

Biomarker patterns in a time-resolved holocene/terminal Pleistocene sedimentary sequence from the Guadiana river estuarine area (SW Portugal/Spain border)

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Abstract

The vertical distribution of terrestrial and phytoplankton biomarker compounds in a sediment core from the coastal estuarine zone of the Guadiana river (southwest border between Portugal and Spain) was determined by gas chromatography–mass spectrometry. Significant downcore fluctuations were observed in the patterns of the most abundant alkyl series (*n*-alkanes and *n*-fatty acids), as well as in several biomarker ratios. In addition, a specific contribution from conifers is reflected in the presence of a resin acid series that first appears 6500 years BP. The observed changes in the lipid assemblage within the Holocene suggest recent variations in the planktonic and terrigenous supply, which are attributed mainly to the evolution of the circulation pattern in the estuary and to the alteration of vegetation cover within the Guadiana drainage basin. Our data suggest that on a 10^3 – 10^4 year time-scale, little or no diagenetic change occurred with respect to the compounds in question.

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1. Introduction

Understanding the biogeochemical processes and environmental conditions that lead to the preservation of organic matter (OM) in marine sediments has been the subject of a wealth of studies over the last decades (Hedges et al., 1999, and references therein). Many of these studies have been carried out on sediments deposited along the continental margins, where more than 90% of all organic carbon burial occurs (Berner, 1989).

Estuarine zones are good environments for studying the origins, pathways and fates of sedimentary organic

material (Hedges and Keil, 1999) due to the rapid accumulation of fine sediments and consequent sealing of these materials from bacterial remineralization. In such semi-enclosed environments the period of climatic warming and sea level rise that occurred since the last glacial maximum may be reflected in the characteristics of organic carbon supplied to and buried in the sediments.

Marces, among which detritus from photoautotrophic biota, components of vascular plants and anthropogenic inputs are the most important, contribute to the chemical composition of coastal estuarine sedimentary OM. The study of the bulk properties of this sedimentary organic material, and in particular the study of its lipid fraction at a molecular level, can contribute in significant ways to a better delineation of the multistage geochemical processes that occur in

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estuarine sediments (Henrichs, 1992; Hedges and Keil, 1999). Biomarker assemblages found in the lipid fraction of sedimentary organic matter are specific and sensitive to the tracing of biogenic sources (Killops and Killops, 1993; Ishiwatari et al., 1994; Yunker et al., 1995; Goñi et al., 1998; van Dongen et al., 2000; Ternois et al., 2001), thereby providing valuable information on the structure of the trophic system involved in the formation of the organic fraction of estuarine sediments (Laureillard and Saliot, 1993; Harvey and Johnston, 1995; Canuel et al., 1997). The biomarker approach has also been widely used to interpret changes that occurred within terrestrial ecosystems and to explore the possibility of short-term environmental changes and geochemical processes (Volkman et al., 1980; Kawamura and Ishiwatari, 1981; ten Haven et al., 1987a; Kohnen et al., 1991; Ohkouchi et al., 1997; Meyers, 1997). Consequently, molecular geochemistry has become an independent climatic proxy for low-resolution climate reconstruction, offering information complementary to the classical climatic proxies, such as the study of pollen, macroscopic remains and organism assemblages, isotopic analyses, etc. (Ficken et al., 1998; Filley et al., 2001; Schwark et al., 2002).

A multidisciplinary study of the diagenetic processes based on these premises was conducted in the area of the Guadiana River Estuary and its submerged delta, where a sedimentary sequence of up to 50 m (Boski et al., 2002) has accumulated in the period from the Late Pleistocene Weichselian Pleniglacial to the present Holocene Interglacial. Based on a dataset of sedimentological records, macro and micro paleontological data and ^{14}C dating of carbonate and OM recovered from sediment samples gathered from four continuously cored boreholes that were drilled in the area of the Guadiana estuary (Boski et al., 2002), the biomarkers can be related to the timescale at approximately centennial resolution. The main goals of this research were: (i) to determine the origin of OM deposited in the area during the last climatic cycle (over the last 13–14 kyears BP), (ii) to investigate whether there are significant changes in the lipid assemblage during this period, and (iii) to assess whether it is possible to relate documented climate and environmental fluctuations that occurred in the S. Iberian ecosystems during the Holocene (Dias et al., 2000; Zazo et al., 1992, 1999; Yll et al., 2002) with the molecular proxies provided by the biomarker approach.

2. Study area and geological setting

Guadiana is a major river on the Iberian Peninsula; its total length is 730 km, the last 200 of which forms a natural border between Portugal and Spain. In this section the river bed is cut into Upper Carboniferous

schists and greywackes and follows a N–S path defined during the Quaternary (Morales, 1997). The resistant character and faulting of the basement rocks contributed to the narrow and deep pattern of the river valley incision during marine lowstands. Boreholes and seismic profiles indicate that the palaeo-valley is 600 m wide and 70 m deep below mean sea level, ca 7 km inland from the mouth (Boski et al., 2002). These characteristics distinguish the Guadiana estuary from the other estuaries in the Guadalquivir basin, which are cut into soft non consolidated Plio-Pleistocene sediments in the form of broad and shallow structures (Borrego et al., 1993). Therefore, in contrast to other estuaries in the region, the Guadiana offers insights into the sedimentary record on a high resolution timescale. Several cored boreholes that reached the pre-Holocene sub-stratum were drilled in recent years in order to identify the architecture of sedimentary facies and to quantify the organic carbon content trapped in sediments accumulating during the drowning of the Guadiana fluvial valley by marine waters (Boski et al., 2002).

