Hydrogen isotopes in individual alkenones from the Chesapeake Bay estuary

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Abstract

Hydrogen isotope ratios of individual alkenones from haptophyte algae were measured in suspended particles and surface sediment from the Chesapeake Bay (CB) estuary, eastern USA, in order to determine their relationship to water δD values and salinity. δD values of four alkenones (MeC37:2, MeC37:3, EtC38:2, EtC38:3) from particles and sediments were between −165‰ and −221‰ and increased linearly (R² = 0.7–0.9) with water δD values from the head to the mouth of the Bay. Individual alkenones were depleted in deuterium by 156–188‰ relative to water. The MeC37 alkenones were consistently enriched by 12‰ relative to the EtC38 alkenones, and the di-unsaturated alkenones of both varieties were consistently enriched by 20‰ relative to the tri-unsaturated alkenones. All of the increase in alkenone δD values could be accounted for by the water δD increase. Consequently, no net change in alkenone–water D/H fractionation occurred as a result of the salinity increase from 10 to 29. This observation is at odds with results from culture studies with alkenone-producing marine coccolithophorids, and from two field studies, one with a dinoflagellate sterol in the CB, and one with a wide variety of lipids in saline ponds on Christmas Island, that indicate a decline in D/H fractionation with increasing salinity. Why D/H fractionation in alkenones in the CB showed no dependence on salinity, while D/H fractionation in CB dinsoterol decreased by 1‰ per unit increase in salinity remains to be determined. Two hypotheses we consider to be valid are that (i) the assemblage of alkenone-producing haptophytes changes along the Bay and each species has a different sensitivity to salinity, such that no apparent trend in alkenone–water occurs along the salinity gradient, and (ii) greater osmoregulation capacity in coastal haptophytes may result in a diminished sensitivity of alkenone–water D/H fractionation to salinity changes.

1. INTRODUCTION

The hydrogen isotopic composition of algal lipids closely co-varies with the hydrogen isotopic composition of the water in which the organisms grew (Sessions et al., 1999; Englebrecht and Sachs, 2005; Zhang and Sachs, 2007; Schwab and Sachs, 2009; Sachs and Schwab, 2011). Algal lipid D/H ratios in sediments have thus been increasingly used to reconstruct the water cycle in past environments (e.g., Sauer et al., 2001; Huang et al., 2002; Pahnke et al., 2007; Van der Meer et al., 2007, 2008; Sachs et al., 2009). However, beside the biosynthetic pathways (Sessions et al., 1999) and species-specific differences (Schouten et al., 2006; Zhang and Sachs, 2007), environmental parameters including temperature (Wollhowe et al., 2009; Zhang et al., 2009), nitrogen limited growth rate (Zhang et al., 2009), growth phase (Wollhowe et al., 2009) and salinity (Schouten et al., 2006; Sachse and Sachs, 2008; Sachs and Schwab, 2011) have been shown to influence hydrogen isotope fractionation during lipid synthesis by algae, potentially complicating paleohydrologic interpretations.

For instance, culture (Schouten et al., 2006) and field studies (Sachse and Sachs, 2008; Sachs and Schwab, 2011) demonstrated that increased salinity resulted in decreased D/H fractionation between environmental water and the
biosynthesized lipids. Paleosalinity and paleohydrologic reconstructions based on lipid $\delta D$ values soon followed (Pahnke et al., 2007; Van der Meer et al., 2007, 2008; Sachs et al., 2009; Smittenberg et al., 2011). Internal cell water is the main donor of hydrogen atoms in all positions of the lipid chain (Duan et al., 2002; Baillif et al., 2009), and thus its hydrogen isotope composition is likely to be directly reflected in the biosynthesized lipids (Kreuzer-Martin et al., 2006). It has been thus proposed that the D-enrichment in algal lipids as salinity increases reflects progressive D-enrichment of internal cell water due to its continual enrichment as biosynthetic reactions draw D-depleted hydrogen from the internal water pool in combination with reduced exchange with external water (Sachse and Sachs, 2008; Sachs and Schwab, 2011). A refinement put forth by Sachs and Schwab (2011) is that turgor regulation via increased osmolyte production siphons off isotopically depleted H, leaving internal water used for lipid synthesis enriched in D. Nevertheless, the mechanisms controlling the sensitivity of lipid $\delta D$ values to salinity are still being evaluated and a general theory to explain the phenomenon is lacking.

Prymnesiophyte algae are the unique producers of alkenones (Volkman et al., 1980; Marlowe et al., 1984). In the modern ocean, they are produced primarily by the species Emiliania huxleyi and Gephyrocapsa oceanica, (Marlowe et al., 1984, 1990; Conte et al., 1995; Volkman et al., 1995) while in coastal and lacustrine environments, the main producers are likely the species Isochrysis galbana and Chryso-tila lamellosa (e.g., Volkman et al., 1988; Li et al., 1996; Thiel et al., 1997; Schulz et al., 2000; Chu et al., 2005; Sun et al., 2007).

Heretofore, the measurement of alkenone $\delta D$ values has been performed on a mixture of the C$_{37}$:3 and C$_{37}$:2 alkenones owing to the inability to achieve adequate separation between the two compounds during gas chromatography (Englebrecht and Sachs, 2005; Schouten et al., 2006; Pahnke et al., 2007; Van der Meer et al., 2007, 2008). Separation techniques using column argentation chromatography or recently developed high-performance liquid chromatography-mass spectrometry (HPLC-MS) permit the purification of each individual alkenone for stable hydrogen isotope analysis based on the number of carbon atoms and double bonds (D’Andrea et al., 2007; Schwab and Sachs, 2009). Using the HPLC-MS technique, a systematic ca. 20%$_{\text{iso}}$ difference in the $\delta D$ values of C$_{37}$:3 and C$_{37}$:2 alkenones (where $\chi = 37$ or 38) was measured in sediments and particles from a site in the CB estuary (Schwab and Sachs, 2009). An identical hydro- gen isotopic offset between C$_{37}$:3 and C$_{37}$:2 alkenones ($\chi$C$_{37}$:3-C$_{37}$:2 = $(\delta D_{C_{37}:3}+1)/(\delta D_{C_{37}:2}+1) = 0.97$) was recently measured in cultured E. huxleyi and G. oceanica harvested at different growth stages (Wolhowe et al., 2009). The similar isotopic offset between C$_{37}$:3 and C$_{37}$:2 alkenones in both studies, despite the likely presence of alkenone producers in the Chesapeake Bay (CB) other than E. huxleyi and G. oceanica, led Wolhowe et al. (2009) to propose that a simple isotopic mass balance calculation could be used to determine $\delta D$ values for individual alkenones measured from total C$_{37}$ alkenones (i.e., the combined C$_{37}$:2 + C$_{37}$:3 alkenone peak eluting from a GC) without labor-intensive chemical separations.

