profiles, which may indicate a consistent contribution of diatoms to Toe accumulated in these sapropels. In contrast, haptophyte and eustigmatophyte algal abundances decrease relative to the other two algal classes, except at Site 964, where relatively high amounts of haptophytes are maintained during sapropel formation, possibly resulting from less nutrient-rich conditions.

The presence of both loliolide isomers was previously reported from sediments underlying highly productive surface waters e.g. Peruvian shelf (Repeta, 1989) and from Mediterranean sapropels (ten Haven *et aI.,* 1987b). Based on isorenieratene data and diatom patterns, nutrient supply probably triggered high productivity at the easternmost part of the Levantine basin, subsequently spreading to the western part of the Levantine basin. Consequently, excessive oxygen depletion resulted in euxinic conditions up into the upper water column. Continued high productivity was controlled by the release of regenerated phosphate from the sediment resulting in high C_{org} burial. In addition, the increase of the abundances in diatoms and dinoflagellates relative to the haptophytes and eustigmatophytes in the standing crop during sapropel deposition can be considered as evidence for nutrient rich and highly productive episodes. Based on reconstructed SST data, changes in the phytoplankton composition were not influenced by SST variations, which is consistent with SST data reported by Rinna *et ai.* (2002) for another Pliocene, TOC-rich sapropel.

$\delta^{13}C$ results of alkenones and loliolide-palaeoproxy of enhanced biological productivity?

To further investigate the increase in productivity during sapropel formation, changes in the carbon isotopic compositions of alkenones and loliolide (only at Site 969) were studied. The isotopic signature $\delta^{13}C$ depends on factors such as light, concentration and $\delta^{13}C$ of DIC, growth rate, cell size and morphology, and species composition (Rau *et aI., 1996;* Laws *et aI.,* 1995; Popp *et ai.,* 1998; Rost *et aI.,* 2002). Because of the absence of carbonate skeletons in the sapropel by overprinting or dissolution caused by the prevailing palaeoenvironmental conditions, the $\delta^{13}C_{CO2(aq)}$ could not be estimated. Consequently, it was not possible to calculate the carbon isotopic fractionation effect during photosynthesis ($\varepsilon_p = \delta^{13}C_{CO2(aq)} - \delta^{13}C_{biomass}$). However, two features were observed related to the $\delta^{13}C_{37:2}$ alkenone records measured in the homogeneous ooze marls and sapropels. Firstly, measurements in the homogeneous intervals at Sites 967 and 964 showed approximately equal values. In the sapropels a slight depletion of 0.3-1‰ in $\delta^{13}C$ is observed. Secondly, Site 969 showed a general depletion in of the $C_{37:2}$ alkenones of 1‰ reaching values of -27.4‰. $\delta^{13}C$ measurements of loliolide/isololiolide also showed a depletion of ca. 1‰ through the sapropel, similar to the observation of the $\delta^{13}C$ of $C_{37:2}$ alkenones at all three sites. The anticipated increase in $^{13}C/^{12}C$ of the phytoplankton population resulting from an increased rate of uptake of C02 (relative to the C02 supply) during times of high productivity was not found. Assimilation of isotopically light DIC (e.g. from riverine and anoxic deep water) in foraminifera and $\delta^{13}C_{(TOC)}$ was reported during deposition of sapropel S5 at Site 967 (Struck *et ai.,* 2001). Extensive recycling of respired CO_2 close to the chemocline during enhanced productivity could provide an explanation for the depleted $\delta^{13}C$ values. The isotopic fractionation (ε_p) depends on growth rate/[CO_2]aq, (Bidigare *et aI.,* 1997; Riebesell *et aI.,* 2000) and would thus be expected to decrease upon increased productivity. This would, however, be counteracted by increased CO_2 concentrations and isotopic depletion of DIC by substantially increased recycling of isotopically depleted, respired CO_2 formed by OM mineralisation and advection to the photic zone by occasional erosion of the chemocline during storms. Based on the lighter $\delta^{13}C$ values of $C_{37:2}$ alkenones at Site 969, recycling of respired CO_2 was probably more intensive at this site. This is consistent with the highest

isorenieratene ARs at this site compared with Sites 967 and 964, indicating a shallower chemocline enabling a more substantial delivery of respired, isotopically depleted CO_2 to the photic zone.

CONCLUSIONS

Biomarkers representing four classes of phytoplankton were used to obtain insights into the changes in export productivity during sapropel fonnation in the eastern Mediterranean basin. Enhanced productivity resulted in increased export production and consequently led to oxygen depletion in the pore waters and water column. High relative abundances of diatoms at the lower part of sapropel, Site 967 and the increasing tendency at Site 969 indicated that most likely nutrients were transported from the Nile and surrounding rivers into the eastern basin, which spread across to the western part of the Levantine basin. Based on the $U_{37}^{k'}$ data, productivity was not influenced by changes in sea surface temperature, but was mainly nutrient-controlled. Compound specific measurements of diatom and haptophyte biomarkers showed no significant changes during sapropel fonnation most probably implying enhanced recycling of CO_2 from below the chemocline, which probably buffered the growth rate effect on isotope fractionation in lipids.

ACKNOWLEDGEMENTS

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Chapter 5

Membrane tetraether lipids of planktonic Crenarchaeota in Pliocene sapropels of the eastern Mediterranean Sea

Diana Menzel, Ellen C. Hopmans, Stefan Schouten and Jaap S. Sinninghe Damste

ABSTRACT

The distribution of glycerol dibiphytanyl glycerol tetraethers (GDGTs), biomarkers of planktonic crenarchaeota, was studied in three time-equivalent Pliocene sapropels as well as in the homogeneous intervals, below and above the sapropel. In both the homogeneous intervals and sapropels the dominance of GDGT-O and crenarchaeol among the GDGTs indicated planktonic crenarchaeota as their source similar to what was found in many other marine sediments. In the homogeneous intervals highest GDGT abundances (normalized to TOe) were measured at the easternmost study Site 967 of the eastern Mediterranean Sea and decreased at the central and western Sites 969 and 964. Within the three studied sapropels large variations in GDGT abundance were observed. The newly established sea surface temperature (SST) proxy, the TEX_{86} index, derived from GDGTs of planktonic crenarchaeota was used to estimate past SST. Comparison with previous obtained SST data using the alkenones from haptophyte algae revealed substantial differences. Whereas the $U^{K'}_{37}$-based SSTs showed almost constant values of ca. 25°C in both the homogeneous intervals and the sapropels, the TEX_{86}-based SSTs were 26-29°C in the homogeneous intervals and decreased to 15-17°C in the sapropels. The TEX86-based SST values outside the sapropel probably reflect summer SST based on a comparison with the present-day Mediterranean Sea. The surprisingly low TEX_{86}-based SST estimates for the sapropels showed similarities with those obtained for the contemporary euxinic Black Sea. The marine crenarchaeota of the modern Black Sea thrive at the deeper and colder chemocline, leading to a reduction in TEX_{86} which in turn would result in artificially low SST estimates. A similar situation is inferred for the Pliocene eastern Mediterranean during sapropel deposition since the SST anomaly co-occurs with the build-up of a shallow chemocline.

INTRODUCTION

Life on Earth can be classified into three fundamentally different domains: Bacteria, Archaea and Eukaryota (Woese, 1987). Archaea are known to live under extreme environmental conditions, such as high temperature, high salinity, high pressure, anoxia, and low and high pH, that are hostile to most other forms of life. Archaea are divided into three kingdoms, Crenarchaeota, Euryarchaeota and Korarchaeota (Woese *et al.*, 1990; Barns *et al.*, 1996; Takai and Sako, 1999). Since the application of molecular techniques to microbial

ecology, in particular the use of 16S rRNA as a molecular marker, archaea have revealed to be widespread in different ecosystems and have been shown not to be restricted to extremophilic environments as was previously thought (Ward *et al.,* 1992). A particularly exciting result was the discovery of the widespread occurrence of planktonic archaea in marine plankton in the Pacific Ocean and coastal waters of North America (Fuhrmann *et al.,* 1992; de Long, 1992). Among marine archaea, two major groups originating from the euryarchaeal and crenarchaea1 group were identified in oceans, lakes and polar waters (e.g. Hershberger *et al.,* 1996; McGregor *et al.,* 1997; Massana *et al.,* 1998). Pelagic euryarchaeota and crenarchaeota showed different patterns of abundance in the open sea. High abundances of crenarchaeota accounting for up to 40% of total DNA-containing picoplankton in meso - (150-1,000 m) and bathypelagic waters (1,000-3,500 m) (Karner *et al.,* 2001; Church *et al.,* 2003) were detected. In contrast, pelagic euryarchaeota showed higher abundances in coastal surface waters (Massana *et al.,* 1997; Murray *et al.,* 1999b; de Long *et al., 1999).*

Membrane lipids of cultivated hyperthermophilic crenarchaeota consist of diphytanyl glycerol diethers and glycerol dibiphytanyl glycerol tetraethers (GDGTs), (de Rosa and Gambacorta, 1988). The occurrence of ether-bound acyclic and cyclic biphytanes in particulate organic matter (POM) from the water column and surface sediments (e.g. Hoefs *et al.,* 1997; King *et al.,* 1998) as detected by chemical degradation studies, hinted to the presence of planktonic crenarchaeota, and, thus confirmed the molecular ecological studies (e.g. Karner *et al.,* 2001). The development of a high performance liquid chromatography/mass spectrometry technique has enabled the characterization of intact GDGTs (Hopmans *et al.,* 2000). Using this technique, marine crenarchaeota were found to contain GDGTs with zero to four cyclopentane rings (Schouten *et al.,* 2000). A novel GDGT containing four cyclopentane rings and one cyclohexane ring was exclusively found in planktonic crenarchaeota and was called crenarcheaol (Schouten *et al.,* 2000, Sinninghe Damste *et al.,* 2002a). The biosynthesis of this GDGT, in particular the cyclohexane ring, is thought to be a membrane adaptation to allow these descendants of (hyper)thermophilic crenarchaeota to cope with the relatively low temperatures of the ocean (Sinninghe Damste *et al.,* 2002a). The dominant presence of these membrane lipids confirmed their high contribution to the marine picoplankton in modern oceans (Schouten *et al.,* 2000; Sinninghe Damste *et al.,* 2002a).

The number of cyclopentane rings in GDGTs of cultivated (hyper)thermophilic archaea increases with increasing growth temperature (Gliozzi *et al.,* 1983). A study of marine surface sediments showed that non-thermophilic crenarchaeota also adjust the number of cyclopentane rings in their GDGT membrane lipids according to temperature (Schouten *et al.,* 2002). This response was quantified in the so-called TEX86 index (Schouten *et al., 2002),* which revealed a strong linear correlation with sea surface temperature (SST) and can be used to reveal past SST (Schouten *et al.,* 2003). In this study we analysed GDGTs in three time-equivalent Pliocene sapropels to obtain insight in temporal and spatial changes in the abundances and distribution pattern of this biomarker. Additionally, the newly established palaeo proxy for SST reconstruction, the TEX86, was compared with previous results (Menzel *et al.,* 2003) obtained from a more commonly used proxy for SST reconstruction in palaeoceanography, the $U_{37}^{K'}$, which derives from the long-chain unsaturated alkenones from

haptophyte algae (Brassell *et ai.,* 1986). These two SST palaeo proxies give divergent results for the three studied Pliocene sapropels. Whereas the $U^{k'}_{37}$-based SST showed values of ca. 25°C, the TEX_{86} revealed a significant trend to lower temperatures down to IS-17°C. Possible causes are discussed.

MATERIAL AND METHODS

Samples

Core samples were taken during ODP Leg 160 in the eastern Mediterranean Basin (Emeis *et at.,* 1996). Site 967 (Eratosthenes Seamount) and Site 969 (Mediterranean Ridge) are located in the Levantine Basin. Site 964 is located in the Ionian Basin (Fig. 5.1a). The detailed chronology for these cores obtained by astronomical tuning enabled us to select three laterally equivalent sapropels with an age of 2.943 Ma (Lourens *et ai.,* 1996). Relevant sections (967C-8H-4, 111-134 em; 969E-6H-6, 23-43 cm; 964D-IOH-l, 100.5 -113 cm) were sub-sampled in 0.5-1.5 em slices above, through and below the sapropel. Total organic carbon (TOC) was reported previously and showed distinct differences between these sapropels (Nijenhuis *et at.* 1998; Fig. 5.1b).

Lipid analyses

Ground, freeze-dried sediment samples (0.1 to 3 g) were Soxhlet extracted with dichloromethane/methanol (1:1, v/v) mixture for 24 h. The extracts were concentrated with a rotary evaporator at 30°C. An aliquot of the total extract (1.5 to 3 mg) was fractionated using a Pasteur capillary pipette (150 mm length) packed with Al_2O_3 (activated for 2.5 h at 120°C). The apolar fraction (Fl) was eluted using a mixture of hexane/dichloromethane (9: 1, v/v; 4 ml). Subsequently, the ketone fraction (F2) was eluted using hexane/dichloromethane (1:1, v/v; 4 ml). Finally, the polar fraction was obtained using dichloromethane/methanol (1:1, v/v; 4 ml). The polar fraction was used to identify and quantify glycerol dibiphytanyl glycerol tetraethers (GDGTs) by high performance liquid chromatography/mass spectrometry analyses (HPLC/MS). Absolute GDGT amounts (μg gol dry weight sediment) were used to calculate accumulation rates (ARs), assuming (i) a synchronous start and end of sapropel deposition at the three locations, (ii) a duration of sapropel deposition of 7000 years (Nijenhuis and de Lange, 2000) and (iii) a constant sedimentation rate during sapropel deposition (Menzel *et at.,* 2002). ARs in the homogeneous intervals were not calculated because sedimentation rates are expected to be different during times of deposition of sapropel and the homogeneous intervals.

High performance liquid chromatography/mass spectrometry (HPLCIMS)

Analyses were performed using an HP (Palo-Alto, CA, USA) llOO series LC-MS equipped with an auto-injector and Chemstation chromatography manager software. Separation was achieved on a Prevail Cyano column (2.1 x 150 mm, 3 μm; Alltech, Deerfield, IL, USA), maintained at 30°C. Injection volumes varied from 1 to 5 μl. Tetraethers were eluted isocratically with 99% A and 1% B for 5 min, followed by a linear gradient to 1.8% B in 45 min, where A = hexane and B = propanol. Flow rate was 0.2 ml/min. After each

analysis the column was cleaned by back-flushing hexane/propanol (90:10, v/v) at 0.2 ml/min for 10 min. Detection was achieved using atmospheric pressure positive ion chemical ionization mass spectrometry (APCI-MS) of the eluent. Conditions for APCI-MS were as follows: nebulizer pressure 60 psi, vaporizer temperature 400°C, drying gas (N_2) flow 6 l/min and temperature 200°C, capillary voltage -3 kV, corona 5 μA (~ 3.2 kV). Positive ion spectra were generated by scanning *m/z* 950-1450 in 1.9 s. Mass spectra typically represented the peak-apex spectrum and are corrected for background.

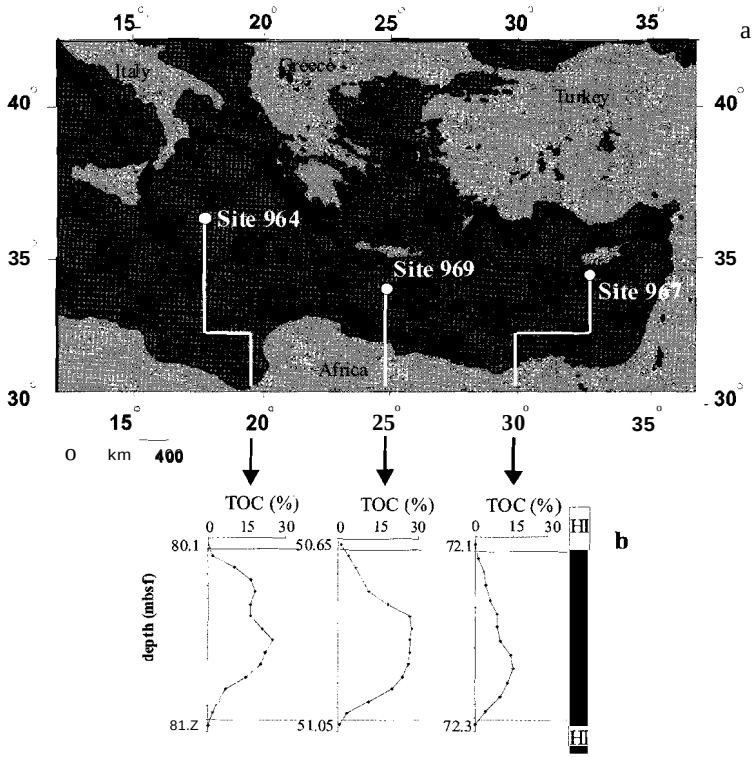

Figure 5.1 (a) Location of ODP drilling sites and (b) lithological description (HI = homogeneous calcareous ooze, S = sapropel) and Toe profiles of the investigated Pliocene sapropel (data from Nijenhuis *et al., 1998).*

RESULTS

GDGT distribution

The HPLC/MS base peak chromatograms revealed the presence of the GDGTs I-VI in both, the sapropels and the homogeneous sediments below and above the sapropel at the three sites (Fig. 5.2). GDGT-O (I) and crenarchaeol (V) were the dominant GDGTs (Fig. 5.2). The GDGT distribution between the homogeneous interval and sapropel of Site 967 indicated internal variations in the relative distribution of the analysed GDGTs. In the homogeneous

intervals of the sapropels (Fig. 5.2a) higher relative abundances of GDGT-2, GDGT-3 and crenarchaeol (V+VI) were detected, compared with the GDGT distribution in the sapropel (Fig. 5.2b). Smaller variations in the relative distribution of GDGTs within the three time-equivalent sapropels were also observed as is expressed by variations in TEX_{86} values (see below).

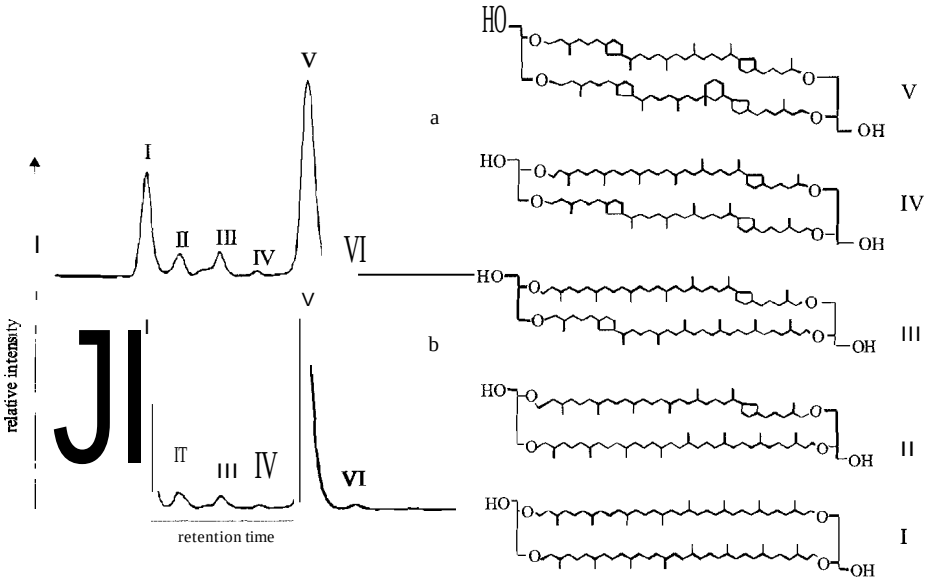

Figure 5.2 *HPLC/MS* base peak chromatograms of (a) the homogeneous interval of ODP Site 160-967C-8H-4, 130-134 cm and (b) the sapropel of ODP Site 160-967C-8H-4, 121.5-122.5 cm series were identified as: GDGT-O=I, GDGT-1=II, GDGT-2 = III, GDGT-3 = IV, crenarchaeo1 = V and an isomer of crenarchaeo1 =VI (cf. Hopmans et al., 2000).

Temporal and spatial distribution pattern of tetraether lipid abundance

The total GDGT content showed below sapropel deposition at Site 967 values of 1670 μg/g TOe and reached a maximum of 2790 μg/g TOe when the Toe content was the highest (Fig. 5.3). After the maximum was reached, the GDGT content decreased substantially in the central part of the sapropel to values of about 100 TOe and increased again in the upper part of the sapropel to 1600 TOe. Above the sapropel the GDGT is 550 μg/g TOe. Site 969 showed below the sapropel GDGT amounts of 640 TOe (Fig. 5.3). In the lower and central part of the sapropel the values remained relatively constant and then increased to maximum value of 2150 TOe. After the maximum was reached, the GDGT content decreased noticeably towards the top of the sapropel to values of about 150 TOe. Above the sapropel the GDGT content decreased to values of 40 TOe. Site 964 revealed below the sapropel GDGT values of 160 μg/g TOe (Fig. 5.3). At the base of sapropel the GDGT content increased to values of 1200 μg/g TOe, but then decreased considerably to values of about 100 μg/g TOe in the central part of the sapropel. Maximum concentrations of GDGT occur in the upper part of the sapropel with 1900 TOe. Above the sapropel the GDGT content was negligible.

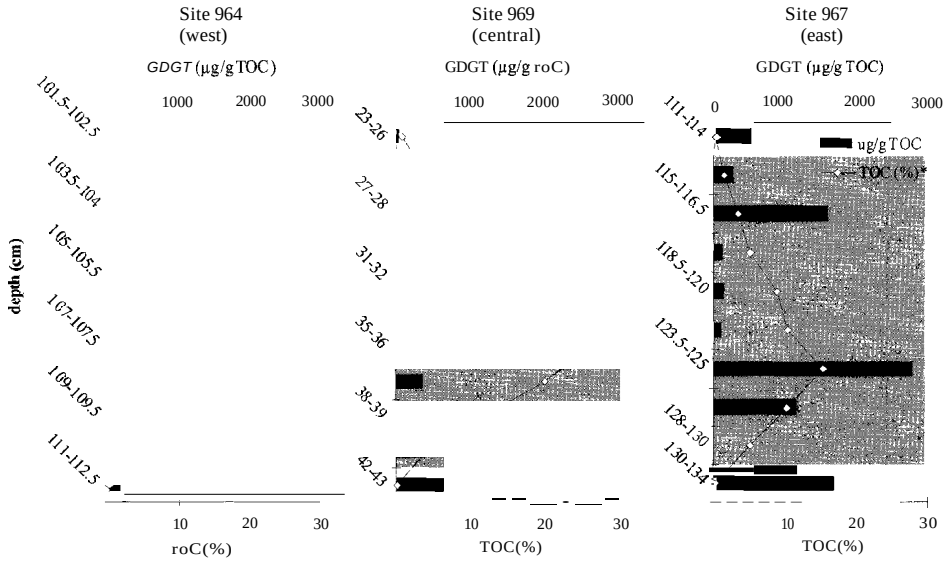

Figure 5.3 Summed GDGT concentrations (normalized to TOe) and TOe content (*data from Nijenhuis *et ai"* 1998) plotted against depth for the three sapropels at ODP Sites 967, 969 and 964, Light grey shaded regions represent the sapropeL White regions represent the homogeneous calcateous ooze.

In the lower part of sapropel at Site 967, GDGT accumulation rates (ARs) (see experimental for details) reached a maximum value of 12.3 µg m'2 yr-! and reduced to values ranging between 0.2 and *004* µg m^{-2} yr'l at the central part of the sapropel were these low values remained up to the upper part of the sapropel (Fig. *504).* At Site 969, GDGT ARs increased steadily reaching a maximum value at the central part of the sapropel with values of 17.2 µg m'2 yr'! (Fig. *504).* After the maximum was reached, GDGT ARs decreased considerably to values of 0.7 µg m'2 yr-! at the upper part of the sapropeL Site 964 showed the lowest GDGT ARs with a slight increase in the lower part of the sapropel of 0.8 µg m^{-2} yr'!. Through the entire central part of the sapropel low ARs of about 0.3 µg m^{-2} yr'l were shown, whereas in the upper part of the sapropel a maximum value of 4.6 µg m'2 yr-I was reached (Fig. *504).* After the GDGT AR has reached the maximum it decreased dramatically as was observed at the other two sites.