The samples were obtained from a borehole (CM5) drilled to ca. 53 m near the confluence of the Beliche and Guadiana rivers and located in the intertidal zone of the latter (Fig. 1). The stratigraphic column from borehole CM5 and the inferred depositional environment are shown in Fig. 2. With the exception of the seven lowermost meters, which are mostly sandy and accumulated during a mixed fluvial/intertidal regime, the sedimentary column is fine-grained. The predominating fine, silty/argillaceous granulometry of the sediments indicates a low energy intertidal sedimentation regime that prevailed throughout most of the Holocene infilling of this semi-enclosed valley adjacent to the principal estuarine channel.

Table 1 shows the specific horizons that were ^{14}C -dated for borehole CM5 and the materials used. Assuming constant accumulation rates for the intervals between dated horizons and over the remainder of the core, the ^{14}C dates indicate that the sedimentary sequence accumulated over a period of ca 13–14 kyears, representing one of the longest postglacial sedimentary records in the non glaciated areas.

As shown by the changes in organic carbon (OC) accumulation rates (Fig. 3), about 80% of the present Holocene sedimentary sequence accumulated during the fast sea level rise, which terminated ca. 6000 years BP and was followed by a much slower rate of vertical accretion.

Thirty-two samples taken every 1–3 m were collected from the centre of the sectioned sediment cores in order to avoid contamination. Each sampled interval was 2–3 cm thick. Samples were stored at $-25\text{ }^{\circ}\text{C}$ to prevent microbial growth and were freeze-dried before analysis.

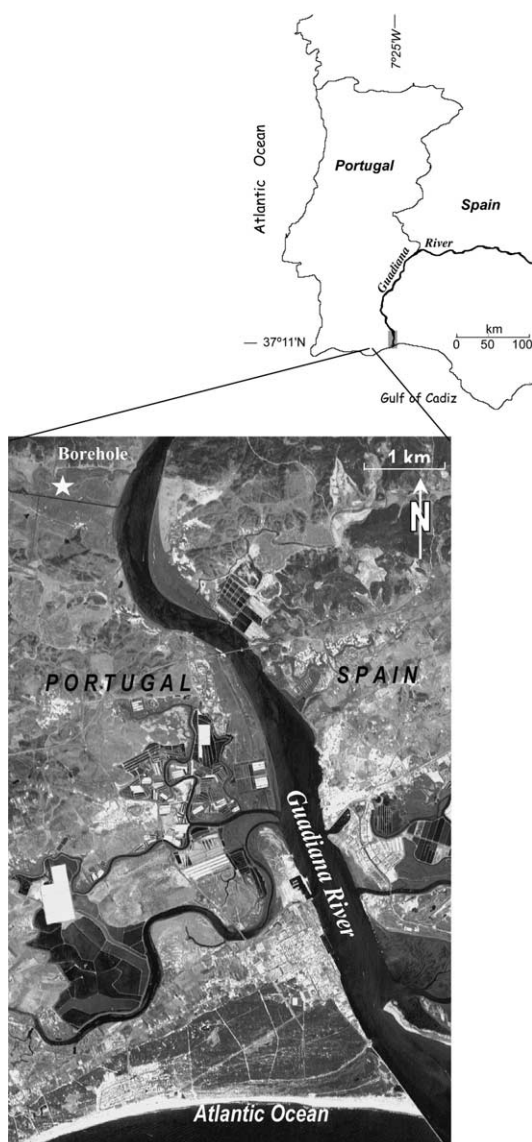


Fig. 1. Satellite panchromatic image of the Guadiana Estuary (supplied by the Indian Remote Sensing Service, IRS). CM5 is the site of Beliche borehole (Geographical coordinates: latitude $37^{\circ}16' N$, longitude $7^{\circ}26'50'' W$).

3. Experimental

3.1. Bulk analysis

Sediment samples were finely ground and homogenised in an agate mill. In order to determine total organic carbon (TOC), nitrogen (TN) and sulfur (TS), two aliquots of about 20 mg were packed into silver cups. One was submitted to decarbonation by using 25% HCl prior to analysis in an EA1108 Carlo Erba C/H/N/S elemental analyser and another was analysed

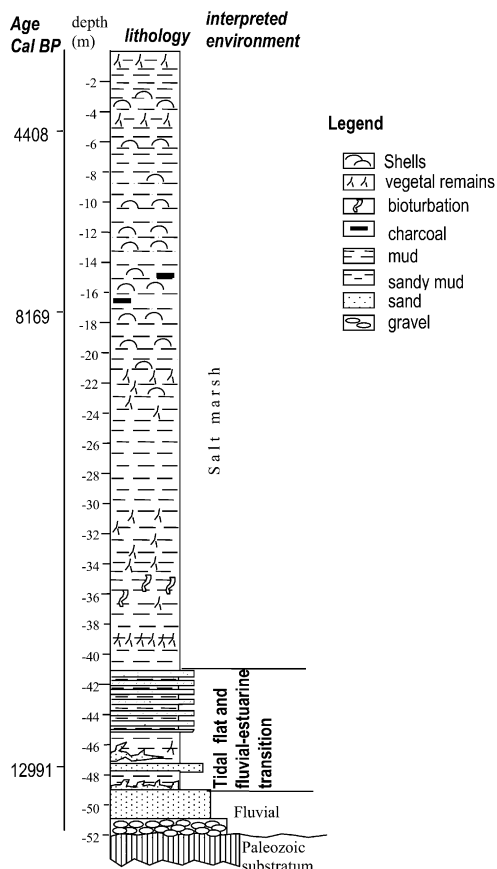


Fig. 2. Stratigraphic column from borehole CM5 and inferred depositional environments. Depth referred to the borehole mouth elevated at 1 m above MSL.