To test the fidelity with which individual alkenones record water $\delta D$ values and to evaluate the influence of salinity on $D/H$ fractionation in alkenones, we measured the hydrogen isotope composition of these compounds along the salinity (10–29) and water $\delta D$ ($\sim 37.6^\circ_{\text{SMOW}}$ to $\sim 10.6^\circ_{\text{SMOW}}$) gradient in the CB estuary in Maryland and Virginia, Eastern USA. The same set of samples was used by Sachs and Schwab (2011) to demonstrate that about half of the increase in dimosterol $\delta D$ values along the CB resulted from increasing water $\delta D$ values, and the other half from decreasing $D/H$ fractionation as salinity increased. Our results are then used to test the hypothesis of Wolhowe et al. (2009) that the hydrogen isotope offsets between individual alkenones are constant in an estuarine environment characterized by large salinity variations.

2. MATERIALS AND METHODS

2.1. Geological setting and water chemistry

Located in the Mid-Atlantic region of North America, the CB is a N–S elongate (300 km), shallow (average depth 8.2 m) estuary with an axial channel that is 12–30 m deep (Fig. 1). Freshwater from the Susquehanna River at the head of the Bay supplies 48% of the total riverine flux of water to the CB and creates typical partially mixed estu-arine circulation with an outflow of freshwater in the upper layers and an inflow of seawater at depth. A salinity gradient along the length of the CB, and as a function of depth is thus formed (e.g., Pritchard, 1967; Austin, 2004).

Multi-decadal time series of monthly physical, chemical and biological parameters in the CB are available on the website of the CB Monitoring Program http://www.chesapeakebay.net/data_waterquality. Salinity, water $\delta D$ values, and water temperature at multiple depths along the N–S transect of the Bay in May 22–25, 2006 are published in Sachs and Schwab (2011). Nutrient, Chl $\alpha$ concentration and primary production at the same sampling locations are available on the CB Monitoring Program website. Briefly, surface (0–2 m) salinity and $\delta D$ values increased from 0 to $\sim 56.8^\circ_{\text{SMOW}}$ (station CB1.1) at the head of the CB, where the Susquehanna River enters, to 28.9 and $\sim 11.4^\circ_{\text{SMOW}}$ (station CB7.4N) at the mouth of the Bay (Fig. 1). The water $\delta D$ values, previously published in Sachs and Schwab (2011), were linearly correlated with salinity (increasing with depth along and the length of the estuary) according to the relationship $\delta D_{\text{water}} = 1.63 \pm 0.04 \times \text{Salinity} - 52.3 \pm 0.7$, $R^2 = 0.91$, with uncertainties represented as the standard errors of the slope and intercept of the regression. The observed relationship reflects fractionation due to evaporation toward more saline media and the mixing line between marine and freshwater (Craig, 1961; Craig and Gordon, 1965). Surface (0–2 m) water temperatures were between 14.9 and 20.1 °C. Lower temperatures occurred in the upper-bay (CB3.2) and lower-bay (CB7.4 N) and higher temperature in the mid-bay (CB6.2) (Fig. 1). A halocline and a thermocline were observed at all CB stations, with bottom waters as much as 15 saltier and 2 °C colder than surface waters. As a result, since freshwaters were depleted in deuterium by 50%$_{\text{iso}}$ relative to seawater, water $\delta D$ values
increased with depth at all stations, with bottom waters as much as 15% heavier than surface waters. Total dissolved nitrogen and phosphate ranged from ca. 0.14 to 0.88 mg L\(^{-1}\) and from 5.0 to 23.6 µg L\(^{-1}\), respectively, with both nutrients most abundant at the head of the Bay and least abundant at the mouth. In May 2006, surface Chl \(a\) concentrations were between 1.07 and 31.4 µg L\(^{-1}\), with lowest values at the mouth (station CB7.4 N) of the Bay and highest values in the mid-Bay region. Maximum chlorophyll concentrations occurred at 3–5 m water depth throughout the Bay. Higher primary production in the mid-Bay resulted in hypoxic (1–30% saturation of dissolved oxygen) or anoxic bottom waters.

2.2. Water, particle and sediment sampling in the Chesapeake Bay

Surface sediment and suspended particles were collected at 26 of the CB Monitoring Program stations along the central axis of the Bay from the R/V Kerhin during May 22–25, 2006 (Fig. 1). Sample collection was timed to coincide with the haptophyte bloom that was expected to occur in late May, after peak runoff fluxes occurred (Mercer et al., 2005), and within two days of both the Maryland and Virginia Monitoring Program’s monthly sampling of the Bay. The coordinates of each station and the detailed sampling procedure are published in Sachs and Schwab (2011). In brief, near surface sediments, collected with a grab sampler, were sub-sampled – typically at 0–2 and 0–10 cm – and subsequently frozen at –20 °C. Water and suspended particle samples were collected at multiple depths at each sampling site – typically 0–2, 2–4, 4–5, 14 and 30 m – by tubing attached to a conductivity–temperature–depth continuous profiling instrument and filtered through glass fiber filters immediately frozen at –20 °C. Water for hydrogen isotope analysis was collected with each filter and between stations. Salinity and temperature were continuously measured on board the vessel.

2.3. Coccolithophore identification

Samples were taken to determine coccolithophore taxonomic composition at each sample depth by vacuum filtering 1–2 L of water through cellulose nitrate and poly-carbonates filters (0.45 µm pore size, 47 mm diameter). Each filter was rinsed with buffered water (63 mL of NH\(_4\)OH in 500 mL of distilled water) to remove salt, then dried at 40 °C. Filters were analyzed by polarized light microscopy at the University of Washington (Seattle, USA) and by scanning electron microscopy (SEM) at the University of Lausanne, Institute of Geology and paleontology (Switzerland).

2.4. Lipid extraction and pre-treatment

The detailed procedure for extracting and purifying alkenones is reported in Schwab and Sachs (2009). In brief, \(n\)-C\(_{37}\) alkane, 2-nonadecanone (C\(_{19}\)-ketone) and cholesterol

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**Fig. 1.** Maps of the CB showing surface (0–2 m) water (a) salinity, (b) \(\delta D\) values and (c) temperature at sample locations (red stars green circles). The station locations from which surface sediment and suspended particle were taken for this study are shown with red stars. Maps were produced with ArcGIS Spatial Analyst, with inverse distance weighted output cell size of 0.001°, 131 salinity points, 105 water \(\delta D\) values, and 122 temperature points. Salinity and temperature data are from the CB monitoring program (http://www.chesapeakebay.net/data_waterquality). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
(Sigma–Aldrich) were added as internal standards to freeze-dried sediment and filters prior to extraction with an Automated Solvent Extractor (ASE-200, Dionex Corp., Sunnyvale, CA, USA). The solvent was removed under a stream of nitrogen using a Turbovap system (Caliper, Hopkinton, MA, USA). The extracts were separated with column chromatography on pre-combusted Al2O3 into four fractions: F1 was eluted with hexane/DCM (9:1, v/v) and contained hydrocarbons, F2 was eluted with hexane/DCM (1:1, v/v) and contained aromatic lipids and ketones (including alkenones), F3 was eluted with DCM/MeOH (1:1, v/v) and contained alcohols and F4 was eluted 100% MeOH and contained acids and polar lipids. Elemental sulphur was removed from F1 and F2 using activated Cu powder.