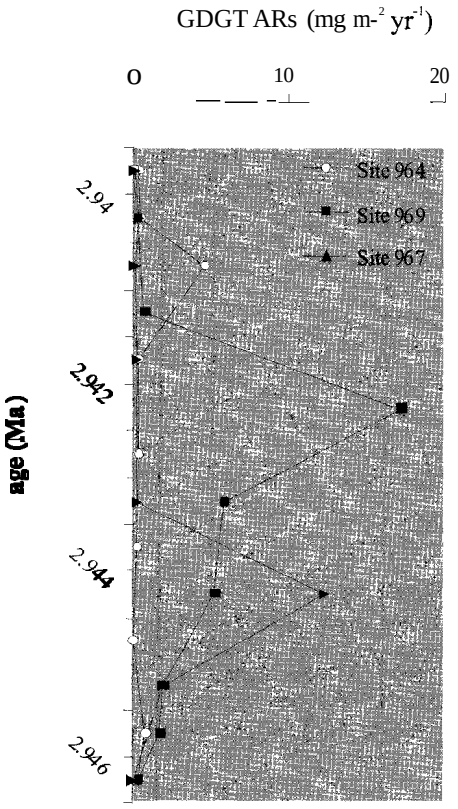

Figure 5.4 Summed GDGT accumulation rates in the three sapropels of the three ODP Sites 967,969 and 964.

Sea surface temperature estimation based on using the TEX_{S6}

The TEXS6 index (TetraEther indeX of GDGTs consisting of 86 carbon atoms) is defined as:

$$TEX_{86} = \frac{([III]+[IV]+[VID}{([II]+[III]+[IV]+[VI])}$$

and can be used to reveal past SST (Schouten *et at.,* 2002). The distribution of GDGTs correlates with SST, i.e. with higher SST the number of cyc10pentane rings of the tetraether membrane lipids increases, i.e. GDGT-2, GDGT-3 and crenarchaeol increase in abundance relative to tetraether lipids GDGT-O and GDGT-I (Fig. 5.2). The TEXs6 showed distinct temperature changes between the homogeneous intervals and Pliocene sapropels at all three studied ODP sites (Fig. 5.5). In the homogeneous intervals below the sapropel, the TEXS6 revealed highest SST at Site 969 of 29°C and slightly lower SST's at Site 967 and 964 of 27°C and 25°C, respectively. In all sapropels, the $TEX_{S}6$-based SST showed a substantial decrease. The largest drop (l5°C) is observed at Site 967 in the lower part of the sapropel (Fig. 5.5). In the upper part of the sapropel the SST slowly increased to values of 22°C. In the homogeneous interval, above the sapropel the SST reached 26°C. At Site 969, the SST

dropped within the sapropel to values around 17°C in the central part of the sapropel and increased also slowly towards the top of the sapropel to values of 21°C. In the homogeneous interval above the sapropel the SST remained at 21°c (Fig. 5.5). In the sapropel at Site 964 the SST at the base of the sapropel was the highest with 33°C and then decreased towards the top of the sapropel to values of 18°C. In the homogeneous interval above this sapropel, some GDGT isomers were below the detection limit and no TEX_{86} values could be obtained (Fig. 5.5).

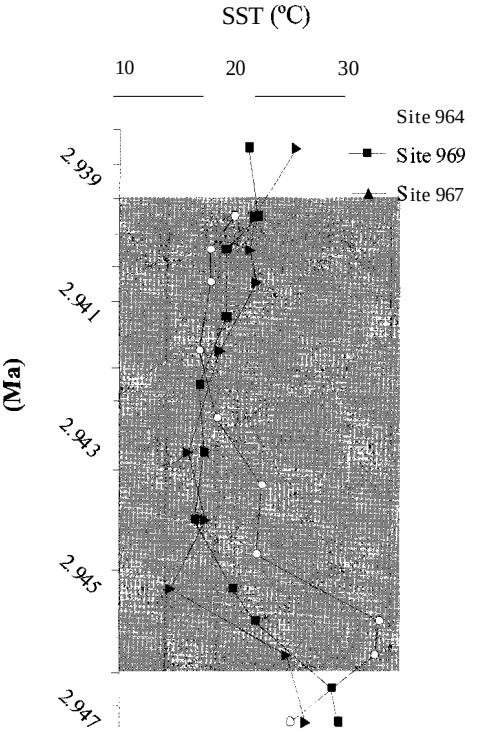

Figure 5.5 SST (ºC) reconstruction based on the TEX_{86} proxy (after Schouten *et al.,* 2002) of the three time-equivalent Pliocene sapropels of the eastern Mediterranean Sea. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

DISCUSSION

Origin of GDGT lipids

GDGT-O (1) is a general archaeal core membrane lipid (e.g. Koga *et aI.,* 1998) and occurs not only in planktonic crenarchaeota but also in methanogens (Koga *et al., 1993).* Crenarchaeol (V) has been assigned as a specific core membrane lipid of planktonic crenarchaeota (Schouten *et aI.,* 2000; Sinninghe Damste *et at.,* 2002a). The dominance of GDGT-O and crenarchaeol in both the homogeneous intervals and the sapropels (Fig. 5.2) is characteristic for the GDGTs of planktonic crenarchaeota (Sinninghe Damste *et aI.,* 2002b; de Long *et al.,* 1998). Such GDGT membrane lipid distributions were also observed in many other marine sediments (Schouten *et aI.,* 2002) and marine POM from the North Sea

(Wuchter *et ai.,* 2003) and the Arabian Sea (Sinninghe Damste *et al.,* 2002b). GDGTs in the water column of the Black Sea also showed a predominance of GDGT-O and crenarchaeol in the upper 400 m (Wakeham et al., 2003). By contrast, GDGTs derived from anaerobic, methane-oxidizing archaea, which reside in the deeper anoxic zone (> 1000 m water depth) have a distinct distribution of carbon isotopically depleted GDGTs. A dominance of GDGT-O and crenarchaeol in GDGT distributions was found in sinking particles collected with sediment traps in the Black Sea. The GDGTs derived from methane-oxidizing archaea were not detected in sediment traps or underlying surface sediments, showing that the sedimentary GDGTs are predominately derived from surface waters. This is apparently caused by efficient transport mechanism (e.g. grazing and production of fecal pellets) of GDGTs in surface waters (Wakeham *et ai.,* 2003).

Coolen *et al.* (2002) proposed that metabolically active marine chemoorganotrophic crenarchaeota are present in late Pleistocene sapropels of the eastern Mediterranean Basin. These authors suggested that, in comparison with the homogeneous intervals, sapropels exhibit elevated microbial cell numbers with crenarchaeota constituting a fraction of ca. 16%. Increased enzyme activities of anaerobic microbial degradation using carbon substrates originating from the sapropel organic matter were measured and ascribed, in part, to metabolically active crenarchaeota (Coolen *et al.,* 2002). This indicates that the GDGTs found in our sapropels could derive from physiologically active crenarchaeota in the sapropels and do not represent chemical fossils derived from planktonic crenarchaeota. However, the GDGT abundances of a sapropel showed large fluctuation on a cm scale (Fig. 5.3), which is inconsistent with chemoorganotrophic crenarchaeota using sapropel organic matter as carbon source as the source of these GDGTs. The ^{13}C contents of biphytanyl moeities of crenarchaeol from both present-day and ancient marine environments (Hoefs *et al.,* 1997; Kuypers *et al.,* 2001) and their ^{14}C content (Pearson *et aI.,* 2001) indicated that marine crenarchaeota probably fix bicarbonate. ^{13}C label experiments confirmed a light-independent bicarbonate uptake (Wuchter *et al.,* 2003), indicating chemoautotrophy and not chemoorganotrophy (cf. Coolen *et al.,* 2002) by marine crenarchaeota. In addition to the large variations in the absolute abundances of GDGTs, also large variations in the GDGT distributions within the sapropel are observed. As these represent physiological adaptation of their membrane to temperature (see detailed discussion below), this provides an additional argument for their origin from planktonic crenarchaeota and not from chemoorganotrophic crenarchaeota within the sapropel, which would encounter identical sedimentary temperature conditions.

SST reconstructions using $U_{37}^{K'}$ and TEX_{86}

$U_{37}^{K'}$-based SST derived from the haptophyte algae revealed almost similar SST values of 24-26°C obtained in the three time-equivalent Pliocene sapropels and their corresponding homogeneous intervals (Fig. 5.6) (cf. Menzel *et aI.,* 2003). In contrast, the TEX86-based SST estimates showed substantial differences between the homogeneous intervals and the sapropels as well as between these sapropels (Fig. 5.6). The reason for this large difference must lie in the fact that both SST proxies originate from two different organisms occupying different habitats in the eastern Mediterranean Basin.

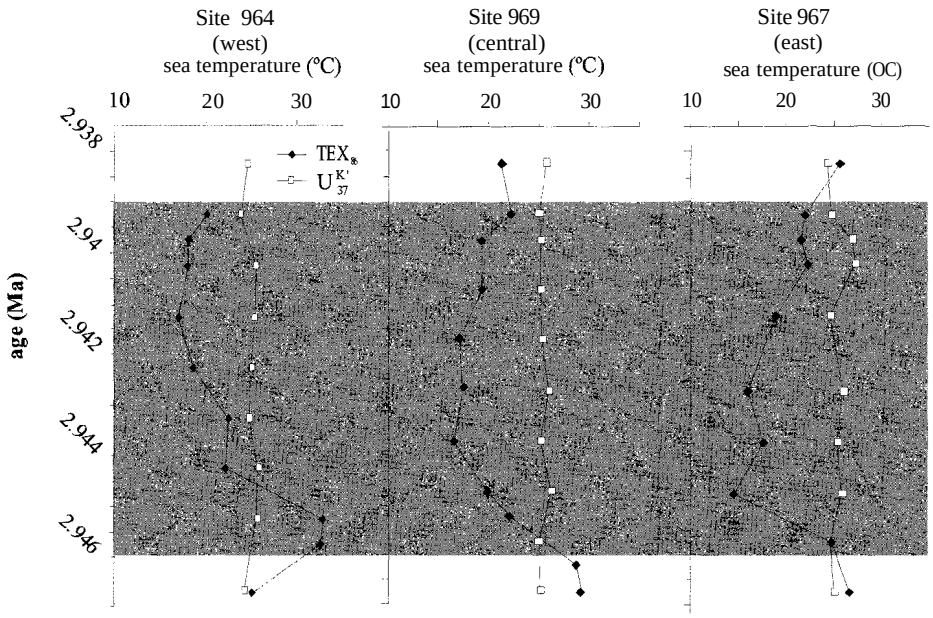

Figure 5.6 Reconstruction of the SST (°C) during Pliocene sapropel deposition using two different SST proxies $U_{37}^{K'}$ and TEX_{86}, in the sapropels at ODP Sites 967,969 and 964.

In the contemporary oligotrophic eastern Mediterranean Sea the annual phytoplankton bloom occurs in winter as soon as deep water mixing occurs, providing nutrients to the photic zone, and ceases when phosphate is depleted (Krom *et aI.,* 2003). After the winter bloom, water column stratification occurs in March-April, which results in the formation of a deep chlorophyll maximum that is characteristic for the remainder of the year (Krom *et al., 2003).* Haptophytes live in the upper 200 m and are the most common eukaryotes followed by diatoms and dinoflagellates (Kimor *et aI.,* 1987). An $U_{37}^{K'}$-based SST of ca. 19°C was estimated from suspended matter collected in sediment traps (Ternois *et al.,* 1997) and surface sediments of the Mediterranean Basin (Emeis *et aI.,* 2000), reflecting the SST in winter when haptophyte production is most pronounced. Planktonic crenarchaeota typically have their main growth phase during the annual cycle outside the main period of phytoplankton blooms. A negative correlation was obtained between the abundance of chlorophyll *a* and particulate organic carbon and the abundance of crenarchaeota for Antartic coastal waters and Southern Ocean (polar regions) (Murray *et aI.,* 1998; Murray *et aI.,* 1999b) and the Santa Barbara Channel (Murray *et al.,* 1999a). Schouten *et al.* (2002) also inferred different growing seasons for haptophytes and marine crenarchaeota in the eastern South Atlantic. Indeed, TEX86 values in surface sediments of the eastern Mediterranean Sea indicate an SST of ca. 26°C (Sinninghe Damste *et al.,* unpublished data), which reflects the SST during summer in the Mediterranean Sea. Thus, the $U_{37}^{K'}$- and TEX_{86}-based SSTs in the homogeneous intervals (thought to reflect periods in the Pliocene comparable with the modem oligotrophic eastern Mediterranean Sea)

are likely reflecting the winter and summer SST, respectively. These SST estimates are realistic values considering the warmer climate conditions during the Pliocene, compared with the present-day conditions (e.g. Poore and Sloan, 1996).

During sapropel deposition, the precession minimum caused warmer and especially more humid climate conditions with a stronger seasonal climate contrast (e.g. Rossignol-Strick *et al.,* 1982; Rossignol-Strick, 1985; Prell and Kutzbach, 1987; Hilgen, 1991). Due to warmer summer and colder winter periods, lower U^{K}_{37}-based and higher TEX_{86}-based SSTs could be expected, if the timing of the bloom season did not change. The $U^{K'}_{37}$ values in the sapropel, compared with those in the homogeneous intervals, indicated a maximum increase in SST of l°e. U^{K}_{37}-based SST's obtained for other Pliocene sapropels (3.058 Ma, Lourens *et al.,* 1996) showed a slight decrease of 1°C in SST (Rinna *et aI.,* 2002). U^{K}_{37} values of late Pleistocene to Holocene sapropels, relative to those of the homogeneous intervals also only revealed a small change in SST of a few degrees (Emeis *et al.,* 1998). In strong contrast with the $U^{K'}_{37}$-based SST estimates in the Pliocene sapropels, the TEX_{86}-based SST revealed an unexpected cooling, i.e. a drop of 1O-12°C in SST (Fig. 5.6), which appears in contrast with the reported climatic change. This must indicate that either the ecological niche of the planktonic crenarchaeota or the timing of their growth season must have been fundamentally different at the time of sapropel deposition.

During Pliocene sapropel deposition a substantial increase in primary productivity, compared to the situation before and after sapropel deposition (represented by the homogeneous intervals), is indicated by the enhanced Ba flux (Nijenhuis and de Lange, 2000) and was caused by increased nutrient delivery to the basin (Passier *et al.,* 1999a). The increased primary productivity is also revealed by increasing accumulation rates of specific biomarkers for the major groups of phytoplankton (diatoms, haptophytes, dinoflagellates, eustigmatophytes) towards the centre of the sapropel (Menzel *et al.,* 2003). The enhanced organic matter flux to the sea floor led to oxygen depletion, sulphate reduction and ultimately to the presence of sulphide in the photic zone of the water column. The presence of such a shallow chemocline was indicated by the presence of isorenieratene derivates (Passier *et al.,* 1999a) and intact isorenieratene (Menzel *et aI.,* 2002), the characteristic pigment of anaerobic, photolithotrophic green sulfur bacteria, which require both sulphide and light. Such conditions are currently found in the Black Sea (Repeta *et al.,* 1989), the world's largest euxinic basin. Therefore, the Black Sea is good model to compare environmental situations related to sapropel deposition in the Mediterranean Sea. Marine primary productivity in the Black Sea (210 gC Karl and Knauer, 1991) is substantially higher than in the oligotrophic eastern Mediterranean Sea (26 gC m,2yr,ı, Bethoux, 1989), and similar to that estimated for the eastern Mediterranean Sea during Pliocene sapropel deposition (ca. 150 gC m'2yr'I), based on Ba profiles (Passier *et al.,* 1999a). The Black Sea shows in general two major phytoplankton blooms, a diatom and silicoflagellate bloom in spring and a haptophyte bloom in autumn (Honjo *et al., 1987).*

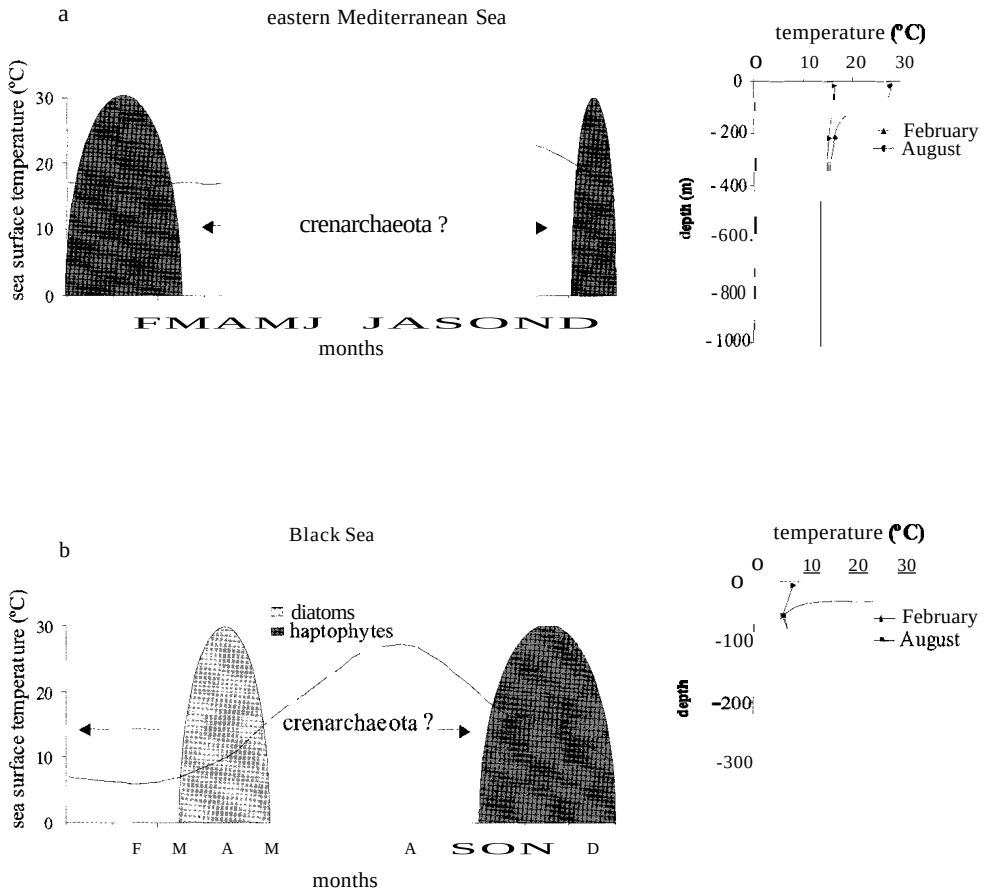

Figure 5.7 Illustration of monthly (average) sea surface temperature (SST, 0C), water temperature depth profile and the seasonal cycle of the major phytoplankton bloom in (a) the eastern Mediterranean Sea and (b) the Black Sea based on data from (www.nemoc.navy.mil/images/climo/med).

The $U_{37}^{K'}$-based SST of 13°C obtained from the Black Sea surface sediments (Freeman and Wakeham, 1992) is reflecting autumn SST's. A TEX_{86}-based SST of SoC was determined for settling particles collected with a sediment trap and 11°C for surface sediment (0-1 em) (Schouten *et at.,* 2002). This TEX_{86} value gives an even lower SST than observed with the $U_{37}^{K'}$-based SST. Both values are low considering the means of the annual range in SST for the Black Sea (Fig. 5.7). The low TEX_{86}-based SST, compared with the $U_{37}^{K'}$-based SST, shows similarities of SST data obtained from the studied Pliocene sapropels (Fig. 5.8). It should be noticed that the SSTs reported from the Black Sea show in general colder SSTs, compared with the Mediterranean Sea, because of its location at higher latitude.

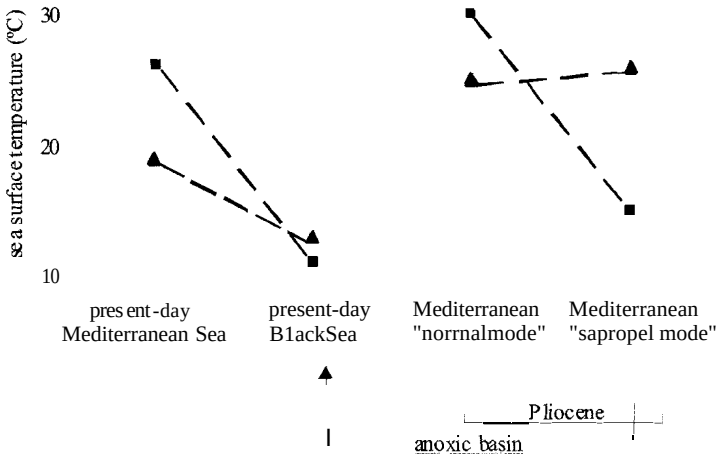

Figure 5.8 Sea surface temperature reconstruction using the $U^{K'}_{37}$ (▲) and TEX_{86} (.) between the Mediterranean Sea at present-day, during homogeneous intervals and sapropel deposition as well as the present Black Sea.

Water column studies of the Black Sea could provide insight in the observed TEX_{86}-based SST "anomaly". In the Black Sea water column, a sharp peak in GDGT concentrations at the chemocline (ca. 100 m) was revealed for both summer (July 1988) (Wakeham *et at., 2003)* and winter (December 2001) without a substantial difference in absolute abundance between the summer and winter situation (Sinninghe Damste *et ai.,* unpublished data), suggesting that there is no large contrast in the seasonal abundance of the crenarchaeota. If the GDGT distribution would reflect the average annual SST, temperatures of ca. 17°C would be expected. However, average TEX_{86}-based SSTs of 9°C in July 1988 (Schouten *et at.,* 2002) and 6°C in December 2001 (Sinninghe Damste *et aI.,* unpublished data) for water column POM from *0-400* m were measured. This is in accordance with the relatively low TEX_{86}-based estimates for sediment traps and the surface sediment. The relatively low TEX_{86}-based SST estimates may be related to the specific distribution of crenarchaeota in the Black Sea (i.e. peaking at the chemocline) in combination with the strong thermal stratification in summer (Fig. 5.7). This stratification results in low temperatures (ca. 8°C) at the chemocline throughout the annual cycle. It is likely that this population of crenarchaeota also contributes to the GDGT flux to the sediments since, in contrast to the much deeper dwelling population of methane-oxidizing crenarchaeota, these archaea can still be grazed upon. This could explain the anomalously low TEX_{86}-based SST estimates in the Black Sea.

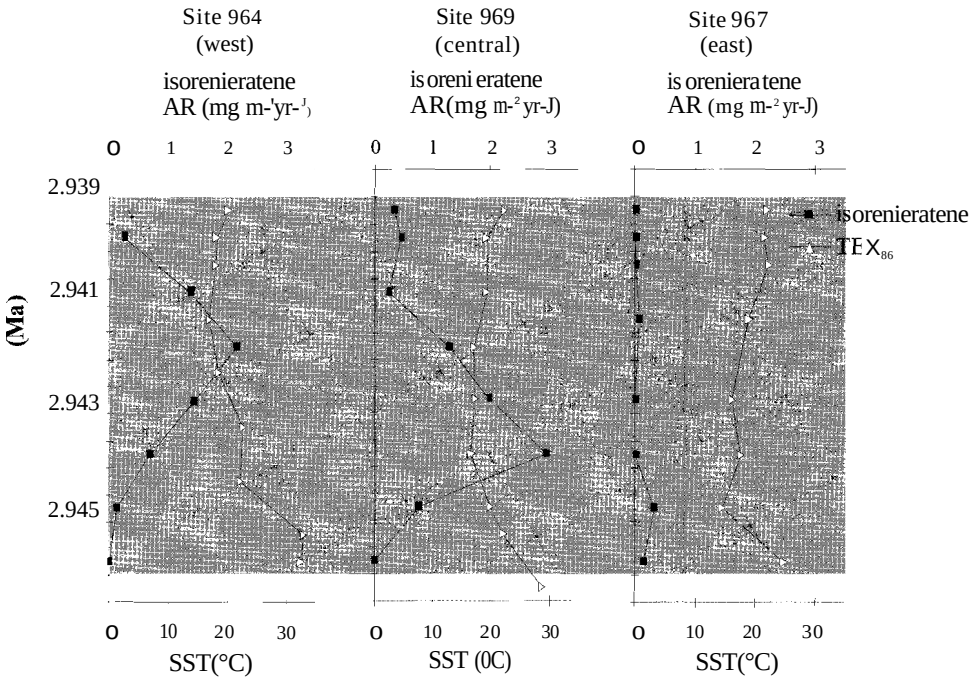

Figure 5.9 Comparison of isorenieratene accumulation rates and TEX_{s6}-based SST estimates in the sapropel at Sites 967, 969 and 964.

