van Soelen et al., (2014) *Organic Geochemistry* 70, 20–28

Unusual C_{35} to C_{38} alkenones in mid-Holocene sediments from a restricted estuary (Charlotte Harbor, Florida)

E.E. van Soelen[a,b*], J.M. Lammers[a], T.I. Eglinton[c,d], J.S. Sinninghe Damsté[a,b], G.J. Reichart[a,b]

[a] Department of Earth Sciences – Geochemistry, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands
[b] NIOZ Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg, The Netherlands.
[c] ETH, Geological Institute, Sonneggstrasse 5, 8092 Zürich, Switzerland
[d] Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

As published in: *Organic Geochemistry* 70, 20–28 (2014)

Abstract

Unusual C_{35} to C_{38} alkenones were identified in mid-Holocene (8–3.5 kyr BP) sediments from a restricted estuary in southwest Florida (Charlotte Harbor). The distribution was dominated by a C_{36} diunsaturated (ω15,20) ethyl ketone, identical to the one present in Black Sea Unit 2 sediments. Other unusual alkenones were tentatively assigned as a $C_{35:2}$ (ω 15,20) methyl ketone, a $C_{37:2}$ (ω 17,22) methyl ketone and a $C_{38:2}$ (ω 17,22) ethyl ketone. In late Holocene sediments < 3.5 kyr BP, the common C_{37} to C_{39} alkenones were found. Compound-specific ^{14}C, ^{13}C, and D isotope measurements were used to constrain the possible origin of the alkenones. Conventional radiocarbon ages of alkenones and higher plant-derived long chain *n*-alcohols indicated no significant difference in age between mid-Holocene alkenones and higher plant *n*-alcohols. Both alcohols and alkenones were offset vs. calibrated ages of shell fragments in the same sediment core, which suggests they were pre-aged by 500–800 yr, implying resuspension and redistribution of the fine-grained sedimentary particles with which they are associated. The hydrogen isotopic (δD) composition (-190‰ to -200‰) of the C_{37} and C_{38} alkenones in the late Holocene sediments is in line with values for coastal haptophytes in brackish water. However, the unusual C_{36} and C_{38} alkenones from the mid Holocene sediments were enriched in D (by ca. 100‰) vs. the late Holocene alkenones. Also, $\delta^{13}C$ values of mid-Holocene alkenones were consistently offset compared with late Holocene alkenones (-21‰ to -22‰ and -22‰ to -23‰, respectively). We suggest that the alkenones in Charlotte Harbor were produced by unknown alkenone-producing haptophyte.

*Corresponding author. Department of Earth Sciences – Geochemistry, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands. Tel.: +31 30 2535050. E-mail address: evansoelen@gmail.com (E.E. van Soelen).

1. Introduction

Alkenones are well established as an important group of lipids for paleoclimate reconstruction, as past surface water temperatures are recorded in their degree of unsaturation (Brassell et al., 1986; Prahl and Wakeham, 1987; Müller et al., 1998). The stable hydrogen and carbon isotopic compositions of these compounds have also been linked to past surface ocean salinity (Schouten et al., 2006; Schwab and Sachs, 2011) and pCO_2 conditions (Bidigare et al., 1997; Pagani et al., 2002), respectively. However, correct interpretation of these alkenone-based proxies requires detailed knowledge of the precursor organisms as well as development of robust calibrations. In open ocean waters, the haptophyte *Emiliania huxleyi* is the dominant source for C_{37}, C_{38} and C_{39} alkenones, followed by the closely related *Gephyrocapsa oceanica* (Conte et al., 1995; Volkman et al., 1995). In coastal settings, alkenones may also be produced by other haptophyte species, such as Isochrysis spp. and *Chrysotila lamellosa* (Marlowe et al., 1984; Rontani et al., 2004). In particular, alkenone distributions in coastal freshwater or hypersaline environments may deviate from those in open marine sediments (e.g. Zink et al., 2001), yet the biological origin of the alkenones in these systems remains elusive (e.g. Zink et al., 2001; Lopez et al., 2005).

An unusual C_{36} diunsaturated alkenone was identified by Xu et al. (2001) in Holocene sediments from the Black Sea. This compound, which was shown from nuclear magnetic resonance (NMR) spectroscopy to be an ethyl ketone with double bonds at ω15,20 (from now on $C_{36:2}$ EK), was the most abundant alkenone in Unit II of the Black Sea sediments (7.3–3.6 kyr BP). The finding was confirmed by Rontani et al. (2006b), who also recognized other unusual alkenones in these sediments, including $C_{35:2}$ (ω15,20) and $C_{37:2}$ (ω17,22) methyl ketones and a $C_{38:2}$ EK (ω 17,22). Furthermore, the $C_{36:2}$ EK was also detected in Black Sea particulate matter, indicating that the source organism still exists today (Rontani and Wakeham, 2008), although the alkenones in Black Sea surface sediments are dominated by those derived from *E. huxleyi* (Coolen et al., 2006). In addition to the Black Sea, the unusual $C_{36:2}$ EK has been recognized, albeit in relatively small amount, in late Pleistocene sediments from the Japan Sea (Fujine et al., 2006) and in particulate matter from the Ligurian Sea (Rontani et al., 2001). Its occurrence in sediments of Aptian age has also been suggested (Brassell and Dumitrescu, 2004), although no information was provided on the double bond positions.

The biological source for the unusual series of alkenones remains unknown. Fujine et al. (2006) suggested a relationship with low salinity conditions, although a true freshwater origin is unlikely according to Xu et al. (2001) since the $C_{36:2}$ EK in the Black Sea occurred only after the introduction of marine water. Association with low salinity conditions is also inconsistent with its present-day occurrence in the Ligurian Sea (Rontani et al., 2001). Nor-alkenones have also been detected in a culture of *E. huxleyi* (Prahl et al., 2006), but with double bond positions different from the $C_{36:2}$ EK detected in the Black Sea Unit II sediments. C_{35} and C_{36} alkenones with the same double bond positions as the Black Sea alkenones, were tentatively assigned in a cultured strain of *G. oceanica* (Rontani et al., 2006b), but the distribution differed markedly from those in Black Sea Unit II sediments. In an effort to identify the haptophytes responsible for alkenone production in the Black Sea sediments, Coolen et al. (2006, 2009) used fossil DNA to reconstruct haptophyte assemblages occurring in the water column during the Holocene. In Unit I Black Sea sediments, only haptophyte DNA belonging to *E. huxleyi* was detected (Coolen et al., 2006), whereas in Unit II sediments, DNA from both *E. huxleyi* and *Isochrysis* spp. was detected (Coolen et al., 2009). *Isochrysis* spp. were ruled out as a source for the unusual alkenones since, from 5000 yr

onwards, no *Isochrysis* spp. DNA was detected, whereas C_{36} alkenones were abundant in the sediments. Coolen et al. (2009) proposed three strains of *E. huxleyi* as a possible source of the $C_{36:2}$ EK, since the presence of their fossil DNA coincided with, and was unique for, a period during which the abundance of $C_{36:2}$ EK in the Black Sea sediments was greatest.

We have also detected unusual C_{36} alkenones in sediments of two Florida estuaries: Tampa Bay (van Soelen et al., 2010) and Charlotte Harbor (van Soelen et al., 2012). As in Black Sea mid- Holocene sediments, $C_{36:2}$ EK is the predominant alkenone in the mid-Holocene sediments in these estuaries, while late Holocene sediments are dominated by the more common C_{37} and C_{38} alkenones. To place further constraints on the origin of the alkenones in Charlotte Harbor, compound-specific stable (δD and $\delta^{13}C$) and radiocarbon isotopic compositions of alkenones were determined. The isotopic data shed light on the production, transport and deposition of these compounds, and thus directly and indirectly yield new information on the source of the alkenones.

2. Setting

Charlotte Harbor is a coastal system located in the southwest of Florida, consisting of a central estuary and two lagoons that are sheltered from the Gulf of Mexico by a series of barrier islands. The average water depth is around 2 m, but can reach over 6 min some parts of the estuary (Evans et al. 1989). Freshwater is supplied by three rivers (Peace, Myakka and Caloosahatchee rivers) and supply is largest during the rainy season between June and October (Taylor, 1974). During the dry season, salinity in the harbor is relatively high; in the upper part, values can be over 32 (Stoker, 1992). During May and July, increased runoff can lead to vertical density stratification, and anoxic bottom water conditions can develop in the harbor estuary (Fraser, 1986; Heyl, 1998). During the wet season, near surface salinity may drop below 5, while near bottom water salinity is between 15 and 20 (Stoker, 1992). Water temperature in Florida coastal waters, including Charlotte Harbor and Tampa Bay, is generally around 16°C in winter (January, February) and 30°C in summer (June until September) (Fraser, 1986 and www.noaa.gov). Winds are strongest in the winter months when they have a north/northeast direction; in the summer months the prevailing wind direction is southeast (Evans et al., 1989). Average wave height is between 30 and 50 cm (Tanner, 1960; Hine et al., 1987) and the average tidal range for estuaries at the Florida west coast is generally < 1 m (NOAA: http://tidesandcurrents.noaa.gov/).