The alkenones and alkenoates contained in the F2 were identified by Gas Chromatography-Mass Spectrometry (GC–MS) using an Agilent (Santa Clara, CA, USA) 6890 N gas chromatograph equipped with an Agilent 5983 autosampler, a split-splitter injector operated in splitless mode, and a HP-5 ms column (30 m, 0.25 mm i.d. and 0.25 μm film thickness, Agilent) interfaced to an Agilent 5975 quadrupole mass selective detector (MSD). They were quantified with a Gas Chromatograph–Flame Ionization Detector (GC–FID). The Agilent 6890 GC was equipped with an Agilent 5983 autosampler and a programmable temperature vaporization inlet (PTV) operated in splitless mode. A 60 m Varian Chrompac CP-Sil 5 capillary column (0.25 mm × 0.25 μm) was used with helium as the carrier gas (1.6 mL/min).

2.5. Alkenone purification by HPLC-MS

Detailed methods for alkenones purification by HPLC are given in Schwab and Sachs, 2009. Briefly, an Agilent (Santa Clara, CA, USA) high performance liquid chromatograph (HPLC) 1100 series, equipped with an autoinjector, a quaternary pump, an integrated fraction collector, and Chemstation chromatography manager software was used. Typically, one injection of 100 μL of the concentrated F2 (alkenone) fraction was enough for subsequent hydrogen isotope analyses of each individual alkenone. A Prevail Cyanopropyl column (250 × 4.6 mm × 5 μm; Alltech, Deerfield CA) maintained at 30 °C was used for separation. Compound classes were separated using an isocratic mixture of hexane and 4% DCM in hexane. Eluting compounds were identified in real-time with an Agilent 1100 SL mass spectrometer equipped with a multimode source operated in positive atmospheric pressure chemical ionization (APCI+) mode on ca. 2% of the total flow that was split from the eluent using an adjustable flow splitter (ASI, El Sobrante, CA, USA). A Waters (Milford, MA, USA) 510 pump was used to add a flow of 0.3 mL/min 2,2,4-trimethylpentane to the 2% split in order to supply sufficient solvent to the APCI source to achieve optimal ionization efficiency.

2.6. Hydrogen isotope analyses

Alkenones were quantified by comparing their integrated peak areas with that of the n-C37 standard using GC–FID operating at the same conditions previously described. The target compounds were dissolved in toluene to reach the adequate concentration (250–300 ng/μL of the individual alkenones) needed for sufficient signal intensity on the GC–IRMS system (Trace Ultra GC and DELTA V from Thermo Scientific, Waltham, MA, USA). The GC was equipped with a split-splitless injector operated in splitless mode at 300 °C, a TRIPLUS autosampler (Thermo Scientific, Waltham, MA, USA), and a DB-5ms capillary column (60 m × 0.32 mm × 0.25 μm, Agilent). Helium was used as the carrier gas at a constant flow of 1 mL/min.

Instrument performance and the H1+ factor were determined on a daily basis using a tank of H2 reference gas (Sessions et al., 2001) and a mixture of n-alkanes (n-C14 to n-C38) of known isotopic composition. Hydrogen isotopic composition of n-C14 to n-C36, n-alkane was determined off-line using a thermochemical elemental analyzer (TC/EA) (Thermo-Fisher, Bremen, Germany) interfaced to the DELTA V PLUS irMS system via a Confo III combustion interface (Thermo-Fisher, Bremen, Germany). The n-C38, n-C40 and n-C44 standards used for δD corrections and added to the n-alkane mixture for H1+ factor calculation were purchased from A. Schimmelmann, (Indiana University, Bloomington, Indiana). The average offset between GC–irMS δD values and those determined off-line was 3.9‰ (n = 3630, 165 runs with 22 peaks). The H1+ factor was lower than four and stable during the measurements reported here. The precision of alkenone δD measurements, as determined by the pooled standard deviation, was 1.3‰.

3. RESULTS

3.1. Alkenone producers in the Chesapeake Bay

No evidence of calcified haptophyte species, such as the common marine coccolithophorids E. huxleyi and G. oceanica, was found in 10 samples investigated by light microscopy or two samples investigated by SEM despite both those species having been reported in the Bay (Marshall, 1994; Marshall et al., 2005). Although the two samples investigated by SEM (station CB4.4 and station CB7.4) do not represent a comprehensive set of all sites in the Bay, they did contain substantial concentrations of alkenones. Light microscopy revealed the occurrence of uncultivated spherical cells in all samples tentatively identified as L. galbana or C. lamellosa. Both of those species have been identified in the Bay (Marshall, 1994; Marshall et al., 2005) and are known to produce alkenones (Versteegh et al., 2001; Sun et al., 2007). However, since E. huxleyi and G. oceanica may lose their coccoliths under certain environmental conditions and/or phases of growth (Green et al., 1996; Paasche, 2002), we cannot definitively exclude their presence in the CB. Doing so would require DNA analyses...
since taxonomic differentiations on naked cells are nearly impossible by SEM (Coolen et al., 2004).

3.2. Alkenone distributions along the bay

Alkenones were detected throughout the Bay in suspended particles from a variety of depths (Electronic annex Table EA-1) and in surface sediments (Electronic annex Table EA-3) collected between station CB3.1 and CB7.4N at salinities ranging from 3.7 to 30.9 (Fig. 1). Alkenone concentrations in water ranged from 0.01 to 0.36 \( \mu g \) L\(^{-1}\) in the Upper-Bay, from 0.06 to 2.1 \( \mu g \) L\(^{-1}\) in the Mid-Bay and from 0.04 to 1.3 \( \mu g \) L\(^{-1}\) in the Lower-Bay (Table EA-1). Converted to mass of suspended matter, alkenone concentrations were between 0.4–23.6 \( \mu g \) g\(^{-1}\) in the Upper-Bay, 1.8–271.4 \( \mu g \) g\(^{-1}\) in the Mid-Bay and 3.2–86.3 \( \mu g \) g\(^{-1}\) in the Lower-Bay (Table EA-1). At individual stations highest alkenone concentrations usually occurred between 1 and 5 m, close to the depth of maximum of Chl \( a \) concentration. Alkenone concentrations in sediments averaged a factor of four lower than in filters, but tracked concentrations in the overlying water, with higher concentrations of 42.5 \( \mu g \) g\(^{-1}\) at station CB6.1, and lower concentrations of 0.8–0.6 \( \mu g \) g\(^{-1}\) at station CB3.3E and CB7.4 in the Lower- and Upper-Bay, respectively (Table EA-3).