It is likely that the observed TEX_{s6}-based SST "anomaly" in the Pliocene sapropels results from the same mechanism as in the Black Sea. Indeed, the large drop in TEX_{s6}-based SST is in all sapropels associated with the shoaling of the chemocline (Fig. 5.9). It seems that the availability of nutrients drives the planktonic creanarchaeota to a different ecological niche, in contrast with the oligotrophic Mediterranean Sea, where the TEX_{s6}-based SST derives from the upper 200 m during the summer (Fig. 5.7). However, at present, the ecophysiological factors determining the abundance of planktonic crenarchaeota are still unknown to fully understand this relationship.

CONCLUSIONS

The dominance of GDGT-0 and crenarchaeol among the GDGTs, large variations in absolute abundances of GDGTs and their GDGT distribution (expressed as TEXs6-index) within the three Pliocene sapropels and their corresponding homogeneous intervals indicated the presence of fossil GDGTs derived from planktonic crenarchaeota. Differences in $U^{K'}_{37}$-and TEXs6-based SST estimates obtained in both, the homogeneous intervals and sapropels are due to the fact that both SST proxies originate from two different organisms occupying different habitats in the eastern Mediterranean Basin. Whereas the $U^{K'}_{37}$-based SST estimates showed hardly any differences between the homogeneous intervals and the sapropels, the

TEX_{86}-based SST estimates revealed a cooling of lO-12°C during sapropel deposition. TEXs6-based SST estimates obtained in the homogeneous intervals showed similarities with the modem oligotrophic Mediterranean Sea, while the TEX_{86}-based SST estimates in the sapropels showed similarities with the contemporary euxinic Black Sea. It is assumed that this TEXs6-based SST trend is caused by a shallowing chemoc1ine during sapropel deposition, which has driven planktonic crenarchaeota to a different ecological niche compared with the situation during the deposition of homogeneous intervals. Ecophysiological studies on planktonic crenarchaeota are required to further unravel this TEX_{86}-based SST "anomaly".

ACKNOWLEDGEMENTS

We would like to thank lort Ossebaar for analytical help and **IPPU** for funding a Ph.D. position.

Chapter 6

Higher plant vegetation changes during Pliocene sapropel formation

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ABSTRACT

The $\delta^{13}C$ values of higher plant wax C_{27-33} n-alkanes were determined in three, time-equivalent Pliocene (2.943 Ma) sapropels and homogeneous calcareous ooze from three different sites forming an east-west transect in the eastern Mediterranean Basin to study the composition of the vegetation on the continents surrounding the Mediterranean Sea. A two-end member mixing model transformed the measured $\delta^{13}C$ values into the contribution of C_4 plants to the terrestrial vegetation. These calculations indicated a high C_4 plant contribution (i.e. 40-50%) in the periods just before and just after sapropel formation. During sapropel deposition the C_4 plant contribution increased by up to 20% at all sites. This is interpreted to record the increased overall plant coverage of the Mediterranean borderlands resulting from the change of formerly barren desert areas into C4 grass-dominated savannah's as a response to the wetter climate during sapropel deposition. Enhanced accumulation rates (ARs) of long-chain n-alkanes (C_{27-33}) and n-alkan-l-ols (C_{26-30}) towards the middle of the sapropel in concert with a decrease in the *Til*Al ratio confirm an increased delivery of terrigenous organic matter at all sites. These biomarkers were probably predominantly fluvially transported to the Mediterranean Sea, not only by the Nile but by fossil wadi river systems on the northern African continent.

INTRODUCTION

Pliocene Mediterranean sapropels are characterized by extremely high total organic carbon (TOe) contents (over 30%, Emeis *et at.,* 1996), much higher than those of Quaternary sapropels. The actual mechanism responsible for the deposition of these extremely TOC-enriched sediments is still widely debated. Enhanced productivity and/or improved preservation (e.g. Canfield, 1994; Bouloubassi *et aI.,* 1999; Calvert and Pedersen, 2001) or a combination of both (e.g. Passier *et at.,* 1999a) are all considered. Recently we have shown that oxygen depletion of the water column was so extreme that "Black Sea" conditions (overlapping photic and euxinic zones) prevailed in the eastern Mediterranean Sea during Pliocene sapropel formation (Menzel *et at.,* 2002). These conditions were optimal for the

preservation of marine organic matter (OM) but were probably induced by an increase in the marine primary productivity. This increased productivity resulted from the increased influx of nutrients from the continents induced by the climatic change leading to more humid conditions in the Mediterranean area.

Terrestrial OM is generally thought to represent <5% of the OM in the sapropels and to playa trivial role in the formation of sapropels. In contrast, in the homogeneous intervals just below and above the sapropels, the TOC content is much lower «0.1%) and the terrestrial OM contribution is expected to be much higher due to its greater resistance to oxidative degradation relative to marine OM (Hoefs *et al.,* 2002). However, the values in the homogeneous intervals are higher than those of the sapropels (Fig. 6.1c; Nijenhuis and de Lange, 2000). This is in contrast to what would be expected if the homogeneous calcareous ooze contained a higher terrestrial OM fraction since Quaternary terrestrial OM is commonly isotopically depleted relative to marine OM (Tyson, 1995). This apparent contradiction may be explained if the terrestrial OM was not only comprised of C_3 plant material ($\delta^{13}C \approx$ -27%0), but also contained a substantial C_4 plant component ($\delta^{13}C \approx$ -12%0). A distinct east-west trend of increasingly more negative $\delta^{13}C_{TOC}$ values in both the homogeneous and sapropel intervals was also noted for the same set of sapropels (Fig. 6.lc; Nijenhuis and de Lange, 2000). This may be explained if the C_4 plant material was delivered to the eastern Mediterranean Basin predominantly by the Nile river.

At present, the tropical and subtropical climate zones of northern and central Africa covering mainly the Sahara, Sahel and Savannah zones are characterized by predominantly a C_4 plant vegetation (White, 1983). During the Pliocene the African continent was exposed to generally warmer and wetter climate/vegetation zones than at present (Haywood *et al., 2000).* However, around 3 Ma ago, northern African environments appear to have shifted towards a drier and somewhat cooler state (deMenocal, 1995). At this time interval, pollen and other evidence suggest more widespread grassland in eastern Africa and the extension of deserts in northern Africa (Leroy and Dupont, 1994). C_4 biomass was generally of minor importance in the Miocene, but expanded substantially in the Pliocene and Pleistocene (Ceding, 1992). Subtropical African climate varied primarily at precessional periodicities (19-23 kyr), responding to precipitation changes controlled by monsoon intensity (deMenocal, 1995). Sapropel formation is directly correlated with precession minima (Northern Hemishere insolation maxima), resulting in the increase in monsoonal activity (Rossignol-Strick, 1983).

Based on $\delta^{13}C_{TOC}$ values measured in the homogeneous intervals, we anticipated a substantial contribution of C_4 plants to the terrestrial OM before, during and after Pliocene sapropel formation. In addition, we would expect climate-induced changes in plant vegetation during formation of these sapropels favouring C_3 plant over C_4 plant vegetation with increasing humid climate conditions. To verify this hypothesis, samples from three time-equivalent Pliocene sapropels and adjacent homogeneous calcareous ooze obtained from three different sites forming an east-west transect in the eastern Mediterranean Basin were studied. Long-chain odd-carbon-numbered C_{27} to C_{33} n-alkanes, constituents of terrestrial higher plant epicuticular waxes (Eglinton and Hamilton, 1967), were used for compound-specific carbon isotope analyses to evaluate C_4/C_3 plant vegetation changes during sapropel formation. Additionally, accumulation rates of terrestrial n-alkanes and n-alkan-l-ols were

determined to obtain insight into the temporal and spatial contribution of terrestrial OM during sapropel deposition.

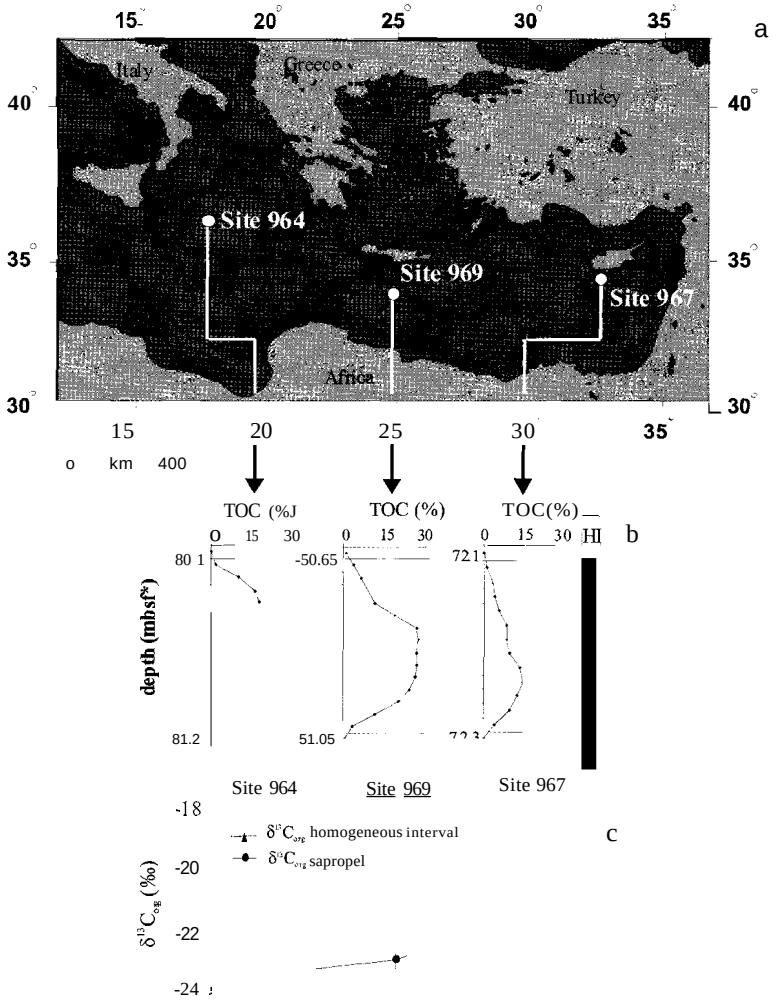

Figure 6.1 (a) Location of ODP drilling sites, (b) lithological description (HI = homogeneous interval, S = sapropel) and TOC profiles of the investigated Pliocene sapropel (data from Nijenhuis et aI., 1998, * metres below seafloor) and (c) $8^{13}C_{TOC}$ (%0) of the homogeneous intervals and the sapropels of the eastern Mediterranean Sea (data are from Nijenhuis and de Lange (2000)). Error bars represent the standard deviation.

MATERIAL AND METHODS

Samples

Core samples were taken during ODP Leg 160 in the eastern Mediterranean Basin (Emeis *et al.*, 1996). Site 967 (Eratosthenes Seamount) and Site 969 (Mediterranean Ridge) are located in the Levantine Basin. Site 964 is located in the Ionian Basin (Fig. 6.1a). The detailed chronology for these cores obtained by astronomical tuning enabled us to select three laterally equivalent sapropels with an age of 2.943 Ma (Lourens *et al.*, 1996). Relevant sections (967C-8H-4, 111-134 cm; 969E-6H-6, 23-43 cm; 964D-lOH-l, 100.5 -113 cm) were sub-sampled in 0.5-1.5 cm slices above, through and below the sapropel. Total organic carbon (TOC) was reported previously and showed distinct differences between these sapropels (Nijenhuis *et al.*, 1998; Fig. 6.1b).

Extraction and fractionation of biomarkers

Ground, freeze-dried sediment samples (0.1 to 3 g) were Soxhlet extracted with a mixture of dichloromethane/methanol (1: 1, v/v) for 24 h. The extracts were concentrated with a rotary evaporator at 30°C. An aliquot of the total extract (1.5 to 3 mg) was fractionated using a Pasteur capillary pipette (150 mm length) packed with (activated for 2.5 hat 120°C). The apolar fraction was eluted using a mixture ofhexane/dichloromethane (9:1, v/v; 4 mI). Subsequently, the saturated hydrocarbon fraction was obtained using a Pasteur capillary pipette (150 mm length) packed for *1/3* with $AgNO_3$-impregnated silica and for *2/3* with (activated for 2.5 h at 120°C). This fraction was eluted with hexane. All fractions were dried under a flow of nitrogen and analysed by gas chromatography (GC), GC-mass spectrometry (MS) and isotope-ratio monitoring (irm)-GC-MS. Quantification was performed by integration of the relevant peak areas of interest and the internal standard (squalane). *n*-Alkan-I-ols were identified from the total lipid fraction (see Menzel *et ai.*, 2003 for details), which was derivatised using N,O-bis(trimethylsilyI)trifluoroacetamide (30 min at 60°C).

Instrumental analysis

GC was performed on a Hewlett-Packard 6890 series gas chromatograph equipped with a fused silica capillary column (50 m x 0.32 mm or 25 m x 0.32 mm) coated with CP-Sil 5 (film thickness 0.12 μm). Helium was used as the carrier gas. The samples were injected on-column at 70°C and the oven was subsequently programmed to 130°C at *20°C/min,* followed by an increase of *4°C/min* to 320°C, where it remained isothermal for 10 min. Compounds were detected using a flame ionisation detector (FID).

GC-MS analyses were performed using a Hewlett-Packard 5890 series II gas chromatograph connected to a Fisons instruments VG platform II mass spectrometer operated at 70 eV, with a mass range *m/z* 50-650 and cycle time of 0.65 s (resolution 1000). The capillary column and temperature programme were as described for the GC analyses.

Isotope-ratio-monitoring gas chromatography-mass spectrometry (irm-GC-MS) was performed using a Finnigan Delta-plus XL-irm-GC-MS system, equipped with an on-column injector and fitted with a 25 m x 0.32 mm fused silica capillary column coated with CP-SiI5 (film thickness 0.12 μm). Helium was used as carrier gas and the oven was programmed as

described for the GC analyses. Isotopic values were calculated by integrating the *m/z* 44, 45 and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and those of C02-spikes produced by admitting C02 with a known 13C content at regular intervals into the mass spectrometer. They are reported in permil versus the VPDB standard. Duplicate analyses were carried out for each sample and the results were averaged to obtain a mean value. $\delta^{13}C$ values typically have an error of <0.5 %0.

Quantitation

Quantitation was performed by integration of the peak areas of interest and that of the internal standard (squalane for n-alkanes, nonadecan-lO-one for n-alcohols). C_{26}, C_{28} and C_{30} n-alkan-I-ols, which were sometimes co-eluting with other lipids, were quantified by integration of individual peak areas in mass chromatograms using characteristic *m/z* values (i.e. M+-15). Typical quantitation errors are <10%. Calculations of accumulation rates (ARs) were performed using absolute biomarker amounts (µg g-! dry weight sediment), assuming (i) a synchronous start and end of sapropel formation at the three locations, (ii) a duration of sapropel formation of 7000 years (Nijenhuis and de Lange, 2000) and (iii) a constant sedimentation rate during sapropel formation (Menzel *et at.,* 2002). ARs in the homogeneous intervals were not calculated because sedimentation rates are expected to be different during times of deposition of sapropel and the homogeneous intervals.

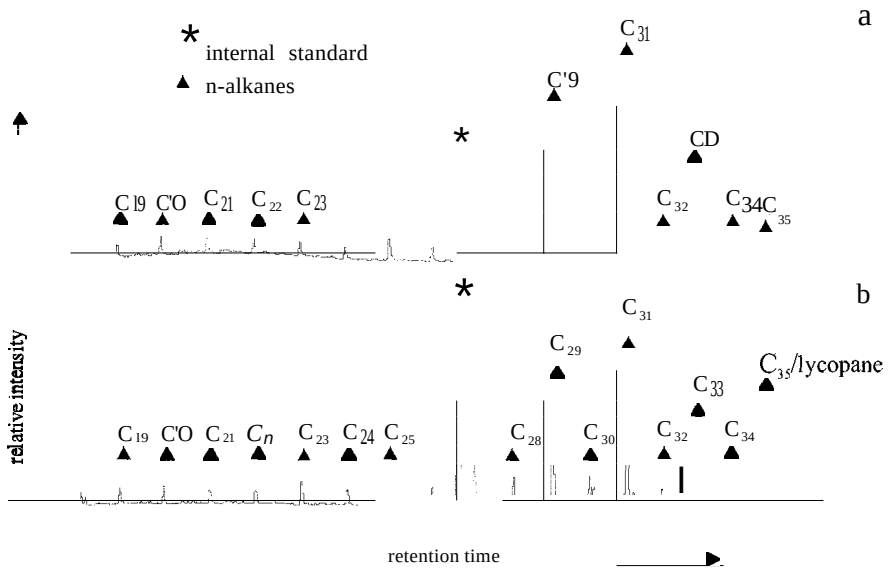

Figure 6.2 Partial gas chromatograms of typical saturated hydrocarbon fractions of solvent extracts of (a) a homogeneous interval of Site 967, 111-114 cm and (b) a sapropel of Site 967, 115 - 116.5 cm.

RESULTS

n-Alkane and n-alkan-l-ol accumulation rates

Biomarkers in the saturated hydrocarbon fractions of the organic solvent extracts of the sediments were dominated by a homologous series of n-alkanes ranging from C_{17} to C35 in both the homogeneous intervals and the sapropels (Fig. 6.2). The C_{35} n-alkane co-elutes with lycopane, which is especially abundant in the sapropels due to the anoxic bottom waters during sapropel deposition (Fig. 6.2b; *cf* Sinninghe Oamste *et ai.,* 2003). The n-alkanes in the *n-C24* to *n-C35* range possess a strong odd-over-even carbon number predominance values of 5-8), indicative of a predominant higher plant origin of the n-alkanes (Eglinton and Hamilton, 1963). In the homogeneous interval below the sapropel the organic carbon-normalized abundances of C_{27-33} n-alkanes were 1040 TOC at OOP Site 967, 280 TOC at OOP Site 969 and 450 TOC at OOP Site 964 (Fig. 6.3a). In the sapropel C_{27-33} n-alkanes contents were lower with mean values of 72 TOC at OOP Site 967, 70 TOC at OOP Site 969 and 215 TOC at OOP Site 964 (Fig. 6.3a). In the homogeneous interval above the sapropels the C27-33 n-alkane contents were higher than in the sapropel with values of 225 TOC at OOP Site 967, 110 TOC at OOP Site 969 and 370 TOC at OOP Site 964 (Fig. 6.3a).

The C_{26-30} n-alkan-ols with a strong even-over-odd carbon number predominance are likely also derived from terrestrial higher plants (Logan *et ai.,* 1995). Their concentration profiles were similar to those of the n-alkanes. In the homogeneous interval below the sapropel the n-alkanol contents were 290 TOC at OOP 967, 330 TOC at OOP Site 969 and 90 TOC at OOP Site 964 (Fig. 6.3b). Within the sapropel n-alkanols are less abundant: 86 TOC at OOP Site 967,50 TOC at OOP Site 969 and 80 μg/g TOC at OOP Site 964. **In** the homogeneous interval above the sapropel, n-alkan-l-01 abundances increased to 260 TOC at OOP Site 967, and 60 TOC at OOP Site 969; the highest abundance occured at OOP Site 964 with 1500 TOC.

Assuming a constant sedimentation rate within the sapropel and duration of sapropel formation of 7000 years, accumulation rates (ARs) for these terrestrial biomarkers were calculated (see Menzel *et ai.,* 2003 for a detailed discussion) and compared between the three sites. Oue to the likely substantially increased sedimentation rates in the homogeneous interval and poor preservation of OM no ARs were calculated for these intervals. C_{27-33} *n*-alkane (Fig. 6.4a) and C_{26-30} n-alkan-l-01 ARs (Fig 6.4b) at all three sites generally showed an increase from the base of the sapropel towards the middle section and sunsequently a drop towards the top with exceptions from this general trend for some individual samples. The highest ARs for the n-alkanes were found in the middle section of sapropel OOP Site 969 with values of about 600 $\mu g.m^{-2}.yr^{-1}$. The ARs for n-alkanes for the other two sites were substantially lower. The n-alkan-l-01 ARs at the three sites showed less variation; the maximum AR was noted for OOP Site 969 with 550 $\mu g.m^{-2}.yr^{-1}$.

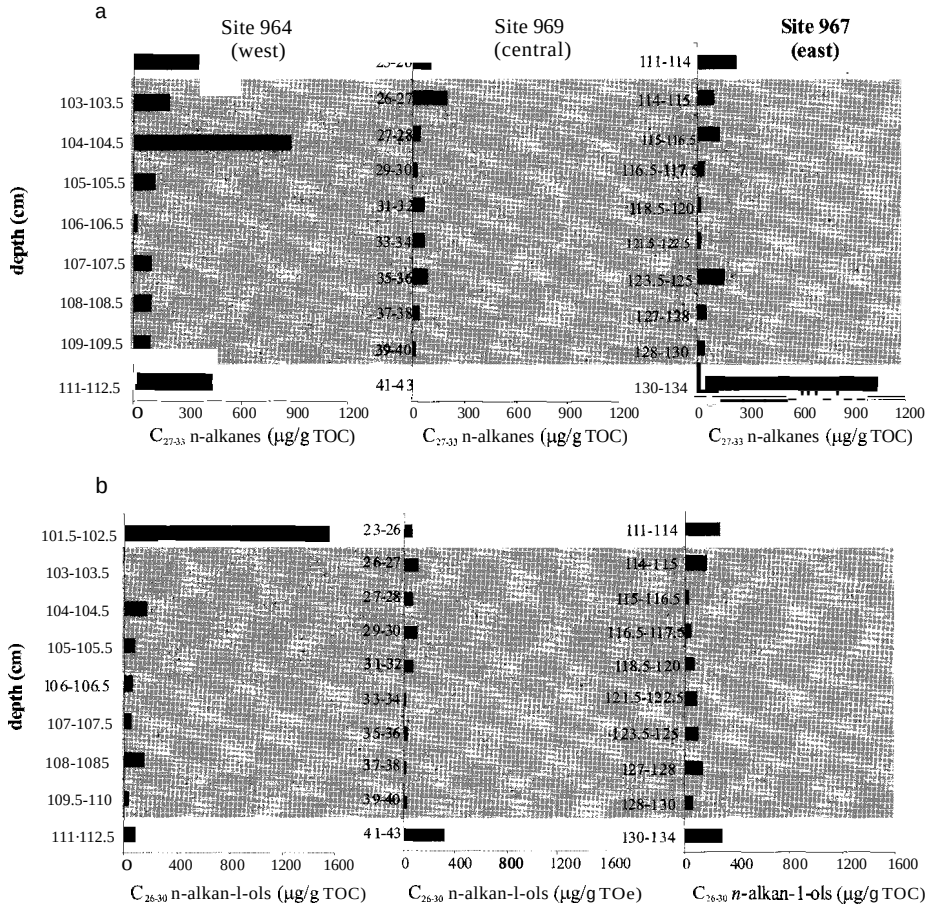

Figure 6.3 Bar plots showing the absolute abundance of (a) C_{27-33} n-alkanes and (b) C_{Z6-30} n-alkan-l-ols in the homogeneous intervals and sapropels at Site 967, 969 and 964. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

Compound-specific isotopic analyses **of long-chain n-alkanes** (C_{27-33})

The $\delta^{13}C$ values of the C27-C33 n-alkanes varied between -24 ‰ and -31.5 ‰ (Fig. 6.5). Generally, the C_{27} and C_{33} n-alkanes are more enriched in 13C relative to the C_{29} and C33 n-alkanes, *n-C33* is more enriched relative to *n-C27* in the sapropel than the homogeneous interval. The weighted average $\delta^{13}C$ values of the C_{27-33} n-alkanes (Table 6.1) in the homogeneous intervals were more negative than those in the sapropels. ODP Site 967 showed $\delta^{13}C_{27-33}$ n-alkane mean values of -28.8 ‰, ODP Sites 969 and 964 values of -30.3 ‰ and -29.6 ‰, respectively. In the sapropels Il-alkane values at ODP Site 967 were -27.4 ‰ and more negative at ODP Site 969 (-28.8 ‰) and at ODP Site 964 (-28.3 ‰) (Table 6.1).