Table 1
Information from ^{14}C datings

Depth (cm)	^{14}C age (4 B.P.)	Cal. age (4 B.P.)	Material	Method ^a	Lab. code ^b
579	4295 ± 35	4408	<i>Venerupis</i> sp. shell	AMS	KIA 15211
1775	7725 ± 45	8169	<i>Cardium</i> sp. shell	AMS	KIA15210
4767	10 990 ± 40	12 991	wood	AMS	Beta-137110

^a Accelerator mass spectrometry technique.

^b KIA—Institut Royal du Patrimoine Artistique, Brussels, Belgium; Beta—Beta Analytic Radiocarbon Dating Laboratory, Miami, FL, USA.

directly without any pre-treatment. Both silver cups were pinched closed before instrumental analysis. TOC content was obtained from the instrumental reading for a decarbonated sample and total inorganic carbon (TIC) was calculated from the difference between the total carbon reading for an untreated sample and TOC

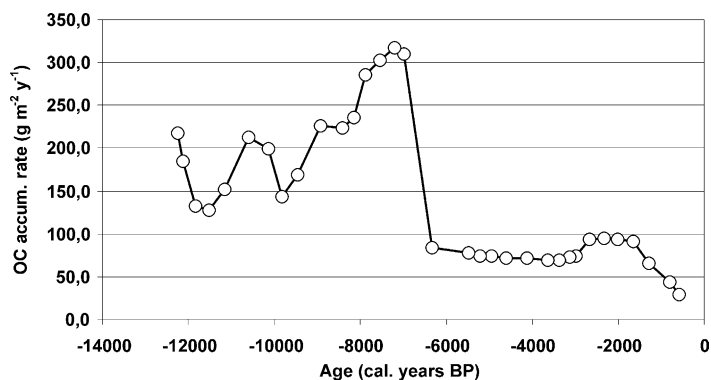


Fig. 3. Changes in organic carbon (OC) and OC accumulation rate during sediment formation.

(Nieuwenhuize et al., 1994). The instrumental readings were checked against aliquots of the sulfanilamide standard analysed every tenth sample.

3.2. Lipid analysis

Aliquots (10 g) of sediment were Soxhlet-extracted with a dichloromethane–methanol (2:1) mixture for 48 h. Total extracts were saponified with 0.5 mol/l KOH/methanol for 2 h under reflux. In order to remove elemental sulfur, activated (2 M HCl) copper curls were added. Neutral lipids were isolated by extraction with hexane and acidic lipids were isolated with dichloromethane after acidification to pH < 1. Neutral lipid classes were further separated into sub-fractions by column chromatography (silica gel: alumina 1:1, deactivated with 1% water). Sub-fractions were successively eluted with hexane, toluene, toluene:methanol (3:1) and methanol, respectively. The acidic lipids and polar fractions were methylated with trimethylsilyldiazomethane and silylated with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) before analysis by gas chromatography (GC; Hewlett - Packard 5730A) and gas chromatography–mass spectrometry (GC–MS; Hewlett-Packard GCD).

Separation of acidic and neutral compounds was achieved using a SE-52 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). The column oven temperature was programmed to increase from 40 to 100 °C at 30 °C min⁻¹ and then to 300 °C at 6 °C min⁻¹. Helium was used as carrier gas at a flow rate of 1.5 ml min⁻¹. Mass spectra were measured at 70 eV ionizing energy. Individual compounds were identified by inspecting mass fragments, by key single ion monitoring (SIM) for different homologous lipid series, by low resolution mass spectrometry and by comparison with published mass spectra libraries (NIST and Wiley libraries).

Total lipid contents were determined by gravimetry and expressed relative to TOC. Due to the limited

amount of sediment samples available, most biomarkers series occurred in very low concentration in the lipid sub-fractions and hence absolute concentrations were subject to considerable inaccuracy. Therefore, compound abundance was assessed using the SIM traces only on a semiquantitative basis, assuming that a constant relationship exists between the reconstructed ion current response and the amount of a component in the corresponding sub-fraction.

4. Results and discussion

4.1. Bulk geochemical parameters

Downcore values for sediment TIC, TOC, TN, TS and lipid content as well as the atomic ratios C/N and C/S are plotted in Fig. 4. TOC content varies through the core from 0.7 to 2.3%, which is within a range typical for coastal sediments (Nieuwenhuize et al., 1994). The core section comprising the upper 10 m gives TOC values (mean 1.4 ± 0.3%) very similar to those in the lower section (mean 1.2 ± 0.4%).

The palaeoenvironmental significance of the C/N ratio, and its usefulness as an OM identifier have been emphasised by Meyers (1994). C/N ratios have been used to distinguish between OM inputs in estuaries, since autochthonous marine organisms rich in protein material have C/N values (4–10) much lower than terrestrial plants (20 and greater, Kawamura and Ishiwatari, 1981; Meyers et al., 1984; Uzaki and Ishiwatari, 1986; Meyers, 1994), although the presence of clay-bound inorganic N in sediments (Muller, 1977) should be also taken into consideration. The downcore C/N ratio values in the borehole (Fig. 4) range between 8 and 16, indicating an input of different mixtures of land and aquatic OM. These values are similar to those found in marine sapropel, where OM is composed of a mixture of vascular plant debris and algal plus bacterial remains (Meyers, 1994). The variability in the C/N ratio