All particulate and sediment samples were characterized by similar alkenone distribution patterns to those shown in Schwab and Sachs (2009), with a predominance of methyl C\(_{37}\) alkenones over ethyl C\(_{38}\) alkenones, a higher relative concentration of tri- over di- and tetraunsaturated C\(_{37}\) and C\(_{38}\) alkenones, a small proportion of C\(_{39}\) alkenones, and trace amounts of tetraunsaturated C\(_{38}\) and C\(_{40}\) alkenones (Fig. 2). Only methyl and ethyl C\(_{36:3}\) and C\(_{36:2}\) alkenoates were detected, with ethyl C\(_{36:2}\) alkenoate predominating. A distinct characteristic of all alkenone distributions was the lack of C\(_{38}\) methyl alkenones.

The relative abundance of the tetra-unsaturated C\(_{37}\) alkenone, expressed as \( %_{C37:4} = \frac{C_{37:4}}{C_{37:4} + C_{37:3} + C_{37:2}} \) (Marlowe et al., 1984), was between 2.3–9.5% in particles (Table EA-2) and 2.0–10.5% in surface sediments (Table EA-3), and increased with salinity along the N–S transect of the Bay and with depth (Fig. 3A). For instance, between 0.5–3 m and 23 m at the station CB3.3C, salinity increased from 10.2 to 20.7 and \( %_{C37:4} \) increased 3.5 and 5.7 (Fig 3A).

The relative abundance of C\(_{37}\) alkenones relative to C\(_{38}\) alkenones, expressed as \( C_{37}/C_{38} = \frac{(C_{37:3} + C_{37:2})}{(C_{38:3} + C_{38:2})} \) (Marlowe et al., 1984) was between 1.2–4.5 in particles and 1.1–2.1 in sediments (Tables EA-2 and EA-3). Samples between stations CB3.1 and CB7.4 indicated a decrease of C\(_{37}/C_{38}\) (from 3.1 to 1.2) as salinity increased from 3.7 to 28.9 along the N–S transect of the Bay and with depth (Fig. 3B). A different trend was observed at station CB7.4N, where two particle samples collected at 5 and 11 m displayed much higher C\(_{37}/C_{38}\) ratios of 4.2 and 4.5, respectively, in the salinity range 29.7 at 5 m to 30.9 at 11 m. No correlation between either \%_{C37:4} or C\(_{37}/C_{38}\) with water temperature or nutrient concentration was observed.

In particles and sediments, alkenone indices such as \( U_{K37}^{C} = \frac{C_{37:2}}{C_{37:4} + C_{37:3} + C_{37:4}} \) (Brassell et al., 1986) and \( U_{K0}^{C} = \frac{C_{37:2}}{C_{37:2} + C_{37:3}} \) (Prahl and Wakeham, 1987) displayed large variations throughout the Bay. Contrary to an earlier report by Mercer et al. (2005) \( U_{K37}^{C} \) and \( U_{K0}^{C} \) in particles and surface sediment showed no correlation with temperature (e.g. Fig. 4). In suspended particles, \( U_{K37}^{C} \)

![Fig. 2. Partial gas chromatogram showing representative alkenone distribution in surface sediments of the CB; with Me and Et being Methyl and Ethyl alkenone, respectively and Me-and Et-oate being Methyl and Ethyl alkenoate, respectively.](image-url)
and U\textsubscript{17}\textsuperscript{K} ranged from 0.06 to 0.22 and from 0.12 to 0.26, respectively (Table EA-2). In sediment, U\textsubscript{17} and U\textsubscript{17}\textsuperscript{K} ranged from 0.12 to 0.28 and from 0.18 to 0.33, respectively (Table EA-3). Water surface temperatures derived from the relationship U\textsubscript{0} = 0.034T + 0.039 (Prahl et al., 1988) were between 2.3–8.4°C in particles (Table EA-1) and 4.2–8.7°C in sediment (Table EA-3), all 7.7–17.1°C (average 12.4°C) colder than the measured water temperature. Improved estimates of the water temperature were obtained using the alkene temperature calibrations $U_{17} = 0.0377T - 0.5992$ (Sun et al., 2007) and $U_{17} = 0.016T - 0.0607$ (Versteegh et al., 2001) calibrated with C. lamellosa and I. galbana, respectively. Applying the C. lamellosa calibration, U\textsubscript{17}\textsuperscript{K}-derived water temperatures were between 17.6–21.7°C in particles and 15.9–23.3°C in sediments, which together averaged 2.7 ± 1.9°C warmer than measured temperatures. Applying the I. galbana temperature calibration, U\textsubscript{17}\textsuperscript{K}-derived water temperatures were between 11.0–24.1°C in particles and 15.1–24.7°C in sediments, on average just 0.3 ± 2.9°C colder than measured temperatures (Tables EA-1 and EA-3).

### 3.3. Alkenone δD values

Alkenone δD values were measured in sediment and particle samples with sufficient alkene abundance, which in this study was approximately greater than 1 μg of the total lipid extract. Such abundances occurred between stations CB3.3C and CB7.4N where surface salinities were between 10–29 and water δD values were between −37.6‰ and −10.6‰. The δD values of individual alkenones (MeC\textsubscript{37}:2, MeC\textsubscript{37}:3, EtC\textsubscript{38}:2, EtC\textsubscript{38}:3) in suspended particles collected at different depths are shown in Table EA-4A along with the associated water δD, salinity and temperature values. Individual alkene δD values from surface sediments (0–10 cm) are listed in Table EA-4B along with the salinity and water δD values from water depths of 2–5 m, where highest alkene concentrations occurred (Table EA-1).

δD values of alkenones from particles and sediments were between −207‰ to −182‰ for MeC\textsubscript{37}:3, −185‰ to −165‰ for MeC\textsubscript{37}:2, −221‰ to −196‰ for EtC\textsubscript{38}:3, and −197‰ to −175‰ for EtC\textsubscript{38}:2. No significant difference in the δD value of individual alkenones was observed between surface sediments and particles in the overlying water column. Individual alkenones were depleted in deuterium relative to water by an amount that was lipid-specific but relatively constant throughout the Bay. That amount was −177 ± 2.9‰ (n = 35) for MeC\textsubscript{37}:3, −156 ± 3.2‰ (n = 25) for MeC\textsubscript{37}:2, −188 ± 3.2‰ (n = 28) for EtC\textsubscript{38}:3, and −168 ± 3.0‰ (n = 30) for EtC\textsubscript{38}:2. These isotopic fractionations are expressed as δalkene-water values ($\delta_{\text{alkene-water}} = (\delta_{\text{alkene}} - 1)/((\delta_{\text{water}} + 1) - 1)$ in Tables EA-4A and EA-4B.