A reconstruction of Holocene environmental conditions in Charlotte Harbor has been published previously (van Soelen et al., 2012). The sediments of the harbor were divided into three different units on the basis of sedimentological differences inferred from whole core X-ray fluorescence (XRF) scanning data. Unit II sediments (mid-Holocene) consist of organic-rich clay [up to 10% total organic carbon (TOC)] interbedded with some quartz sand layers. Visual inspection showed distinctly to indistinctly laminated intervals. Unit I (late Holocene) is characterized by coarser grained sediments (clay to fine sand) with TOC content up to 2%, interbedded with some shell debris layers. Details of whole core XRF scanning and accelerator mass spectrometry (AMS) radiocarbon dating of shell (fragments) from the cores, as well as high resolution TOC, nitrogen and biomarker data for core CH1, can be found in van Soelen et al. (2012). Based on these data, it was inferred that, during the mid-Holocene period (8–3.5 kyr BP), the estuary was eutrophic. High runoff resulted in extensive terrestrial organic matter (OM) input and high primary productivity, which resulted (at least

periodically) in stratified water column conditions. From 3.5 kyr BP onwards, the terrestrial OM input decreased and conditions became more oligotrophic.

3. Material and methods

3.1. Sediment samples

Two cores, CH1 and CH3, were recovered from Charlotte Harbour (26°520N, 82°070W, Fig. 1) at a water depth of ca. 7 m during a field campaign in April 2008, using a vibracorer. Selected samples were analysed for compound-specific stable hydrogen and carbon isotopic compositions of alkenones (Fig. 2). Alkenone fractions from CH1 were obtained in an earlier study (van Soelen et al., 2012). Additional samples from core CH3 were extracted to perform compound-specific radiocarbon dating of long chain *n*-alcohols and C_{36} to C_{38} alkenones (sample CH3-B at 120–150 cm, and CH3-D at 270–300 cm), and for a detailed study of alkenone distributions (sample CH3-A at 0–10 cm and CH3-C at 260–270 cm).

3.2. Extraction and isolation of alkenones and alcohols

Large sediment samples (ca. 300 g for CH3-B and CH3-D and ca. 30 g for CH3-A and CH3-C) (Fig. 2) were freeze-dried, after which the powdered sediments were extracted with a Soxhlet extractor using dichloromethane (DCM)/MeOH (9:1, v/v). Extracts were separated into three fractions using large columns (17 x 2 cm) packed with activated Al2O3. Three eluents were used: (i) hexane/DCM (9:1 v/v), (ii) DCM and (iii) DCM/MeOH (1:1 v/v), resulting in a hydrocarbon fraction (F1), a ketone fraction (F2) and a polar fraction (F3), respectively. Alkenones were isolated from new and previously acquired ketone fractions (F2) by way of a series of purification steps as described by Ohkouchi et al. (2005). First, straight chain components were isolated using urea adduction. The purity of these adducts was sufficient to allow stable hydrogen and carbon isotope analyses of alkenones. Radiocarbon measurements required two further purification steps. Column chromatography with $AgNO_3$-impregnated SiO_2 was carried out, using DCM and EtOAc as eluents, to obtain saturated and unsaturated ketone fractions, respectively. The unsaturated ketone fraction was eluted from a silica gel column (1 g, 1% H_2O deactivated), using hexane/DCM (7:3, v/v) and hexane/ DCM (6:4, v/v), with the latter fraction exclusively containing $C_{36\text{-}38}$ alkenones.

Alcohols for compound specific radiocarbon measurements were isolated from F3 (samples CH3-B and CH3-D). F3 was saponified after dissolution in 0.5 M KOH in MeOH (10 ml) and heating for 2 h at 75°C. After addition of double distilled water (250 µl), fractions containing *n*-alcohols were obtained using liquid–liquid extraction with hexane. Residual water was removed using Na_2- SO_4. *N*-alcohols were acetylated (1 ml Ac_2O, 1 ml pyridine; 70°C, 30–60 min) to adjust the polarity, after which branched and/or cyclic components were removed using urea adduction.

The purity of the fractions was checked between each cleaning step using gas chromatography (GC), with an HP gas chromatograph fitted with a CP-Sil 5CB fused silica column (30 m x 0.32 mm i.d.) and flame ionization detection (FID). Samples were injected on-column, with He as carrier gas at constant pressure (100 kPa). The oven was heated from 70°C to 130°C at 20°C min^{-1} and then to 320°C (held 20 min) at 4°C min^{-1}. GC–mass

spectrometry (GC–MS) was performed with a Thermo Finnigan Trace instrument, using the same column and oven program as for GC, only with a constant flow for He.

Selected alcohols (C_{24}, C_{26}, C_{28}, C_{30} and C_{32} *n*-alkanols) were separated from the acetylated alcohol fraction using preparative capillary GC (PCGC) at Woods Hole Oceanographic Institution (WHOI). Sample CH3-B was analyzed using an Agilent 7890A series gas chromatograph equipped with FID and an Agilent 7683B series automatic injector. Sample CH3-D was analyzed with an HP 5890 Series II gas chromatograph equipped with FID and HP7673 injector. For both cases, the GC oven program was: 50°C (1 min) to 100°C at 25°C min^{-1}, then to 320°C (held 10 min) at 5°C min^{-1}. Both samples were run in hexane. After PCGC, the trapped compounds were eluted with hexane and measured using GC-FID to determine yield and purity. The fraction containing the C30 *n*-alcohol of sample CH3-B was not sufficiently pure for isotopic measurement and was not further analysed.

3.3. Structural assignment of unusual alkenones

Dimethyl disulfide (DMDS) was used to locate the double bond positions (Leonhardt and DeVilbiss, 1985) in sample CH1-DMDS (309.5–310.5 cm, Fig. 2): The alkenone fraction was dissolved in 100 µl hexane and 100 µl DMDS and 20 µl of I_2 solution in Et_2O (60 mg ml^{-1}) were added. The solution was heated at 40°C for ca. 20 h before addition of 200 µl $Na_2S_2O_3$ in water (5%) to deactivate the iodine. The solution was washed (3x) with hexane to extract the thioether derivatives of the ketones. Hexane fractions were combined and dried under N_2. The ketones in EtOAc were measured using GC and GC–MS (as described above).

3.4. Compound-specific stable and radiocarbon analysis of long chain n-alcohols and alkenones

Radiocarbon analysis was performed on purified C_{24}, C_{26}, C_{28}, C_{30} and C_{32} *n*-alcohols and C_{36}–C_{38} alkenone samples at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility, according to established procedures (e.g. Ohkouchi et al., 2005). Radiocarbon data are presented as fraction modern (fM) values (Table 1), which were corrected for isotopic fractionation based on $\delta^{13}C$ values measured directly on the samples prepared for radiocarbon analysis (also presented in Table 1; Stuiver and Polach, 1977). No correction was made for potential blank contributions. Fraction modern (fM) values of *n*-alcohols were corrected for the addition of two carbons during acetylation. Fraction modern values were then converted to conventional 14C age [14C = -8033 * LN(fM)] and calibrated into calendar years before present (BP) (i.e. before 1950 AD) using the CALIB6.0 program (Stuiver and Reimer, 1993).

Stable hydrogen and carbon isotopes ratios, presented in Table 2, were measured on purified samples from core CH1 and CH3. Stable hydrogen and carbon isotopic compositions of long chain alkenones were measured using GC-isotope ratio mass spectrometry (GC-IRMS; DeltaplusXP) at Utrecht University, using the same type and size of column, and temperature program as for GC-FID. Each day, the H_3^+ factor was determined and standards were measured in order to calibrate against international references (Schimmelman, Biogeochemical Laboratories, Indiana University). Hydrogen isotopic composition values are reported in ‰ relative to Vienna Standard Mean Ocean Water (VSMOW). Carbon isotopes are reported in ‰ relative to Vienna Peedee Belemnite (VPDB). Replicate analyses,

conducted when the concentration allowed duplicate measurements, gave a standard deviation of better than 0.3‰ for $\delta^{13}C$ and better than 2‰ for δD.