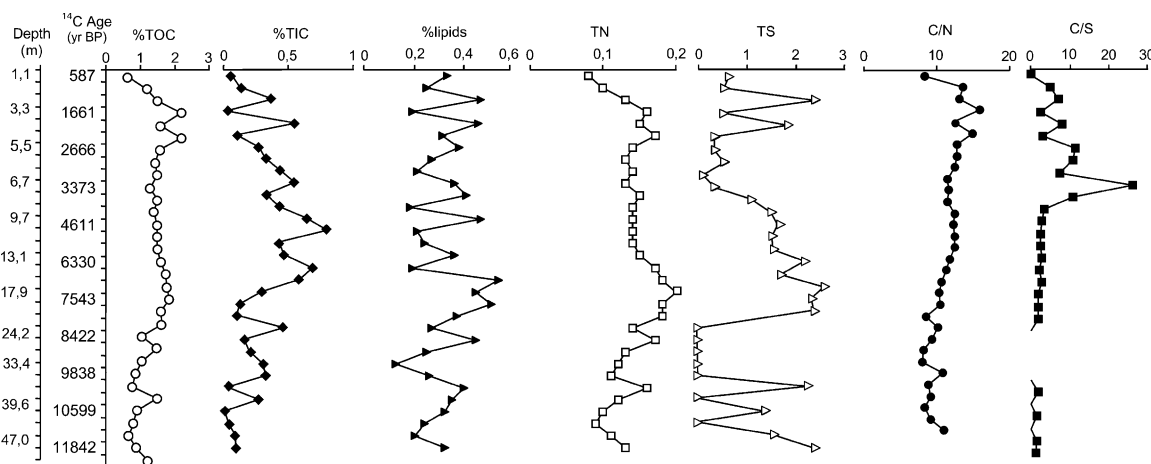


Fig. 4. Downcore plots of core bulk characteristics. TOC: total organic carbon; TIC: total inorganic carbon; lip: total lipid extract relative to TOC; TN: total nitrogen; TS: total sulfur; C/N: atomic carbon/nitrogen ratio; C/S: atomic carbon/sulfur ratio.

throughout the core seems also to indicate estuarine mixing of marine and terrestrially derived OM inputs, with seasonal variations being a consequence of a balance between fluvial and tidal influence. The slight downcore decreases in the C/N values may suggest preferential utilization of carbon and bacterial immobilization of nitrogen, as reported for lacustrine sediments (Meyers and Ishiwatari, 1993).

A similar trend is observed for total nitrogen (TN, Fig. 4), which is considered indicative of the contribution from aquatic flora. Due to some high peak values, the total sulfur content (TS, Fig. 4) seems to be more abundant in the lower 30 m section (mean $2.0 \pm 0.4\%$) than in the upper 10 m (mean $0.97 \pm 0.7\%$). In the latter, microscopic observation revealed a frequent presence of sub-millimetre crystals of gypsum, which form through the process of evaporitic precipitation. In the lower horizon, the gypsum crystals are absent but occasional microcrystals of pyrite were observed instead. This trend indicates a more efficient trapping of volatile sulfur produced during diagenesis. The presence of molecular sulfur in the extracts throughout the core (its removal by Cu before chromatographic analysis was necessary), and the detection of S_8 in direct pyrolyzates of the whole sediment (data not shown), indicate that sulfate reduction and sulfur incorporation into the early diagenetic products were important processes throughout the Holocene cycle of sedimentation.

The decrease in the atomic C/S ratio in the lower sections (> 10 m; Fig. 4) may be considered as an indication of a strengthening of sulfate reduction, probably related to a transfer of larger quantities of reactive organic matter to the anoxic zone (Leventhal, 1983; Hadas et al., 2001).

4.2. Biomarker distributions

The samples displayed a lipid content accounting for between 0.2 and 0.5% of the TOC (Fig. 4). Scrutiny of Fig. 4 also indicates that in several segments of the profile, the lipid content co-varies with the content of carbonate, which accumulated mostly in the form of foraminifera tests, as determined by regression analysis. Thus, significant positive correlations were observed from 0 to 9.7 m, $R^2 = 0.589$, and from 9.9 to 42.9 m, $R^2 = 0.425$ (data not shown). Acidification in the lowest part of the core probably led to poor conservation of carbonate, i.e., absence of macrofauna (Boski, unpublished data). The oscillations in the content of both components may indicate a primary productivity variability in the estuary.

Table 2 shows the variety of biomarker series detected in the methylated and silylated lipid extracts, along with a semi-quantitative assessment of their abundances. Although we cannot provide absolute concentrations, considerable environmental information is still revealed by the biomarker distributions. The approach used limits the possibilities for comparison with results obtained for other systems, although it allows us to detect small qualitative inter-sample differences and to determine trends in organic composition through the core.

Fig. 5 shows SIM traces corresponding to the most abundant biomarker series, i.e. *n*-alkanes (m/z 85) and *n*-fatty acid methyl esters (m/z 74) in core sections collected at different depths. A homologous series of C_{14} – C_{36} *n*-alkanes was found throughout the core. A bimodal distribution with maxima at C_{17} and C_{29} was only evident at 8420 years. The clear odd/even predominance indicates that the sampled material did not pass through extensive diagenesis that could indicate a rapid burial

Table 2
Relative abundance^a of biomarker classes found in the total extracts of lipids from samples taken at different depth