Particulate and sedimentary alkene δD values were linearly correlated with water δD values with R² values of 0.8–0.9 in particles and 0.7–0.9 in sediments (Table 1, Fig 5A). As salinity was linearly correlated with water δD values (Sachs and Schwab, 2011), alkene δD values were linearly correlated with salinity with R² values of 0.6–0.8 in particles and 0.8 in sediments (Table 1, Fig 5B). Linear regression analyses of $\delta_{\text{alkene-water}} = x_{\text{slope}}\delta_{\text{water}} + x_{\text{intercept}}$ relationships indicated similar slopes ($x_{\text{slope}}$) of 0.703 ± 0.07 (n = 8) for each type of alkene in both particles and sediments. But, intercepts ($x_{\text{intercept}}$) differed for each type of alkene: −178‰ for
Table 1
Equations of the relationships between individual alkenones and water $\delta D$ values, individual alkenone $\delta D$ values and salinity, and $\delta_{\text{alkenone-water}}$ and salinity in sediment and water particle samples. Relationships between coeluting alkenones and water $\delta D$ from others studies are shown for comparison.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type of samples</th>
<th>Equation line $\delta D$ alkenone versus $\delta D$ water and salinity</th>
<th>$R^2$</th>
<th>$n$</th>
<th>$\delta_{\text{alkenone-water}}$ − slope</th>
<th>$\delta_{\text{alkenone-water}}$ − intercept</th>
<th>Equation line $\delta_{\text{alkenone-water}}$ versus salinity</th>
<th>$R^2$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeC$_{37:3}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 0.793\delta D_{\text{water}} - 177.2^*$</td>
<td>0.79</td>
<td>16</td>
<td>0.793</td>
<td>0.823</td>
<td>$\delta_{\text{alkenone-water}} = 0.0000\text{salinity} + 0.824$</td>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>MeC$_{37:3}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.710\delta D_{\text{water}} - 179.5^*$</td>
<td>0.85</td>
<td>19</td>
<td>0.710</td>
<td>0.821</td>
<td>$\delta_{\text{alkenone-water}} = -0.0002\text{salinity} + 0.827$</td>
<td>0.2</td>
<td>19</td>
</tr>
<tr>
<td>MeC$_{37:2}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 0.585\delta D_{\text{water}} - 164.4^*$</td>
<td>0.72</td>
<td>11</td>
<td>0.585</td>
<td>0.836</td>
<td>$\delta_{\text{alkenone-water}} = -0.0004\text{salinity} + 0.849$</td>
<td>0.5</td>
<td>11</td>
</tr>
<tr>
<td>MeC$_{37:2}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.701\delta D_{\text{water}} - 158.0^*$</td>
<td>0.87</td>
<td>16</td>
<td>0.701</td>
<td>0.842</td>
<td>$\delta_{\text{alkenone-water}} = -0.0002\text{salinity} + 0.848^*$</td>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>EtC$_{38:3}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 0.776\delta D_{\text{water}} - 190.6^*$</td>
<td>0.85</td>
<td>13</td>
<td>0.776</td>
<td>0.809</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.812$</td>
<td>0.1</td>
<td>13</td>
</tr>
<tr>
<td>EtC$_{38:3}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.618\delta D_{\text{water}} - 191.1^*$</td>
<td>0.77</td>
<td>15</td>
<td>0.618</td>
<td>0.809</td>
<td>$\delta_{\text{alkenone-water}} = -0.0002\text{salinity} + 0.817^*$</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>EtC$_{38:2}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 0.680\delta D_{\text{water}} - 172.5^*$</td>
<td>0.78</td>
<td>14</td>
<td>0.680</td>
<td>0.828</td>
<td>$\delta_{\text{alkenone-water}} = -0.0003\text{salinity} + 0.835$</td>
<td>0.2</td>
<td>14</td>
</tr>
<tr>
<td>EtC$_{38:2}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.761\delta D_{\text{water}} - 168.3^*$</td>
<td>0.83</td>
<td>16</td>
<td>0.761</td>
<td>0.832</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.834^*$</td>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>MeC$_{37:3}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 1.307\text{sal} - 218.3^*$</td>
<td>0.81</td>
<td>16</td>
<td>1.307</td>
<td>0.782</td>
<td>$\delta_{\text{alkenone-water}} = -0.0002\text{salinity} + 0.817^*$</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>MeC$_{37:3}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.915\text{sal} - 212.3^*$</td>
<td>0.63</td>
<td>19</td>
<td>0.915</td>
<td>0.788</td>
<td>$\delta_{\text{alkenone-water}} = -0.0003\text{salinity} + 0.835$</td>
<td>0.2</td>
<td>14</td>
</tr>
<tr>
<td>MeC$_{37:2}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 0.900\text{sal} - 193.9^*$</td>
<td>0.85</td>
<td>11</td>
<td>0.900</td>
<td>0.806</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.812$</td>
<td>0.1</td>
<td>13</td>
</tr>
<tr>
<td>MeC$_{37:2}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.958\text{sal} - 192.0^*$</td>
<td>0.65</td>
<td>16</td>
<td>0.958</td>
<td>0.808</td>
<td>$\delta_{\text{alkenone-water}} = -0.0002\text{salinity} + 0.817^*$</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>EtC$_{38:3}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 1.121\text{sal} - 228.0^*$</td>
<td>0.78</td>
<td>13</td>
<td>1.121</td>
<td>0.772</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.834^*$</td>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>EtC$_{38:3}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 0.911\text{sal} - 221.1^*$</td>
<td>0.78</td>
<td>15</td>
<td>0.911</td>
<td>0.779</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.812$</td>
<td>0.1</td>
<td>13</td>
</tr>
<tr>
<td>EtC$_{38:2}$</td>
<td>Sediment</td>
<td>$\delta D_{\text{alk}} = 1.102\text{sal} - 207.3^*$</td>
<td>0.85</td>
<td>14</td>
<td>1.102</td>
<td>0.793</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.834$</td>
<td>0.2</td>
<td>14</td>
</tr>
<tr>
<td>EtC$_{38:2}$</td>
<td>Filter</td>
<td>$\delta D_{\text{alk}} = 1.172\text{sal} - 206.3^*$</td>
<td>0.83</td>
<td>16</td>
<td>1.172</td>
<td>0.794</td>
<td>$\delta_{\text{alkenone-water}} = -0.0001\text{salinity} + 0.834$</td>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>MeC$<em>{37:3}$ + MeC$</em>{37:2}$</td>
<td>$\delta D_{\text{alk}} = 0.732\delta D_{\text{water}} - 225.2$</td>
<td>0.99</td>
<td>5</td>
<td>0.732</td>
<td>0.775</td>
<td>From Englebrecht and Sachs (2005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EtC$<em>{38:3}$ + EtC$</em>{38:2}$</td>
<td>$\delta D_{\text{alk}} = 0.745\delta D_{\text{water}} - 232.8$</td>
<td>0.99</td>
<td>5</td>
<td>0.745</td>
<td>0.767</td>
<td>From Englebrecht and Sachs (2005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeC$<em>{37:3}$ + MeC$</em>{37:2}$</td>
<td>$\delta D_{\text{alk}} = 2.60\delta D_{\text{water}} - 215$</td>
<td>0.85</td>
<td>11</td>
<td>2.600</td>
<td>0.785</td>
<td>From Schouten et al. (2006) (E. huleyi)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeC$<em>{37:3}$ + MeC$</em>{37:2}$</td>
<td>$\delta D_{\text{alk}} = 2.90\delta D_{\text{water}} - 242$</td>
<td>0.76</td>
<td>6</td>
<td>2.900</td>
<td>0.758</td>
<td>From Schouten et al. (2006) (G. oceanica)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\delta_{\text{alkenone-water}}$ − intercept = 1 − ($-1/1000$).

