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Alkenone distribution in Lake Van sediment over the last 270 ka: influence of temperature and haptophyte species composition

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ABSTRACT

Fossil long-chain alkenones have been used for several decades to reconstruct past ocean surface water temperatures and gained recent interest as a paleotemperature proxy for continental lake settings. However, factors besides temperature can affect alkenone distributions in haptophyte algae, and alkenone compositions can differ between haptophyte species. Alkenone-biosynthesizing haptophyte algae are genetically much more diverse in lakes than in the marine realm, and species-level variations in alkenone compositions could have implications for alkenone paleothermometry. Here, we performed a paired analysis of alkenone distributions and haptophyte species compositions using ancient DNA in up to 270 ka-old sediments of Lake Van in Turkey to reveal a possible species-effect on fossil alkenone distributions and paleotemperature estimates. The same predominant haptophyte in Lake Van today prevailed also since the last ~100 ka. However, a calibration of alkenone paleotemperature especially in the oldest analyzed intervals is complicated due to a more complex haptophyte species composition predominated by a haptophyte (LVHap_6), which is phylogenetically different from sequences recovered from currently existing lakes including Lake Van and from haptophyte species existing in culture. The predominance of LVHap_6 coincided with the presence of alkenone MeC38:3 and relatively high MeC37:3/4 (2.4) and MeC38:4/5 ratios (3.0). Uk37 index values in the sediment core over the last 270 ka reflect relative changes in past temperature and are additionally linked to haptophyte species composition. A sustained period of high salinity, as indicated by pore-water salinity measurements, could potentially have triggered the succession of haptophytes as sources of alkenones in Lake Van.

1. Introduction

Long-chain alkenones with 37–39 carbon atoms are of great interest to paleoclimatologists because of the strong empirical relationship between the degree of unsaturation in alkenones (e.g. Uk′37 index) and growth temperature (Brassell et al., 1986; Herbert et al., 2003). In marine settings, the phylogenetically closely related coccolithophorid haptophytes *Emiliania huxleyi and Gephyrocapsa oceanica* are the main sources of alkenones (Volkman et al., 1980; Marlowe et al., 1984, 1990) and Uk′37 analysis on fossil alkenones has been widely used since the last three decades as a molecular proxy for past sea surface temperature (SST) reconstructions e.g. (Prahl and Wakeham, 1987; Martrat et al., 2007). In lakes, recent studies have also supported the idea of a linear dependency between temperature and the degree of alkenone unsaturation. The alkenone distribution in lakes differs from the alkenone distribution in the marine realm. For example, the methyl ketone MeC37:3 predominates the alkenone distribution in marine environments

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whereas MeC37:4 is a more abundant alkenone in lake environments (Castañeda and Schouten, 2011). This resulted in the development of alternative alkenone unsaturation indexes such as Uk37, Uk38, Uk′38 and Uk3738 (Zink et al., 2001; Chu et al., 2005; Sun et al., 2007; Pearson et al., 2008; Toney et al., 2010), which have been used to reconstruct temperature within lake sediment cores from Asia (Liu et al., 2006; Chu et al., 2012) and Greenland (D'Andrea et al., 2011).

However, the high genetic diversity of alkenone-biosynthesizing haptophyte algae in lakes is indicative of the presence of a more complex array of biological sources of alkenones than in the marine realm (Coolen et al., 2004; D'Andrea et al., 2006; Theroux et al., 2010). This may complicate the applicability of alkenone paleothermometry in lakes since culture experiments have shown that the relationship between temperature and the alkenone unsaturation index differs between haptophyte species. For example, *Isochrysis galbana* responds to temperature in a way described by the Uk′37 index (Versteegh et al., 2001) whereas the Uk37 index can best be used when alkenones are produced by *Chrysotila lamellosa* (Sun et al., 2007). Despite the availability of multiple temperature calibrations derived from modern lake environments (ex: Pearson et al., 2008; Toney et al., 2010), uncertainty concerning the effect of biological sources on alkenone distribution limits the application of those calibrations, both spatially and temporally (Theroux et al., 2010). For instance, ancient DNA analysis has shown that the haptophyte species composition varied over the course of the Holocene in the Antarctic Ace Lake (Coolen et al., 2004) and in the Black Sea (Coolen et al., 2009). In these studies, the alkenone distribution co-varied with salinity-driven changes in the haptophyte species composition. In contrast, other studies have shown that genetically distinct haptophyte species displayed very similar alkenone distributions (Theroux et al., 2010). Thus, additional DNA based studies are needed to increase our knowledge of the genetic diversity of alkenone-producing haptophytes in modern-day lakes and to perform lake-specific paleotemperature calibrations. Similar genetic profiling of the past haptophyte composition in the sedimentary record is necessary to verify if the alkenone-inferred past temperature can be calibrated.

The main purpose of our study was to generate a 600-ka record of alkenone-inferred paleotemperature from Lake Van (Turkey). The sedimentary record was recovered from Lake Van in 2010 as part of the ICDP project PALEOVAN which aims to reconstruct continental climate changes in the Near East (Litt et al., 2012). Possible parallel changes in the haptophyte composition were studied from 18S rDNA signatures in the photic zone and in the sedimentary record of Lake Van in order to reveal a possible species-effect on alkenone distribution and paleotemperature estimates. Temperature calibration curves from the literature were compared with data points obtained from Lake Van sediment trap material. Alkenone index Uk37 was qualitatively linked to past temperatures and haptophyte species composition over the last 270 ka, focusing on contrasting conditions characterizing glacial and interglacial intervals. Past changes in surface water salinity as a possible causative for the observed variability in alkenone distributions and haptophyte species compositions was inferred from parallel pore water salinity measurements.

2. Material and methods

2.1. Regional setting

Lake Van is an endorheic lake extending for 130 km West South West – East North East (WSW–ENE), situated on a high plateau in eastern Anatolia, Turkey (38°N, 43°E, 1650 m a.s.l.) with a maximum water depth of 460 m. The modern water chemistry is characterized by low calcium and magnesium concentrations, sulfate concentrations comparable to those found in seawater, high alkalinity (pH 9.8) and brackish waters with salinity ranging between 19 and 22 ppt (Reimer et al., 2009). Surface water temperatures varied between 4 and 26 °C between 2006 and 2008, with maximum values generally observed between July and September during summer and autumn (Stockhecke et al., 2012). In the Lake Van region, maximum precipitation occurs during spring and autumn (Sariş et al., 2010) whereas maximum air temperatures are recorded between July and September (IAEA/WMO, 2014). See Supplementary Information for details about precipitation and temperature in Lake Van region.