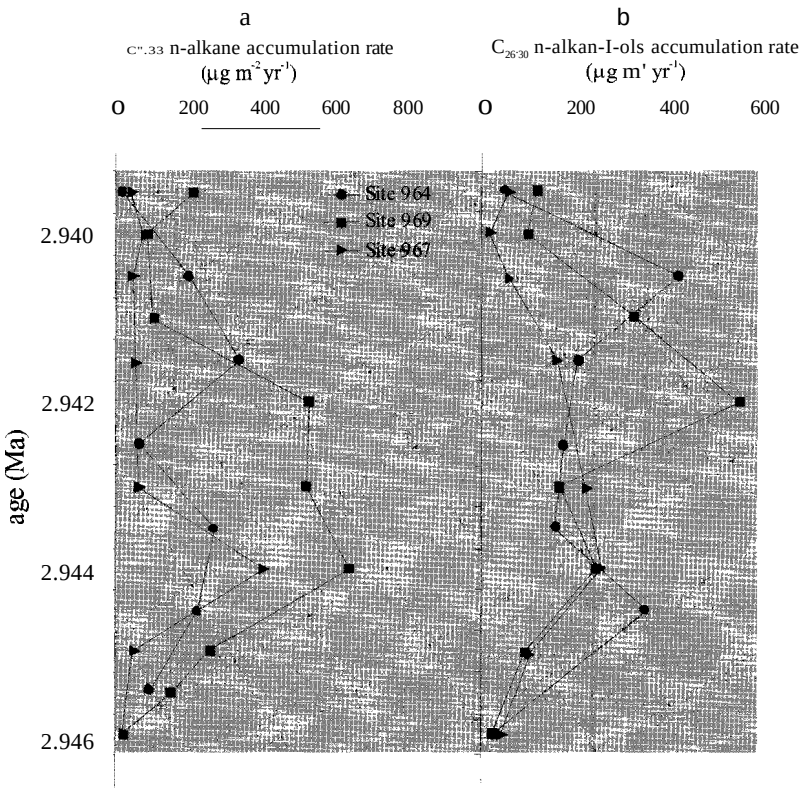

Figure 6.4 Accumulation rates of (a) C_{27-33} n-alkanes and (b) C_{26-30} n-alkan-l-ols in the sapropel sections at Sites 967, 969 and 964.

DISCUSSION

Origin of n-alkanes

The strong odd-over-even carbon number predominance of the long-chain n-alkanes (Fig. 6.2) provides evidence that they derive from waxes of terrestrial higher plant vegetation. The $\delta^{13}C$ values of the individual n-alkanes (C27-33) ranged between -24 %0 and -31.5 %0. This wide range revealed that they originate from a mixture of C3 plants ($\delta^{13}C$ leaf wax lipids C_3 plants = -36 %0; Rieley *et ai.,* 1991; Collister *et ai.,* 1994) and C4 plants ($\delta^{13}C$ leaf wax lipids C_4 plants = -21.5 %0; Rieley *et ai.,* 1991; Collister *et ai.,* 1994) both during times of sapropel formation and deposition of the homogeneous intervals in the Pliocene (Fig. 6.5). An additional indication for the substantial contribution of C4 plant waxes is the characteristic trend of increasing $\delta^{13}C$ values of the n-alkanes with increasing chain length (Fig. 6.5). Generally, C_3 plant leaf wax lipids become more depleted in 13C with increasing number of carbon number, whereas the values of C4 plant individual leaf wax components are constant with increasing carbon number or even increase slightly (Rieley *et ai., 1991;* Collister *et ai.,* 1994; Kuypers *et ai.,* 1999). The fact that *n-C_{33}* is substantially enriched in l3C

relative to *n*-C_{31}, thus, also indicates a contribution from C4 plant wax lipids. This relative enrichment is stronger in the sapropels than in the homogeneous sediments, suggesting a larger C_4 plant wax contribution in the sapropels, consistent with the more enriched ^{13}C content of all odd n-alkanes (Fig. 6.5).

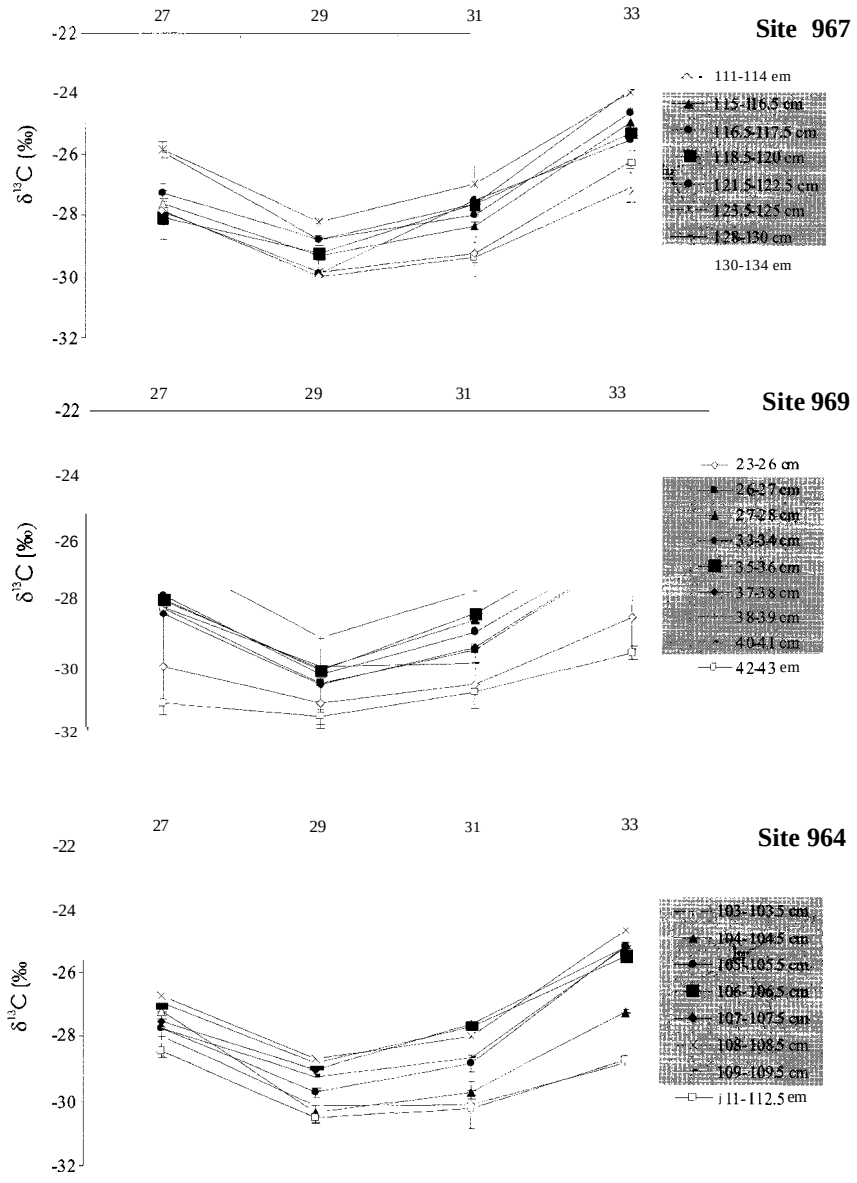

Figure 6.5 $\delta^{13}C$ values *versus* carbon chain length of n-alkanes. Error bars represent the standard deviation. Symbols in the light grey shaded boxes represent measurements made of the sapropel samples. Symbols in the white regions represent the homogeneous calcareous ooze.

Table 6.1 Weighted average C values (%0) of the C_{27-33} n-alkanes and their transformation to C_4 plant contribution (%)[a].

	Site 964	Site 969	Site 967
813C27_33 (%0) homogeneous (n=2)	-29.6	-30.3 (0.4)	-28.8 (0.2)
013C27_33 (%0) sapropel (n=7)	-28.3 (0.8)	-28.8 (0.6)	-27.4 (0.6)
C_4 plant vegetation (%) homogeneous (n=2)	44	39 (3)	49 (1)
C_4 plant vegetation (%) sapropel (n=7)	54 (6)	50 (4)	59 (4)

a values are averages; values in parentheses represent the standard deviation

The C4 plant contribution of the leaf-wax n-alkanes was estimated with a two end-member mixing model, assuming the C4 leaf-wax lipids = -21.5 %0 and $\delta^{13}C_3$ leaf-wax lipids = -36.0 %0 (Collister *et al.,* 1994). These calculations showed that in the homogeneous intervals the average percentage of C4 plant vegetation ranged between 40 and 50%, with the highest contribution at ODP Site 967, located closest to the river Nile (Fig. 6.6; Table 6.1). In the sapropels the C4 plant contribution to the wax lipids increased by up to 20% to reach values between 50-70% for all three sites, with the highest contribution again at ODP Site 967 (Fig. 6.6, Table 6.1).

These data reveal a substantial contribution of C_4 plants to the terrestrial vegetation on the continent surrounding the Mediterranean in the Pliocene and, surprisingly, a higher contribution at times of sapropel deposition. The Pliocene (ca. 3 Ma) palaeoclimate in the European and Mediterranean region was warmer, wetter and less seasonal than at present, particularly at the middle to high latitudes (Haywood *et al.,* 2000). $U^{K'}_{37}$ values from alkenones in the same sapropels provide estimates for sea surface temperature of 24-27°C (Menzel *et al.,* 2003). Since it is generally believed that alkenones in the Mediterranean Sea are produced during the winter season (Ternois *et ai.,* 1996), these estimates likely reflect winter temperatures, indicating that the annual air temperature during periods of insolation maximum (i.e. sapropel formation) were rather high.

Plants possessing C_3 and C_4 photosynthesis respond differently to ambient conditions of light, temperature, pCO_2, paz and humidity (e.g. Collatz *et ai.,* 1992). C4 photosynthesis is insensitive to temperature whereas increasing temperature alters the kinetics of Rubisco (the primary COz-fixing enzyme of plants utilising the Calvin-cycle) and thus photorespiration and COz fixation in C_3 plants (Collatz *et ai.,* 1998). Growth studies on *C3/C4* grasses have shown that higher temperatures increase the frequency of C4 photosynthesis within the grass taxa, whereas precipitation was not a critical parameter (Ehleringer *et al.,* 1997). On the other hand, Huang *et ai.* (2001) have shown that the summer rainfall can be an important factor for the dominance of C4 plants in certain environments. Schefuβ *et ai.* (2003) showed that the decreased air humidity, controlled by decreased sea surface temperature, was a decisive factor in the expansion of C4 grass vegetation in southern Africa during the Mid-Pleistocene transition.

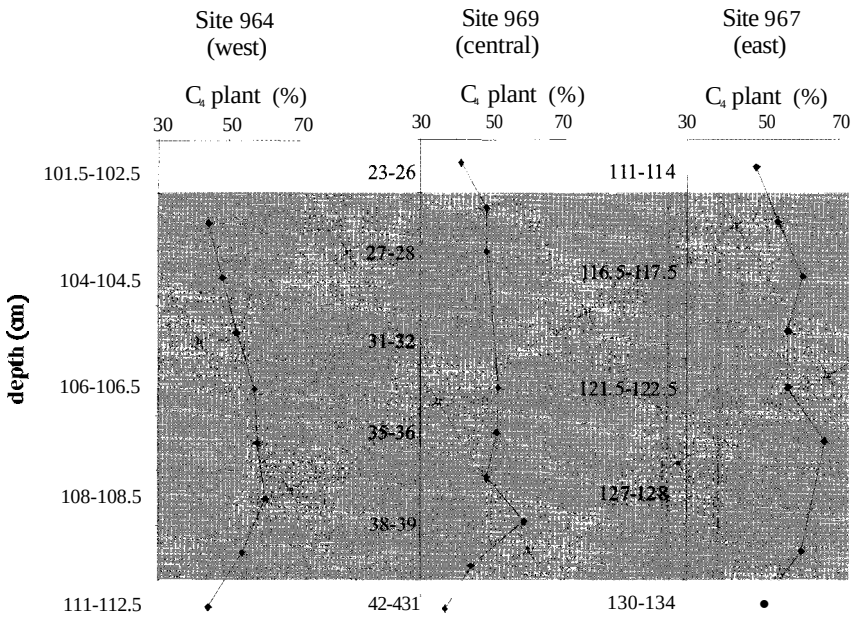

Figure 6.6 Calculated C_4 plant contribution (%) based on the weighted average $\delta^{13}C$ values of the C_{27-33} n-alkanes. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

The increase (up to 20%) in C4 plants relative to C3 plants during time of sapropel formation may thus have been caused by the increase in air temperature. On the other hand the increased rainfall during times of sapropel formation may have substantially increased the overall plant coverage of the Mediterranean Sea borderlands by transforming formerly barren deserts of the North African continent into savannah's dominated by C4 grasses. Such a process could also explain the increased contribution of C4 plants relative to C3 plants in the plant-wax n-alkanes. This interpretation is supported by the diminished delivery of Sahara dust evident from the lowered Ti/AI ratios of the sapropels (Wehausen and Brumsack, 1999; Nijenhuis and de Lange, 2000) and the increasing ARs of plant lipids towards the centre of the sapropel (see below).

Mode of transport of terrestrial plant n-alkanes

Both aeolian and fluvial transport brings terrestrial plant n-alkanes and n-alkan-l-ols to marine settings. Leaf wax n-alkanes, the major lipid constituent of the epicuticular wax layer of terrestrial plants (Eglinton and Hamilton, 1963), can easily be removed from the leaf surface by rain or wind, especially by sandblasting dust storms. They are common organic components of aerosols (Poynter et aI., 1989, Schefuβ et aI., 2003). n-Alkan-l-01s are not as abundant in aerosols and are, therefore, mainly fluvially transported to marine settings.

The absolute abundances of C_{27-33} n-alkanes and C_{26-30} n-alkanols normalised to TOC were higher in the homogeneous interval than in the sapropels (Fig. 6.3). This difference is

probably the effect of the changing redox conditions during deposition. The sapropel was deposited in euxinic waters (Menzel *et al.,* 2002), resulting in a minimal oxygen exposure time of OM during settling through the water column and burial in surface sediments and yielding optimal preservation of biomarker lipids and OM *(ef* Hoefs *et al.,* 2002; Sinninghe Damste *et al.,* 2002c). The homogeneous intervals below and above the sapropel were, however, deposited in oxic bottom waters resulting in substantially longer oxygen exposure times. Under these conditions terrestrial n-alkanes are selectively preserved (Hoefs *et al.,* 2002; Sinninghe Damste *et al.,* 2002c), which explains the elevated TOC-normalized concentrations outside the sapropel layer. Long-chain n-alkan-I-ols are slightly more labile towards oxygen exposure than n-alkanes (Hoefs *et al.,* 2002) but the same mechanism applies. Within the sapropels labile marine OM is preserved due to the excellent conditions for preservation and the terrestrial biomarkers are "diluted", resulting in lower concentrations.

Within the sapropels the ARs of the C_{27-33} n-alkanes and C_{26-30} n-alkanols increased from the base of the sapropel towards the middle section and then decreased towards the top (Fig. 6.4). This is opposite to the trend in the Ti/Al ratios in the same sapropel with the lowest TilAl ratio is in the middle of the sapropel, suggesting a decreasing aeolian dust delivery during sapropel formation (Wehausen and Brumsack, 1999; Nijenhuis and de Lange, 2000). This difference seems to indicate that the terrigenous lipids were predominantly of fluvial rather than aeolian origin. Low *KJ*Al and Mg/Al ratios in the sapropels confirm that fluvial input during sapropel formation indeed increased (Wehausen and Brumsack, 1999, Nijenhuis and de Lange, 2000). Interestingly, both n-alkane and n-alkan-l-ol ARs at ODP Site 967, located closest to the mouth of the Nile river are not higher than those at ODP Sites 969 and 964. This observation may indicate that not only the Nile river had increased fresh water discharge during sapropel formation, but also the discharge of the fossil river (wadi) of the North African margin increased as has been proposed for the Quaternary 85 sapropel (Rohling *et al.,* 2002). The increased flux of land-plant derived lipids towards the middle section of the sapropel is consistent with the suggested increased overall plant coverage of the Mediterranean borderlands.

Implications for $\delta^{13}C_{TOC}$ valnes

Assuming that the long-chain n-alkanes were mostly fluvially transported into the eastern Mediterranean Basin during Pliocene sapropel formation, $\delta^{13}C$ values of C_{27-33} *n*-alkanes can be used, with care, as a proxy for the isotopic composition of bulk terrestrial OM, which predominantly originated from fluvially transported terrigenous OM. The $\delta^{13}C$ values of C_{27-33} n-alkanes in the homogeneous intervals suggest a contribution of 40-50% of C_4 plants to the terrestrial OM (Fig. 6.6), implying that bulk terrestrial OM may also be composed of a substantial part of C_4 plants. Assuming $\delta^{13}C$ bulk C_3 -27 ‰ and bulk C_4 plants ≈ -12 ‰ (Tyson, 1995), we can estimate that terrestrial OM derived for ca. *50%* from C_4 plants will have a $\delta^{13}C$ value of ca. -19.5 ‰. This is exactly the $\delta^{13}C_{TOC}$ value measured at ODP Site 967 in the homogeneous intervals (Fig. 6.lc). In these sediments with a TOe content smaller than 0.1%, it is likely that a substantial part of the TOC is derived from more refractory terrestrial OM and the substantial contribution of C_4 plants explains the

counter intuitive shift towards more enriched $\delta^{13}C$ values from sapropel to the homogeneous interval. The distinct east-west trend of increasingly more negative $\delta^{13}C$ values in the homogeneous intervals (Fig. 6.1c) suggests that C_4 plant material was transported predominantly by the Nile river, consistent with the higher calculated C_4 plant contribution for the terrestrial wax lipids at ODP Site 967 (Fig. 6.6). In the sapropel, $\delta^{13}C_{roc}$ values are predominantly determined by more ^{13}C-depleted marine OM relative to terrestrial OM. The east-west trend for these values (Fig. 6.1c) is to some extent also seen in the $\delta^{13}C$ of alkenones (Menzel *et al.*, 2003) but may, in part, also be caused by the higher contribution of C_4 plant material to the relatively small terrestrial OM contribution to ODP Site 967.

CONCLUSIONS

$\delta^{13}C$ values of C_{27-33} n-alkanes reveal a contribution of a mixture of C_3 and C_4 land plant OM to the eastern Mediterranean Basin before, during and after sapropel deposition in the Pliocene. The $\delta^{13}C$ values of C_{27-33} n-alkanes in the homogeneous intervals revealed an eastward increase in the C_4 plant contribution, perhaps explaining the increased enriched $\delta^{13}C_{roc}$ values towards the east by apparently discharges of the Nile river. Based on a two-end member mixing model, the contribution of C_4 plants to the wax lipids increased by up to 20% in the sapropels at all sites. This somewhat unexpected shift from C_3 to C4 plant vegetation may be explained by the increased air temperature during the Northern hemisphere insolation maximum associated with sapropel deposition. Alternatively, and perhaps more likely, the increased rainfall at times of sapropel deposition resulted in an increase of overall plant coverage of the Mediterranean borderlands resulting from conversion of formerly barren desert areas into C_4 plant dominated-savannah's. This scenario is supported by the decreased Ti/AI ratio's recorded in sapropels pointing to a decreased delivery of Sahara dust and the increased terrigenous OM delivery (based on n-alkane and n-alkanol ARs) during sapropel deposition. This delivery was caused by an increased fluvial transport resulting from the wetter conditions and was most probably delivered not only by the Nile river system but also by fossil river systems of the North African coast (e.g. Libyan Basin) into the Mediterranean Sea.

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Chapter 7

The molecular composition of kerogen in Pliocene Mediterranean sapropels and associated homogeneous calcareous ooze

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ABSTRACT

The organic matter of three time-equivalent Pliocene sapropels was characterized as Type II kerogen being dominated by amorphous organic matter, originating mainly from marine organisms. This was witnessed by the relatively high abundances of algaenan- and chlorophyll-derived pyrolysis products in the Curie-point pyrolysates of the kerogens of the sapropels. However, a small amount of terrigenous organic matter was also observed in the form of pollen and spores that most probably yielded the oxygenated aromatic pyrolysis products known to derive from sporopollenin. Lignin pyrolysis products, such as methoxyphenol and catechol (1,2-benzenediol), which are commonly used as unequivocal evidence for contribution of terrestrial higher plants, were absent in the kerogen pyrolysates of the sapropels. No major spatial and temporal differences in the kerogen composition of the three time-equivalent sapropels were observed. Palynological studies revealed that the homogeneous intervals, below and above the sapropels, consisted of pollen, spores and amorphous organic matter. This is confirmed by Curie-point pyrolysis - *GC/MS* results showing a relatively high abundance of algaenan-derived compounds and the presence of oxygenated aromatic pyrolysis products. The differences observed between the kerogen pyrolysates of the sapropels and their homogeneous intervals are suggested to be governed primarily by enhanced preservation conditions, resulting in increased preservation of labile marine organic matter in the sapropels.

INTRODUCTION

The sedimentary record of the eastern Mediterranean Basin is characterized by the occurrence of so-called sapropels, organic-rich sediments containing >2% total organic carbon (TOC) (Kidd *et ai.,* 1978). Presently, sediments deposited in the eastern Mediterranean Sea have TOC contents of 0.3% at maximum (Calvert, 1983; Calvert and Pedersen, 1992). The switch between the deposition of sapropels and homogenous TOC-lean oozes must reflect periodical changes in depositional setting (Olausson, 1961; Kidd *et ai.,* 1978; Cita and Grignani, 1982). These changes are thought to be caused by the changing

climate conditions at the pace of the astronomical precession cycle (Rossignol-Strick *et al.*, 1982; Rossignol-Strick, 1985; Hilgen, 1991; Lourens *et al.*, 1996). A wetter climate results in an increased delivery of nutrients to the basin and consequently increased marine productivity and consequently to enhanced preservation. Ultimately, this leads to the deposition of sediments with a high organic matter (OM) contents (2-30% TOC, Emeis *et al.*, 1996). Pliocene sapropels are characterized by very high TOC contents (> 26%, e.g. Emeis *et al.*, 1996; Nijenhuis and de Lange, 2000) and can be considered as extremes and are thus worth to be studied in this respect. In addition to the analysis of biomarkers (e.g. Bosch *et al.*, 1998; Rinna *et al.*, 2002; Menzel *et al.*, 2002, 2003), the characterization of macromolecular OM and the identification of its precursors can be important for the reconstruction and understanding of the mechanisms resulting in sapropel deposition.