3.5. Stable carbon and oxygen isotope ratios of benthic foraminifera

Sediment samples were taken every 20–40 cm over the whole length (4.3 m) of core CH1 and freeze dried. Samples were subsequently wet sieved and disaggregated over a 150 lm mesh sieve. Tests of the benthic foraminiferal species *Ammonia* sp. were picked from the > 150 μm size fraction. From each sample 30–60 wellpreserved specimens were selected and further ultrasonically (2 min) cleaned using ultra-pure water and MeOH rinsing steps. Oxygen and carbon isotope ratio measurements were carried out using an automated common acid bath (IsoCarb) coupled online to a VG SIRA 24 mass spectrometer. Precision and accuracy based on replicate analyses of international and in-house standards is better than 0.1‰ and 0.07‰ for oxygen and carbon isotopic composition respectively. All values are reported in ‰ relative to VPDB.

4. Results

4.1. Alkenones in Charlotte Harbor sediments

The most abundant alkenone in Unit II sediments is a $C_{36:2}$ EK with a concentration up to 145 μg g^{-1} TOC (or 6 μg g dw^{-1}) (Fig. 5; van Soelen et al., 2012). C_{36} alkenones were not detected in intervals shallower than 130 cm (or 3.5 kyr BP onwards). GC–MS analysis after DMDS derivatization revealed that the C_{36} alkenone possessed two double bonds at positions ω15 and ω20 and the $C_{36:2}$ EK co-occurred with C_{35}, C_{37} and a C_{38} alkenones, with the latter being the next most abundant (Fig. 3b). Based on the mass spectra, these alkenones were assigned as $C_{35:2}$ and $C_{37:2}$ methyl ketones and a $C_{38:2}$ ethyl ketone (Fig. 3c–f). Unfortunately, due to the low abundance of the compounds, the double bond positions could not be confirmed using DMDS derivatization. Generally, because double bonds may migrate during ionization in the MS source, it is not possible to confirm double bond positions based on ion fragments in mass spectra of the ‘‘classical’’ alkenones. However, Rontani et al. (2006a) suggested that diagnostic ion fragments are formed during ionization of those alkenones possessing a three methylene unit separation between double bonds. Based on these fragments (i.e. m/z 250 and 278 for the $C_{36:2}$ EK x15,20; Fig. 3d), it was possible to assign the double bond positions of the $C_{38:2}$ EK in Unit II sediments as x17,22 (Fig. 3f), while the mass spectral characteristics of the $C_{37:2}$ alkenone show the characteristics of the more common alkenone with double bond positions at x15,22 (Fig. 3e). From 3 kyr BP onwards, C_{37} and C_{38} alkenones with a ‘‘normal’’ distribution (Fig. 3a) predominated (Fig. 6). The concentration was initially around 20 lg g^{-1} TOC (or 0.1 lg g/dw), but from 2000 yr BP onwards, it increased up to 75 lg g^{-1} TOC (or 0.9 lg g/dw).

4.2. Compound-specific radiocarbon compositions

Radiocarbon ages of alkenones and *n*-alcohols are reported in Table 1. Alkenones were not measured individually, but as a combined fraction containing C_{36}, C_{37} and C_{38} alkenones.

Conventional ^{14}C ages of isolated long chain *n*-alkanols were 4990–4600 yr BP (core CH3-B, 120–150 cm) and 5700–5240 yr BP (core CH3-D, 270–300 cm; Table 1 and Fig. 4). Conventional ages of alkenones were older than the average conventional ages of the *n*-alkanes by ca 530 yr (CH3-B) and 980 yr (CH3-D; Fig. 4).

4.3. Compound specific carbon and hydrogen isotope compositions

Compound specific stable carbon isotopic ($\delta^{13}C$) and hydrogen isotopic (δD) compositions of alkenones are presented in Table 2 and Fig. 6. The $\delta^{13}C$ values of C_{36} alkenones and $C_{38:2}$ alkenones in Unit II sediments were on average -21‰ and -22‰, respectively. In Unit I, $\delta^{13}C$ values were slightly more negative, averaging -23‰ and -24‰ for the $C_{37:2}$ and $C_{38:2}$ alkenones, respectively. Average δD values of C_{36} alkenones were -106‰, and -103‰ for C_{38} alkenones in Unit II. In Unit I, δD values were more depleted (-193‰ for C_{37}; -197‰ for C_{38}).

4.4. Benthic foraminiferal stable isotopes

Oxygen and carbon isotopic data for the benthic foraminifera are presented in Figs. 6d–g. Foraminifera were found in most of the selected samples, except for an interval between 6200 and 8200 yr BP (355–425 cm). The oxygen isotope record ($\delta^{18}O$) of the benthic foraminifera Ammonia spp. showed an overall decrease from 0.2‰ to -1.3‰, from 8000 yr BP to the present. Superimposed on this trend, between 6000 and 4000 yr BP, d18O values were more variable, oscillating between 0.5‰ and -1.5‰. Carbonate $\delta^{13}C$ values showed relatively little variability (from -2.5‰ to -3.5‰) between 8000 and 6000 yr BP and between 4000 yr BP and present. Between 6000 and 4000 yr BP, values were more variable, ranging from -2.5‰ to -4.5‰.

5. Discussion

The distribution of the C_{37} and C_{38} alkenones in late Holocene sediments of Charlotte Harbor (Fig. 3a) is typical for haptophytes that produce carbonate skeletons, such as *E. huxleyi* or *G. oceanica* (Volkman et al., 1980; Conte et al., 1995). The low abundance of alkenones with more than two double bonds is also consistent with the relatively high temperature of these subtropical waters (Brassell et al., 1986). Although in coastal areas alkenones can also be produced by non-calcifying or ‘‘naked’’ haptophyte species like Isochrysis spp. (Marlowe et al., 1984) or *C. lamellosa* (Rontani et al., 2004), the observed distribution is different. We therefore conclude that a cosmopolitan species, like *E. huxleyi* or *G. oceanica* is likely the major source of alkenones in Charlotte Harbor sediments during the late Holocene.

The alkenone distribution in mid-Holocene sediments in Charlotte Harbor also strongly differs from that generally attributed to coastal haptophytes species. The presence of C_{36} alkenones in these sediments was established in earlier studies (van Soelen et al., 2010, 2012); however until now double bond positions had not been determined. We now confirm that these alkenones are identical to the $C_{36:2}$ EK reported earlier in mid-Holocene sediments of

the Black Sea by Xu et al. (2001). The co-occurring $C_{38:2}$ EK in the mid-Holocene interval is, based on its mass spectrum, most likely also identical to the one reported in the Black Sea, with double bond position ω17,22 (Rontani et al., 2006a,b). The origin of these C_{36} alkenones remains unknown.

Radiocarbon measurements may provide valuable constraints on the origin of the alkenones. Radiocarbon ages of long chain nalcohols and C_{36}–C_{38} alkenones were compared at two selected depth intervals. Comparison of radiocarbon ages of individual compounds from the same depth interval can reveal differences in age due to pre-aging during transport, re-suspension in the water column, or biological reactivity (Mollenhauer and Eglinton, 2007). Long chain *n*-alcohols derive from the leaf wax of higher plants (Eglinton et al., 1962; Eglinton and Hamilton, 1967) and thus have a terrestrial origin. The $C_{36:2}$ and $C_{38:2}$ ethyl ketones in Unit II have an unknown source, although they are most likely produced by haptophytes that grow (i) in the brackish estuary, (ii) in an open marine setting (Gulf of Mexico) after which they are transported to the estuary, or (3) in a freshwater environment (river or lakes) after which they are transported to the estuary by runoff.