2.2. Sampling

A 220-m-long sediment record covering ~600 ka of deposition (i.e., Marine Isotope Stages [MIS] 1–15) (Stockhecke et al., in press) was obtained from a drilling platform in summer 2010 at the Ahlat Ridge (AR) drill site (38°40′N 42°40′E) at a water depth of 350 m (Litt et al., 2012). A one-meter-long core was also obtained using a short gravity corer in the vicinity of the AR, in order to recover the most recent (Holocene) sediments disturbed by the drilling operations. A sequential sediment trap (Technicap-PPS4-3) was installed in the main Tatvan Basin (38.64°N, 42.76°E) at a water depth of 100 m to collect sinking material monthly from August 2010 until August 2011. In addition, particulate material was collected from 30, 135, 230, and 335 m depth by filtration of water (between 80 and 300 L) in summer (between June and August) using a McLane *in situ* pump equipped with glass fiber filters (Whatman).

2.3. Alkenone analyses

From the 220-m-long record, a total of 93 samples consisting of 60 core catchers and 33 undisturbed drilled core sections were used for alkenone analyses. The majority of the samples analyzed for alkenones had a total organic carbon (TOC) content of more than 1% and samples were also selected based on expected shifts in depositional conditions (i.e., from changes in TOC content and elemental compositions as inferred from X-Ray Fluorescence [XRF] scanning) and based on climate conditions (i.e., pollen-inferred vegetation changes). In addition, four samples from the short gravity core, eleven samples from the sediment traps (September 2010–August 2011) and four water filter samples were used for alkenone analyses. All samples were freeze-dried and the sediment samples were homogenized.

Alkenones were extracted using 1–2 g of sediment from the cores and 10–90 mg of sediment from the sediment traps, with a mixture of 10 mL of dichloromethane (DCM) and methanol (MeOH) (DCM:MeOH 7:3 v/v) in microwave Teflon bombs, and by applying a program of 2 minutes (min) at 300 watts (W) and 5 min at 500 W. Alkenones collected on the filtered POM were extracted using Sohxlet at 70 °C reflux with 50 mL of DCM:MeOH (7: v/v) for 24 h. One quarter of the filtered POM samples was saved for subsequent DNA extraction as outlined below. The total lipid extract (TLE) obtained after extraction was transferred to a separation funnel containing 20 mL of nanopure water (H_2O) with 5% sodium chloride (NaCl) to remove the salts from the sample. The lipids were separated from the saline aqueous phase using 3 × 10 mL DCM and free elemental sulfur was eliminated with HCl pre-activated copper powder. Fatty acids were separated from the neutral lipids by saponification in approximately 3 mL of 6% potassium hydroxide (KOH) solution in MeOH at 80 °C for 3 h. The lipids were recovered by extracting the KOH/MeOH with 3 × 1 mL hexane, then separated over a silica column (230–400 mesh Merck, 4 cm length, 0.6 cm

diameter) into 3 fractions of various polarity using 4 mL of the following eluents: 1) 100% hexane; 2) hexane: DCM mixture (1:2 v/v) and 3) DCM:MeOH mixture (95:5 v/v). The second fraction containing the alkenones was analyzed quantitatively on a gas chromatograph (GC, Shimadzu) and qualitatively on a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 Ultra, Shimadzu). The GC column used (ZB-5MS, Phenomenex) had a film of 0.25um, an internal diameter of 0.25 mm and a length of 30. The temperature program was: 70 °C (0 min hold), 20 °C min^{-1} to 130 °C and 4 °C min^{-1} to 320 °C (5 min hold). Compound identification was based on a mass to charge ratio of typical peaks (e.g. $m/z = 43$ for methyl and $m/z = 57$ for ethyl alkenones) and on comparison of mass spectra available in the literature (e.g. MeC38:5 and MeC38:4 reported by Jaraula et al. (2010)). The quantification was based on a calibration using C36 n-alkane as a standard and peak areas of a flame ionization detector (GC-FID). Alkenones concentrations are reported in micrograms per gram of dry sediment ($\mu g\ g^{-1}_{sed}$, Supplementary Information).

The indexes Uk37=(C37:2 − C37:4)/(C37:2 + C37:3 + C37:4) and Uk′37 = C37:2/(C37:2 + C37:3) (Prahl and Wakeham, 1987) were determined by considering only methyl (Me) alkenones. The indexes Uk3738 = (C38:2 + C37:2 − C38:4 − C37:4)/(C37:4 + C37:3 + C37:2 + C38:4 + C38:3 + C38:2), Uk38 = (C38:2 − C38:4)/(C38:2 + C38:3 + C38:4) and Uk′38 = C38:2/(C38:2 + C38:3) (Pearson et al., 2008) were determined by considering C37 methyl and C38 ethyl compounds. The reproducibility of those indexes was tested by analyzing 16 duplicate samples on two different GC-FID instruments (Agilent Technologies 7890A GC System and Shimadzu GC-200 Plus) during different days. The averaged standard deviation, measured on duplicate analysis for Uk37, Uk′37, Uk3738, Uk38 and Uk′38 were ±0.04; ±0.02, ±0.05, ±0.09 and ±0.02 respectively.