In thermally immature sediments, including Mediterranean sapropels, the largest fraction (>90%) of sedimentary organic matter (OM) consists of OM insoluble in common organic solvents, so-called kerogen (Durand, 1980). Over the last 30 years, a number of kerogen formation pathways have been proposed, including the neogenesis model (Tissot and Welte, 1984), the selective preservation pathways (e.g. Tegelaar *et al.*, 1989) and the *in-situ* polymerization pathway (Collinson *et al.*, 1998; Stankiewicz *et al.*, 1998, 2000). The neogenesis model is based on depolymerisationJre-condensation reaction that results in the preservation of amorphous organic matter (AOM), the composition of which strongly depends on the source organisms and more importantly on the diagenetic processes involved in its formation (Tissot and Welte, 1984). In the selective preservation model a substantial fraction of OM is derived from mixtures of selectively preserved, sometimes altered, resistant biomacromolecules (Tegelaar *et al.*, 1989a). These resistant macromolecular biomolecules become concentrated during mineralisation of the more abundant but more easily hydrolysable biopolymers such as proteins and carbohydrates. An alternative source for the occurrence of high-molecular-weight sedimentary OM in this model results from the incorporation of inorganic sulphur species into low-molecular-weight lipids and carbohydrates (Sinninghe Damste *et al.*, 1998). In particular, depositional environments favouring the activity of sulphate-reducing bacteria and with a limited supply of available iron induce the formation of these highly resistant sulphur-containing substances (Sinninghe Damste *et al.*, 1988, 1989; Sinninghe Damste and de Leeuw, 1990). Stankiewicz *et al.*, (2000) have proposed an alternative model of kerogen formation: conversion of free aliphatic molecules into a resistant aliphatic macromolecule via *in-situ* polymerisation.

Many types of resistant aliphatic biopolymers have been identified in organisms. These macromolecules have been encountered in cell walls of chlorophyceae and eustigmatophytes (as algaenans, de Leeuw and Largeau, 1993; Gelin *et al.*, 1997; Blokker *et al.*, 1998), in higher plant cuticles (as cutans, Nip *et al.*, 1986a,b; Tegelaar *et al.*, 1989b), in periderm tissues (as suberans, Tegelaar *et al.*, 1995; Collinson *et al.*, 1994), in inner seed coats of freshwater plants (as tegmens, van Bergen *et al.*, 1994) and in spores and pollen grains of higher land plants (as the major part of sporopollenins, van Bergen *et al., 1993*, 2004). Aromatic biopolymers, such as lignins with their phenolic structure, occur in higher land plants (Sarkanen and Ludwig, 1971), phlorotannins in brown macroalgae (e.g. Grosse-Darnhues and Glombitza, 1984) and, partly, in sporopollenins (Schenk *et al.*, 1981; van

Bergen *et ai.,* 1993, 2004). These biopolymers can be traced back in the geological record, e.g. in coals (e.g. McKinney and Hatcher, 1996) and freshwater sediments (Goth *et ai., 1988).* In marine sediments, algal aliphatic biopolymers, i.e. algaenans, are the most abundant macromolecules derived from the primary producers living in the euphotic zone. This has been confirmed by geochemical analysis of particulate organic matter (POM) in sediment traps in the Mediterranean Sea (Peulve *et ai., 1996).*

In this study, macromolecular OM of three time-equivalent Pliocene sapropels, as well as sediments above and below the sapropel, were studied using Rock-Eval pyrolysis, palynology and Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Using Rock-Eval pyrolysis the hydrogen index (HI) and oxygen index (01) can be determined. These parameters are related to the origin and thermal maturity of the total OM (Tissot and Welte, 1984). Palynological analysis is applied to identify microscopically recognizable macromolecular OM (e.g. spores, pollen, algae material) to determine its origin. Curie-point pyrolysis of macromolecular OM in combination with GCIMS is able to characterize the composition of macromolecular OM at the molecular level by providing a semi-quantitative distribution of various molecular types in the kerogen macromolecule (Larter and Horsfield, 1993). With the combination of these different methods the composition of the macromolecular structure of the kerogen can be thoroughly studied. The present study aims to determine the source materials for the kerogen fraction in time-equivalent sections from three sites in the eastern Menditerranean reflecting the time before, during and after sapropel deposition.

MATERIAL AND METHODS

Samples

Cores at different locations in the eastern Mediterranean Basin were taken during ODP Leg 160 (Emeis *et ai.,* 1996). Site 967 (Eratosthenes Seamount), and Site 969 (Mediterranean Ridge) are located in the Levantine Basin. Site 964 is located in the Ionian Basin (Fig. 7.1a). The detailed chronology of these cores, obtained by astronomical tuning (see Lourens *et ai.,* 1996 for details), enabled us to select three laterally equivalent sapropels (967C-8H-4, 111-134 cm, 969E-6H-6, 23-43 em, 964D-I0H-l, 105-113 cm) with an age of 2.943 Ma. These sapropels have TOC contents up to 30% (Fig. 7.1b; Nijenhuis *et ai., 1998).* The sapropels were sub-sampled in 0.5 - 1.5 em slices. The homogeneous intervals above and below the sapropels (Nijenhuis and de Lange, 2000) are composed of light-coloured, organic-poor, nannofossil and foraminiferal marl ooze (Calvert, 1983) and were samples in slices of several em. Ground, freeze-dried sediment samples (0.1 to 3 g) were Soxhlet extracted with dichloromethane/methanol (1:1, v/v) for 24 h to remove the solvent soluble fraction from the sediments.

Rock-Eval pyrolysis

Rock-Eval pyrolysis was performed on freeze-dried, powdered whole sediments using a Rock-Eval VI instrument following procedures described by Lafargue *et ai. (1998).*

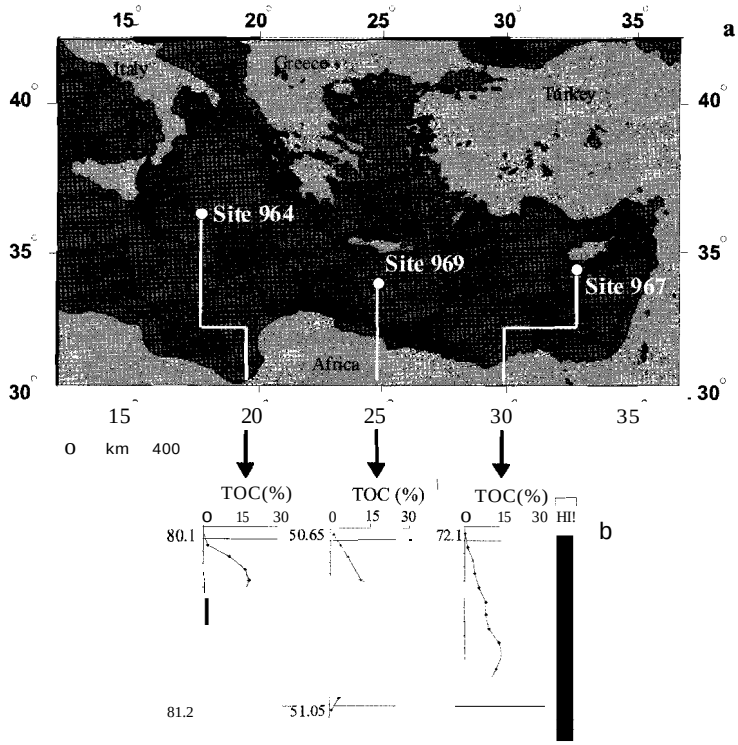


Figure 7.1 (a) Location of ODP drilling sites, (b) lithological description (HI = homogeneous calcareous ooze, S = sapropel) and TOe profiles of the investigated Pliocene sapropel (data from Nijenhuis *et at., 1998)*

Palynological sample preparation

Extracted sediments (ca. 1 g of the sapropels, ca. 3 g of the sediments of the homogenous interval) were treated with 30% hydrochloric acid (HCI) to remove carbonates. After the initial treatment H_20 was added to the suspension. The sample in HCIIHzO was left at room temperature overnight. Subsequently, the liquid was decanted, H20 was added and the sample was centrifuged (7 min, 2000 rpm). The liquid was decanted and 38% hydrofluoric acid (HF) was added to dissolve silicates. The samples were agitated for 2 h, H_20 was added and the mixture left overnight. The HCLlHF treatment was repeated once again. The liquid was then decanted, 30% HCI was added to dissolve the silica-gel. The gel dissolved immediately after adding HCI, and the liquid was separated from the residue by way of decanting after settling overnight. The samples were washed until a pH of 4-5 was reached. The size of the palynomorphs ranges from 5 μm to > 200 μm. A 250 μm mesh sieve was used to remove oversized organic material. Sieving with a 10 μm mesh allowed the collection of the fraction containing pollen, spores and dinoflagellates. After sieving, a small part of the residue was mounted on a slide, embedded with glycerine jelly, covered, sealed with paraffin wax and studied using a microscope.

Isolation of kerogen from calcareous oozes for Py-GCIMS analysis

Extracted sediments (typically ca. 3 g) were treated following the protocol "palynological sample preparation" (see above) up to and including the washing procedure to pH of 4-5. Thereafter, samples were extracted using a vortex sample tube shaker with methanol (3x) and subsequently with methanol/dichloromethane (1:1, v/v) (3x). After each addition of solvent the liquid was removed by centrifugation (15 min, 2000 rpm). Finally, the samples were freeze-dried and ground. The yield of kerogen obtained from the homogeneous intervals of ODP Site 967 above the sapropel, and below and above the sapropel from ODP Site 964, was extremely low and the kerogen fraction could, therefore, not be studied using Curie-point pyrolysis-GCIMS.

Curie-point pyrolysis-gas chromatography/mass spectrometry

The freeze-dried, extracted sapropels and isolated kerogens from the calcareous oozes were pressed onto a flattened ferromagnetic wire with a Curie temperature of 610°C. The interface temperature of the pyrolysis unit was set at 200°C. Flash pyrolysis was conducted by inductive heating of the sample-coated wires to the Curie temperature at which it was held for 10 s. Py-GCIMS analyses were performed using a FOM-5LX Curie-Point pyrolyser connected to a Hewlett-Packard 5890 series II gas chromatograph (GC) and a Fisons instruments VG platform II mass spectrometer (MS). The GC was equipped with a CP-sil 5CB-MS silica column (50 m x 0.32 mm, film thickness 0.4 μm). The GC oven temperature was programmed from 40°C (5 min) to 310°C (isothermal for 10 min) at a rate of 5°C.min^{-1}. Helium was used as carrier gas. The MS was operated at 70 eV, scanning the range *m/z 50*-650 with a cycling time of 0.65 s.

Identification and quantification of flash pyrolysis products

Compound identification was based on comparison of mass spectral and retention time data with literature data (Sinninghe Damste *et ai.,* 1988, 1992b; Hartgers *et ai., 1992;* van Bergen *et ai.,* 1994). Relative amounts of the individual compounds in the pyrolysates were determined by integration of individual peak areas from summed mass chromatograms using relevant *m/z* values. These *m/z* values represent only part of the total ion count of the mass spectrum. Therefore, the measured peak areas were multiplied by a correction factor to compensate for this (Table 7.1). Correction factors, which were not available from literature, were determined using authentic standards as described by Hartgers *et ai., 1992.*

Table 7.1 Characteristic *m/z* values and correction factors used to calculate the relative abundances and internal distribution in the pyrolysates.

Compound class	Characteristic mass fragments *(m/z)*	Corr. factor
n-alkanes	55+57	2.9*
n-alkenes	55+57	4.9*
unsaturated isoprenoids	55+57	4.9
saturated isoprenoids	55+57	2.9
Methylketones	58	3.6#
Alkylbenzenes	78+91+92+105+106+119+120+133+134	1.6*
Alkylthiophenes	84+97+98+111+112+125+126+139+140	2.5*
Alkylphenols	94+107+108+121+122	2.2*
Alkylpyrroles	80+81+94+95+108+109+122+123+136+137	1.5
oxygenated aromatics	78+91+92+105+106+119+120+133+134	2.6
alkylindenes	115+116+129+130+143+144	1.8*
Indoles	117+132	7.0
phytadienes/phytenes	55+57+68+70+82+95+97+123	2.4
prist-I-ene/prist-2-ene	55+57	4.9
3-ethyl-4-methyl-1H-pyrrole-2,5-dione	53+67+96+110+124+139	2.9
6,10,14-trimethyl-2-pentadecanone	58	7.5
C_{20} isoprenoid thiophenes	98+111+125	2.6

* from Hartgers *et al.,* 1992, 1994b
# from Hoefs *et al., 1996*

RESULTS

Rock-Eval pyrolysis

Sapropels are characterized by hydrogen index (HI) values between 200 and 460 mg HC/g TOe and oxygen index (01) values between 80 and 205 mg C02/g TOC, indicating a mixture of Type II and III kerogen in the pseudo-van Krevelen diagram (Fig. 7.2a). The HI and 01 of the homogeneous intervals are not reliable because TOC contents were <0.5% (Whelan and Thompson-Rizer, 1993). A plot of the TOe contents versus the HI values shows that generally the sapropels with a higher TOC content also have a higher HI (Fig. 7.2b). This is true for all three sites.

Palynological characterization of the kerogen

The kerogen of the homogeneous intervals are characterized by (not "fluffy") AOM and palynomorphs. The major palynomorphs are bisaccate pollen, other pollen, spores and different dinocysts.

The sapropels kerogen are dominated by "fluffy" AOM. The major palynomorphs are bisaccate pollen, other pollen and few spores. Also a few dinocysts of *Polyspaeridium zoharyii* were found.

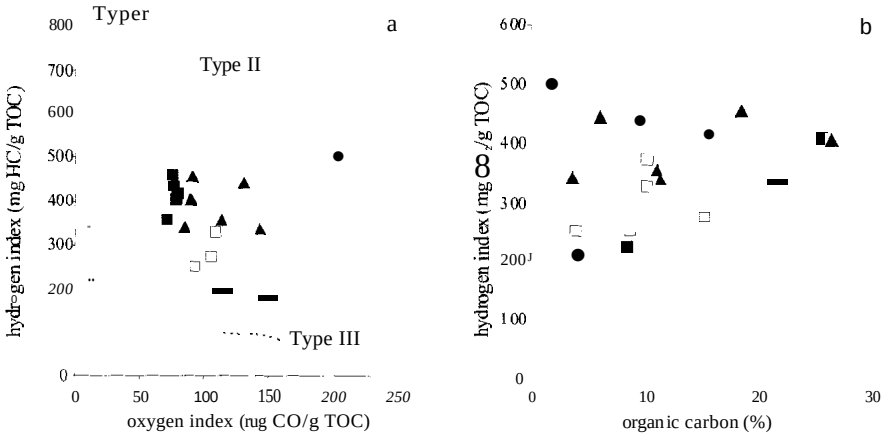


Figure 7.2 (a) Rock-Eval pyrolysis data of bulk sediments plotted in a pseudo van Krevelen diagram for sedimentary organic Thermal alteration pathways of organic matter Types I, II and II are indicated by dashed lines and (b) cross-plot of TOe content and Rock-Eval hydrogen index values of the sapropels. The different symbols indicate the different sites: 967 (O), 969 (▲) and 964 (.).

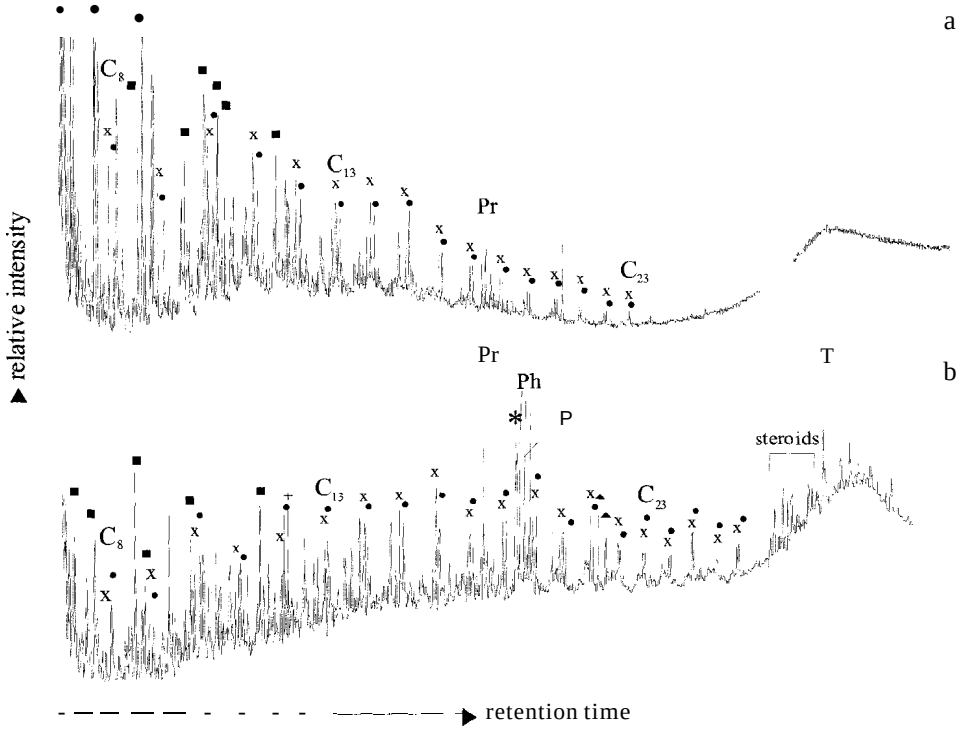


Figure 7.3 Total ion current (TIC) traces of the flash pyrolysates of the kerogens from Site 967, (a) homogeneous interval (967C-8H-4, 130-134 em) and (b) sapropel (967C-8H-4. 127-128 em) Key: • = n-alkanes, x = n-alk-I-enes, • = alkylbenzenes, + = 3-ethyl-4-methyl-IH-pyrrole-2,5-dione, Pr = prist-I-ene, * = 6, 10, 14-trimethyl-2-pentadecanone, Ph = phytadienes, P = phytene. ▲ = C_{20} isoprenoid thiophene. T = a-tocopherol.

Pyrolysis GC-MS

General composition of the kerogen pyrolysates

Sub-samples of the sapropels and homogeneous intervals from the three different ODP sites with total organic carbon (TOC) contents ranging between 0.2 and 27% were studied using flash pyrolysis-GC/MS. The molecular composition of the kerogen pyrolysates of the homogeneous intervals and sapropels differs substantially as is illustrated by the total ion current (TIC) traces in Fig. 7.3. The relative abundance of the various compound classes encountered in the pyrolysates of the homogeneous intervals and sapropels are compiled for the three ODP sites in Fig. 7.4. These data represent the average for the sapropels and the homogenous intervals. As an indication for the variability within the sapropel the standard deviation is also indicated in Fig. 7.4.

The pyrolysates of the kerogens of the homogeneous intervals of the Sites 967 and 969 deposited below and above the sapropel are dominated by alkylbenzenes, n-alkanesln-alk-l-enes and alkylthiophenes (Fig. 7.4). Acyclic isoprenoids (including prist-l-ene/prist-2-ene), methylketones (including 6,10,l4-trimethyl-2-pentadecanone), oxygenated aromatics and alkylphenols show relatively minor contributions and alkylpyrroles are absent (Fig. 7.4). The pyrolysates of the sapropel kerogens from the three studied ODP sites show an increase in the relative abundance of the compound classes of n-alkanes/n-alk-l-enes, acyclic isoprenoids, methylketones, oxygenated aromatics, alkylphenols and alkylpyrroles when compared with those of the homogeneous intervals (Fig. 7.4). A relative decrease by approximately a factor of 2 is observed in the alkylbenzenes and alkylthiophenes. The relatively high abundances of prist-l-ene/prist-2-ene, phytene, phytadiene and 6,10,14-trimethyl-2-pentadecanone (i-CI8 MK) in the pyrolysates of the sapropel kerogens are notable (Fig. 7.3b). The relative abundance of the individual compound classes within the sapropel show only minor changes. The pyrolysates of the kerogens of the three different ODP sites show similar compositions (Fig. 7.4). Other pyrolysis products, such as 3-ethyl-4-methyl-lH-pyrrole-2,5-dione, *Czo* isoprenoid thiophene, a-tocopherol, cholest-3,5-diene, tris-norhopane, hop-17(2l)-ene and 17P,21P(H)-homohopane were only detected in kerogen pyrolysates of the sapropels (Fig. 7.3b) but were not considered in the calculation of the relative abundance of compound classes.

Detailed observations for the distribution pattern of the various major compound classes is given below and will be illustrated using pyrolysis data obtained with sediments from Site 967. These data are representative for the observations at all three sites.

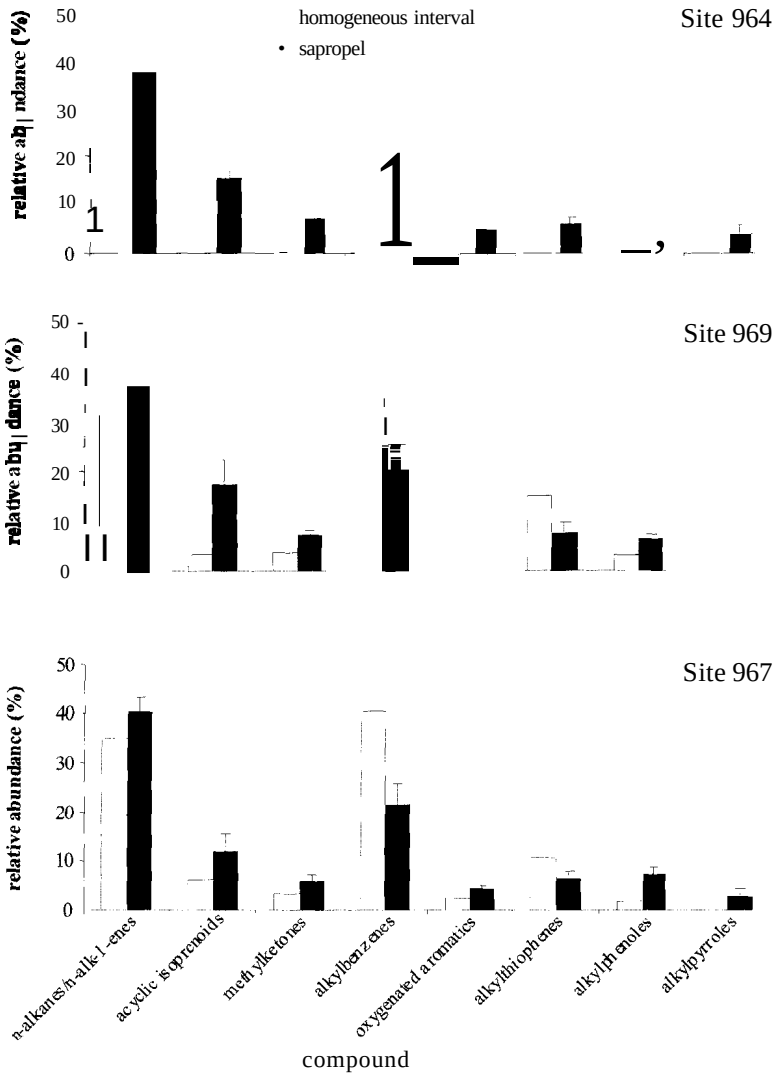


Figure 7.4 Bar plots showing relative abundances of the various compound classes in the kerogen pyrolysate of the homogeneous intervals and sapropels of Site 964, Site 969 and Site 967. Kerogen pyrolysate data of sapropels represent the average of all samples and error bars reflect standard deviations *(n* = 7).

Aliphatic pyrolysis products

n-Alkanesln-alk-l-enes. The kerogen pyrolysates of the homogeneous intervals show relatively higher abundances of n-alkanes compared with the n-alk-l-enes (Fig. 7.5a). The distribution pattern of the n-alkanes in the homogeneous interval shows a dominance of short-chain members, ranging from Cg to C_{26} and maximising between Cg to *CIS* with a relative dominance of C_S, C_{IO} and CI2 n-alkanes. This is clearly reflected in the average chain

length (ACL) of 13.5 and 13.9 in the kerogen pyrolysates of Sites 967 and 969, respectively (Table 7.2). The carbon number distribution of the n-alkanes in the pyrolysates of the sapropel kerogens shifts towards increasing carbon chain length, resulting in higher ACLs of 14.3 in Site 967, 15.5 in Site 969 and 15.9 in Site 964 (Table 7.2). The relatively high abundances of the C_{15} and C_{18} *n*- alkanes in the kerogen pyrolysates of the three sapropels are worth noting.