Compound classes	Core depth (m)				
	1–7	7–16	16–28	28–38	38–50
	¹⁴ C Age (yr BP)				
	3650	7200	9100	10 200	12 500
<i>n-Alkanes</i>					
<C ₂₄	++	++	+++	+++	+++
>C ₂₄	+++	+++	+++	+++	+++
Isoprenoids	+/-	+/-	+/-	+/-	+/-
Cyclic alkanes	+/-	+/-	+/-	+/-	+/-
<i>n-Fatty acids</i>					
<C ₂₂	+++	+++	+++	+++	+++
>C ₂₂	+++	+++	+++	++	++
Branched fatty acids	+	+	+	+	+
Unsaturated fatty acids	+	+	+	+	+
β-Hydroxy fatty acids	-	+/-	+/-	+/-	+/-
ω-Hydroxy fatty acids	-	+/-	+/-	+/-	+/-
<i>n</i> -Alkanols	+	+	+	+	+
<i>n</i> -Alkan-2-ones	+	+	+	+	+
Resin acids	+	+	-	-	-
<i>Other compounds:</i>					
Aromatic hydrocarbons	+/-	+/-	+/-	+/-	+/-
Head to head isoprenoids	+/-	+/-	+/-	+/-	+/-
Organic sulphur fatty acids	+/-	+/-	+/-	+/-	+/-

^a +++ high content, ++ abundant, + present, +/- trace and - absent.

during a phase of decreasing vegetation cover and enhanced soil erosion.

A strong odd predominance in the *n*-C₂₇–*n*-C₃₁ range is usually related to the occurrence of wax lipids derived from higher plants, particularly angiosperms, although longer chain alkanes may also reflect inputs of sea grasses (Jaffé et al., 1995). They can also originate from diatoms, although our inability to detect brassicasterol, a much more typical marker for these organisms (Volkman, 1986), suggests that these compounds do not derive from this source. Lower molecular weight alkanes with maxima at C₁₅, C₁₇ and C₂₁ are derived from lipids synthesised by phytoplankton and benthic algae (Cranwell, 1982; Wakeham et al., 1991; Canuel et al., 1997). They are less abundant than the higher homologues due, presumably, to their preferential degradation during early diagenesis.

Similar spatial variations in aliphatic hydrocarbons in surficial lake sediments have been associated with transport of riverine sediments in a series of resuspension-deposition cycles. During these cycles, fine-grained sediments of terrestrial origin accumulate in the lakes deep basin, whereas planktonic hydrocarbons are thought to be depleted by microbial degradation during transport to the deep basin (Doskey, 2001). Similar shifts in OM input to Holocene lake sediments have

been recently reported by Filley et al. (2001), based on isotopic OM source proxies.

Fig. 6 shows the downcore plots of the alkane C₃₁/C₁₇ and C₃₁/C₂₉ ratios, as well as the *n*-alkane source indicators employed by Bourbonniere and Meyers (1996) to identify changes in the terrigenous/aquatic mixture of hydrocarbons (TAR_{HC} index) calculated as the ratio C₂₇+C₂₉+C₃₁/C₁₅+C₁₇+C₁₉.

The alkane C₃₁/C₁₇ or C₃₁/C₁₉ ratios can serve as a measure of the relative proportions of allochthonous and autochthonous organic matter inputs. High values (>0.4), such as those seen in this study, are thought to be diagnostic of non-marine sources, whereas lower values are encountered in all kinds of marine sedimentary environments (Moldowan et al., 1985).

The C₃₁/C₂₉ ratio may be used as a palaeoclimatological proxy. Recently, Schwark et al. (2002) pointed out that centennial climate trends and reconstruction of Holocene vegetation history are consistently reflected in the palynological proxy and the variability within the *n*-C₂₇–*n*-C₃₁-alkane distribution. The predominance of the C₂₉ and C₃₁ homologues found in the Guadiana river sediments is typical of the input for a pine assemblage.

The downcore plot of the TAR_{HC} index indicates that higher plant sources predominate in the core sections above 13.5 m (mean = 25.6 ± 16.1). However, decreasing