2.4. DNA analyses

Total DNA was extracted from the filtered POM samples using the PowerWater™ DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA). Total DNA from Holocene and Pleistocene sediments was extracted from 2 g of wet weight sediment using the PowerMax™ Soil DNA Isolation Kit (MO BIO Laboratories Inc.). The twenty sediment intervals were selected for DNA extraction and subsequent analysis of haptophyte-specific 18S rDNA signatures based on their alkenone content (>0.2 $\mu g\ g^{-1}_{sed}$). Extraction of sedimentary DNA was performed inside the ancient DNA-dedicated lab at WHOI using necessary precautions to prevent contamination with foreign DNA and controls for contamination (Coolen et al., 2009). For example, to avoid cross-contamination, sediment subsamples were obtained with heat-sterilized knives from the center of each core slices. DNA extracts were tested for the presence of polymerase chain reaction (PCR)-inhibiting impurities and, if necessary, subjected to further purification as described previously (Coolen et al., 2009). The concentration of extracted DNA was determined fluorometrically using PicoGreen® ds DNA quantification reagent (Molecular Probes, Eugene, OR). The extracted and purified POM and sedimentary genomic DNA served as template for SYBR®green-based quantitative polymerase chain reaction (qPCR) with haptophyte-specific primers targeting a 498-bp-long fragment spanning the 18S rDNA-V4 region (Coolen et al., 2004) and using a Realplex qPCR cycler (Eppendorf, Beverly, MA). qPCR ingredients and conditions were similar to those described previously (Coolen et al., 2009) and the reactions were stopped at the end of the exponential phase after 32–38 cycles depending on the starting amount of haptophyte template DNA present in the samples. Haptophyte 18S rDNA abundance was reported in gene copy numbers per gram of wet sediment (copies g^{-1}_{sed}). The quality of the PCR products was analyzed by agarose gel electrophoresis and samples with PCR products of the expected length were subjected to denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993) after Coolen et al. (2009). In short, the polyacrylamide gel (6%, wt/vol) contained a denaturing gradient of 20–50% (with 100% denaturant equaling 7 M urea and 40% formamide). The gel was run for 5 h at 12.5 V cm^{-1} at 60 °C using a PhorU2 system (Ingeny, Leiden, Netherlands). Afterward, the gels were stained for 20 min by covering the gels twice with 10 mL of 1× TAE buffer (pH 8.3), containing 2 μl undiluted SYBR®Gold (Invitrogen) followed by destaining for 60 min in 1× TAE buffer (pH 8.3). In order to prevent DNA damage by UV, we used a Dark Imager (Clare Chemicals Research Inc., Dolores, CO) which uses visible light instead of UV in order to visualize the SYBR®Gold-stained DNA. Digital gel images were made using a "Foto/Analyst® Express System" (Fotodyne, Hartland, WI) and ImageJ software. TotalLab TL100 v2006 1D-gel analysis software (Nonlinear Dynamics, Durham, NC) was used to determine the pixel density and vertical position of each band. This information was used to determine the relative abundance of each band within a given sample and to identify the exact vertical position of each band in order to characterize unique vs. identical bands between samples e.g. (Díez et al., 2001). A total of 20 DGGE bands were excised from the gel and prepared for subsequent bi-directional capillary sequencing with the same haptopyte-specific primers as used for PCR using the facilities of Agencourt, Beverly, MA. The paired forward and reverse sequences were aligned and checked for sequencing errors using CLC Main Workbench version 6.8.1 software (CLC Bio, Cambridge, MA). Using CLC Main Workbench, primer sequences were trimmed and closely related haptophyte 18S rDNA sequences were identified through a BLAST search (Altschul et al., 1990) against the NCBI-nr database. A phylogenetic bootstrap tree (1000 replications) was reconstructed also in CLC Main Workbench based on the overlapping region of the aligned 460 base pair (bp)-long Lake Van and the selected most similar sequences using the Neighbor-Joining algorithm (Saitou and Nei, 1987).

2.5. Temperature recordings and alkenone fluxes from sediment traps

Sediment trap material was collected between September 2010 and August 2011 at 5, 15 and 30 m water depth. Temperature was recorded at one minute resolution using a thermistor Vemco Minilog with a resolution of 0.1 °C and an accuracy of ±0.2 °C. The thermistors were attached on the same mooring as the sediment traps. Temperature values were averaged daily as well as averaged for the total duration at which each trap was opened (~one month).

Alkenone fluxes were determined using the surface area (500 cm^2) of the traps and the period of opening, which was approximately one month. Alkenone fluxes were normalized to TOC content as follows: Homogenized freeze-dried samples were analyzed for total carbon (TC) using an elemental analyzer (HEKAtech Euro EA). Total inorganic carbon (TIC) content was determined using a titration coulometer (Coulometric Inc., 5011, CO_2-Coulometer) and subtracted from the TC to yield the TOC content.

2.6. Chronostratigraphy

The age model for the 220-m-long Lake Van sediment record was constructed using climatostratigraphic alignment, varve chronology, tephrostratigraphy, argon–argon single-crystal dating, radiocarbon dating, magnetostratigraphy, and cosmogenic nuclides (Stockhecke et al., 2014). The presented aligned chronology is based on the Greenland Ice Core Chronology 2005 (GICC05)

(NGRIPmembers, 2004; Steffensen et al., 2008; Svensson et al., 2008; Wolff et al., 2010) for the last 116 ka of sedimentation, the speleothem-based synthetic Greenland record (GLT-syn) (Barker et al., 2011) for the interval 116–400 ka BP and the same synthetic Greenland record, but on the timescale from Antarctica Dome C ice cores, for sediments aging between 400 and 600 ka (GLT-syn) (Barker et al., 2011). Eight geomagnetic tie points (from ~32 to ~250 ka), based on minima in the relative paleointensity record, and nine $^{40}Ar/^{39}Ar$ ages confirm the age model. However, uncertainties increase with depth as tectonic activity affected the drill site and the sedimentary regime was completely different during the early evolution of Lake Van (Stockhecke et al., in press). The nomenclature of the Marine Isotope Stages follows Lisiecki and Raymo (2005) and the nomenclature substages were adapted from Jouzel et al. (2007). The majority of the analyzed samples from Lake Van represent lacustrine background sediment, but 7 out of 87 samples are close to volcaniclastics or within event deposits with approximated ages of 171; 203, 261, 267, 400, 583 and 604 ka BP.

2.7. Porewater salinity

Pore-water was obtained from 125 core catchers sampled (i.e., every ~3 m) during the drilling operation, and from 11 samples obtained from the short core. Pore-water was extracted onsite from the core catchers within a few hours after sampling using a Carver two column manual hydraulic press with a maximum clamping force of 22 tons, and a pore-water extraction system IODP style Manheim squeezers. All parts of the squeezer in contact with the sample were either Teflon or titanium. Porewater salinity was measured using a portable refractometer (VWR item N°635-0171) with a salinity range of 0–28‰. The refractometer was calibrated with nanopure water after every ~10 measurements.

3. Results

3.1. Sediment trap material: alkenone flux rates and associated temperatures

Over the course of sampling the daily averaged temperatures ranged from 4 to 22 °C at 5 and 15 m water depth (Fig. 1A), while temperatures at 30 m remained relatively constant. Monthly alkenone fluxes varied widely from 0.1 to 47 mg of alkenone per gram of organic carbon per square meter per day ($mg \cdot g^{-1}_{Corg} \cdot m^{-2} \cdot day^{-1}$) (Fig. 1B) and were highest during the spring and summer of 2011. The alkenone unsaturation indexes Uk37, Uk3738 and Uk38 could be determined in each sediment trap sample (Fig. 1A). Uk37, Uk3738 and Uk38 values were highest during the winter months of November 2010 and February 2011 and relatively low and stable from March to August 2011 when alkenone fluxes were high (Fig. 1B).