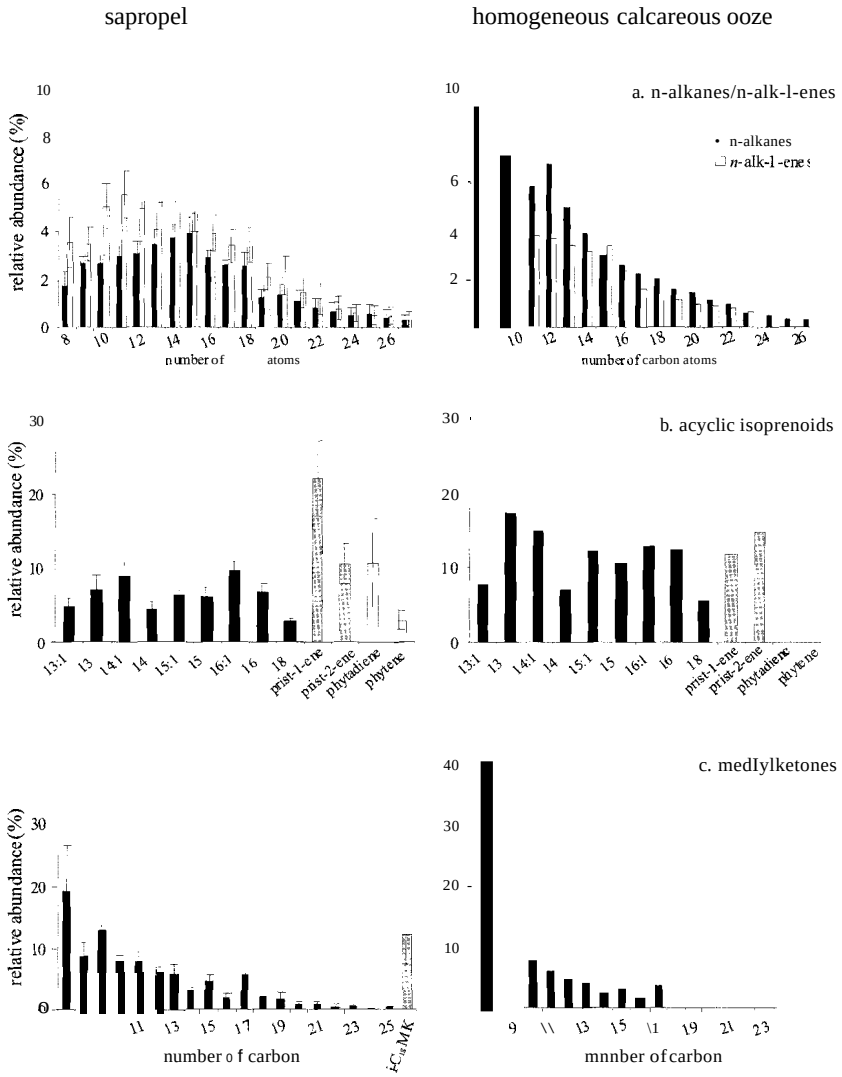


Figure 7.5 (a-c) Internal distribution patterns of different aliphatic compound classes between the homogeneous, calcareous ooze and sapropel of Site 967. Kerogen pyrolysate data of the sapropels represents means and error bars are standard deviations *(n* = 7) (i-C_{18} MK = 6,10, 14-trimethyl-2-pentadecanone).

The distribution pattern of n-alk-I-enes in the homogeneous interval is similar to that of the n-alkanes ranging from C_S to C_{23} with a relative dominance of C_9 and CIS n-alk-I-enes (Fig. 7.5a). The ACLs are generally lower than those for the n-alkanes, with values of 12.5 and 12.6 in the homogeneous intervals of Sites 967 and 969, respectively (Table 7.2). In the kerogen pyrolysates of the sapropels n-alk-I-enes show a dominance of short carbon chain members, maximizing between C_{IO} to CIS (Fig. 7.5a). The ACLs are 13.7 at Site 967, 15.4 at Site 969 and 15.7 at Site 964 (Table 7.2).

Table 7.2 Average chain length (ACL) of various compound classes in the flash pyrolysate of the homogeneous intervals and the sapropels of ODP Sites 967, 969 and 964. ACL = $\sum(iX_i)/\sum X_i$, where X is abundance and i ranges from 8 to 27.

Kerogen pyrolysate	Compound class	ODP 964	ODP 969	ODP 967
homogeneous intervals	n-alkanes	n.d.	13.9	13.5
	n-alk-l-enes	n.d.	12.6	12.5
	methylketones	n.d.	8.8	9.3
Sapropels	n-alkanes	15.9	15.5	14.3
	n-alk-I-enes	15.7	15.4	13.7
	methylketones	12.3	12.3	11.0

Acyclic isoprenoids. Prist-l-ene/prist-2-ene are present in the kerogen pyrolysate of both the homogeneous interval and sapropel, but show relatively higher abundances in the sapropel with a concomitant increase in the prist-I-ene/prist-2-ene ratio (Fig. 7.5b). Phytadiene and phytene are only present in the pyrolysates of the sapropel kerogens (Fig. 7.5b). The Cl3, Cl3:I, C_{14}, $C_{14:1}$, CIS, $C_{15:1}$, C_{16}, and CIS isoprenoid alkanes/alkenes are especially abundant in the pyrolysates of the sapropel kerogens and show a relative increase of the C_{13} and C_{16}:1 members in the kerogen pyrolysates of the homogeneous interval (Fig. 7.5b).

Methylketones. Methylketones are present in the kerogen pyrolysates of both the homogeneous intervals and sapropel (Fig. 7.5c). The odd-over-even carbon number predominance is more pronounced in the kerogen pyrolysate of the sapropels: in all kerogen pyrolysates a predominance of C_{l3}, CIS and Cl7 is observed. The ACLs show the lowest values in the homogeneous interval with values of 8.8 at Site 969 and 9.3 at Site 967 (Table 2). In the pyrolysate of the sapropels the ACL increases from 11.0 at Site 967 to 12.3 at Sites 969 and 964 (Table 2). i-CIS MK is relatively abundant in the sapropel kerogen pyrolysates, but absent in the kerogen pyrolysates of the homogeneous interval (Fig. 7.5c).

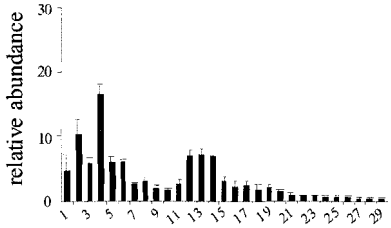
Aromatic pyrolysis products

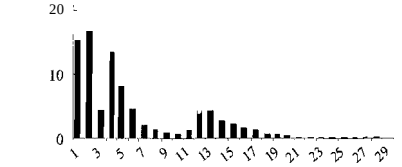
Alkylbenzenes. A wide variety of alkylbenzenes is identified in the pyrolysates. In the kerogen pyrolysates of the homogeneous intervals, benzene, toluene and styrene are most abundant (Fig. 7.6a). In the sapropels, a relative increase in the abundance of 1,3+1,4 dimethylbenzene and 1,2,3,4-tetramethylbenzene (TMB) is observed (Fig. 7.6a).

sapropel

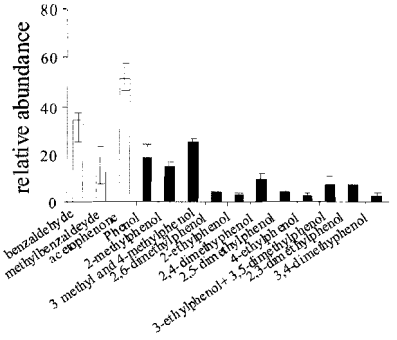
homogeneous calcareous ooze

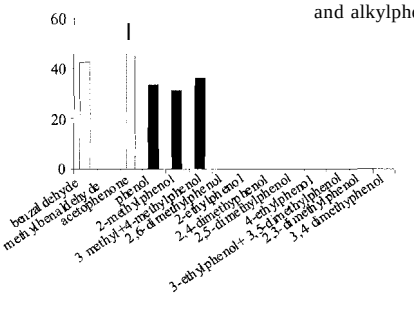
a. alkylbelllenes



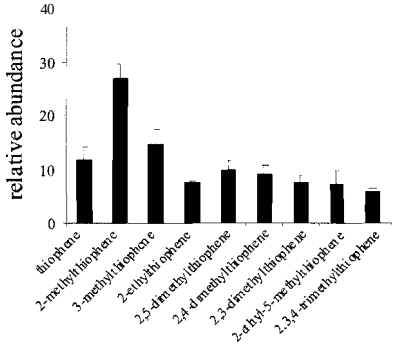


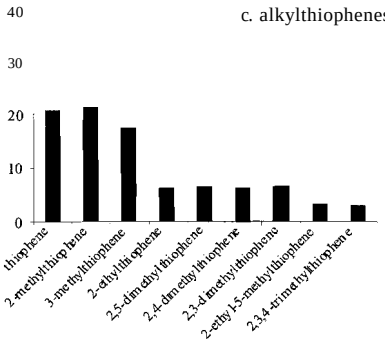
b. aromatics and alkylphenols



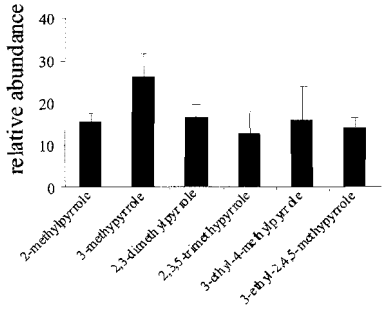


c. alkylthiophenes





d. alkylpyrroles



not present

Figure 7.6 (a-d, page 90) Comparison of the internal distribution pattern of different aromatic compound classes between the homogeneous, calcareous ooze and sapropel of Site 967. Kerogen pyrolysate data of the sapropels are means and error bars are standard deviations (n = 7). (a) Key to alkybenzenes: I-benzene, 2- toluene, 3 - ethylbenzene, 4 - 1,3+1,4-dimethylbenzene, 5 - styrene, 6 - 1,2 - dimethylbenzene, 7 - n-propylbenzene, 8 - I-methyl-3-ethylbenzene, 9 - l-methyl-4-ethylbenzene, 10 - 1,3,5-trimethylbenzene, 11 l-methyl-2-ethylbenzene, 12 - 1,2,4-trimethylbenzene, 13 - 1,2,3-trimethylbenzene, 14 - 1,2,3,4-tetramethylbenzene, 15 - n-butylbenzene, 16 - n-pentylbenzene, 17 - n-hexylbenzene, 18 - n-heptylbenzene, 19 - n-octylbenzene, 20 - *n*-nonylbenzene, 21 - n-decylbenzene, 22 - n-undecylbenzene, 23 - n-dodecylbenzene, 24 - *n*-tridecylbenzene, 25 - n-tetradecylbenzene, 26 - n-pentadecylbenzene, 27 - n-hexadecylbenzene, 28 - n-heptadecylbenzene, 29 - n-octadecylbenzene.

Oxygenated aromatics and alkylphenols. Among the oxygenated aromatics, only benzaldehyde and acetophenone are common in the kerogen pyrolysates of the homogeneous intervals. In the pyrolysates of the sapropel kerogens methylbenzaldehyde is also present, albeit in relatively low abundance (Fig. 7.6b). Phenol, 2-methylphenol and the co-eluting 3- and 4-methylphenol were identified in the pyrolysates of the kerogens of the homogeneous intervals. In the sapropel kerogen pyrolysates, these alkylphenols are still dominant, but C_2 alkylated phenols were also detected (Fig. 7.6b).

Alkylthiophenes. In the kerogen pyrolysates of the sapropels and homogeneous intervals nine alkylthiophenes were identified. Thiophene and C1.alkylated thiophenes are dominant in the pyrolysates of the kerogen of the homogeneous intervals (Fig. 7.6c). In the pyrolysates of the sapropel kerogens, thiophene showed a relative decrease whereas C2 and C3 alkylthiophenes increased, in relative abundance.

Alkylpyrroles. They appear exclusively in the sapropels with 3-methylpyrrole and 3-ethyl-4-methylpyrrole relatively abundant (Fig. 7.6d). Distributional variations of this compound class in the pyrolysates of the sapropel kerogens are small.

DISCUSSION

OM sources: Rock-Eval pyrolysis and palynological observations

Based on the results obtained from Rock-Eval pyrolysis, the OM present in the three time-equivalent sapropels is a mixture of Type II and Type III kerogen (Fig. 7.2a; Espitalie *et al.,* 1977; Tissot and Espitalie, 1975). HI and 01 values simiar to those measured here have been reported for other Pliocene/Pleistocene sapropels of the eastern Mediterranean Sea (Bouloubassi *et al.*. 1999). Type II kerogen is characterized mainly by aliphatic, but also aromatic OM, originating predominantly from marine organisms. Type III kerogen is characterized mainly by aromatic material derived from terrestrial plants (Tissot *et al., 1974).* Alternatively, this type of kerogen may also derive from oxic degradation of marine OM (Kenig *et al.,* 1994). Reworking of hydrogen-rich marine OM during settling in the water column and burial in the sediment, typically results in an increase in 01 and decrease of HI (Pratt, 1984), specifically with long residence time at the oxic water-sediment interface. Hollander *et al.* (1990) observed, in a modem lacustrine setting, that OM deposited in an anoxic water column had higher atomic *HIC* ratios and lower atomic O/C ratios than OM deposited in an oxic water column.

The high OIs and the only moderately high HIs (200-500 mg HC/g TOC) compared with other TOC-rich sediments (Espitalie *et al.,* 1977; Kenig *et al.,* 1994) is somewhat surprising for these sapropels with TOC contents up to 30%. Previous studies of these Pliocene sapropels revealed anoxic conditions up into the photic zone during sapropel deposition (Menzel *et al.,* 2002), implying optimal conditions for preservation of relatively labile marine OM. A reason for the high OIs and relatively low HIs of the sapropels may be that sediments, rather than isolated kerogen were analysed by Rock-Eval pyrolysis. A number of workers have reported that certain minerals, particularly the clay minerals smectite and illite, cause catalytic effects with whole rock pyrolysis and give artificially low HIs, when compared with isolated kerogen (e.g. Orr, 1983; Katz, 1983; Peters, 1986). This observation is particularly evident for sediments off the Louisiana Coast in the Gulf of Mexico, known to contain high amounts of smectite (Whelan and Thompson-Rizer, 1993). It is known that the Pliocene sapropels contain substantial amounts of smectite as a result of the wet climate conditions during their deposition (Foucault and Melieres, 2000). Therefore, the presence of smectite is likely responsible for the high OIs and only moderately high HIs of the sapropels.

Palynological studies of the sapropels indicate the dominance of AOM of marine origin (Tyson, 1995). The minor contribution of pollen and spores embedded in the AOM is evidence for only a small contribution to the OM derived from terrestrial higher plant material. This indicates that the Rock-Eval pyrolysis data indicating Type WIll kerogen is likely not related to the admixture of Type III kerogen from the continent with kerogen of a marine origin but more likely due to the effects of oxic degradation or, more likely, the artificial low **HI** values due to the presence of smectite. The positive relationship of Rock-Eval HIs and the TOC content in the sapropels at all three sites (Fig. 7.2b) appears to imply a progressive enrichment and preservation of hydrogen-rich aliphatic components with higher accumulation of OM. This has been observed before for many other sediments (e.g. Herbin *et aI.,* 1986; Hue *et al.,* 1992; Kenig *et a!',* 1994; Bouloubassi *et aI., 1999).*

Palynological observations in the homogeneous intervals showed pollen, spores, dinocysts and AOM, which is different to the kerogen composition obtained of the sapropels. Based on these palynological observations, the kerogen of the homogeneous intervals should be described as a mixture of resistant marine and terrestrial OM, which has persisted aerobic decomposition.

OM sources: molecular evidence

The molecular composition of the flash pyrolysates is in agreement with a predominant marine source for the OM in the sapropels. The composition is similar to that of pyrolysates of other marine kerogens (e.g. Hoefs *et al.,* 1995, 1996) and settling of macromolecular OM in the marine water column (Peulve *et al., 1996).*

Algaenan-derived compounds

n-Alkanes/n-alk-l-enes and methylketones are relatively abundant compound classes in the kerogen pyrolysates of the sapropels, and to a lesser extent, also in those of the homogeneous intervals (Fig. 7.4). Temporal and spatial distributional variations of these compound classes in the kerogen pyrolysate of the sapropels were only minor. These

abundant pyrolysis products have been described earlier as being derived from non-hydrolyzable aliphatic macromolecules, possibly algaenan, biosynthesized by marine algae (e.g. Derenne *et al.,* 1989, 1992; Behar *et ai.,* 1995; Gelin *et al.,* 1996). Thermal rearrangement reactions of the ether bridges during pyrolysis account for the formation of the methy1ketones (Gelin *et ai.,* 1993). To date, algaenans have only been found as outer cell wall constituents in eustigmatophytes and some marine ch1orophytes (Gelin *et al., 1999).* None of the diatoms and prymnesiophytes studied to date and only one dinoflagellate were shown to contain algaenan (Gelin *et al.,* 1999). Therefore, the origin of a1gaenans seems limited to only two classes, eustigmatophytes and chlorophyte microalgae of phytoplankton among the wide range of primary producers. The presence of eustigmatophytes in the water column is confirmed by the high abundances in the sapropels of its characteristic lipid biomarker, the C_{30} 1,15-alkyldiol (Menzel *et ai., 2003).*

The origin of the series of C_{13}-C_{18} isoprenoid alkanes/alkenes is less clear; they have been proposed to derive from non-hydrolyzable aliphatic macromolecules with isoprenoid carbon skeletons that form an ether-linked macromolecule biosynthesized by an as yet unknown marine algae (Derenne *et ai.,* 1989, 1990; *et ai.,* 1998). The presence of 6,10,14-trimethyl-2-pentadecanone in kerogen pyrolysates of the Madeira Abyssal Plain (MAP) turbidites was also proposed to originate from isoprenoid algaenans (Hoefs *et ai.,* 1996). Alternative sources are discussed below.

Chlorophyll- and tocopherol-derived compounds

Phytadiene and phytene are distinct products in the kerogen pyrolysates of the sapropels (Fig. 7.5b). Phytadiene and phytene are derived from the phytyl side chain of chlorophyll (van de Meent *et al.,* 1980a,b; Ishiwatari *et ai.,* 1991). C_{20} isoprenoid thiophenes and phytenes presumably originate from pyrolysis of sulphur-bound phytol (Sinninghe Damste *et ai.,* 1987; Schouten *et al.,* 1993). A1kylpyrro1es, present only in the pyro1ysates of the sapropel kerogens, are likely derived from chlorophyll and/or other tetrapyrrole pigments (Sinninghe Damste *et ai.,* 1992b).

Both pristene isomers (prist-1-ene and prist-2-ene) have been demonstrated to derive primarily from kerogen-bound tocophery1 moieties through an intramolecular rearrangement reaction, rather than from chlorophyll-a (Goosens *et al.,* 1984). This is supported by the presence of intact a-tocopherol in the kerogen pyrolysate of the sapropels (Fig. 7.3b; cf. van Bergen *et ai.,* 1994). Tocopherol is relatively abundant in chloroplasts of most autotrophic organisms.

The presence of chlorophyll and tocopherol pyrolysis products in the kerogen pyrolysates of the sapropels but not in those of the homogeneous intervals just below and above the sapropel implies optimal preservation conditions for these relatively labile lipids. Both compound classes are likely of marine origin. They can be produced in the terrestrial realm but their labile nature prevents their transport (with concomitant exposure to oxygen) over long distances.

Higher-plant-derived compounds

Py-GCIMS analysis of the kerogen pyrolysates revealed the presence of oxygenated aromatics, i.e. benzaldehyde, methylbenzaldehyde and acetophenone (Fig. 7.6b) for both the sapropels and the homogeneous intervals. These pyrolysis products, can be derived from sporopollenin expected to be selectively preserved (i.e. the structural constituents in the outer wall of pollen and spores from vascular plants) (van Bergen *et aI.,* 1993,2004), confirmed by the presence of pollen and spores in the sapropels and the homogeneous intervals. There is no evidence for the presence of pyrolysis products of cutans and suberans, biopolymers also derived from terrestrial higher land plants, since in that case a range of n-alkanes/n-alk-1-enes up to C_{35} would be expected (Nip *et al.,* 1986a,b; Tegelaar *et al.,* 1989b). Analysis of the biomarker lipids in these samples confirm the presence of some terrigenous OM in both the homogeneous intervals and in the sapropels as witnessed by the presence of long-chain *n*-alkanes and n-alcohols (Menzel *et aI.,* in press).

The presence of the aromatic biopolymer lignin can also be indicative for a contribution of terrigenous OM. If so, the kerogen pyrolysates should contain methoxyphenols and, in case of more degraded lignin, catechols and ultimately phenols (Hatcher and Clifford, 1997; van Bergen *et aI.,* 2000). Diagenetic transformation of methoxypheno1 moieties into phenol moieties typically takes tens of million of years (Ewbank *et al.,* 1996; Edwards *et al.,* 1997; Abbott *et al.,* 1998). Neither methoxyphenols nor catechols were present in the kerogen pyrolysates of the sapropels or homogeneous intervals (Fig. 7.6b). This indicates, at most, only a minute contribution of lignin. Some alky1phenols are present in the kerogen pyrolysates of the sapropels but these are most likely derived from marine organisms (cf. van Heemst *et al..* 1999), as previously proposed from the distribution pattern of phenols obtained in the kerogen pyro1ysate of OM collected in sediments traps in the Mediterranean Sea (Peulve *et aI., 1996).*

Origin of other pyrolysis products

Alkylthiophenes can be formed during the early stage of diagenesis by the incorporation of reduced inorganic sulphur, originating from bacterial sulphate reduction, interacting with organic material (presumably carbohydrates) (van Kaam-Peters *et aI., 1998;* Kok *et al.,* 2000). It has been proposed that the degree of OM sulphurization is primarily controlled by the supply of reactive iron (Sinninghe Damste and de Leeuw, 1990). Sulphate reduction rates were high during sapropel formation based on the euxinic conditions in Mediterranean waters (Passier and de Lange, 1998; Passier *et al.,* 1999b), which explains the presence of alkylthiophenes in the kerogen pyrolysates of the sapropel.

Alkylbenzenes in kerogen pyrolysates can have many precursors in the kerogen (Hartgers *et aI.,* 1994a). One group of potential precursors is formed by the aromatic carotenoids biosynthesized by photosynthetic green sulphur bacteria, which, upon pyrolysis, yield high amounts of 1,2,3,4-tetramethybenzene (TMB), (Hartgers *et aI.,* 1994b). Menzel *et al.* (2002) showed the presence of intact isorenieratene, an aromatic carotenoid, in these but it was absent in the homogenous intervals. In accordance with this, the kerogen pyrolysates of the sapropels indeed contained relatively higher amounts of TMB than those of the homogeneous intervals (Fig. 7.6a). TMB was, however, not absent in the kerogen

pyrolysates of the homogeneous intervals. This can be explained by the findings of Hoefs *et a!.* (1995), who showed that there are other precursors than kerogen-bound aromatic carotenoids for TMB in flash pyrolysates of marine kerogens.

Implication for sapropel formation

Palynological studies showed differences in the kerogen composition between the homogeneous interval and sapropels. The oxic degradation of OM during the formation of homogeneous intervals may have altered the palynomorph assemblage because some taxa are more susceptible to aerobic decay than others (Cheddadi *et a!.,* 1991; Cheddadi and Rossignol-Strick, 1995; Versteegh and Zonneveld, 2002). Pollen and spores are highly resistant against oxygen because of the presence of sporopollenin (Zetzsche and 1931). This explains the dominant presence of palynomorphs relative to the AOM in the homogeneous intervals. The opposite result was found in the sapropels where AOM was the predominant macromolecular OM. These palynological results are consistent with the Py-GC!MS data.