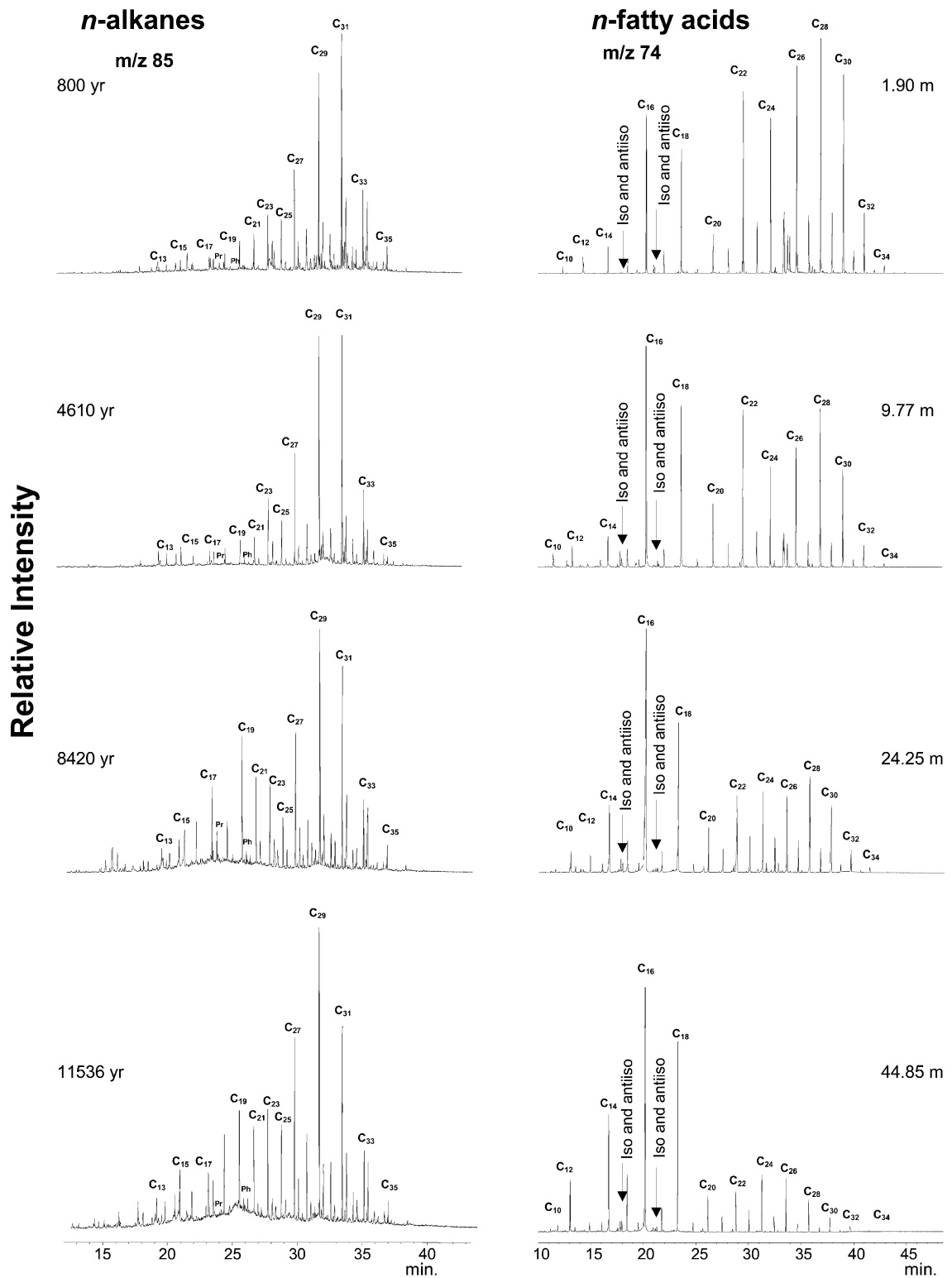


Fig. 5. Reconstructed ion traces of *n*-alkanes (m/z 85) and *n*-fatty acid methyl esters (m/z 74) series in sediment samples taken at different depth. Number on the peak refers to carbon chain length.

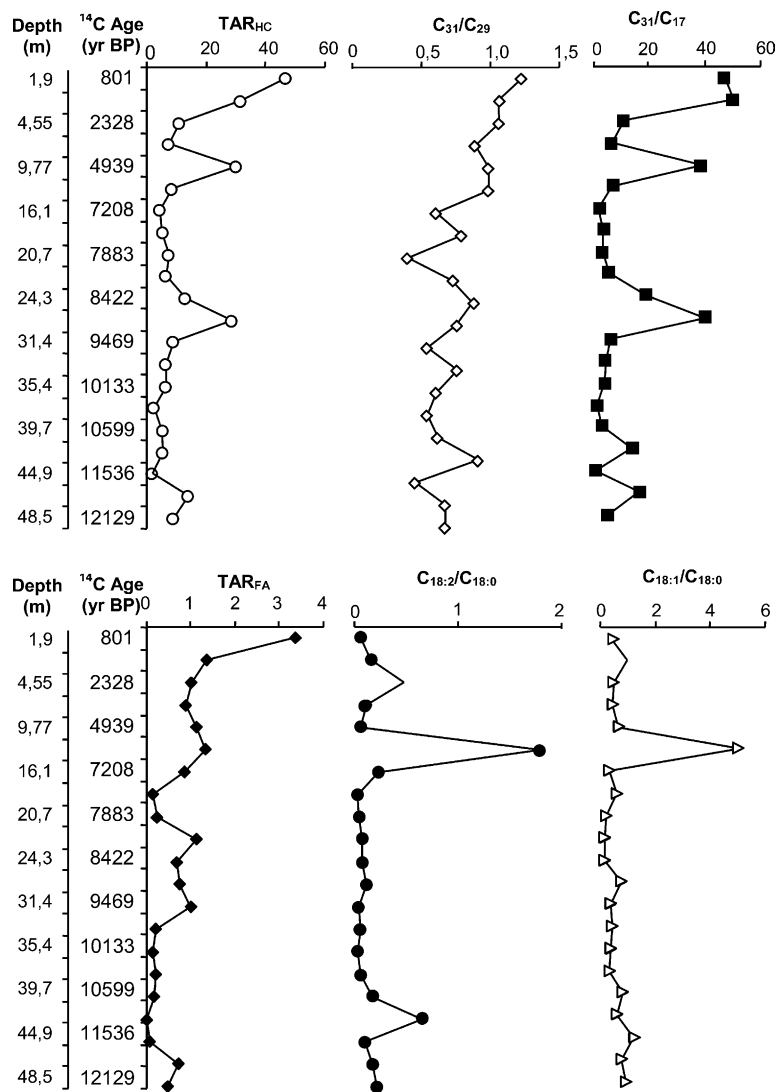


Fig. 6. Downcore plots of *n*-alkane ($\text{C}_{31}/\text{C}_{29}$, $\text{C}_{31}/\text{C}_{17}$, TAR_{HC}) and *n*-fatty acids ($\text{C}_{18:2}/\text{C}_{18:0}$, $\text{C}_{18:1}/\text{C}_{18:0}$, TAR_{FA}) ratios.

values in the lower half of the core (mean 8.4 ± 6.2) suggest a progressively minor contribution from terrestrial OM input.