Alkenone radiocarbon age assignments are expected to be reliable within about 500 ^{14}C yr (Mollenhauer et al., 2005a). However, this is a conservative estimate considering the additional uncertainties (e.g. ^{14}C age and size of blank contributions) associated with the processing of small samples (Mollenhauer et al., 2005a). Assuming a similar accuracy for alcohols, there are no statistically significant age differences between alcohols and alkenones found in the Charlotte Harbor sediments at the individual compound level. After calibration and applying the 400 yr standard reservoir age correction of the Calib program (as the specific local reservoir age is unknown), alkenones and alcohols in sample CH3-B are of similar age. In sample CH3-D, alkenones are comparable in age with the C_{24} alcohol, but are up to 800 yr older than the other alcohols. Plant wax alcohols are, in turn, on average 500–800 yr older than the calibrated ages of AMS ^{14}C-dated and shell fragments in the sediments (Fig. 5). The apparent older ages of the organic compounds vs. the carbonate ages implies pre-aging of the alcohols. Such pre-aging might occur when they are temporarily stored in terrestrial reservoirs (e.g. soil) and also during transport to the estuary. Previous studies using compound specific radiocarbon ages of alkenones have shown that alkenones in continental margin and ocean basins sediments can be substantially pre-aged (up to several 1000s of yr), compared with marine carbonates from the same depth interval in a sediment core (e.g. Ohkouchi et al., 2002; Mollenhauer et al., 2005b). These age differences have been attributed to long distance transport of alkenones as a consequence of re-suspension and re-distribution of fine grained particulate matter in the water column. Although long distance transport in Charlotte Harbor is unlikely, repeated re-suspension/re-deposition cycles due to wave and tidal action in the estuary might induce aging of alkenones (and *n*-alcohols) in the sediments, resulting in the apparent offset in the calibrated ages of alkenones and shell fragments as the latter are less prone to resuspension. Also, offshore marine sediments might be transported to the estuary during storm surges caused by tropical storms or cyclones (e.g. Lane et al., 2011), thereby introducing pre-aged alkenones into the system.

Stable carbon isotopic values of the alkenones, between -20‰ and -24‰, are in line with values for marine phytoplankton lipids (Hoefs, 1980), although δ^{13}C values of the unusual ethyl ketones in Unit II sediments are consistently enriched vs. Unit I alkenones by ca. 1–2‰ (Fig. 6 and Table 2). If alkenones in both Unit I and Unit II sediments were produced in the water column of the estuary, shifts in δ^{13}C must reflect differences in fractionation by the precursor species, changes in δ^{13}C of the dissolved inorganic carbon (DIC) that is used for alkenone production, or changes in pCO_2 (Popp et al., 1999). Changes in local δ^{13}C DIC

related to, for example, variability in runoff and thus input of depleted riverine DIC would impact on the alkenone $\delta^{13}C$ record. This can be estimated by comparing the alkenone $\delta^{13}C$ record with the benthic foraminiferal carbon isotopic record, since in (shallow) coastal settings the $\delta^{13}C$ of the benthos reflects DIC $\delta^{13}C$ values (e.g. Diz et al., 2009).

Within Unit I, d13C values of the benthic foraminifera show only minor variability (Fig. 6d). In contrast, the large shifts in d13C during the mid-Holocene (especially around 5.7 and 4.8 kyr BP) indicate more variable conditions. The consistent carbon isotopic enrichment of Unit II vs. Unit I alkenones by ca. 1–2‰, contrasts with the more depleted d13C values of the benthic foraminifera in Unit I. Thus, changes in DIC $\delta^{13}C$ cannot explain the enriched d13C alkenone values in Unit II. These results are consistent with Xu et al. (2001), who also found more enriched carbon isotopic values for the $C_{36:2}$ ethyl ketone, confirming that the origin of the C_{36} alkenone is distinct from other ($C_{37:2}$ and $C_{37:3}$) alkenones.

As for $\delta^{13}C$, there is a distinct and consistent offset in δD between alkenones from Unit I and Unit II. The D values of the $C_{36:2}$ and $C_{38:2}$ ethyl ketones from Unit II are unusual, since D enriched values of between -79‰ and -115‰ have only rarely been observed in natural systems (Giosan et al., 2012) and are offset from δD values in Unit I sediments by ca. 100‰. Controlled growth experiments with *E. huxleyi* have shown that the hydrogen isotopic composition of alkenones is linearly correlated with the hydrogen isotopic composition of the water they grow in (Englebrecht and Sachs, 2005). Schouten et al. (2006) showed that the degree of fractionation between water and alkenone δD values by *E. huxleyi* and *G. oceanica* decreases when salinity increases. However, this salinity effect on δD was not found for alkenones produced in Chesapeake Bay by coastal haptophytes (Schwab and Sachs, 2011). Schwab and Sachs (2011) suggested that the coastal haptophytes might have a greater osmoregulation capacity than marine haptophyte species, which would result in a different fractionation. Alternatively, a number of different haptophyte species may contribute alkenones in Chesapeake Bay, each with a different sensitivity to salinity (Schwab and Sachs, 2011).

The δD values of the ‘‘regular’’ alkenones in Charlotte Harbor Unit I (Fig. 6) are close to those for alkenones in Chesapeake Bay water in the salinity range 10–28 (Schwab and Sachs, 2011), the range of salinity in Charlotte Harbor estuary today (e.g. Miller and McPherson, 1991; Surge and Walker, 2005). Since there are no experimental data for $C_{36:2}$ ethyl ketones, it is not possible to directly calculate corresponding salinity values. Based on the regression between δD of C_{37} alkenones and salinity established by Schouten et al., 2006 for *E. huxleyi* and *G. oceanica*, measured δD values of around -100‰ suggest unrealistically high salinity values in excess of 50. Although chain length is known to affect δD values, and may explain ca. 15‰ of the observed difference in δD (Schwab and Sachs, 2009), the remaining offset implies that other mechanisms are involved. This could be due either to a difference in the relationship between δD and seawater salinity, or to changes in fractionation related to the source organism.

A marked increase in estuarine water δD can only be explained by enhanced net evaporation, since the range in the δD of the precipitation is limited (between -10‰ and -30‰; Bowen, 2008). Water in the Charlotte Harbor estuary is a mix of relatively D enriched marine water from the Gulf of Mexico (δD 0‰) whereas rivers and rain supply more depleted water. The enhanced net evaporation required to explain the observed 100‰ difference in δD between mid and late Holocene sediments would involve an increase in salinity in the bay. To test whether such an increase in salinity was viable during the mid-Holocene, oxygen isotopes were analyzed on benthic foramn *Ammonia* sp. A potential vital effect is expected to be small

for oxygen isotopes for this species (e.g. Diz et al., 2012). The oxygen isotopic composition ($\delta^{18}O$) of the foramn thus primarily reflects water temperature and $\delta^{18}O$ of the surrounding water (McCrea, 1950), which in turn reflects salinity. Irrespective of the underlying mechanism responsible, the observed changes in $\delta^{18}O$ are modest compared with the variations in alkenone δD.

The δD values of the alkenones in mid-Holocene sediments of Charlotte Harbor strongly deviate from δD values for alkenones from *E. huxleyi* (Schouten et al., 2006) and from values found for alkenones in other modern brackish environments studied thus far (Schwab and Sachs, 2011). The C_{36} alkenones in sediments from the Black Sea were also relatively enriched in deuterium with reported values between -112‰ and -162‰ (Giosan et al., 2012) corresponding, based on co-occuring C_{37} alkenones, to salinity between 20 and 30. The offset in δD between C_{36} and C_{37} alkenones in the Black Sea of ca. 100‰ seems unrelated to the salinity changes. This suggests that the two types of alkenones derive from different organisms, which – although living in the same environment – fractionate differently. Although Coolen et al. (2009) suggest that *E. huxleyi* in the Black Sea is the most likely source for the C_{36} alkenones, it is difficult to reconcile the differences in isotopic fractionation with this explanation. Overall, the unusual double bond positions, in combination with the significantly enriched δD values for the alkenones, suggest that the C_{36} alkenones do not derive from any known alkenone-producing haptophytes, but are instead produced by an unknown species.

6. Conclusions

Unusual C_{35} to C_{38} alkenones were identified in mid-Holocene sediments from an estuary in southwest Florida (Charlotte Harbor). The $C_{36:2}$ EK (ω15,20) is the most abundant and is identical in structure to the C_{36} alkenone found in, amongst others, Holocene sediments of the Black Sea. Conventional radiocarbon ages of alkenones are 530 and 980 yr older than co-eval and higher plant-derived long chain *n*-alcohols, although the offset may not be significant considering the reservoir effects and ^{14}C measurement uncertainty. Both alkenones and *n*-alcohols are older than calibrated ages of shell (fragments) in the same sediment core, indicating pre-aging of OM likely through cycles of fine-grained sediment re-suspension and re-deposition. The δD values of alkenones in Charlotte Harbor late Holocene sediments are consistent with those of alkenones produced by coastal haptophytes (Schwab and Sachs, 2011), whereas δD values for the mid-Holocene C_{36} alkenones are strongly (ca. 100‰) enriched in the heavy isotope. These enriched δD values cannot be explained by differences in salinity alone. We conclude that the unusual C_{36} alkenones in the mid Holocene sediments are produced by an unknown haptophyte species which exhibits different fractionation effects associated with alkenone biosynthesis relative to the known alkenone producers.