Comparison of the composition the kerogen pyrolysates of the homogeneous intervals and the sapropels showed similarities with changes in kerogen composition of the Madeira Abyssal Plain (MAP) turbidites (Hoefs *et ai.,* 1996). The sedimentary record of the MAP contains a large number of OM-rich turbidites, which were formed by re-deposition of sediments deposited at the northwestern African Shelf. Due to mixing during transport these turbidites contain uniformly distributed OM in the up to 50 cm thick turbidites. The upper part of the turbidite is subjected to oxic conditions due to the diffusion of oxygen, allowing a study of the effects of post-depositional oxidation on OM composition (Hoefs *et a!.,* 1996). A number of differences observed between the compositions of the kerogen pyrolysates of the oxidized and non-oxidized MAP turbidites, agree with those observed in this study for the kerogen pyrolysates of the homogenous intervals and sapropels, respectively.

Compound classes, such as isoprenoid alkanes/alkenes and alkylphenols revealed relatively low abundances in the homogeneous intervals and relatively high abundances in the kerogen pyrolysates of the sapropels, which was also observed in the kerogen pyrolysates of the oxidized and non-oxidized MAP turbidites, respectively. In addition, (i) a shift to a lower n-alkaneln-alk-l-ene ratio, (ii) an increased ACL measured ACL for n-alkanesln-alk-l-enes and methylketones (Table 2), and (iii) relatively higher abundance of pristenes in the kerogen pyrolysates of the sapropel compared with that of the homogeneous interval are observed (Fig. 7.5a). The degree of alkylation (not specified in detail here) of the alkylphenols, alkylthiophenes, alkylbenzenes and alkylpyrroles in the kerogen pyrolysates of the sapropels, was also higher compared than in those kerogen pyrolysates of the homogeneous intervals (Fig. 7.6a-d). Chlorophyll pigments are generally labile natural products. Earlier studies on chlorophyll have shown that pigments are much more stable under anoxic than oxic conditions (Sun *et a!',* 1991; Sun and Wakeham, 1994). This agrees with the presence of chlorophyll-derived compounds, such as phytene and alkylpyrroles, reflecting anoxic preservation conditions during sapropel deposition (Fig. 7.5b, 6d). Phytadienes were absent in the homogeneous interval, which is consistent with their absence in kerogen pyrolysates of

particulate OM collected in sediment traps deployed in the Mediterranean Sea (Peulve *et ai.,* 1996).

The interpretation that the OM of the homogeneous intervals was exposed much longer to oxygen during deposition than the OM of the sapropels is in good agreement with other data. The presence of isorenieratene in the sapropels indicates that sapropels were deposited under euxinic conditions (Menzel *et ai.,* 2002), in contrast with the homogeneous intervals. In surface sediments of the eastern Mediterranean this preferential degradation of labile marine OM also occurs (van Santpoort *et ai.,* 2002). Surprisingly, however, values in surface sediments clearly revealed more enriched values with increasing depth (van Santvoort *et ai.,* 2002). This $\delta^{13}C_{org}$ trend indicates that in the eastern Mediterranean Sea marine OM is more depleted in ^{13}C than terrestrial OM, which usually contains less ^{13}C than marine OM (Tyson, 1995).

More negative values were also observed in the upper Pleistocene-Quaternary sapropels S1 to S9 and the three studied Pliocene sapropels compared to their corresponding homogeneous intervals (Bouloubassi *et ai.,* 1999; Nijenhuis *et al.,* 2000). These apparently more positive values of terrestrial OM, which is enriched in the homogeneous intervals, could be explained if terrestrial OM is derived from a mixture of C_3 and C_4 plants, which have values of ca. -27 and -12 ‰, respectively (Tyson, 1995). Indeed, studies of higher plant wax C_{27-33} n-alkanes revealed, a substantial C_4 plant contribution (i.e. 40-50 %) in the homogeneous intervals (Menzel *et al.,* in press). This could explain the more enriched $\delta^{13}C_{org}$ values (ca. -20 ‰) in the homogeneous intervals (mixture of marine/terrestrial OM), compared to the sapropels (ca. -22 ‰) (predominantly marine OM) (Nijenhuis *et al.,* 2002), where the terrestrial signal is overprinted. Although, the terrestrial OM has revealed an enrichment in $\delta^{13}C$ in the sapropels, the contribution of terrestrial OM is poor, compared with the marine OM. The more positive $\delta^{13}C_{org}$ values in the homogeneous intervals compared with the sapropels is caused by the high contribution of C_4 plants to the OM. The substantial terrestrial input of C_4 plant material was probably derived from the North African continent and transported via the river Nile and fossil wadi systems (cf. Rohling *et ai.,* 2002) into the eastern Mediterranean Sea.

CONCLUSIONS

Differences in kerogen composItIOn between the homogeneous intervals and sapropels were obtained by palynological and Curie-point pyrolysis results. The kerogen pyrolysates of homogeneous intervals consisted of a mixture of resistant marine and terrestrial macromolecular OM, which have persisted aerobic decomposition during their deposition. The kerogen pyrolysates of the sapropels originated predominately of marine and only minor terrestrial macromolecular OM, which were deposited under anoxic preservation conditions. Therefore, these differences in kerogen composition between the sapropels and their homogeneous intervals were caused by different preservation condition, rather than changes in autochthonous versus allochthonous sources. It is assumed that the unusual shift of $\delta^{13}C_{org}$ values, being more depleted in $\delta^{13}C$ in the sapropels, in contrast to the

homogeneous intervals is resulting from the higher C_4 plant relative to C_3 plant contribution of the terrestrial OM in the homogeneous intervals, which has caused the more enriched $\delta^{13}C_{org}$ values. However, the more enriched values of the terrestrial OM measured in the sapropels is overprinted by the predominately marine OM.

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Summary

The present-day Mediterranean Sea is a nutrient poor and, consequently, low productive marine basin. The sediments are grayish, homogeneous and calcareous, containing low organic carbon (ca. 0.1 %), because the organic matter (OM) produced by primary productivity is mineralized through the water column and at the seafloor by oxic degradation processes. However, a Swedish expedition in 1952 discovered black layers of a few centimeters thick imbedded in these grayish sediments in the entire Mediterranean basin. These black layers are laminated and have a high organic carbon content up to ca. 30 % and are called 'sapropels'.

Sedimentologists found a relationship between the presence of sapropels and the astronomical changes in the solar radiation onto the Earth, known as 'Milankowitch cycles'. The precession cycle (21 ka) showed a strong correlation with the occurrence of Mediterranean sapropels from the late Miocene (5.5 Ma ago) to the Holocene (last 10 ka). Precession minima caused a stronger seasonal contrast of insolation at the Northern Hemishere, resulting in colder winters and warmer summers. Additionally, the monsoon inflow into low-latitude Africa resulted in an enhanced transport of moisture from the Atlantic to the African continent leading to a northwards shift of the Intertropical Convergence Zone (ITCZ). The substantially increased rainfall caused the enhanced delivery of freshwater and nutrients to the Mediterranean Sea via the river Nile and other rivers. Consequently, enhanced primary productivity may have led to enhanced accumulation rates of OM to the sea floor inducing anoxic bottom waters and eventually the deposition of sapropels. The inflow of freshwater on the top of the more dense seawater may have caused reduced deep water circulation and water stagnation preventing to renew the bottom water with oxygen. Sapropel deposition thus results from a delicate interplay of climatic, biological and physical factors.

In this thesis, three time-equivalent Mediterranean sapropels of the late Pliocene (2.943 Ma ago) spanning an east-west transect of the entire eastern Mediterranean Sea were studied to obtain detailed insight in past climatic and palaeo-environmental conditions, which have caused their deposition. The sediments originate from a depth of about 80 meters below the seafloor and were recovered during Leg 160 of the Ocean Drilling Program (ODP). The sapropels and a few centimeters of sediments above and below the sapropels were investigated using different lipid biomarkers. These lipid biomarkers or so-called molecular fossils are specific biochemicals biosynthesized by a class of organisms or even specific organisms, which are useful tools to reconstruct palaeo-environmental conditions, e.g. changes in terrestrial *versus* marine input, sea surface temperature, phytoplankton assemblage, the presence of photic zone euxinia.

Isorenieratene is a carotenoid, originating from green sulfur bacteria, Chlorobiaceae and is indicative for an overlap of the photic and sulfidic zone. This biomarker was used to study periods of photic zone euxinia during Pliocene sapropel formation (Chapter 2). The amount of intact isorenieratene (summed *all-trans* and *cis* isomers), ranged from non-detectable at the base and top of a sapropel up to 140 sediment in the central parts.

Isorenieratene accumulation rates at the central and western site are remarkably similar and increase sharply to levels of up to 3.0 mg m^{-2} yr^{-1} in the central part of the sapropel and then drop to low levels. This pattern indicates an expansion of euxinic conditions reaching into the photic zone, followed by deepening of the chemocline during deposition of this Pliocene sapropel. The sapropel from the easternmost site of the basin, which contains less organic carbon, showed much lower isorenieratene accumulation rates and even absence of isorenieratene in the central part of the sapropel. *Bal*Al ratios indicate enhanced palaeoproductivity during sapropel formation, supporting previously proposed models, according to which increased productivity is the driving force for the generation of euxinic conditions.

Besides isorenieratene, a novel unknown carotenoid was found, which showed temporal and spatial differences in relative abundances between the three sites (Chapter 3). Highest relative abundances of the unknown carotenoid were measured at Site 967, located closest to the Nile, and coincided with changes in isorenieratene abundances. Based on its molecular weight, mass spectrum, and its UVIVIS absorption maximum this unknown pigment reflects either (i) spheroidenone or (ii) thiothece-474, likely derived from photosynthetic purple nonsulfur bacteria, i.e. the family Rhodospirillaceae. Based on the wide range of physiological adaptations of these Rhodospirillaceae, the palaeo-ecological implication of the presence of these bacteria is uncertain but probably related to the temporal occurrence of shallow photic zone anoxia (i.e. even more shallow than indicated by the presence of pigments of the Chlorobiaceae).

Biomarkers (alkenones, loliolide/isololiolide, dinosterol and C30 1,15 -dioUketo-ol) representative of four major classes of marine primary producers (haptophytes, diatoms, dinoflagellates, eustigmatophytes, respectively) were studied for changes in phytoplankton export production before, during and after sapropel deposition (Chapter 4). Biomarker contents showed substantial differences between non-sapropelic and sapropelic sediments. In non-sapropelic sediments, small contents are observed, reflecting extensive oxic degradation in oxic pore waters during deposition. In the sapropels, biomarker accumulation rates (ARs) showed a substantial increase from the base to the centre, likely resulting from increased biological productivity since bottom waters were continuously anoxic and preservation conditions are similar. The diatom biomarker, loliolide/isololiode became distinctly more abundant in the sapropels indicating that the eastem Mediterranean basin was enriched in nutrients. This caused enhanced export production by the major phytoplankton classes leading to periods of sapropel deposition. Alkenone-based SST reconstructions indicated that this change in phytoplankton composition was not influenced by changes in sea surface temperature, but was mainly nutrient-controlled. Compound-specific measurements did not provide additional evidence for substantially increased primary production rates: $\delta^{13}C$ values of C37:2 alkenones and lolioloide/isololiolide showed a slight negative shift instead of the anticipated positive shift. This was probably caused by the enrichment of dissolved inorganic carbon in the upper water column, caused by enhanced recycling of respired C02 due to the shallow chemocline.

The distribution of glycerol dibiphytany1 glycerol tetraethers (GDGT's), biomarkers of planktonic crenarchaeota showed in both, the homogeneous intervals and sapropels the

dominance of GDGT-O and crenarchaeol among the GDGT's indicating their origin from planktonic crenarchaeota as it was found in many other marine sediments (Chapter 5). In the homogeneous intervals highest GDGT abundances (normalized to TOC) were measured at the easternmost study Site 967 of the eastern Mediterranean Sea and decreased at the central and western Site 969 and 964. Within the three studied sapropels large variations in GDGT abundance was observed. The newly established sea surface temperature (SST) proxy, the TEX_{86} index, derived from GDGT's of planktonic crenarchaeota was used to estimate past SST. Comparison with previous obtained SST data using the alkenones from haptophyte algae revealed substantial differences. Whereas the $U^{K'}_{37}$-based SST showed almost constant values of ca. 25°C in both the homogeneous intervals and the sapropels, the TEX_{86}-based SST were 26-29°C in the homogeneous intervals and decreased up to l5-17°C in the sapropels. The TEX_{86}-based SST values outside the sapropel probably reflect summer SST based on a comparison with the present-day Mediterranean Sea. The surprisingly low TEX86-based SST estimates for the sapropels showed similarities with those obtained for the contemporary euxinic Black Sea. The distribution of marine crenarchaeota the modem Black Sea, i.e. thriving at the deeper and colder chemocline, suggests that this leads to a reduction in TEX86, resulting in artificially low SST estimates. A similar situation is inferred for the Pliocene eastern Mediterranean during sapropel deposition since the SST anomaly co-occurs with the build-up of a shallow chemocline.

The $\delta^{13}C$ values of higher plant wax $C_{27\text{-}33}$ n-alkanes were determined to study the composition of the vegetation on the continents surrounding the Mediterranean Sea (Chapter 6). A two-end member mixing model transformed the measured $\delta^{13}C$ values into the contribution of C4 plants to the terrestrial vegetation. These calculations indicated a high C4 plant contribution (i.e. 40-50%) in the periods just before and just after sapropel formation. During sapropel deposition the C4 plant contribution increased by up to 20% at all sites. This is interpreted to record the increased overall plant coverage of the Mediterranean borderlands resulting from the change of formerly barren desert areas into C4 grass-dominated savannah's as a response to the wetter climate during sapropel deposition. Enhanced accumulation rates (ARs) of long-chain n-alkanes ($C_{27\text{-}33}$) and n-alkan-l-ols ($C_{26\text{-}30}$) towards the middle of the sapropel in concert with a decrease in the TiJAI ratio confirm an increased delivery of terrigenous OM at all sites. These biomarkers were probably predominantly fluvially transported to the Mediterranean Sea, not only by the Nile but also by fossil wadi river systems on the northern African continent.

The OM of the three time-equivalent Pliocene sapropels was characterized as Type II kerogen being dominated by amorphous OM, originating mainly from marine organisms (Chapter 7). This was witnessed by the relatively high abundances of algaenan- and chlorophyll-derived pyrolysis products of the Curie-point pyrolysates of the kerogens of the sapropels. However, a small amount of terrigenous OM was also observed in the form of pollen and spores that most probably yielded the oxygenated aromatic pyrolysis products known to derive from sporopollenin. Lignin pyrolysis products, such as methoxyphenol and catechol (l,2-benzenediol), which are commonly used as unequivocal evidence for contribution of terrestrial higher plants in the form of woody material to the kerogen, were absent in the kerogen pyrolysates of the sapropels. No major spatial and temporal differences

in the kerogen composItIon of the three time-equivalent sapropels were observed. Palynological studies revealed that the homogeneous intervals, below and above the sapropels, consisted of pollen, spores and amorphous OM. This is confirmed by Curie-point pyrolysis - *GC/MS* results showing a relatively high abundance of algaenan-derived compounds and the presence of oxygenated aromatic pyrolysis products. The differences observed between the kerogen pyrolysates of the sapropels and their homogeneous intervals are suggested to be governed primarily by enhanced preservation conditions, resulting in increased preservation of labile marine OM in the sapropels.

This thesis demonstrates the high potential of using a wide range of biomarkers to reconstruct palaeo-climatic conditions at the time of deposition of Mediterranean sapropels. The data obtained are in general consistent with the idea that the increased run-off from the continent resulted in an increased primary production, which in its tum triggered the deposition of sapropels, but provide unprecedented detailed information with respect to the degree of anoxia and stratification and response of the marine and continental biological communities during this time of rapid climate change.

Samenvatting

De hedendaagse Middellandse Zee bevat relatief lage concentraties aan nutrienten en wordt daarom gekenmerkt door een lage primaire productiviteit. Haar sedimenten zijn grijs gekleurd, homogeen en bevatten een laag gehalte aan organisch koolstof (ca. 0.1%) omdat de relatief kleine hoeveelheden organisch materiaal (OM) die geproduceerd worden door het phytoplankton tijdens het transport naar de bodem en in het oppervlaktesediment zelfvrijwel volledig gemineraliseerd worden. In 1952 werd echter tijdens een Zweedse onderzoek-expeditie gevonden dat er in de gehele Middellandse Zee iets dieper in het sediment zwarte lagen voorkomen. Deze lagen zijn gelamineerd en bevatten veel meer organisch koolstof (tot weI 30%) dan de grijze sedimenten en zij werden sapropelen genoemd.

Sedimentologen hebben een relatie gevonden tussen het voorkomen van sapropelen en de astronomische veranderingen in de instraling van de zon op Aarde bekend als de zogenaamde "Milankowitch" cycli. Van het laat Miocene (5.5 Ma geleden) tot het Holoceen (de afgelopen 10 ka) vertoonde de precessiecyclus (met een periodiciteit van 21 ka) een hoge correlatie met het voorkomen van sapropelen. Minima in de precessiecyclus veroorzaakte een sterker seizonaal contrast in instraling op het noordelijk halfrond, hetgeen resulteerde in koudere winters en warmere zomers. Bovendien zorgde de passaatwinden voor een verhoogd transport van vocht van de Atlantische Oceaan naar equatoriaal Afrika, hetgeen tot een verschuiving naar het noorden van de Intertropical Convergence Zone (ITCZ) leidde. De grotere neerslag resulteerde in een sterk toegenomen transport van zoetwater rijk aan nutrienten naar de Middellandse Zee via de Nijl en andere rivieren. Hierdoor nam de primaire productie toe en dit resulteerde waarschijnlijk in een toename van de flux van OM naar de zeebodem. Hierdoor werd het bodemwater anoxisch, hetgeen uiteindelijk leidde tot een verbeterde preservatie van OM in het sediment en de afzetting van sapropelen. Daamaast induceerde de sterke toename van de instroom van zoet water waarschijnlijk een stratificatie van de waterkolom, leidend tot een afname in de circulatie van diep water en een verminderd transport van zuurstof naar het bodemwater. Sapropeelvorming is dus het gevolg van een samenspe1 van klimatologische, biologische en fysische factoren.

In dit proefschrift wordt de studie beschreven van een laat Pliocene sapropeel (2.943 Ma oud) op drie plaatsen in het oostelijk bekken van de Middellandse Zee die samen een oost-west transect vormen. In deze studie is een gedetailleerd inzicht verkregen in de klimaat- en afzettingscondities die leidde tot afzetting van deze sapropeel, die gekenmerkt wordt door extreem hoge organisch koolstofgehalten (tot 30%). De bestudeerde sedimenten liggen op een ongeveer 80 m diepte vanafhet sediment-water oppervlak en zijn verkregen via Leg 160 van het Ocean Drilling Program (ODP). De sapropeel en de grijs gekleurde, homogene sedimenten afgezet voor en na de periode van sapropeelafzetting zijn bestudeerd met behulp van zogenaamde chemische fossielen. Dit zijn organische componenten waarvan de structuur zo specifiek is dat ze aan bepaalde groepen van organismen of soms zelfs soorten gekoppeld kunnen worden. Zij worden gebruikt om palaeomilieu's te bestuderen en kunnen o.a. inzicht verschaffen in veranderingen in de zeewatertemperatuur, de samenstelling van het

fytoplankton, het voorkomen van anoxische condities in de fotische zone en het transport van plantenmateriaal van het continent naar de zee.

Isorenierateen is een carotenoide afkomstig van groene zwavelbacterien (Chlorobiaceae) die zowellicht als sulfide nodig heben. Deze component werd gebruikt om het voorkomen van de overlap van de fotische met de sulfidische zone vast te stellen tijdens sapropeelvorming in het Plioceen (Hoofdstuk 2). De hoeveelheid intact isorenierateen (waarbij de *all-trans* en *cis* isomeren gesommeerd werden) varieerde van niet detecteerbaar in de onder- en bovenkant van de sapropeel tot 140 sediment in het midden van de sapropeel. Accumulatiesnelheden van isorenierateen in de centrale en westelijke monsterlocatie zijn heel vergelijkbaar en nemen sterk toe tot waarden van 3.0 mg m^{o2} en dalen dan tot lage waarden. Deze profielen geven een expansie van de sulfidische zone tot in de fotische zone aan, gevolgd door het dieper worden van de chemocline tijdens afzetting van deze Pliocene sapropeel. De sapropeel van de meest oostelijke monsterlocatie (dichter bij de monding van de Nijl) werd gekenmerkt door veel lagere accumulatiesnelheden van isorenierateen en zelfs door een afwezigheid van isorenierateen in het middelste gedeelte van de sapropeel. Ba/Al ratio's geven een toename in de primaire productie tijdens de sapropeelvorming aan. Dit is in overeenstemming met een eerder gepostuleerd model waarbij de toegenomen primaire productie uiteindelijk leidt tot euxinische condities in de waterkolom.

Naast isorenierateen werd nog een ander, onbekend, carotenoide aangetroffen in de sapropelen (Hoofdstuk 3). De concentraties van dit carotenoide in de sapropeel vertoonde grote variatie zowel tussen de verschillende monsterlocaties als binnen de sapropeel. De hoogste concentraties werden gevonden in Site 967, dichtbij de monding van de Nijl, en veranderingen in de concentraties waren syncbroon met die van isorenierateen. Op basis van het massaspectrum en UVNIS absorptiespectrum werd dit pigment geidentificeerd als (i) spheroidenon, of (ii) thiotece-474. Het pigment is waarschijnlijk afkomstig van foto-synthetische purperen niet-zwavelbacterien (i.e. de familie Rhodospirillaceae). Omdat deze bacterien zich aan tal van condities kunnen aanpassen is de palaeoecologische interpretatie van het voorkomen van dit carotenoide onzeker. Meest waarschijnlijk is het echter dat dit geinterpreteerd moet worden als het tijdelijk voorkomen van een nog grotere overlap van de fotische en euxinische zone (i.e. nog groter dan al aangegeven door het pigment isorenierateen).

Chemische fossielen (alkenonen, loliolide/isololiolide, dinosterol and C30 1,15 - diol/keto-ol) representatief voor vier belangrijke groepen van fytoplankton (haptofyten, diatomeeen, dinoflaggelaten, eustigmatofyten) werden bestudeerd om de verschillen in export productie van deze groepen fytoplankton voor, tijdens en na de afzetting van sapropelen te bestuderen (Hoofdstuk 4). De gehalten aan chemische fossielen vertoonde aanzienlijke verschillen tussen de sapropelen en de grijze, homogene sedimenten. In deze laatste sedimenten werden relatief lage gehalten aangetroffen ten gevolge van de grote mineralisatie in het oxische poriewater. De accumulatiesnelheden van de chemische fossielen in de sapropelen vertoonde een aanzienlijke toename van de onderkant naar het midden van de sapropeel. Dit kan worden geinterpreteerd als een toename in de biologische productiviteit omdat het bodemwater tijdens afzetting van de sapropeel continue anoxisch geweest is en

condities voor preservatie van OM dus optimaal waren. De chemische fossielen afkomstig van diatomeeen (loliolide/isololiolide) werden relatief meer dominant in de sapropeel. Dit reflecteert vermoedelijk de toegenomen concentratie in nutrienten in het oostelijk bekken van de Middelandse Zee dat resulteerde in sapropeelvorming. Reconstructie van de oppervlakte zeewatertemperaturen m.b.v. alkenonen liet zien dat de verandering in fytoplankton-samenstelling niet bei'nvloed werd door veranderingen in de temperatuur van het zeewater maar in belangrijke mate bepaald werd door de nutrientconcentraties. Component-specifieke $\delta^{13}C$ metingen verschafte geen additionele ondersteuning voor de toegenomen primaire productie omdat zowel de C37:2 alkenoon als loliolide/isololiolide een kleine verandering naar lichtere waarden in plaats van de verwachte toename vertoonde in het midden van de sapropeel. Dit resultaat kan mogelijk verklaard worden door het ondieper worden van de chemocline, waardoor isotopisch licht CO_2 gevormd door afbraak van OM hergebruikt kan worden door het fytoplankton.