3.2. Alkenone distribution in sediment cores

Alkenones recovered from the Lake Van record comprised long chain methyl (Me) and ethyl (Et) ketones with 37, 38 and 39 carbon atoms and 5, 4, 3 or 2 carbon–carbon double bonds as displayed within the chromatograms of Fig. 2. The distributions allowed quantifications of Uk37 values, which were usually below zero indicating MeC37:4 predominance over MeC37:2 (Fig. 2A). Uk37 values occasionally reached positive values indicating a MeC37:4 predominance over MeC37:2 (Fig. 2B). The abundance of C37:2 was usually too low to allow reliable quantification of the Uk′37 index. The indexes Uk3738, Uk38 and Uk′38 could be quantified in most but not all sediment samples, given the presence of an ethyl compound (EtC38:4, Fig. 1A) in some samples and the presence of a

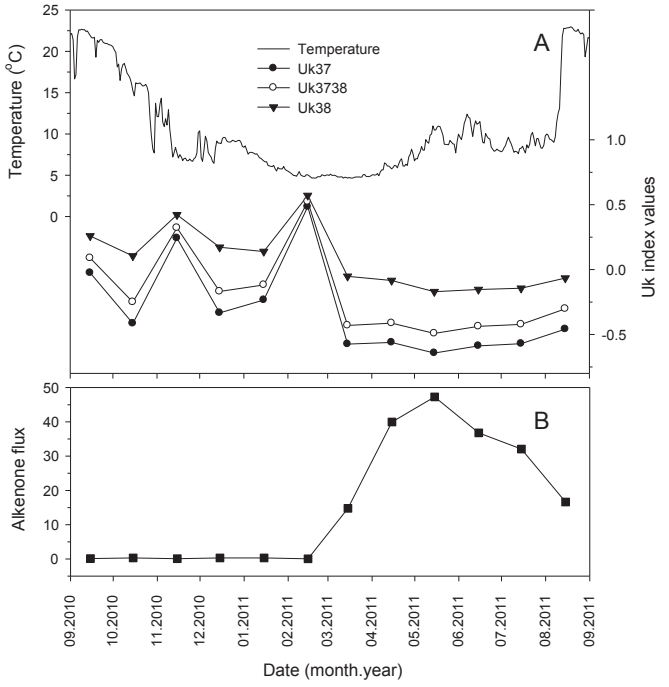

Fig. 1. (A) Water temperature at 5 m and monthly Uk index values as a function of time from the sediment trap material. Date ticks mark every first day of each month. (B) Alkenone fluxes ($mg\ g^{-1}_{Corg}\ m^{-2}\ day^{-1}$) are relatively high between March and August 2011, whereas Uk values are relatively high during the months of November 2010 and February 2011.

methyl compound (MeC38:3*, Fig. 1B) in other samples. The co-eluting compounds MeC38:3 and EtC38:4 were identified based on mass spectrometry analyses, with the presence of the ion $m/z = 43$ indicative for methyl and the ion $m/z = 57$ indicative for ethyl ketones.

Alkenone distribution analyzed in parallel with DNA revealed the presence of similar compounds in all 10 samples, with the exception of the sample deposited 239 ka BP, which contained the compound MeC38:3 (Fig. 3). The sample deposited at 239 ka BP also revealed the highest MeC37:3/4 (2.4) and MeC38:4/5 ratio (3.0) (Table 1). In addition, the Uk37 value of the 239 ka old sample was relatively high compared to most other samples (Table 1).

3.3. Quantitative distribution of fossil haptophyte DNA and relative changes in haptophyte species composition as inferred from fossil 18S rDNA sequencing

Haptophyte 18S rDNA was detected at a water depth of 30 m at the time of sampling, as well as in 9 out of 20 analyzed sediment samples of up to 270 ka in age (Fig. 3). The amount of preserved haptophyte DNA varied between 5×10^3 and 9×10^6 copies g^{-1}_{sed} (Table 1) and sequencing analysis revealed 6 haptophyte operational taxonomic units (OTUs), which are numbered 1–6 in Fig. 3A.

At least three species of haptophytes (OTUs LV_Hap1, 3, and 5) were present in Lake Van at ~270 ka BP (Table 1). Based on the pixel density in the DGGE bands, their relative abundance comprised respectively ~20, ~40, and ~40% of the total haptophyte 18S rDNA pool (Table 1). This haptophyte community was completely replaced 239 ka BP by LV_Hap 6 (100% of detected 18S rDNA sequences, Table 1). We did not detect LV_Hap 3, 5, and 6 in any of the younger sediments. Instead, LV_Hap1 together with a new phylotype (LV_Hap4) represented ~98% and ~2% of the haptophyte 18S rDNA pool between 100 ka BP and 40 ka BP, respectively. LV_Hap1

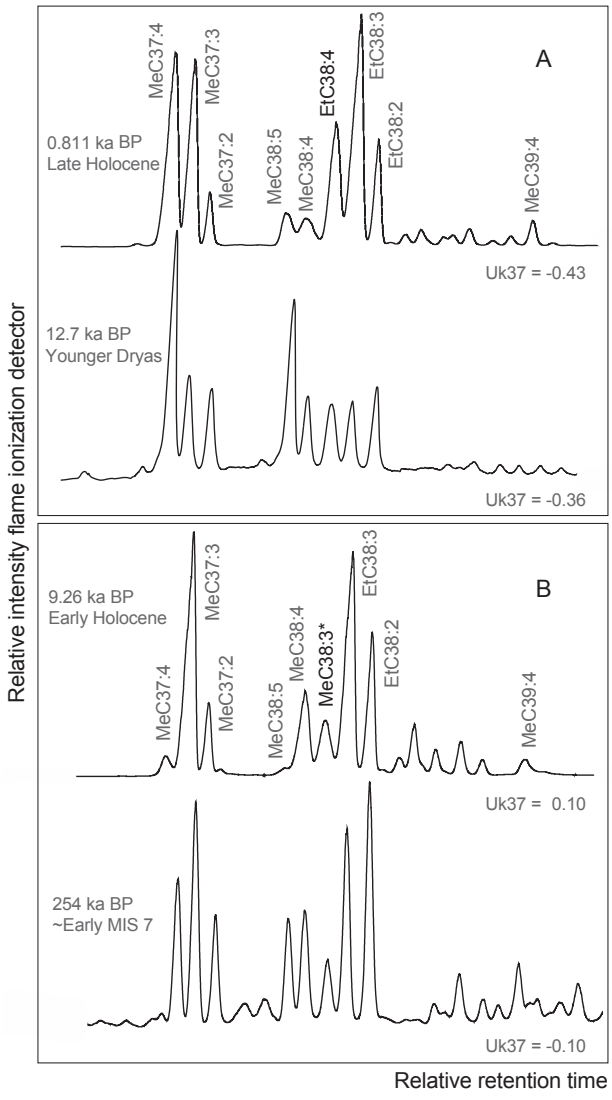

Fig. 2. GC-FID chromatograms displaying alkenone distribution within various sediment samples. (A) Low MeC37:3/4 ratios and EtC38:4 were found in the Late Holocene and the Younger Dryas, whereas high MeC37:3/4 ratios and MeC38:3 (B) were found during the Early Holocene and during the period preceding MIS 7.

predominated (~75% of haptophyte 18S rDNA) together with LV_Hap4 (1%) and a novel type LV_Hap2 (24%) within the youngest (4.17 ka BP) Holocene samples. Over the course of the Holocene, the relative abundance of LV_Hap1 increased to ~94%, whereas LV_Hap 2 decreased from ~24 to ~4% which corresponds to the species composition found in the water column today. Relative abundance of LV_Hap 4 remained relatively constant at ~2% during the last 100 ka.