* = equation in a confidence level of at least 95%.
MeC37:3 = −161 ‰ for MeC37:2, −191 ‰ for EtC38:3, and −170 ‰ for EtC38:2. Calculation of the fractionation factor using the intercept ($x_{\text{intercept}} = 1 - (\delta D_{\text{water}}/\delta D_{\text{H2O}})$) gave 

\[
\delta D_{\text{alk}}/(1 + \delta D_{\text{water}})
\]

values close to the fractionation factors derived from the slope ($x_{\text{slope}}$), with $x_{\text{slope}}$ on average 0.1 higher than $x_{\text{slope}}$ (Table 1). The $D/H$ fractionation between the different alkenones and water ($\delta D_{\text{alkene-water}} = (1 + \delta D_{\text{alk}})/(1 + \delta D_{\text{water}})$) was nearly constant along the salinity gradient of the Bay (Table 2, Fig. 6A). Hydrogen isotopic offsets between di- and tri-unsaturated C37 and C38 alkenones (2C37:2–C37:3 and 2C38:2–C38:3) were $\delta D_{\text{alkene-water}} = (C_{37:7} + 1)/(C_{37:7} + 3)$ with $x = 2$ or 3) and between C37 and C38 alkenones ($\delta D_{\text{alkene-water}} = (C_{37:2} + 1)/(C_{37:3} + 1)$) were similar in all particulate and sediment samples collected in the CB (Table 2 and Electronic annex Tables EA-5A and EA-5B).

### 4. Discussion

#### 4.1. Alkenones and alkenone-producing haptophytes in the Chesapeake Bay

All particulate and sediment samples were characterized by low abundances of C38 methyl alkenones and the C38:4 alkenone, implying minor contributions of alkenones from oceanic haptophyte species such as *E. huxleyi* and *G. oceanica* (Marlowe et al., 1984; Rontani et al., 2004) relative to those favoring fresh or brackish water (Marlowe et al., 1984; Volkman et al., 1995; Li et al., 1996; Thiel et al., 1997; Schulz et al., 2000; Sun et al., 2007). Similar
distributions of alkenones have been reported in marginal and lacustrine environments characterized by unciliated haptophytes such as *C. lamellosa*, *I. galbana* and *Chromulina* sp. (Cranwell, 1985; Volkman et al., 1988; Versteegh et al., 2001; Sun et al., 2007). The light microscopic and SEM-based identification of unciliated haptophyte cells in our samples and reports of *C. lamellosa* and *I. galbana* in the Bay (Marshall, 1994; Marshall et al., 2005) support the notion that these species contributed substantially to the production of alkenones in our samples. The haptophyte bloom was reported to occur in late spring and to account for the bulk of the alkenone production during the year in the CB (Mercer et al., 2005). Therefore, alkenone indices in surface sediments likely represent long-term (i.e., 1–10 years, based on a linear sedimentation rate of 0.1–1 cm yr−1 (Colman and Bratton, 2003; Cronin et al., 2010) averaged surface conditions occurring in late spring when runoff of D-depleted freshwater into the CB is high.

4.1.1. C37/C38 and %C37:4

The ratio of C37/C38 alkenones in the CB was 1.2–4.5 in particles and 1.1–2.4 in sediments, averaging 2.2 (Tables EA-2 and EA-3). Open ocean ratios are typically between 0.5 and 1.5 (Conte et al., 1998; Chu et al., 2005), similar to those found in the most common marine alkenone producers, *E. huxleyi* (0.86–2.16, mean of 1.16 (Conte et al., 1994)) and *G. oceanica* (0.59–0.81, mean of 0.7 (Volkman et al., 1995)). Coastal haptophytes tend to have higher C37/C38 ratios, such as *C. lamellosa* (1.4–9.5, mean of 5.6 (Prahl et al., 1988)) and *I. galbana* (5.4–15.1, mean of 10.9 (Marlowe et al., 1984)), and values in excess of two have been reported in the Baltic Sea (Schulz et al., 2000) and some lacustrine environments (Cranwell, 1985; Thiel et al., 1997; Chu et al., 2005 and references therein) and attributed to alkenone production by species similar to *C. lamellosa* and *I. galbana*. We thus tentatively attribute average C37/C38 ratios of 2.2 in the CB to substantial alkenone production by coastal haptophytes such as *C. lamellosa* and *I. galbana*, noting, however, that Theroux et al. (2010) suggested that C37/C38 ratios better reflect algal growth status than species identity.

Relatively high concentrations of C37:4 compared to (C37:3 + C37:2) have been reported in low salinity marginal, coastal and estuarine environments (Rosell-Mé, 1998; Volkman et al., 1988; Schulz et al., 2000; Blanz et al., 2005; Mercer et al., 2005), while very high %C37:4 values of up to 95% have been found in lacustrine sediments (Cranwell, 1985; Chu et al., 2005 and references therein). It was thus proposed that %C37:4 values could be used as a paleosalinity or water mass proxy (Rosell-Mé, 1998; Schulz et al., 2000; Blanz et al., 2005). Furthermore, analysis of fossil DNA from sediments in hypersaline Ace Lake, Antarctica, showed that higher %C37:4 values are correlated with higher relative abundance of freshwater versus oceanic haptophyte phylotypes (Coolen et al., 2004).

According to these studies, high values of both C37/C38 and %C37:4 are commonly associated with a predominance of freshwater and coastal haptophytes (Chu et al., 2005 and references therein). In the CB, a negative correlation between C37/C38 and salinity (Fig. 3B) supports these observations. But a positive correlation between %C37:4 and salinity (Fig. 3A) is inconsistent with these results. These opposite trends imply that physiological and/or species changes in alkenone producers along the salinity gradient in the CB differ from those in other settings. Furthermore, Toney et al. (2010) found a complete absence of C37:4 in some lacustrine settings that was attributed to the presence of unidentified freshwater alkenone producers. Higher contributions of similar species in the upper reaches (i.e., low salinity region) of the CB may be an alternative explanation for the uncommon trend of %C37:4 as a function of salinity.

4.1.2. U37K and U37K temperature indices

A vast amount of literature demonstrates the high fidelity with which the alkenone unsaturation ratio U37K co-varies with sea surface temperature (SST) in the ocean, and the suitability of a single global temperature calibration equation (U37K = 0.034T + 0.039) (see review by Herbert (2001)). When *E. huxleyi* and *G. oceanica* are not the predominant alkenone producers very different temperature calibrations have been reported (e.g., Rosell-Mé, 1998; Schulz et al., 2000; Chu et al., 2005; Mercer et al., 2005), making alkenone-based SST reconstructions uncertain when the identity of alkenone producers is unknown. Reconstructed SSTs in the CB are closer to measured values when temperature calibrations for *I. galbana* (Versteegh et al., 2001) and *C. lamellosa* (Sun et al., 2007) are used than when marine (Prahl et al., 1988) calibrations are used. This provides further evidence that these coastal species may predominate the assemblage of alkenone-producing haptophytes in the CB. The absence of correlation between U37K or U37K and SSTs (e.g. Fig. 4) may be due to variations in the growth phase or the assemblage of alkenone producers along the CB (see discussion below), both of which are known to influence U37K and U37K (e.g., Conte et al., 1994; Mercer et al., 2005; Toney et al., 2010), or alternatively to the small range of temperature observed during sampling.