Regular isoprenoids were also detected in the range C_{13} – C_{20} although these compounds were present in much lower concentrations than the *n*-alkanes. It is generally assumed that the major source of regular isoprenoids is the phytol side chain of chlorophyll, although chromans and/or tocopherols have been suggested to be additional potential sources (ten Haven et al., 1987b; Li et al., 1995). Therefore, the utility of the pristane/phytane (Pr/Ph) ratio and also pristane/ C_{17} and phytane/ C_{18} ratios as palaeoenvironmental source indicators (Didyk et al., 1978) should be treated with caution. The Pr/Ph ratio varies slightly (0.4–0.7) in these

samples, and it is always smaller than 1. These low values suggest that anoxic conditions in the active sediment layer prevailed at the very early stage of diagenesis (Volkman and Maxwell, 1986). The C_{17}/Pr and C_{18}/Ph values < 1 throughout the core are also indicators of strong microbial activity, independent of the prevalent source and anoxia factors.

The fatty acids constitute the major component of the lipid fractions throughout the core. They are present in the range C_{12} – C_{34} (Fig. 5), showing a bimodal distribution with maxima at C_{16} and C_{28} (C_{24} in the lower sections). The patterns are more similar to those reported previously for sediments rich in marine-derived OM (planktonic algae, diatoms and diatom cultures; Venkatesan, 1988; Volkman et al., 1998), than those typical of

soils rich in longer-chain fatty acids ($>C_{22}$) originating from plant waxes (Almendros et al., 1996).

The downcore plot of the terrigenous/aquatic ratio for fatty acids (TAR_{FA} index, calculated as the ratio $C_{24} + C_{26} + C_{28}/C_{12} + C_{14} + C_{16}$; Bourbonniere and Meyers, 1996; Fig. 6) shows that the distributions are also subject to significant alteration within the sediment sequence. The TAR_{FA} decreases (from 1.5 ± 1.0 in the upper 13 m to 0.5 ± 0.4 in the lower sections) with depth. This trend is similar to that reported by Matsuda (1978) for lacustrine sediments and probably reflects differences in the relative proportions of autochthonous and allochthonous OM delivered to the sediment.

The specific bacterial biomarkers, branched chain *iso*- and *anteiso*- acids and β -hydroxy fatty acids, were detected in all the lipid extracts. In addition, mono-unsaturated C_{16} and C_{18} acids and some poly-unsaturated acids ($C_{18:2}$ and $C_{18:3}$) derived largely from algae (Cranwell et al., 1987), but also from higher plants (Canuel et al., 1997), were also detected. The latter have been also found previously in older lacustrine and marine sediments, and the vertical changes in the unsaturated to saturated acids ratio for the C_{18} species was found to correlate with palaeoclimatic conditions suggested from palynological evidence (Kawamura and Ishiwatari, 1981). As shown in Fig. 6, the $C_{18:1}/C_{18:0}$ and $C_{18:2}/C_{18:0}$ ratios display a maximum at a depth of ca. 15 m, which is difficult to interpret. However, a variety of hypotheses about possible changes in the input of polyunsaturated relative to saturated acids, such as local increases in the population of zooplankton or changes that occurred during early diagenesis, have been described by Kawamura and Ishiwatari (1981).

Traces in Fig. 7 show the distribution of *n*-alkanols (m/z 103), *n*-alkan-2-ones (m/z 58) and resin acids (m/z 163) at 14.5-m depth.

Normal alcohols occurred in the range C_{12} – C_{28} with a strong even-to-odd carbon number predominance and maxima at C_{16} and C_{22} (Fig. 7a); they showed a uniform distribution profile throughout (data not shown). The pattern may derive from a mixture of sources, including terrestrial vascular plant, phytoplankton and aquatic macrophytes. A similar pattern was found by Filley et al. (2001) in a lithological unit of a core from a lake sediment, which showed sharp changes in the *n*-alkanol distributions with depth. It clearly differs from that found in soils, which is dominated by hexacosanol (C_{26}), with a wider range of peripheral *n*-alkanol homologues (C_{18} – C_{34}); (Bull et al., 2000), and also from the unusual pattern (C_{20} – C_{32}), attributed to aquatic macrophytes, reported for freshwater lake sediments (Ficken et al., 1998). In the sediment, the uniformity in the *n*-alkanol distribution and the high values (>1.5) of the ratio of lower to higher homologues ($<C_{20}/>C_{20}$)

throughout the core indicates the occurrence of a continuous autochthonous source.

Phytol (C_{20} isoprenoid alcohol), derived from the phytol chain of chlorophyll, was detected as a component of the alkanol fraction. Its abundance increased slightly with depth throughout the core. It is perhaps the most studied biomarker among those found in modern aquatic environments and, in particular, has been used to track down algal inputs through photosynthesis (Ishiwatari et al., 1999). However, the multiplicity of its sources and the variety of biotic and abiotic degradation processes that it undergoes in different environments (Rontani and Volkman, 2003) limit its usefulness as a biomarker.

A homologous series of alkan-2-ones in the range C_{14} – C_{29} (Fig. 7b) was found in all the sections. The distribution shows a predominance of odd numbered carbon chain lengths with a maximum at C_{25} . Similar distributions have been found in a wide variety of terrestrial and aquatic depositional environments characterised by higher plant or microbial OM inputs (Jaffé et al., 1995; Hernandez et al., 2001 and references therein). The particular *n*-alkan-2-one distribution with a maximum at about the C_{25} homologue, proposed as potential biomarker distribution for seagrass-derived OM (Hernandez et al., 2001) was not observed here. Likewise, we were not able to identify the C_{37} – C_{39} long chain ketones with 2, 3 or 4 double bonds that are specific markers for coccolithophorids (Villanueva et al., 1997, and references therein).

A well-resolved diterpene resin acid pattern (Fig. 7c) belonging to the abietane family was found in the sections from the upper 15 m. These terpenoids have been found to be widely distributed in the geosphere and are considered valuable biomarkers for conifers, although their presence in some environments may be due to long distance aeolian transport (Simoneit, 1977; Otto and Simoneit, 2001 and references therein). The resin acid dehydroabietic acid was present in the greatest concentrations, which is consistent with the fact that other resin acids (e.g. palustric and levopimaric acids) readily undergo transformation into dehydroabietic acid (Ekman and Ketola, 1981; Almendros et al., 1996).