The phylogenetic affiliation of the six OTUs from Lake Van (i.e., LV_Hap1 to 6) with the most similar sequences of cultivated haptophyte species and environmental clones available through GenBank is shown in Fig. 4. LV_Hap1 and LV_Hap 2 clustered with clones from brackish North American (Theroux et al., 2010) and Antarctic (Coolen et al., 2004) lakes and showed 100% sequence similarity with clones recovered from Holocene sediments of the permanently stratified Antarctic Ace Lake (Coolen et al., 2004). The other four phylotypes clustered with cultivated species of *Isochrysis* and *Pseudoisochrysis* as well as clones recovered from Holocene Black Sea sediments (Coolen et al., 2009) when the estimated sea surface salinity of the Black Sea was ~12 ppt (Coolen et al., 2013). Within this cluster, LV_Hap6 was the most distantly related sequence (Fig. 4). None of the sequences from Lake Van clustered with either the coastal marine *C. lamellosa HAP 17* (Rontani et al., 2004) nor with the marine calcified haptophyte species *E. huxleyi* and *G. oceanica*.

4. Discussion

4.1. In-situ calibration of Lake Van UK37-inferred paleotemperatures considering haptophyte species composition

Over the course of the last few years several researchers have used fossil alkenones to reconstruct paleotemperatures in lacustrine environments (ex: D'Andrea et al., 2011; Chu et al., 2012). In Lake Van, only one fossil alkenone record has been described previously (Thiel et al., 1997) and the alkenone distribution was furthermore recently analyzed in sediment trap material from the lake (Huguet et al., 2011). However, no attempt was made in Lake Van to identify the haptophyte sources of these alkenones using molecular biological approaches, although both studies claimed that it is absolutely necessary to determine haptophyte species in Lake Van in order to reliably reconstruct temperatures using alkenone distribution. Combined geochemical and molecular taxonomic studies were for instance performed in North American lakes (Theroux et al., 2010; Toney et al., 2012). In an attempt to overcome this problem we have extracted, amplified, and sequenced haptophyte-specific sedimentary 18S ribosomal RNA genes. These gene sequences were analyzed in parallel with alkenones to reconstruct temporal changes in the haptophyte species composition and to identify the potential source organisms of alkenones in Lake Van.

Another difficulty is that whereas in marine systems few calibration equations could be used worldwide, in lakes this is impossible given the heterogeneity of those systems (ex: Pearson et al., 2008; Toney et al., 2010). Applying temperature calibration from environments which are not similar to the system under investigation can lead to unrealistic results, as shown in Lake Van (Thiel et al., 1997). Hence, it is necessary to establish a calibration at each study site (D'Andrea et al., 2011), or at least in the same region if lakes are similar (Liu et al., 2006).

18S rDNA sequencing analyses revealed that the dominant haptophyte species within the Lake Van water today is the same as the one dominant in all analyzed sediment samples covering the last 100 ka (LV_Hap1, Table 1). These results suggest that it would be possible to apply a paleotemperature calibration for this section of the core based on our sediment trap study (*in-situ* calibration). However, there is no clear correlation between measured water temperatures at a depth of 5 m and the various Uk indexes for the entire duration of sampling (September 2010–August 2011, Fig. 1). High Uk index values observed in November 2010 and February 2011 could be related to a shift in nutrient and light availability as shown elsewhere (Versteegh et al., 2001; Prahl et al., 2006). The input of re-suspended material could also explain the missing correlation.

Over the course of the years 2010 and 2011, alkenone fluxes increased considerably in spring 2011 and remained relatively high through the summer 2011 (Fig. 1). The alkenone flux increase in spring 2011 could potentially be due to high precipitations and nutrients runoff as previously suggested (Huguet et al., 2011). Most importantly, the period of high alkenone fluxes might be linked to faster export rates of alkenone towards deep waters, and therefore constrain a better link between alkenone distribution and surface water temperatures. Hence, linear regression between Uk37, Uk3738 and temperatures are calculated only considering high alkenone fluxes in the period of March–August 2011. This resulted

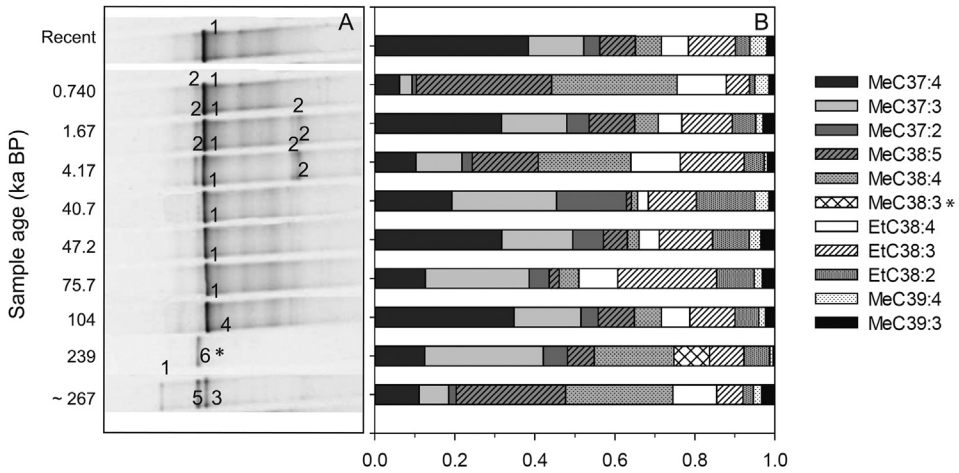

Fig. 3. Haptophyte diagnostic molecular markers. (A) DGGE analysis of PCR-amplified fossil haptophyte 18S rDNA. In total, 20 DGGE bands were excised and sequenced. Note that the gel is displayed at a 90° angle. Bands melting at the same horizontal position, and occasionally also bands at different horizontal positions, represented identical sequences. As a result, the 20 bands comprised only 6 unique OTUs and are numbered 1-6 in the gel. The age of each sediment sample is marked on the left hand side of the gel and "Recent" corresponds to the water sample. (B) Relative abundance of different alkenones. Note that the compound MeC38:3 (*) is detected in the sample where OTU 6 is present.

in a linear regression coefficient of $r^2 = 0.4$ (Table 2). However, this calibration only includes Uk values between −0.6 and −0.4, while Uk values varied between −0.7 and 0.2 in the sediment samples (Fig. 6A).

Hence, despite an encouraging genetic similarity between haptophyte species composition in the modern Lake Van and during the final 100 ka of deposition, the range of Uk values of the *in situ* calibration is different from the Uk values observed in the sediment record.

4.2. Uk values of Lake Van compared to other lake systems

Despite our and other findings in Lake Van (Thiel et al., 1997; Huguet et al., 2011), numerous studies in lakes showed a linear dependency of alkenone distribution to temperature through the indexes Uk37, Uk38 and Uk3738 (references in Table 2). In the following, we combine our results with other studies in order to quantitatively reconstruct temperatures in Lake Van.