Acknowledgements

This research was funded by a HIPO grant to G.J.R., F. Wagner- Cremer and S. Dekker. We thank G. Nobbe, J. Kubiak, M. Kienhuis and D.B. Kasjaniuk (UU), D. Montluçon and S. Sylva (WHOI), and R. Larson and G. Brooks (Eckerd College) for technical support. We thank the staff at the National Ocean Sciences AMS (NOSAMS) facility at WHOI for carbon

isotope measurements and J.-F. Rontani and an anonymous reviewer referee for helpful comments on the manuscript.

References

Bidigare, R.R., Fluegge, A., Freeman, K.H., Hanson, K.L., Hayes, J.M., Hollander, D., Jasper, J.P., King, L.L., Laws, E.A., Milder, J., Millero, F.J., Pancost, R., Popp, B.N., Steinberg, P.A., Wakeham, S.G., 1997. Consistent fractionation of ^{13}C in nature and in the laboratory: growth-rate effects in some haptophyte algae. *Global Biogeochemical Cycles* 11, 279–292.

Bowen, G.J., 2008. The Online Isotopes in Precipitation Calculator, version 2.2. <http://www.waterisotopes.org>.

Brassell, S.C., Dumitrescu, M., 2004. Recognition of alkenones in a lower Aptian porcellanite from the west-central Pacific. *Organic Geochemistry* 35, 181–188.

Brassell, S.C., Eglinton, G., Marlowe, I.T., Pflaumann, U., Sarnthein, M., 1986. Molecular stratigraphy: a new tool for climate assessment. *Nature* 320, 129– 133.

Conte, M.H., Thompson, A., Eglinton, G., Green, J.C., 1995. Lipid biomarker diversity in the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae) and the related species Gephyrocapsa oceanica. *Journal of Phycology* 31, 272–282.

Coolen, M.J.L., Boere, A., Abbas, B., Baas, M., Wakeham, S.G., Sinninghe Damsté, J.S., 2006. Ancient DNA derived from alkenone-biosynthesizing haptophytes and other algae in Holocene sediments from the Black Sea. *Paleoceanography* 21, PA1005.

Coolen, M.J.L., Saenz, J.P., Giosan, L., Trowbridge, N.Y., Dimitrov, P., Dimitrov, D., Eglinton, T.I., 2009. DNA and lipid molecular stratigraphic records of haptophyte succession in the Black Sea during the Holocene. *Earth and Planetary Science Letters* 284, 610–621.

Diz, P., Barras, C., Geslin, E., Reichart, G.J., Metzger, E., Jorissen, F., Bijma, J., 2012. Incorporation of Mg and Sr and oxygen and carbon stable isotopic fractionation in cultured *Ammonia tepida*. *Marine Micropaleontology* 92–93, 16–28.

Diz, P., Jorissen, F.J., Reichart, G.J., Poulain, C., Deharis, F., Leorri, E., Paulet, Y.M., 2009. Interpretation of benthic forminiferal stable isotopes in subtidal estuarine environments. Biogeosciences 6, 2549–2560.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. *Science* 156, 1322–1335.

Eglinton, G., Gonzalez, A.G., Hamilton, R.J., Raphael, R.A., 1962. Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. *Phytochemistry* 1, 89–102.

Englebrecht, A.C., Sachs, J.P., 2005. Determination of sediment provenance at drift sites using hydrogen isotopes and unsaturation ratios in alkenones. *Geochimica et Cosmochimica Acta* 69, 4253–4265.

Evans, M.W., Hine, A.C., Belknap, D.F., 1989. Quaternary stratigraphy of the Charlotte Harbor estuarine-lagoon system, southwest Florida: implications of the carbonate–siliciclastic transition. *Marine Geology* 88, 319–348.

Fraser, T.H., 1986. Long-term Water Quality Characteristics of Charlotte Harbor, Florida. Water-resources Investigations Report, Environmental Quality Laboratory, Inc., Porth Charlotte Harbor 86-4180, 43pp.

Fujine, K., Yamamoto, M., Tada, R., Kido, Y., 2006. A salinity-related occurrence of a novel alkenone and alkenoate in Late Pleistocene sediments from the Japan Sea. *Organic Geochemistry* 37, 1074–1084.

Giosan, L., Coolen, M.J.L., Kaplan, J.O., Constantinescu, S., Filip, F., Filipova-Marinova, M., Kettner, A.J., Thom, N., 2012. Early anthropogenic transformation of the Danube-Black Sea system. *Nature Scientific Reports* 2, 1–6.

Heyl, M.G., 1998. Hypoxia in upper Charlotte Harbor. In: Treat, S.F. (Ed.). Proceedings of the Charlotte Harbor Public Conference and Technical Symposium. *Charlotte Harbor National Estuary Program Technical Report* No. 98-02, pp. 219–227.

Hine, A.C., Evans, R.A., et al., 1987. Depositional response to seagrass mortality along a low-energy, barrier island coast: West-central Florida. *Journal of Sedimentary Petrology* 57, 431–439.

Hoefs, J., 1980. Stable Isotope Geochemistry. Springer, Berlin, 208 pp. Lane, P., Donnelly, J.P., Woodruff, J.D., Hawkes, A.D., 2011. A decadally-resolved paleohurricane record archived in the late Holocene sediments of a Florida sinkhole. *Marine Geology* 287, 14–30.

Leonhardt, B.A., DeVilbiss, E.D., 1985. Separation and double-bond determination on nanogram quantities of aliphatic monounsaturated alcohols, aldehydes and carboxylic acid methyl esters. *Journal of Chromatography* A 322, 484– 490.

Lopez, J.F., de Oteyza, T.G., Teixidor, P., Grimalt, J.O., 2005. Long chain alkenones in hypersaline and marine coastal microbial mats. *Organic Geochemistry* 36, 861– 872.

Marlowe, I.T., Green, J.C., Neal, A.C., Brassell, S.C., Eglinton, G., Course, P.A., 1984. Long chain (*n*-C_{37}–C_{39}) alkenones in the Prymnesiophyceae. Distribution of alkenones and other lipids and their taxonomic significance. *British Phycological Journal* 19, 203–216.

McCrea, J.M., 1950. On the isotope chemistry of carbonates and a paleotemperature scale. *Journal of Chemical Physics* 18, 849–857.

Miller, R.L., McPherson, B.F., 1991. Estimating estuarine flushing and residence times in Charlotte Harbor, Florida, via salt balance and a box model. *Limnology and Oceanography* 36, 602–612.

Mollenhauer, G., Eglinton, T.I., 2007. Diagenetic and sedimentological controls on the composition of organic matter preserved in California Borderland Basin sediments. *Limnology and Oceanography* 52, 558–576.

Mollenhauer, G., Montlucon, D.B., Eglinton, T.I., 2005a. Radiocarbon dating of alkenones from marine sediments: II. Assessment of carbon process blanks. *Radiocarbon* 47, 413–424.

Mollenhauer, G., Kienast, M., Lamy, F., Meggers, H., Schneider, R.R., Hayes, J.M., Eglinton, T.I., 2005b. An evaluation of 14C age relationships between cooccurring foraminifera, alkenones, and total organic carbon in continental margin sediments. *Paleoceanography* 20, PA1016.

Müller, P.J., Kirst, G., Ruhland, G., von Storch, I., Rosell-Melé, A., 1998. Calibration of the alkenone paleotemperature index UK'_{37} based on core-tops from the eastern South Atlantic and the global ocean (60°N–60°S). *Geochimica et Cosmochimica Acta* 62, 1757–1772.

Ohkouchi, N., Eglinton, T.I., Keigwin, L.D., Hayes, J.M., 2002. Spatial and temporal offsets between proxy records in a sediment drift. *Science* 298, 1224–1227.

Ohkouchi, N., Xu, L., Reddy, C.M., Montlucon, D.B., Eglinton, T.I., 2005. Radiocarbon dating of alkenones from marine sediments: I. Isolation protocol. *Radiocarbon* 47, 401–412.

Pagani, M., Freeman, K.H., Ohkouchi, N., Caldeira, K., 2002. Comparison of water column [CO_2aq] with sedimentary alkenone-based estimates: a test of the alkenone-CO_2 proxy. *Paleoceanography* 17, 1069.

Popp, B.N., Hanson, K.L., Dore, J.E., Bidigare, R.R., Laws, E.A., Wakeham, S.G., 1999. Controls on the carbon isotopic composition of phytoplankton: paleoceanographic perpectives. In: Abrantes, F., Mix, A. (Eds.), Reconstructing Ocean History: A Window into the Future. Plenum, New York.