It is noteworthy that the resin acid series was not detected in the sections below 15 m, which corresponds to approximately 6500 years cal BP; this is consistent with the idea of changes in the vegetation cover in the Guadiana drainage basin. However, palynological and geomorphological studies of the palaeoenvironmental characteristics of Southwestern areas of the Iberian Peninsula did not detect significant changes in the Holocene vegetational landscape (Yil et al., 2002).

Our data suggest that, on a 10^3 – 10^4 year time scale, little or no diagenetic change occurred with

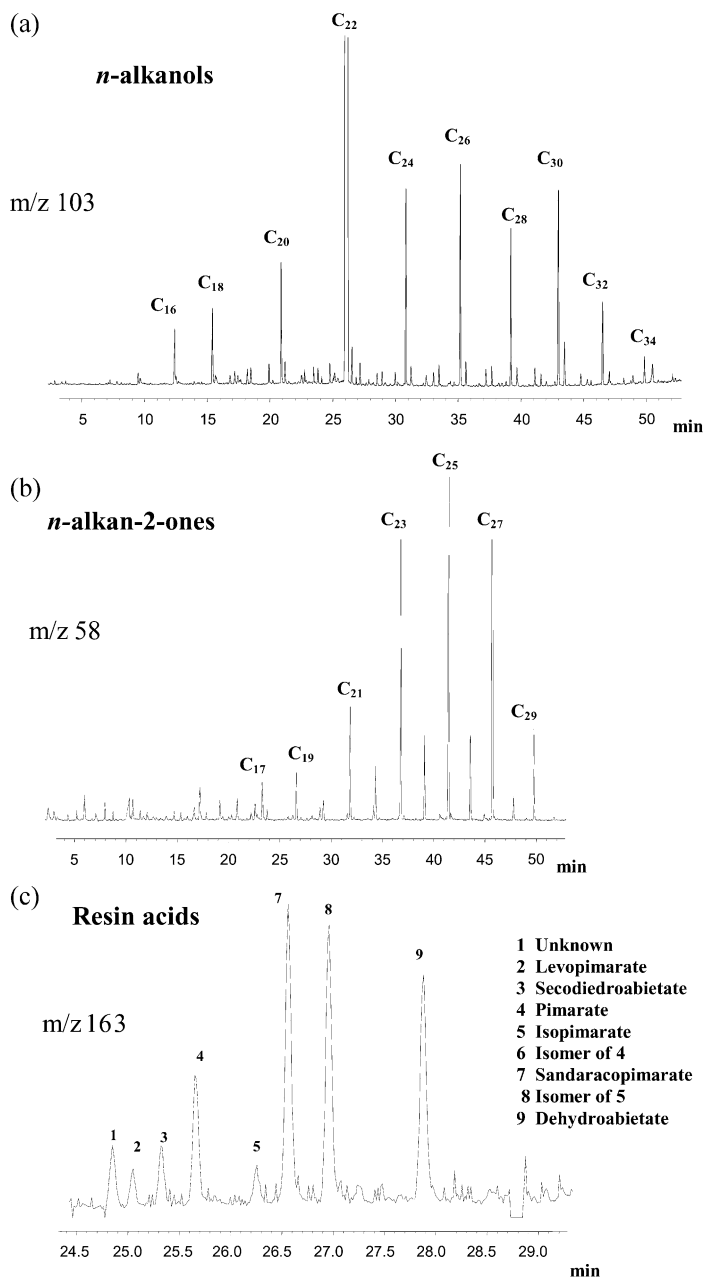


Fig. 7. Reconstructed ion chromatograms of (a) *n*-alkanols (m/z 75), (b) *n*-alkan-2-ones (m/z 58) and (c) resin acids (m/z 163) found in the sedimentary section at 15.5 m. Numbers on the peaks refers to carbon chain lengths. Identifications of resin acids given in the table beside the trace.

respect to the compounds in question, therefore indicating that the evolution of the Holocene landscape is determined basically by the changes in geomorphological conditions in the coast and the marshland systems. In fact, the abrupt transformations registered in the Southeastern sector, where a dramatic deforestation of the territory and the establishment of steppe forma-

tions was observed, does not occur in the western zones, although it is chronologically coincident with transformation of the littoral conditions towards 4450 years BP.

Alternatively, the changes in the biomarker patterns may not have been induced by climate fluctuation, since the transition from a colder Boreal phase to the optimal

warmer and wetter Atlantic climate of the Upper Holocene occurred around 9–6.8 kyears. Instead, the changes may reflect the termination of a wetter-than-modern period that accompanied the deposition of the S₁ sapropel across much of the Mediterranean Sea (Ariztegui et al., 2000).

5. Conclusions

The time-resolved variability in the biomarker distributions in a sedimentary core from the Guadiana estuary provides a good indicator of changes in the supply of terrigenous and marine OM over the past 13 kyears. However, the observed temporal shifts in OM characteristics that are preserved in the sediments do not seem to be caused by climatic variations during the Holocene, which could be responsible for changes in vegetation cover, but rather to documented abrupt transformation of the littoral conditions. It is therefore suggested that changes in OM inputs correspond to (i) soil erosion and the deposition of more refractory continental OM and (ii) a sharp decrease in sedimentation rates in the estuary and consequently, more prolonged exposure of OM to oxic degradation, affecting to a larger extent the labile planktonic marine OM than refractory vascular plant detritus.

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