Since species composition is important, our results were specifically compared to studies where the haptophyte species composition was similar to Lake Van. Phylogenetic analysis of haptophytes using 18S rDNA profiling has been performed in other lakes (D'Andrea et al., 2011; Toney et al., 2012) and allow a direct comparison with the haptophyte sequences recovered from Lake Van. The haptophyte species Hap_A was closely related to OTU_8 (Toney et al., 2012) found in Lakes from China and North America (Theroux et al., 2010), which is closely related to LV_Hap1 i.e. the dominant haptophyte species in Lake Van (Fig. 4). In comparison, the OTU Braya So (Greenland Lake) (D'Andrea et al., 2011) is more distant from LV_Hap1 (Fig. 4). Therefore, given the similarity of OTU_8 and LV_Hap1, a grouping of data points from this study with the one from Toney et al. (2012) is reasonable. However, it only extends our calibration in the negative Uk37 value range to −0.8 (Fig. 5). Since the Uk37 values in the sediment core range from −0.7 to 0.2, an extension of the calibration towards higher Uk37 values is needed. As displayed in Fig. 5, Uk37-temperature data sets from many studies (i.e. Zink et al., 2001; D'Andrea et al., 2011; Toney et al., 2012) and this study show a linear correlation despite heterogenous haptophyte species compositions and various calibration approaches (Table 2). Including a study by Zink et al. (2001) led to an extension of the Uk37 calibration to higher values, i.e., up to −0.2 (Fig. 5). Nevertheless, this is still not enough to cover the whole Uk37 values from our core (Fig. 6A). The range of Uk37 values observed in batch culture experiments of the haptophyte *C. lamellosa* was between −0.2 and 0.2 (Sun et al., 2007, Fig. 5). Although the temperature range covered by Sun et al. (2007) was the same as in the studies of Toney et al. (2012), Zink et al. (2001), and D'Andrea et al. (2011) highest Uk37 values were reached in this study. This nicely demonstrates the influence of different haptophyte types on the UK37 index and reconstructed temperatures. In conclusion, covering the range of Uk37 values found in Lake Van sediments over 270 ka (see Fig. 6A) would only be possible if several haptophyte species such as LV_Hap1, OTU_8, and *C. lamellosa* (Fig. 5) were present over time. However, none of our OTU's were closely related to *C. lamellosa* (Fig. 4), the species responsible for the most positive Uk37 values.

Table 1
Distribution of alkenones and haptophyte sources of alkenones in sediment intervals where both alkenones and haptophyte 18S rDNA were present. Recent: Filtered POC from the photic zone at 30 m, in July 2010. OTU (operational taxonomical unit). The relative abundance of OTUs is based on DGGE pixel density. 18S rDNA abundance reported in copies per gram of wet sediment. The shade indicates the only sample for which OTU 6 is detected.

Age	Haptophyte OTU	18S rDNA abundance	Uk37	MeC37:3/4	MeC38:4/5
(ka BP)	(relative abundance)	Copies g_{sed}^{-1} (10^6)	±0.04	±0.1	±0.6
Recent	1 (94%), 2 (4%), 4 (2%)	–	−0.61	0.4	0.7
0.740	1 (94%), 2 (4%), 4 (2%)	2.46	−0.49	0.5	0.9
1.67	1 (90%), 2 (8%), 4 (2%)	1.75	−0.49	0.5	0.5
4.17	1 (75%), 2 (24), 4 (1%)	0.72	−0.32	1.1	1.4
40.7	1 (98%), 4 (2%)	1.07	−0.03	1.4	1.2
47.2	1 (98%), 4 (2%)	9.50	−0.42	0.6	0.5
75.7	1 (97%), 4 (3%)	0.01	−0.18	2.0	2.0
104	1 (96%), 4 (4%)	0.16	−0.55	0.5	0.7
239	6 (100%)	0.01	−0.13	2.4	3.0
~267	1 (20%), 3 (40%), 5 (40%)	0.02	−0.45	0.7	1.0

MeC37:3/4 = MeC37:3/MeC37:4 and MeC38:4/5 = MeC38:4/MeC38:5.

4.3. Semi-quantitative temperature correlation and comparison to other proxies

Based on the number of DNA analyses, a semi-quantitative reconstruction or qualitative interpretation of Uk37 appears appropriate. A semi-quantitative reconstruction was also done