Prahl, F.G., Wakeham, S.G., 1987. Calibration of unsaturation patterns in long-chain ketone compositions for palaeotemperature assessment. *Nature* 330, 367–369.

Prahl, F.G., Rontani, J., Volkman, J.K., Sparrow, M.A., Royer, I.M., 2006. Unusual C_{35} and C_{36} alkenones in a paleoceanographic benchmark strain of *Emiliania huxleyi*. *Geochimica et Cosmochimica Acta* 70, 2856–2867.

Rontani, J., Wakeham, S.G., 2008. Alteration of alkenone unsaturation ratio with depth in the Black Sea: potential roles of stereomutation and aerobic biodegradation. *Organic Geochemistry* 39, 1259–1268.

Rontani, J., Marchand, D., Volkman, J.K., 2001. $NaBH_4$ reduction of alkenones to the corresponding alkenols: a useful tool for their characterisation in natural samples. *Organic Geochemistry* 32, 1329–1341.

Rontani, J., Beker, B., Volkman, J.K., 2004. Long-chain alkenones and related compounds in the benthic haptophyte *Chrysotila lamellosa* Anand HAP 17. *Phytochemistry* 65, 117–126.

Rontani, J., Prahl, F.G., Volkman, J.K., 2006a. Characterization of unusual alkenones and alkyl alkenoates by electron ionization gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 20, 583–588.

Rontani, J., Prahl, F.G., Volkman, J.K., 2006b. Re-examination of the double bond positions in alkenones and derivatives: biosynthetic implications. *Journal of Phycology* 42, 800–813.

Schouten, S., Ossebaar, J., Schreiber, K., Kienhuis, M.V.M., Langer, G., Benthien, A., Bijma, J., 2006. The effect of temperature, salinity and growth rate on the stable hydrogen isotopic composition of long chain alkenones produced by *Emiliania huxleyi* and *Gephyrocapsa oceanica*. *Biogeosciences* 3, 113–119.

Schwab, V.F., Sachs, J.P., 2009. The measurement of D/H ratio in alkenones and their isotopic heterogeneity. *Organic Geochemistry* 40, 111–118.

Schwab, V.F., Sachs, J.P., 2011. Hydrogen isotopes in individual alkenones from the Chesapeake Bay estuary. *Geochimica et Cosmochimica Acta* 75, 7552–7565.

Stoker, Y.E., 1992. Salinity Distribution and Variation with Freshwater Inflow and Tide, and Potential Changes in Salinity due to Altered Freshwater Inflow in the Charlotte Harbor Estuarine System, Florida. Water-Resources Investigations Report. US Geological Survey, 92-4062, 30pp.

Stuiver, M., Polach, H.A., 1977. Discussion reporting of ^{14}C data. *Radiocarbon* 19, 355–363.

Stuiver, M., Reimer, P.J., 1993. Extended ^{14}C database and revised CALIB radiocarbon calibration program. *Radiocarbon* 35, 215–230.

Surge, D., Walker, K.J., 2005. Oxygen isotope composition of modern and archaeological otoliths from the estuarine hardhead catfish (Ariopsis felis) and their potential to record low-latitude climate change. Palaeogeography, Palaeoclimatology, Palaeoecology 228, 179–191.

Tanner, W.F., 1960. Florida coastal classification. Gulf Coast Association of Geological Societies Transactions 10, 259–266. Taylor, J.L., 1974. The Charlotte Harbor estuarine system. *Florida Scientist* 37, 205– 216.

van Soelen, E.E., Lammertsma, E.I., Cremer, H., Donders, T.H., Sangiorgi, F., Brooks, G.R., Larson, R.A., Sinninghe Damsté, J.S., Wagner-Cremer, F., Reichart, G.J., 2010. Late Holocene sea-level rise in Tampa Bay: integrated reconstruction using biomarkers, pollen,

organic-walled dinoflagellate cysts, and diatoms. *Estuarine, Coastal and Shelf Science* 86, 216–224.

van Soelen, E.E., Brook, G.R., Larson, R.A., Sinninghe Damsté, J.S., Reichart, G.J., 2012. Mid- to late Holocene coastal environmental changes in southwest Florida. *The Holocene* 22, 929–938.

Volkman, J.K., Eglinton, G., Corner, E.D.S., Forsberg, T.E.V., 1980. Long-chain alkenes and alkenones in the marine coccolithophorid Emiliania huxleyi. *Phytochemistry* 19, 2619–2622.

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Sikes, E.L., 1995. Alkenones in Gephyrocapsa oceanica: implications for studies of paleoclimate. *Geochimica et Cosmochimica Acta* 59, 513–520.

Xu, L., Reddy, C.M., Farrington, J.W., Frysinger, G.S., Gaines, R.B., Johnson, C.G., Nelson, R.K., Eglinton, T.I., 2001. Identification of a novel alkenone in Black Sea sediments. *Organic Geochemistry* 32, 633–645.

Zink, K.G., Leythaeuser, D., Melkonian, M., Schwark, L., 2001. Temperature dependency of long chain alkenone distributions in recent to fossil limnic sediments and in lake waters. *Geochimica et Cosmochimica Acta* 65, 253–265.

Fig. 1. Core locations of CH1 and CH3 in Charlotte Harbor estuary, Florida [modified from Evans et al. (1989)].

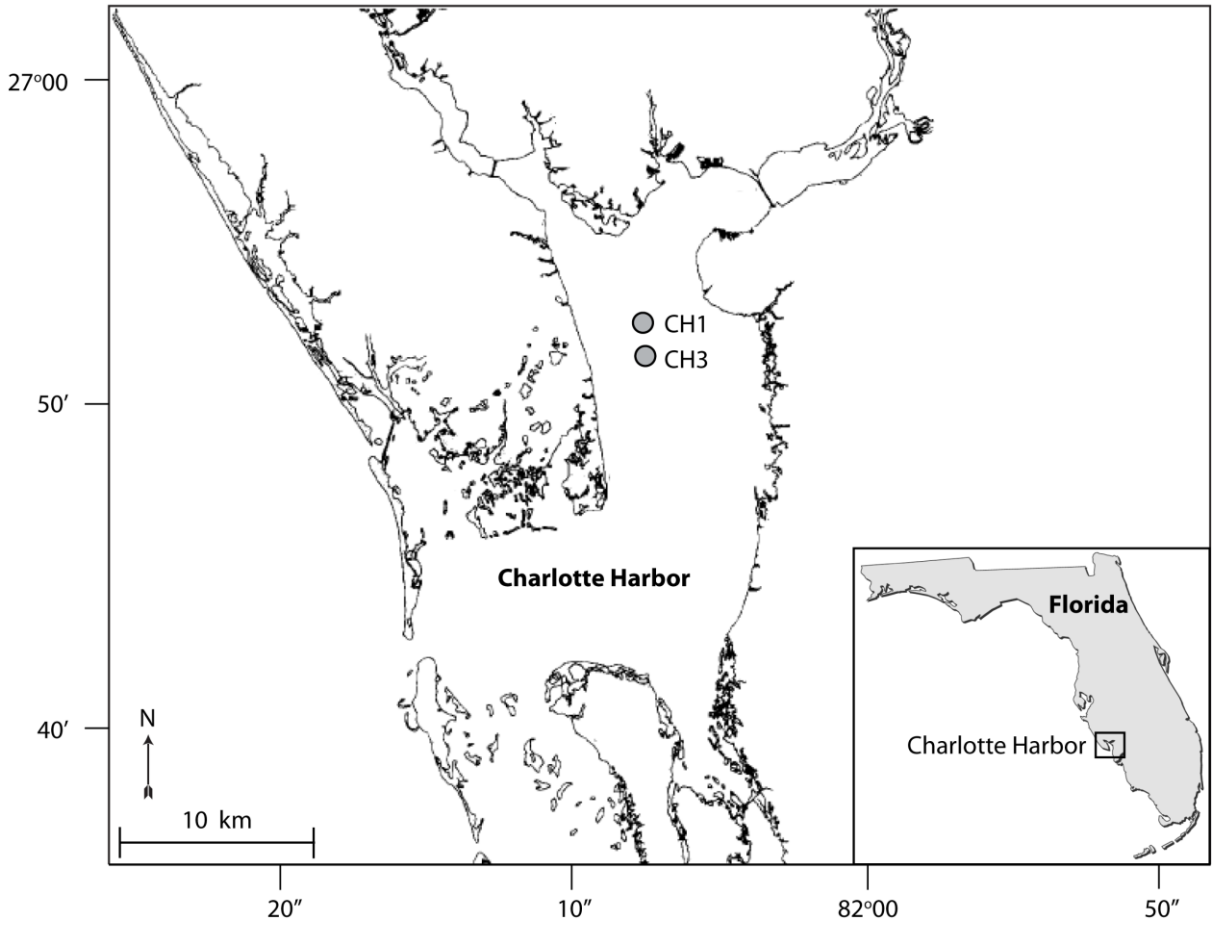

Fig. 2. Core photo and XRF data from cores CH1 and CH3 [modified from van Soelen et al. (2012)]. The dotted lines indicated with (1) and (2) are coarser grained layers used to correlate the two cores. Black lines indicate a transition between sedimentological units. Ages are based on AMS dated shell fragments and presented in calibrated years before present. Gray bars and lines indicate sample depth. Sample CH3-A (0–10 cm) and CH3-C (260–270 cm) were used for alkenone identification, CH3-B (120–150 cm) and CH3-D (270–300 cm) for compound specific radiocarbon dating of alcohols and alkenones, and CH1-I to CH1-VI and CH3-C for compound specific stable hydrogen and carbon isotope measurements.