4.2. CB alkenone δD values

The CB estuary provides an ideal setting to evaluate (i) the fidelity with which alkenone lipids record the hydrogen isotopic composition of water and (ii) the influence of salinity on D/H fractionation in those lipids, because ample alkenone concentrations exist for hydrogen isotope analyses over a ~200 km estuary in water spanning a ΔD range of 27%O (−38‰ O to −11‰ O) and a salinity range of 19 (10.0–28.8). Below we first discuss the relationship between water δD values and δD values in four different alkenones from CB particles and surface sediments, demonstrating that these lipids faithfully track the isotopic composition of water. A discussion of the invariant D/H fractionation between water and alkenones along the salinity gradient of 19 follows.

4.2.1. Relationship between alkenone and water δD values

Our sampling in late May was timed to occur during the maximum of the annual alkenone production that occurs in late spring when runoff of D-depleted freshwater into the
CB is at a maximum ( Mercer et al., 2005 ). Therefore, the similitude of alkenone $\delta D$ values in particulate and sediment samples suggest that alkenone $\delta D$ values in sediment record long-term (i.e., 1–10 years, based on a linear sedimentation rate of 0.1–1 cm yr$^{-1}$ ( Colman and Bratton, 2003; Cronin et al., 2010 ) average surface D-depleted water by late spring freshwater runoff.

Different intercept ($\epsilon$) but similar slope ($\zeta$) values for each of the four alkenones in the equation $\delta D_{\text{alkenone}} = \epsilon + \zeta / C_{17}$ indicate that, while different for each of the four alkenones, the magnitude of lipid-water $D/H$ fractionation for each remains constant along the length of the CB ( Table 1 ). Furthermore, previous culture experiments of the oceanic haptophytes E. huxleyi and G. oceanica showed higher alkenone-water $D/H$ fractionation of 233 ± 6 for C37:2 + 37:3 in Englebrecht and Sachs (2005), 220 ± 16 for C37:2 + 37:3 in Schouten et al. (2006), 198 ± 20 for C37:3 and 180 ± 16 for C37:2 in Wollhoe et al. (2009) than reported in this study in which we find $\epsilon_{\text{C37:3-water}}$ of 180 ± 4 and $\epsilon_{\text{C37:2-water}}$ of 158 ± 3 in water particles ( Table 1 ). As coastal haptophytes are likely predominant in our samples, lower alkenone-water $D/H$ fractionation (by up to 60% relative to Englebrecht and Sachs (2005)) suggests that coastal haptophytes may fractionate less than oceanic haptophytes.

4.2.2. Lack of co-variation between salinity and $D/H$ fractionation in CB alkenones

The $D/H$ fractionation between water and all four alkenones was invariant over the salinity range of 10–29 ( Fig. 6A and Table 2 ). This result is at odds with three recent studies indicating a 1–3% decrease in $D/H$ fractionation in lipids per unit increase in salinity ( Schouten et al., 2006; Sachs and Sachs, 2008; Sachs and Schwab, 2011 ). In batch-cultured E. huxleyi and G. oceanica Schouten et al. (2006) observed a positive linear correlation between salinity and $x_{\text{alkenone-water}}$, the slope of which implied a decrease in $D/H$ fractionation in C37:2 + 37:2 alkenones of 3.3% and 3.0% respectively, per unit increase of salinity over the salinity range 25–35. In the same CB sample set on which we report here we observed a 0.99% decrease in $D/H$ fractionation in dinosterol (4α,23,24-trimethyl-5-α-hydroxy-22E-en-3β-ol) per unit increase of salinity over the salinity range 10–29 ( Sachs and Schwab, 2011 ). And in hypersaline ponds on Christmas Island in the central equatorial Pacific (Republic of Kiribati), a 0.8–1.1% decrease in $D/H$ fractionation per unit increase in salinity was observed in phytene, n-C17 alkane and a mixture of total extractable lipids over the salinity range 35–150 ( Sachs and Sachs, 2008 ). While the 3–4-fold higher sensitivity of $D/H$ fractionation to salinity in Schouten et al. (2006)’s alkenones as compared to these other studies was likely due in (large) part to the associated changes in growth rate, there is strong evidence that $D/H$ fractionation decreases in algal lipids in response to higher salinity.

We consider three hypotheses to explain the lack of co-variation between $x_{\text{alkenone-water}}$ and salinity in the CB. (i) Growth rate and temperature changes cause $D/H$ fractionation in alkenones that counteract the fractionation arising from salinity changes. (ii) The assemblage of alkenone-producing haptophytes changes along the Bay and each species has a different sensitivity to salinity, such that no apparent trend in $x_{\text{alkenone-water}}$ occurs along the salinity gradient. (iii) Coastal haptophytes that likely predominate the assemblage of alkenone producers in the CB may have an enhanced osmoregulation capacity that circumvents the mechanisms that cause a change in alkenone–water $D/H$ fractionation in response to salinity changes in other species of phytoplankton and cyanobacteria.

(i) Growth rate and temperature changes cause $D/H$ fractionation in alkenones that counteract the fractionation arising from salinity changes. Culture and field studies have demonstrated that growth rate and temperature can influence $D/H$ fractionation in lipids ( Schouten et al., 2006; Wollhoe et al., 2009; Zhang et al., 2009 ), but the data are few and the results contradictory. Zhang et al. (2009) showed that acetylated lipids (C14: 16:1; C16; C18 fatty acids) from two species of freshwater green algae (E. unicocca and V. aureus) grown in batch culture at 15 °C were enriched in deuterium by 20–40% relative to those grown at 25 °C. Similarly, Wollhoe et al. (2009) observed that individual alkenones from batch-cultured E. huxleyi and G. oceanica at 17 °C were enriched in deuterium by 40–50% relative to those grown at 25 °C. However, Schouten et al. (2006) observed no significant effect of temperature on alkenone–water $D/H$ fractionation in batch-cultured E. huxleyi and G. oceanica, but growth rate changed along with temperature in these experiments. In the CB, the lack of any trend between alkenone–water $D/H$ fractionation and temperature also suggests that temperature was not a first order influence on individual alkenone $\delta D$ values ( Fig. 6B ). Growth rate has also been shown to influence $D/H$ fractionation. Schouten et al. (2006) observed a substantial increase in $D/H$ fractionation as growth rate increased in batch-cultured E. huxleyi and G. oceanica in response to different temperatures and salinities. In continuous culture experiments (i.e., chemostats), Zhang et al. (2009) found that $D/H$ fractionation also increased substantially as growth rate increased in a sterol from the diatom T. pseudonana, but little or not at all in fatty acids from the same cultures. Growth rate trends from other phytoplankton groups in the CB indicate highest values in the mid-Bay and lowest values near the mouth of the Bay ( Xu and Hood, 2006 ), opposite to those required to counteract the expected decrease of alkenone-water $D/H$ fractionation as salinity increases along the Bay.