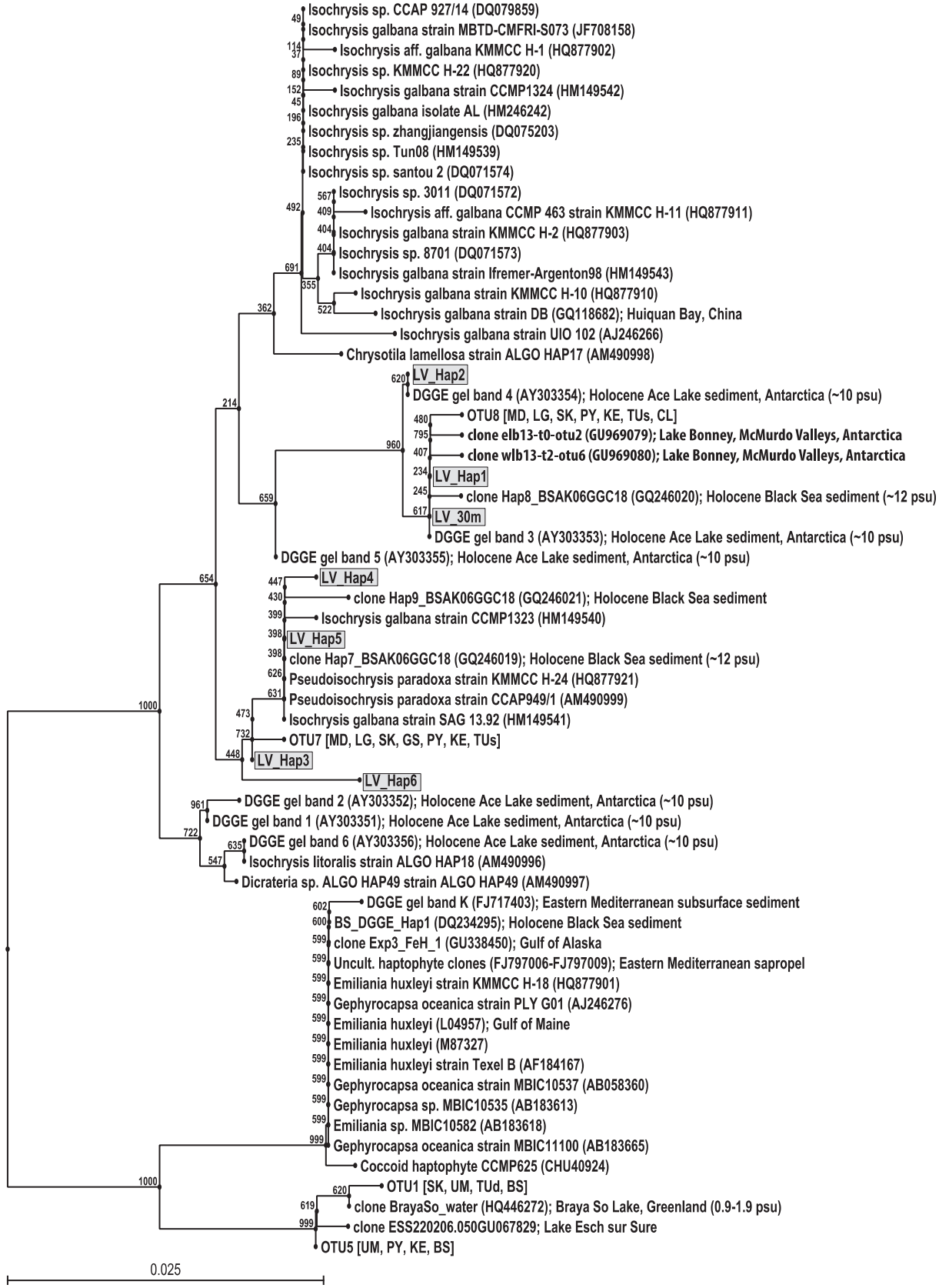

Fig. 4. Neighbor Joining bootstrap tree with haptophyte OTUs from Lake Van in gray boxes labeled (*LV_Hap1-6*) for the sediments and (*LV_30 m*) for the water column as well as the most similar sequences available through GenBank. The scale bar indicates 0.1 fixed point mutations per nucleotide.

Table 2
Alkenone distribution indexes and linear regression coefficient (R^2) describing their correlation to temperatures, from Lake Van sediment trap material (this study) as well as in other published studies. N is the number of data points included within each linear regression. The haptophyte *LV_Hap1* recovered from Lake Van is described thoroughly in Sections 3.3. Calibration approaches include sediment traps (*Sed Trap*) and surface sediments (*Surf Sed*).

Index	R^2, N	Haptophyte	Calibration approach	Reference
Uk37	$R^2 = 0.4$, $N = 6$	LV_Hap1	Sed Trap	this study
	$R^2 = 0.8$, $N = 25$	HapA	Water filters + Cultures	Toney et al. (2012)
	$R^2 = 0.9$, $N = 25$	"brayaSo wat"	Water filters	D'Andrea et al. (2011)
	$R^2 = 0.6$, $N = 21$	HapA and B	Water filters	Toney et al. (2010)
	$R^2 = 1.0$, $N = 14$	*C. lamellosa*	Cultures	Sun et al. (2007)
	$R^2 = 0.6$, $N = 9$	Unknown	Surf Sed	Zink et al. (2001)
Uk3738	$R^2 = 0.4$, $N = 6$	LV_Hap1	Sed Trap	this study
	$R^2 = 0.8$, $N = 13$	Unknown	Surf Sed	Pearson et al. (2008)
Uk38	$R^2 = 0.7$, $N = 12$	Unknown	Surf Sed	Pearson et al. (2008)

recently by estimating relative temperature variation instead of absolute paleotemperature values (Zhao et al., 2013).

In order to explore a semi-quantitative relationship between alkenone distribution and past temperatures, the Uk37 record from Lake Van over 270 ka was compared with independent temperature-sensitive proxies. One of those independent proxies is the SST reconstructed using the Uk′37 index from the Iberian margin (Martrat et al., 2007) (Fig. 6A). Originating from the marine realm, the Iberian margin SST is sensitive to global climate, which is interesting to compare with Lake Van regional climate response. The mean monthly insolation for the month of August at latitude 38°N, which was calculated from Laskar et al. (2004), offers a unique perspective since insolation acts as an external factor on global climate (Fig. 6B). As an indicator of local climate, arboreal pollen abundance in Lake Van sediments is also considered (Fig. 6B). Although sensitive as well to humidity, oak pollen abundance in the Lake Van region is considered to be a robust indicator of past temperatures (Litt et al., 2014). At first glance, Lake Van Uk37 values reach high values during interglacial periods such as MISs 7.5; 7.1; 5.5 and 1. Those interglacial periods also show high oak pollen abundances and high SST (Fig. 6, left). The low sample resolution between 35 and 270 ka (i.e., approximately 1 sample per 3000 years) does not allow further interpretations, however, a closer look at the better resolved 1–35 ka BP timescale (i.e., approximately 1 sample per 300 years) reveals some striking features. Within the transition from the last glacial maximum (LGM) to the Holocene, Uk37 values reached distinctly high values (Fig. 6, right). From the early Holocene towards the late Holocene, Uk37 and insolation are both decreasing. Those decreasing trends are in sharp contrast with pollen abundance and SST values, which were maintained at relatively high values during the Holocene. In summary, Lake Van Uk37 values over the last 270 ka appear to be at least partly dependent on temperature since they are in line with SST as reconstructed by Uk′37 from the Iberian margin (Martrat et al., 2004). Nevertheless, the characteristic trends in Lake Van's Uk37 and temperature over the past 35 ka could have been influenced by temporal shifts in the haptophyte species composition as outlined next.

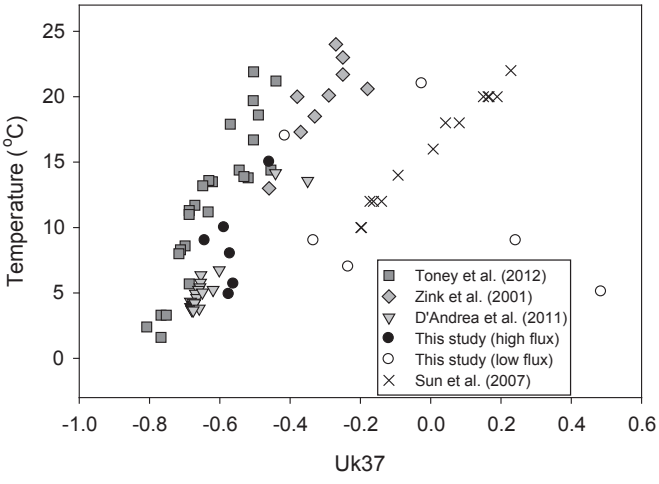

Fig. 5. Uk37 and temperature values obtained from the sediment trap material of Lake Van, compared to Uk37 and temperature values from other studies. Lake Van data points are represented by circles; filled circles corresponding to months of high alkenones fluxes (March–August 2011, Fig. 1) and empty circles corresponding to months of low alkenone fluxes. Data points from other studies are described in Table 2.