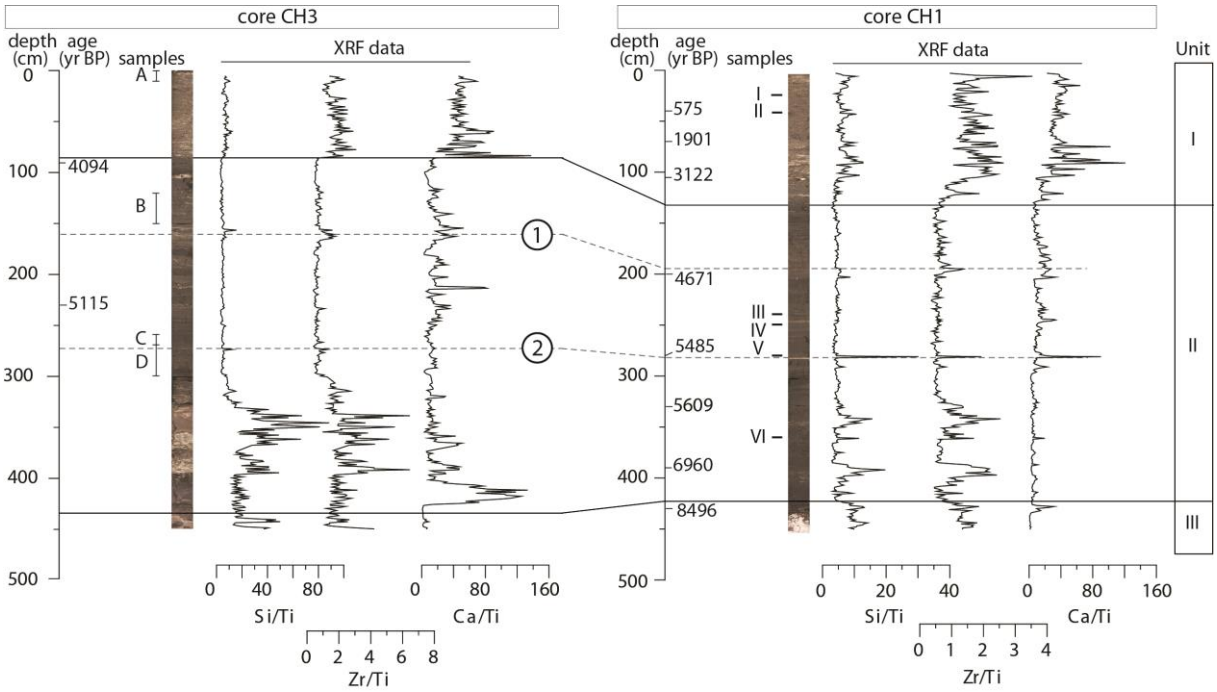

Fig. 3. The left side panels show partial gas chromatograms (FID) of alkenone fractions from CH3-A (a) and CH3-C (b). Other panels (c–f) show mass spectra corresponding to peaks I–IV in the chromatogram of Fig. b.

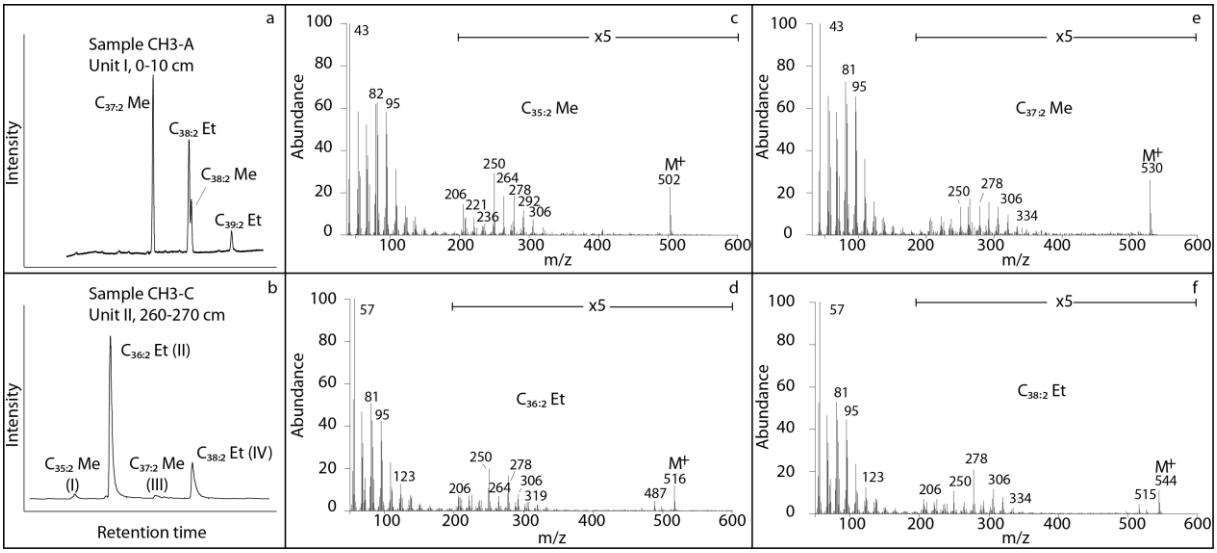

Fig. 4. Conventional radiocarbon ages of n-alcohols and alkenones in CH3-B (120–150 cm) and CH3-D (270–300 cm). Alcohols (circles) were measured individually and alkenones (diamonds) as a combined fraction containing C_{36}–C_{38} alkenones.

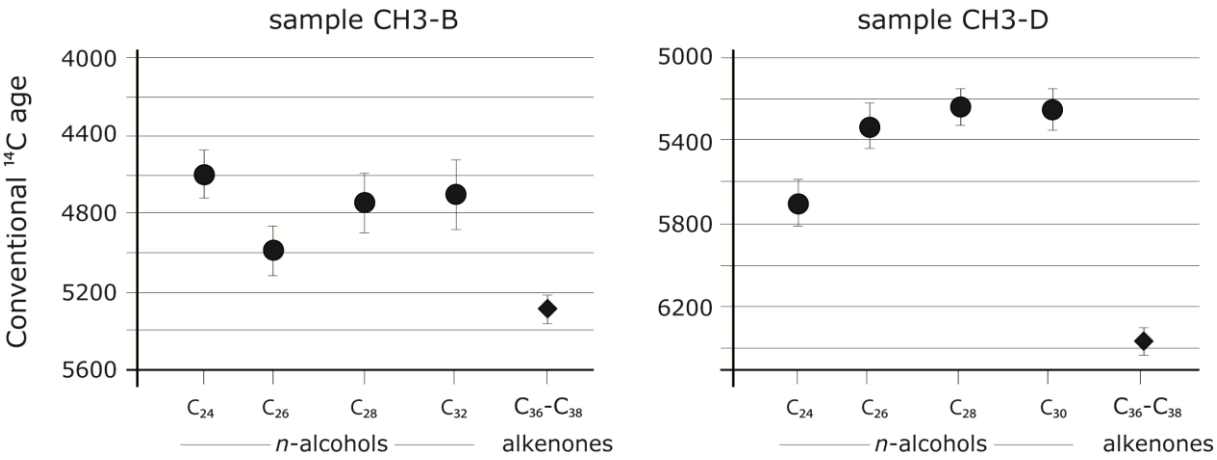

Fig. 5. Calibrated radiocarbon ages. Compounds (*n*-alcohols and alkenones) are from core CH3, shell fragments from core CH1 and CH3. The two cores were correlated based on specific marker beds (see also Fig. 2).