(ii) The assemblage of alkenone-producing haptophytes changes along the Bay and each species has a different sensitivity to salinity, such that no apparent trend in $x_{\text{alkenone-water}}$ occurs along the salinity gradient. Different species of alkenone producers and other phytoplankton are known to produce the same lipids with different $\delta D$ values when grown in water with the same salinity (cf., Schouten et al., 2006) or in water with the same $\delta D$ value (e.g., Zhang and Sachs,
hyper-osmotic stress caused a larger cell volume and a higher concentration of external water into the cell under extreme hypoosmotic stress caused a larger cell volume and a higher concentration of external water into the cell. For instance, Dunaliella tertiolecta, in the marine alga, and White, 2003). Under this condition, the synthesis and their resultant ion concentrations occurring during hyperosmotic stress reduces external water availability and increases harmful ion concentrations in the cell (Epstein, 1980). Sachse and Sachs (2008) and Sachs and Schwab (2011) proposed that algal lipid D-enrichment with increasing salinity results from the progressive D-enrichment of internal cell water as H is preferentially drawn from the internal water pool for biosynthetic reactions, and the flux of external water into the cell slows. Furthermore, since enzyme activities are affected by elevated cytoplasmic ion concentrations (Niu et al., 1995; Kirst, 1989) and ultimately control lipid δD values (Baillif et al., 2009; Chikaraishi et al., 2009; Schwab and Sachs, 2009), it is likely that elevated cytoplasmic ion concentrations occurring during hyperosmotic stress may hamper vital enzymes involved in lipid synthesis and their resultant δD values. As both cellular internal water volume and cytoplasmic ion concentration affect water potential during hyperosmotic stress (Haines, 1994; Jahnke and White, 2003), lipid δD values are expected to be affected by the capacity of algae to reduce water loss and internal concentrations of incompatible ions (i.e., osmoregulate). Conversely, at low external salinities the flux of external water into the cell increases and deleterious ion concentrations in the cytoplasm decrease. For instance, in the marine alga, Dunaliella tertiaolecta, a high flux of external water into the cell under extreme hypoosmotic stress caused a larger cell volume and a higher concentration of x-tocopherol, indicative of heightened stress within the chloroplast membrane (Jahnke and White, 2003). Under this condition, the D/H ratio of the internal cell water is likely to be similar to the D/H ratio of the external water, resulting in lipid δD values that are more likely to reflect those of the environmental water. In CB, such hypo- and

4.3. Paleoenvironmental implications

The difference in sensitivity of D/H fractionation to salinity in CB haptophytes versus CB dinoflagellates indicates that salinity reconstructions based on paleo-lipid δD values may be species dependant. Some species may synthesize lipids with D/H ratios that are sensitive to salinity variations, others may synthesize lipids with δD values that are independent of salinity. The latter are likely to be good targets for water isotopic reconstructions.

Furthermore, the consistency of individual D/H fractionation factors between alkenones and water (alkenone–water) in suspended particles and in sediment of the CB, and the similarity between slope- and intercept-derived fractionation factors (Table 2), suggest that water δD values are the primary control on changing alkenone δD values along the Bay and thus, CB water δD values may be determined using individual alkenone δD values with the equations listed in Table 1.

However, constant hydrogen isotope offsets between alkenones with different chain lengths but the same degree of unsaturation (δC37:2-C38:2 and δC37:3-C38:3 ≈ 1.01) and between those with the same chain length but different degrees of unsaturation (δC37:3-C37:2 and δC38:3-C38:2 ≈ 0.97) suggest that single, well-defined values represent hydrogen isotope fractionation associated with enzymatic dehydrogenation and carboxylation reactions during alkenone biosynthesis in the CB (Rontani et al., 2006; Chikaraishi et al., 2009; Schwab and Sachs, 2009). This observation strengthens the argument of Wolhowe et al. (2009) that these values may be used in isotopic mass balance calculations to determine δD values for individual alkenones without the labor intensive LC-MS separation developed by Schwab and Sachs (2009) and used in this study. However, further evaluation of the universality of the isotopic offset between different alkenones ought to be conducted in the lab and field before extending it to other coastal environments and corresponding species, since different salinity effects on alkenone–water in oceanic and coastal species suggest that physiological or morphological differences between alkenone producers may greatly influence alkenone δD values.

5. CONCLUSION

Alkenone distributions characterized by a high abundance of C37,4 and high C37/C38 ratios, in addition to the occurrence of uncalkified cells, support substantial contributions of coastal haptophyte species, such as C. lamellosa and I. galbana, in all studied surface sediment and suspended particle samples in the CB estuary.

The δD values of individual alkenones (MeC37:2; MeC37:3; EtC38:2; EtC38:3) closely co-varied with water δD values. Constant hydrogen isotope offsets (δC37:2-C38:2 and δC37:3-C38:3 = 1.01 and δC37:2-C37:3 and δC38:2-C38 =
Individual alkenone D/H in Chesapeake Bay estuary

0.97) were observed between the different alkenones in all studied samples along the Bay. This value was used in an isotopic mass balance calculation to determine \( \delta D \) values for individual alkenones in the CB when a mixture of two alkenones is analyzed (e.g., \( C_{37,2} + C_{37,3} \)).

Slope- and intercept-derived fractionation factors of the correlations (\( \delta D_{\text{alkenone}} = \delta D_{\text{alkenone-water}} + \delta D_{\text{water}} + c \)) describing the relationship between water and individual alkenone \( \delta D \) values did not differ significantly. No change in \( D/H \) fractionation (\( \delta D_{\text{alkenone}} \)) was observed along the salinity gradient of the Bay, suggesting that individual alkenone \( D/H \) ratios in the CB were primarily determined by source water \( \delta D \) values and that they may be used as proxy to reconstruct CB water \( \delta D \) values.

The difference in sensitivity of \( D/H \) fractionation to salinity in haptophytes relative to dinoflagellates in the CB indicates that the mechanisms of hydrogen isotope fractionation in different phytoplankton are influenced to a varying extent by salinity. By extension, paleosalinity reconstructions from lipid \( \delta D \) values are expected to be species dependent. Two valid hypotheses we consider to explain the lack of a salinity influence on alkenone-water \( D/H \) fractionation in the CB are: (i) the assemblage of alkenone-producing haptophytes changes along the Bay and each species has a different sensitivity to salinity, such that no apparent trend in \( \delta D_{\text{alkenone-water}} \) occurs along the salinity gradient, and (ii) greater osmoregulation capacity in coastal haptophytes may result in little or no sensitivity of the \( D/H \) fractionation process during alkenone synthesis to salinity changes. Laboratory culture studies with oceanic, coastal and lacustrine alkenone-producing pynmesiosphes ought to provide a test of these hypotheses.

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