De glycerol dibifytanyl glycerol tetraethers (GDGT's), membraanlipiden van crenarchaeota, werden zowel in de homogene intervallen als in de sapropelen gedomineerd door GDGT-O en crenarchaeol (Hoofdstuk 5). Dit indiceerde een herkomst voor de GDGT's van planktonische crenarchaeota zoals voor vele marine sedimenten het geval is. De hoogste concentraties, genormeerd op het totaal organisch koolstof, werden voor de homogene intervallen gemeten in de sedimenten van de meest oostelijke Site 967; de gehalten voor de centrale en meest westelijke monsterlocatie (Site 969 en 964) waren lager. Grote variaties in de GDGT concentraties werden gemeten binnen de sapropelen. De nieuwe proxy voor het bepalen van vroegere oppervlakte zeewatertemperaturen, de TEX_{86} index, die gebaseerd is op variaties in de GDGT distributies, werd toegepast en deze schattingen werden vergeleken met die gebaseerd op de alkenoon proxy ($U^{K'}_{37}$) en vertoonde aanzienlijke verschillen. Terwijl de schatting voor de zeewatertemperatuur op basis van de $U^{K'}_{37}$ ongeveer 25°C bedroeg en vrijwel geen verandering tussen de homogene sedimenten en sapropeel lieten zien, gaven de TEX_{86} bepalingen een zeewatertemperatuur van 26-29°C voor de homogene intervallen en 15-17°C voor de sapropelen. De TEX_{86} schatting buiten de sapropeel geeft waarschijnlijk een schatting voor de zomertemperatuur op basis van een vergelijking met TEX_{86} waarden van oppervlakte sedimenten in de huidige Middellandse Zee. De verassend lage TEX_{86} schatting voor de periode tijdens afzetting van de sapropeel vertoond overeenkomsten met die bepaald voor de huidige, sterk gestratificeerde, euxinische Zwarte Zee. De ecologische niche van de planktonische crenarchaeota in de Zwarte Zee (i.e. de diepere en "koude" chemocline) suggereert dat dit leidt tot lagere TEX86 waarden en daarmee een onderschatting van de oppervlakte zeewatertemperaturen. Een vergelijkbare situatie doet zich waarschijnlijk voor in de Pliocene Middellandse Zee omdat de anomalie in de oppervlakte zeewatertemperatuur tegelijkertijd optreedt met de opbouw van een ondiepe chemocline.

De $\delta^{13}C$ waarden van n-alkanen afkomstig van plantenwassen werden bepaald om veranderingen in de vegetatie van het continent rondom de Middellandse Zee te reconstrueren (Hoofdstuk 6). Met behulp van een eenvoudig mengmodel werden m.b.v deze waarden de bijdrage van C_4 planten aan de vegetatie berekend. Deze berekeningen gaven een relatief hoog gehalte (i.e. 40-50%) aan C_4 planten in de perioden vlak voor en vlak na de tijd van sapropeelvorming aan. Tijdens de afzetting van sapropelen nam deze bijdrage nog eens met

ongeveer 20% toe voor alledrie onderzochte monsterlocaties. Dit werd waarschijnlijk veroorzaakt door de toegenomen plantenbegroeiing op het continent rondom de Middellandse Zee door transformatie van woestijngebieden in door C_4 grassen gedomineerde savannes ten gevolge van het vochtiger klimaat tijdens de periode van sapropeelvorming. De toename in de accumulatiesnelheden van lange n-alkanen (C_{27-33}) and n-alkan-l-olen (C_{26-30}) van de onderkant naar het midden van de sapropee1 samen met een afname in de *Til*Al ratio geeft een toename aan in het transport van terrigeen OM naar de Middellandse Zee tijdens de periode van sapropeelvorming. Deze plantenwassen werden waarschijnlijk voomamelijk fluviatiel getransporteerd, niet aIleen door de rivier de Nijl, maar ook door allerlei fossiele wadi systemen op het Noord-Afrikaanse continent.

Het OM van de drie tijdsequivalente sapropelen werd gekarakteriseerd als Type II kerogeen omdat het voomamelijk amorf OM is atkomstig van mariene organismen (Hoofdstuk 7). Deze vaststelling werd ondersteund door het feit dat de Curie-punt pyrolysaten van deze kerogenen gedomineerd werden door pyrolyseproducten atkomstig van algenaan en chlorofyl. Kleine hoeveelheden terrigeen OM werden ook aangetroffen door de pyrolyseproducten van pollen en sporen (geoxygeneerde aromatische producten). Pyrolyseproducten atkomstig van lignine (zoals methyxyfenolen en catechol), die ondubbelzinnig bewijs leveren voor de bijdrage van houtachtig terrestrisch materiaal, werden niet aangetroffen in pyrolysaten van de kerogenen in de sapropelen. De variatie in samenstelling van de pyrolysaten van de sapropelen was relatief gering. Palynologische karakterisering toonde aan dat het OM van de homogene sedimenten vlak onder en boven de sapropelen vooral uit pollen en sporen en amorf OM bestonden. Dit werd bevestigd middels analyses m.b.v. Curie-punt pyrolyse-GC/MS; de pyrolysaten bestonden voomamelijk uit geoxygeneerde aromaten en producten atkomstig van algenaan. De verschillen in de samenstelling van de pyrolysaten van de kerogeen fracties uit de sapropelen enerzijds en die van de homogene sedimenten anderzijds worden vooral veroorzaakt door de verbeterde preservatie condities tijdens afzetting van de saprope1en, die resulteren in een sterk verbeterde preservatie van labiel marien OM.

Het onderzoek beschreven in dit proefschrift laat de potentie van het gebruik van chemische fossielen voor de reconstructie van palaeoklimaat en -milieu tijdens de periode van sapropeelvorming zien. De verkregen gegevens zijn in algemene zin consistent met de hypothese dat de toename in run-off van het continent leidde tot een toename in de primaire productie en dat dit resulteerde in sapropeelvorming, maar geven zeer gedetailleerde informatie met betrekking tot de mate van anoxia en stratificatie en de respons van de mariene en continentale biologische levensgemeenschappen tijdens deze periode van snelle klimaatverandering.

Zusammenfassung

Das heutige Mittelmeer ist ein marines, niihrstoffarmes Becken und demzufolge durch geringe Primiirproduktion gekennzeichnet. Das Sediment ist griiulich, homogen und kalkreich. Es besitzt einen geringen organischen Kohlenstoffgehalt von ca. 0.1 %, da der vorwiegend von Algen produzierte, geringe Anteil organischen Materials (OM) auf dem Weg durch die Wassersiiule und im Sediment durch Bakterien abgebaut wird. Eine schwedische Expedition entdeckte jedoch 1952 im gesamten Mittelmeer schwarze Schichten (von einigen Zentimetem Miichtigkeit), die in das griiuliche Sediment eingebettet sind. Diese schwarzen Schichten treten periodisch auf, sind geschichtet and besitzen einen hohen organischen Kohenstoffgehalt von bis zu 30% gew. und werden 'Sapropele' genannt.

Sedimentologen fanden einen Zusammenhang zwischen der Anwesenheit von Sapropelen und den astronomischen Variationen in der Sonneneinstrahlung zur Erde, bekannt als 'Milankowitch Zyklen'. Besonders der 'Precession' Zyklus (21000 Jahre) zeigte eine positive Korrelation mit dem Vorkommen von Sapropelen im Mittelmeer vom späten Miocene (vor 5.5 Millionen Jahren) bis zum Holocene (die letzten 10000 Jahre). Das 'Precession' Minimum verursacht dabei, durch die veranderte Sonneneinstrahlung auf der n6rdlichen Halbkugel, einen starkeren jahreszeitlichen Kontrast, was letztendlich zu kiilteren Wintem und warmeren Sommem Zusiitzlich sorgen die Passatwinde für einen erhOhten Transport von Feuchtigkeit vom Atlantik zum nordafrikanischen Kontinent, was zu einer n6rdlichen Verschiebung der Intertropischen Konvergenzzone (ITCZ) führt. Der daraus resultierende erheblich erh6hte Regenfall verursachte eine erh6hte Zufuhr von und Niihrstoffen vom Nil und anderen Fliissen ins Mittelmeer. Foiglich erh6hte sich die Primarproduktion, was wiederum zu erh6hten Akkumulationraten des OM am Meeresboden geführt haben k6nnte, woraufhin anoxisches Bodenwasser und damit Sapropelbildung induziert wurden. AuBerdem sorgte die Zufuhr von SiiBwasser wahrscheinlich zur Reduzierung der Tiefenwasserzirkulation m6glicherweise sogar zur Stagnation und somit Stratifizierung der Wassersiiule, wodurch die Beliiftung des Bodenwassers mit Sauerstoff verhindert wurde. Sapropelbildung ist deshalb das Ergebnis eines Zusammenspiels von klimatischen, biologischen und physikalischen Faktoren.

In dieser Doktorarbeit werden drei Pliocene Sapropele (2.943 Millionen Jahre alt) von drei verschiedenen Stationen im 6stlichen Mittelmeerbecken untersucht, die zusammen ein Ost-West-Transekt bilden. Diese Studie liefert detaillierte Einsichten iiber die friiheren Klima- und Umweltbedingungen, die schlieJ3lich zur Ablagerung der Sapropele haben, die einen extrem hohen organischen Kohlenstoffgehalt besitzen. Das untersuchte Sediment stammt aus einer Tiefe von ca. 80 m unter dem Meeresboden und wurde des Ocean Drilling Program Leg 160 entnommen. Die Sapropele, sowie die griiulich, homogenen Sedimente (homogene Intervalle) wurden mit Hilfe von so genannten Lipid-Biomarkem oder chemischen Fossilien untersucht. Diese Lipid-Biomarker sind organische Verbindungen, deren Struktur so spezifisch ist, dass sie an eine Klasse von Organismen oder sogar eine spezifische Art gekoppelt werden kann. Diese Biomarker k6nnen genutzt werden, um friihere Umweltbedingungen zu rekonstruieren, wie z.B. den Transport von terrestrischem

Pflanzenmaterial ıı ı das Meer, A.nderungen der Oberflachenwassertemperatur, die Algenzusammensetzung, die Anwesenheit von anoxischen Bedingungen in der photischen Zone.

Isorenieratene ist ein Carotenoid, dass in griinen Schwefelbakterien der Klasse Chlorobiaceae vorkommt. Chlorobiaceae benotigen für ein optimales Wachstum geringe Lichtintensitaten und Sulfide. Die Anwesenheit von Isorenieratenen im Sediment weist deshalb auf das Uberlappen der photischen mit der sulphidischen Zone in der Wassersaule hin. Dieser Biomarker wurde genutzt, urn die Perioden der anoxischen/euxinischen Bedingungen in der photischen Zone wahrend der Sapropelbildung zu untersuchen (Kapitel 2). Die Summe der intakten Isorenieratene (summierte *all-trans* und cis-isomers) variierte von nicht ermittelbaren Konzentrationen unterhalb und oberhalb des Sapropels bis zu 140 µg/g Sediment im Zentrum des Sapropels. lsorenieratene Akkumulationsraten an der zentralen und westlichen Station waren bemerkenswert ahnlich und zeigten einen deutlichen Anstieg von der Basis des Sapropels bis zu 3.0 mg m^{-2} yr^{-1} im Zentrum des Sapropels und nahmen dann wieder abo Dieses Muster kennzeichnet die Ausdehnung der euxinischen Bedingungen in die photische Zone. Konzentrationsanderungen im Sapropelprofil spiegeln eine Senkung oder Erhohung der Chemoklinen wahrend der Ablagerung der Pliocene Sapropele wider. Das Sapropel an der ostlichsten Station im Mittelmeerbecken, charakterisiert durch einen geringeren organischen Kohlenstoffgehalt, zeigte im Vergleich zu den anderen zwei Stationen viel geringere Isorenieratene Akkumulationsraten und sogar die Abwesenheit von Isorenieratenen in der Mitte des Sapropels. Ba!Al sind ein Indikator für erhohte Palaoproduktivitat wahrend der Sapropelbildung, wodurch ein bereits früher vorgestelltes Modell unterstUtzt wird, indem die erhohte Primarproduktivitat als der entscheidene Faktor für das Auftreten der euxinischen Bedingungen beschrieben wird.

Neben Isorenieratenen wurde ein neues, unbekanntes Carotenoid entdeckt, das Unterschiede zwischen dem relativen Auftreten der Sapropele der drei Stationen und im individuellen Sapropel selbst anzeigte (Kapitel 3). Die relativen Vorkommen des unbekannten Carotenoids wurden an der Station 967, am dichtesten am Nil liegend, gemessen. Diese Resultate sind synchron mit den Ergebnissen die auch für Isorenieratene gefunden wurden. Basierend auf das Molekulargewicht, das Massenspektrum und sein UVNIS Absorption Maximum, spiegelt dieses unbekannte Pigment entweder (l) Spheridenone, oder (2) Thiotece-474 wider. Dieses Pigment wird moglicherweise von photosynthetisierenden purpur schwefelfreien Bakterien biosynthetisiert, z.B. von der Familie der Rhodospirillaceae. Aufgrund der breiten physiologischen Adaptation dieser Rhodospirillaceae sind die palao-okologischen Auswirkungen dieser Bakterien unsicher. Jedoch konnte man postulieren, dass die Anwesenheit dieses Pigmentes das kurzzeitliche Vorkommen von sulfidischen Bedingungen noch in der photischen Zone (demzufolge als bei der Anwesenheit des Isorenieratenes der Chlorobiaceae) anzeigt.

Biomarker (Alkenone, Loliolide/Isololiolide, Dinosterol und C_{30} 1,15 -Diol/Keto-ol), kennzeichnend für die vier Hauptklassen der marinen Primarproduzenten (Haptophyten, Diatomeen, Dinoflagellaten, Eustigmatophyten) wurden auf ihre Veranderungen bei der Exportproduktion bevor, und nach der Sapropelbildung untersucht (Kapitel 4). Der Biomarkergehalt zeigte betrachtliche Unterschiede zwischen den nicht-sapropelischen und

sapropelischen Sedimenten. In den nicht-sapropelischen Sedimenten konnten nur kleine Biomarkerkonzentrationen gemessen werden, was den oxidativen Abbau in oxischem Porenwasser während der Ablagerung widerspiegelt. In den Sapropelen, zeigten die Biomarker Akkumulationsraten (AR) einen betriichtlichen Anstieg vom der Basis des Sapropels zur Mitte resultierend aus der biologischen Produktivitiit in der Zeit, in der das Bodenwasser anoxisch und die Erhaltungsbedingungen optimal waren. Der Diatomeenbiomarker, LoliolidefIsololiode zeigte in den Sapropelen eine relativ deutliche Dominanz, gegeniiber den anderen drei Phytoplanktonklassen, was auf eine Anreicherung von Niihrstoffen im ostlichen Mittelmeer hinweisen konnte. Diese verursachte eine Exportproduktion der Hauptphytoplanktonklassen was letztendlich zur Ablagerung der Sapropele führte. Die Rekonstruktion der Oberfliichenwassertemperatur, die mit Hilfe der Alkenone bestimmt wurde, zeigte dass die Veriinderungen in der Phytoplanktonzusammensetzung nicht durch die Veriinderungen der Oberfliichentemperatur, sondem durch die Niihrstoffe beeinflusst wurde. $\delta^{13}C$ Messungen an den spezifischen Biomarkem zeigten jedoch keinen zusiitzlichen Hinweis auf erhohte Primiirproduktion. 1m Gegenteil, die $\delta^{13}C$ Werte der $C_{37:2}$ Alkenones und Lolioloide/Isololiolide zeigten eine geringe negative, statt der erwarteten positiven Verschiebung ihrer $\delta^{13}C$ Werte. Diese geringe Verschiebung zu niedrigen $\delta^{13}C$ Werten ist wahrscheinlich durch die Verschiebung der Chemoklinen zur photischen Zone, wodurch das geformte isotopisch leichtere CO_2 durch den Abbau von OM sofort wieder durch das Phytoplankton assimiliert wird, zu erkliiren.

Die Glycerol Dibiphytanyl Glycerol Tetraethers (GDGTs), Membranlipide der Crenarchaeota zeigte sowohl in den homogenen Intervallen als auch in den Sapropelen die Dominanz von GDGT-O und Crenarchaeol unter den GDGTs. Dieses Resultat ist ein Indikator für die Anwesenheit von planktischen Crenarchaeota in den Sapropelen, wie es auch in vielen anderen marinen Sedimenten gefunden wurde (Kapitel 5). In den homogenen Intervallen, an der ostlichen Station 967 des ostlichen Mittelmeeres wurden, vergleichbar mit der westlichen und zentralen Station, die hochsten GDGT Konzentrationen (normalisiert auf TOe) gefunden. In den drei untersuchten Sapropelen konnten groBe Veriinderungen in den GDGT Vorkommen beobachtet werden. Das neu entwickelte Proxy zur Rekonstruktion der friiheren Oberfliichenwassertemperatur (SST), der TEX_{S6} index ist abgeleitet von den Variationen der GDGT Verteilung. Vergleiche der TEX86 Ergebnisse mit dem $U^{K'}_{37}$, der von den Alkenonen der Haptophyten abgeleitet wird, zeigte groBe Unterschiede. der $U^{K'}_{37}$ unveriinderte Werte von ca. 25°C, sowohl in den homogenen Intervallen, als auch in den Sapropelen anzeigte, ergaben die TEXS6 Werte in den homogenen Intervallen hohe Temperaturen von 26-29°C und in den Sapropelen niedrige Temperaturen von 15-17°C. Die TEX_{S6} Werte in den homogenen Intervallen spiegeln höchst wahrscheinlich die Sommer SST, basierend auf den gemessenen TEX_{S6} Werten des heutigen Mittelmeeres, wider. Die iiberraschend niedrigen TEX86 Werte in den Sapropelen zeigten im Gegenteil vergleichbare TEX_{86} Werte, die im heutigen euxinischen, stark stratifizierten Schwarzen Meer gefunden wurden. Die okologische Nische der marinen Crenarchaeota im heutigen Schwarzen Meer (d.h. in der tieferen und "kiilteren" Chemoklinen) konnte die starke Abnahme der TEXS6 Werte erkliiren. Diese Situation wäre vergleichbar für das Mittelmeer während der

Ablagerung des untersuchten Pliocene Sapropel, aufgrund der Anwesenheit einer flachen Chemoklinen, die wahrend der Zeit der Sapropelbildung anwesend war.

Die $\delta^{13}C$ Werte der C_{27-33} n-alkane, Bestandteil der pflanzenwachse wurden auf die Zusammensetzung der Vegetation auf den Kontinenten rund urn das Mittelmeergebiet untersucht (Kapitel 6). Mit Hilfe eines einfachen Mischungsmodells wurden diese gemessenen C_{27-33} Werte in den Anteil der C4 Pflanzen an der Vegetation berechnet. Diese Berechnung zeigten einen hohen Anteil von 40-50% der C4 Pflanzen bevor und nach der Sapropelbildung. Wahrend der Sapropelbildung stieg sogar der C_4 Pflanzenanteil an allen drei Stationen auf bis zu 20% an. Dieses Ergebnis wäre zu interpretieren durch die ansteigende Verbreitung der Vegetation. Das dominierende feuchte Klima verursachte die Transformation des Wiistengebietes zu einem Savannengebiet, dass von C4 Grasem dominierend wurde. Die Akkumulationsraten der langkettigen n-alkanen (C_{27-33}) und n-alkan-I-ols (C_{26-30}) im Zentrum des Sapropels harmonisiert mit der Abnahme der Ti/AI Verhaltnisse und bestatigt hiermit die Zunahme der terrigenen Zufuhr an allen Stationen. Diese Pflanzenwachse wurden wahrscheinlich hauptsachlich fluviatil in das Mittelmeer transportiert, aber nicht allein durch den Nil, sondem auch durch die fossilen Wadi-FluB Systeme, gelegen im nordlichen Teil des afrikanischen Kontinentes.

Das OM der drei zeitgleich abgesetzten Pliocene Sapropele wurde als Kerogentyp II charakterisiert, aufgrund der Dominanz des amorphen OM, welches hauptsachlich von marinen Organismen abstammt (Kapitel 7). Diese Feststellung wurde durch die relativ hohen Vorkommen der Algaenan - und Chlorophyllabstammenden Pyrolyseprodukte des Kerogens der Sapropele Jedoch konnte auch eine kleine Menge des terrigenen OM, in Form von Pollen und Sporen mikroskopisch beobachtet werden. Diese palnologischen Ergebnisse erklaren hochst wahrscheinlich auch die oxidierten aromatischen Pyrolyseprodukte, die vom Sporopollenin abgeleitet werden konnen. Lignin Pyrolyseprodukte, wie Methoxyphenol und Catechol (l,2-benzenediol), die ein eindeutiger Beweis des Eintrages hoherer Pflanzen sind, konnten im Kerogenpyrolysat der Sapropele nicht gefunden werden. Variationen in der Kerogenzusammensetzung zwischen den drei zeitgleich abgesetzten Sapropelen waren gering. Palynologische Untersuchungen zeigten in den homogenen Intervallen, also bevor und nach der Sapropelbildung, die Anwesenheit von Pollen, Sporen und auch amorphem OM. Diese Ergebnisse wurden auch im Kerogenpyrolysat wiedergefunden in Form der Algaenan und oxydierten aromatischen Pyrolyseprodukte. Die Unterschiede im Kerogenpyrolysat zwischen den Sapropelen und den homogenen Intervallen ist wahrscheinlich auf die vorherrschenden verbesserten Erhaltungsbedingungen und damit die Erhaltung von labilem marinen OM in den Sapropelen

Diese Doktorarbeit demonstriert das hohe Potential der Nutzung einer Vielzahl von Biomarkem in der Rekonstruktion Klima- und Umweltbedingungen, die zur Bildung der Sapropele im Mittelmeer haben. Diese Ergebnisse stimmen im Allgemeinen mit der Hypothese, dass der SiiBwasserzufluss, hauptsachlich des Nils, zur Verstarkung der Primarproduktivitat hat, was ausschlaggebend für die Ablagerung der Sapropele war. Zusatzlich liefert diese Arbeit beispiellose, detaillierte lnformationen das Ausmaf3 von Euxinia und Wassererstratifikation sowie der Anwesenheit mariner und kontinentaler biologischer Gemeinschaften wahrend der Zeit eines rapiden Klimawechsels.

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Curriculum Vitae

Diana Menzel werd op 2 april 1968 in Woldegk geboren. Van 1974 tot 1984 bezocht ze de Polytechnischen Oberschule "Arthur Becker" in Strasburg. Van 1984 tot 1987 volgde ze een pedagogische opleiding aan de Medische Fachschule in Prenzlau. Van 1987 tot 1991 werkte ze in de kinderopvang in Strasburg. In oktober 1991 begon ze met de studie biologie aan de Rostock, waar zij in september 1994 het Vordiplom behaalde. Vervolgens legde ze zich primair toe op de mariene biologie, met stages bij het PML (Plymouth, Engeland) en de Universiteit van Amsterdam (vakgroep microbiële ecologie), en Diplomarbeit bij het lOW (Institut für Ostseeforschung, W De studie biologie werd in april 1999 afgesloten. Per september 1999 werd zij door het IPPU bij de Universiteit Utrecht aangesteld als Assistant in Opleiding. **In** deze functie verrichtte ze organisch-geochemisch onderzoek aan sedimenten uit de Middellandse Zee. Na beeindiging van het dienstverband in februari 2004, werd het onderzoek afgerond, resulterend in deze dissertatie.