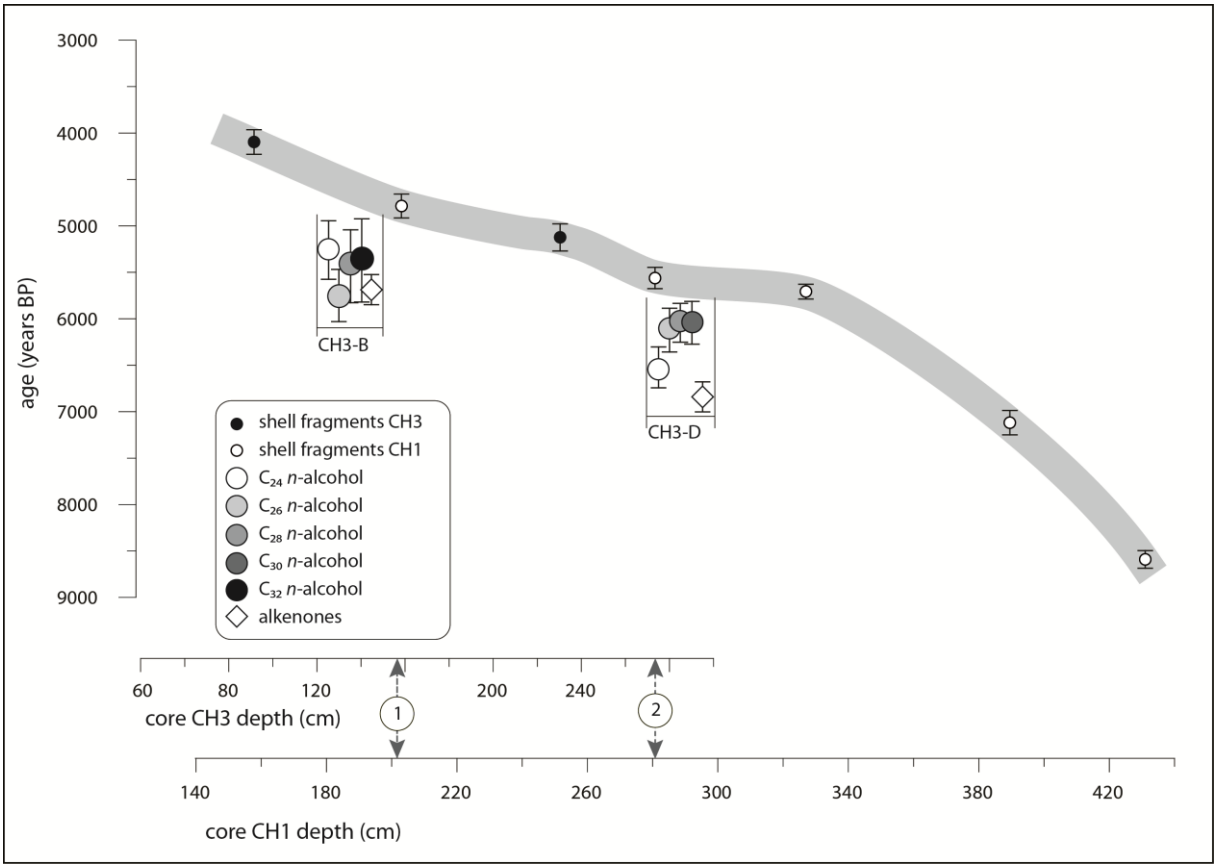

Fig. 6. Concentrations of C_{22}–C_{30} *n*-alcohols (a), C_{37} + C_{38} alkenones (b) and C_{36} alkenones (c) in sediments from core CH1. Carbon and oxygen isotope ratios of the benthic foraminifera *Ammonia* spp. are shown in figures d and e, respectively. Figs. f and g show compound specific hydrogen and carbon isotopes of C_{36}–C_{39} alkenones.

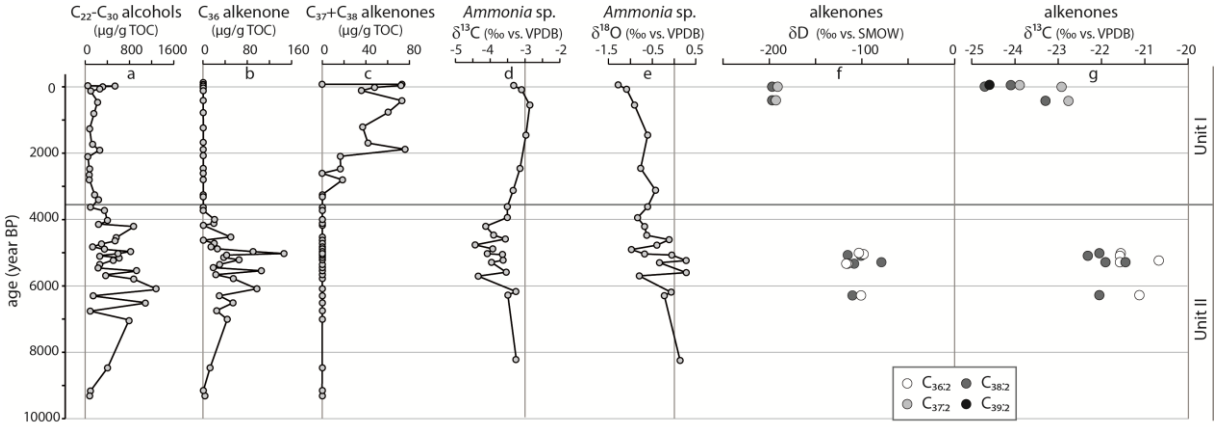

Table 1. Carbon isotopic composition and AMS data for alcohols and alkenones in Charlotte Harbor sediments, reported as fraction modern (fM), conventional radiocarbon ages (Conv. 14 age) and calibrated ages in year before present (Cal. age 2-sigma).

Sample	Depth (cm)	Compound	$\delta^{13}C$ (‰)	fM	Conv. ^{14}C age (year BP)	Calibration curve	Cal. age 2-sigma (year BP)
CH3-B	120—150	C_{24} *n*-alcohol	-25.0[a]	0.564±0.009	4600±120	INTCAL09	5250±310
		C_{26} *n*-alcohol	-29.2	0.537±0.009	4990±130	INTCAL09	5740±280
		C_{28} *n*-alcohol	-30.2	0.554±0.011	4750±150	INTCAL09	5420±390
		C_{32} *n*-alcohol	-25.0[a]	0.557±0.013	4700±180	INTCAL09	5360±440
		Alkenones (C_{36}-C_{38})	-22.4	0.518±0.005	5290±70	MARINE09	5680±170
CH3-D	270—300	C_{24} *n*-alcohol	-29.3	0.492±0.007	5700±110	INTCAL09	6510±220
		C_{26} *n*-alcohol	-29.7	0.515±0.007	5330±110	INTCAL09	6110±230
		C_{28} *n*-alcohol	-31.5	0.521±0.006	5240±90	INTCAL09	6030±210
		C_{30} *n*-alcohol	-30.4	0.520±0.007	5250±100	INTCAL09	6030±230
		Alkenones (C_{36}-C_{38})	-23.4	0.453±0.003	6360±60	MARINE09	6830±160

[a] assumed values

Table 2. Compound specific stable hydrogen and carbon isotopic composition of alkenones in Charlotte Harbor sediments (δD in ‰ vs. SMOW, $\delta^{13}C$ in ‰ vs. PDB).

Sample	Depth (cm)		$C_{36:2}$ EK	$C_{37:2}$ MK	$C_{38:2}$ EK+MK	$C_{38:2}$ EK	$C_{39:2}$ EK
CH1-I	24	δD		-191	-196		
		$\delta^{13}C$		-22.9	-24.7		
CH1-II	42	δD		-195	-198		
		$\delta^{13}C$		-22.8	-23.3		
CH1-III	240	δD	-103			-114	
		$\delta^{13}C$	-21.5			-22.1	
CH1-IV	250	δD	-98			-102	
		$\delta^{13}C$	-21.6			-22.3	
CH1-V	280	δD	-114			-108	
		$\delta^{13}C$	-20.7			-21.9	
CH1-VI	360	δD	-101			-111	
		$\delta^{13}C$	-21.1			-22.1	
CH3-A	0—10	δD					
		$\delta^{13}C$		-23.9	-24.1		-24.6
CH3-C	260—270	δD	-114 (stdv. 1.0)			-79 (stdv. 1.0)	
		$\delta^{13}C$	-21.5 (stdv. 0.3)			-21.6 (stdv. 0.2)	