4.4. Special alkenone distribution in LV_Hap6 and its relation to salinity

The unique sample containing 100% LV_Hap6 also had a unique alkenone distribution characterized by the presence of MeC38:3 and relatively high ratios of MeC37:3/4 and MeC38:4/5. However, this unusual alkenone distribution was also apparent in samples where we were unable to detect LV_Hap6. Therefore, we cannot claim with absolute certainty that LV_Hap6 is entirely responsible for this unique alkenone composition. However, some speculation is valid in this respect. Uk37 values were often higher than −0.2 in up to 270 ka-old Lake Van sediments (see line in Fig. 6A). Since relatively high Uk37 values and high MeC37:3/4 ratios were observed in the co-presence of LV_Hap6, and since no LV_Hap1 nor OTU_8 seem to be responsible for Uk37 values greater than −0.2 (Fig. 5), it is possible that LV_Hap6 was present but below the detection limit in other sediment layers of Lake Van.

Although DNA analyses remains the most recognized tool for confirming specific haptophyte species (Theroux et al., 2010), this fact should not discourage organic geochemists from investigating novel molecular tracers. For instance, it was pointed out only recently that the haptophyte species Hap_B thriving in Lake Georges surface water produces the compound MeC38:3, which is absent in the genetically distinct haptophyte species Hap_A found in the deep anoxic water of the same lake (Toney et al., 2012).

MeC38:3 was also found in many sediment layers of Lake Van over the last 270 ka (Fig. 6A, white symbols). The reason for the presence of this compound is not clear, but a comparison to the pore water salinity profile of Lake Van reveals an interesting feature (Fig. 7). Despite pore water diffusion, the porewater salinity profile should reflect the lakes salinity condition at the time of sediment deposition during the last 200 ka (Tomonaga et al., 2013). Following this argument, MeC38:3 was absent at times of high surface water salinity in Lake Van (~100 and 20 ka BP). MeC38:3 was occasionally detected in other sections of the core even in intervals where alkenones were generally lower in concentration. The most recent occurrence of MeC38:3 is seen at around 9 ka BP, which corresponds to the early Holocene. Given the evidence for glaciation of the Bitlis massif near Lake Van during the LGM (Akçar and Schlüchter, 2005) it is likely that the occurrence of MeC38:3 is linked to a haptophyte species shift triggered by freshening of the surface water following glacier melting during the early Holocene. Similar links were established in the Black Sea, where a gradual increase in salinity during the early and mid-Holocene caused simultaneous shifts in haptophyte populations and their alkenone compositions (Coolen et al., 2009).

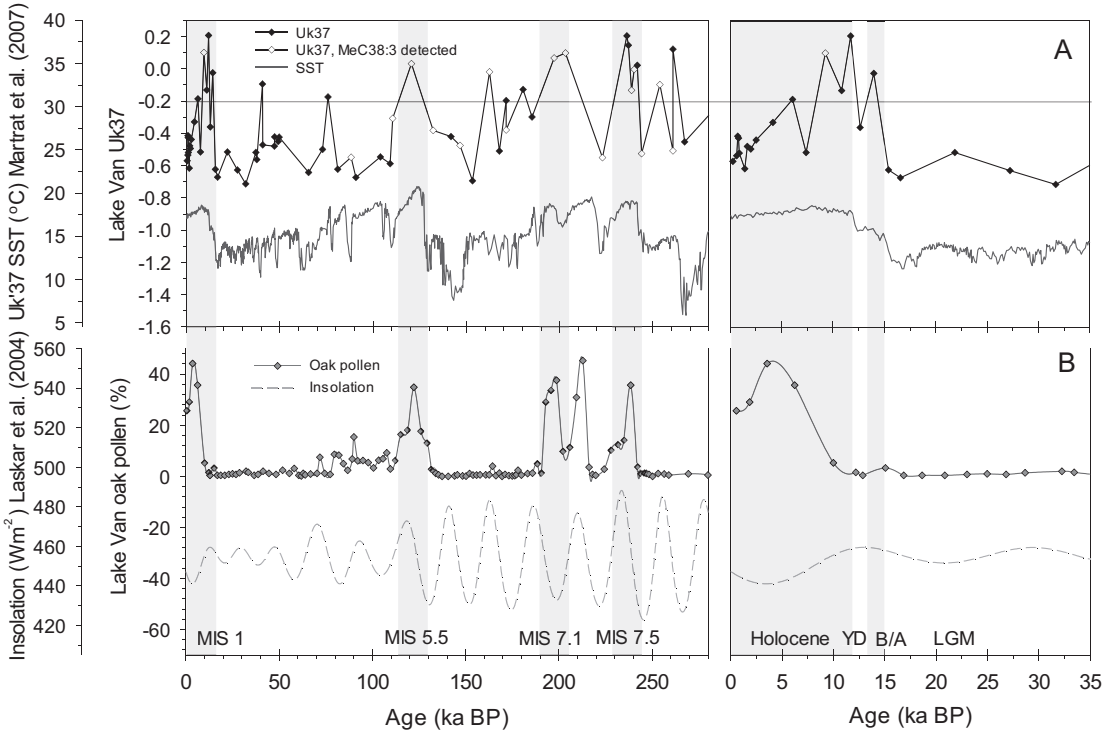

Fig. 6. (A) Lake Van Uk37 proxies compared with alkenone derived sea surface temperatures from the Iberian margin (Martrat et al., 2007) within the sediment core over the last 270 ka (left) and over the last 35 ka (right). The line marks the separation between Uk37 values that are higher and lower than 0.2 (see text). (B) Lake Van arboreal (oak) pollen abundance and mean monthly insolation for the month of August at latitude 38 °N derived from Laskar et al. (2004).

5. Conclusions

The observed shifts in the Uk37 values over the last 270 ka may have been influenced by both temperature and past haptophyte species composition. We were able to reconstruct haptophyte communities from the analysis of fossil DNA as far back as 270 ka in an attempt to establish an *in-situ* temperature calibration for Lake Van. The results imply that species-specific variations in alkenone distributions complicate the quantitative paleotemperature reconstructions. Future studies involving a higher resolution survey of fossil haptophyte 18S rDNA in Lake Van will be helpful to further test this claim. Nevertheless, attempts to link alkenone distributions to haptophyte species in lake settings in general are highly encouraged. Our results further imply that changes in lake salinity coincided with haptophyte community shifts and high molecular MeC37:3/4 and MeC38:4/5 ratios along with the occurrence of the compound MeC38:3. Alkenone deuterium to hydrogen (D/H) ratios as a quantitative salinity proxy (van der Meer et al., 2008) combined with DNA analysis would confirm the role of salinity in regulating historical haptophyte species compositions and would be useful in assessing the suitability of other lakes for reconstructing continental climate using alkenone paleothermometry.

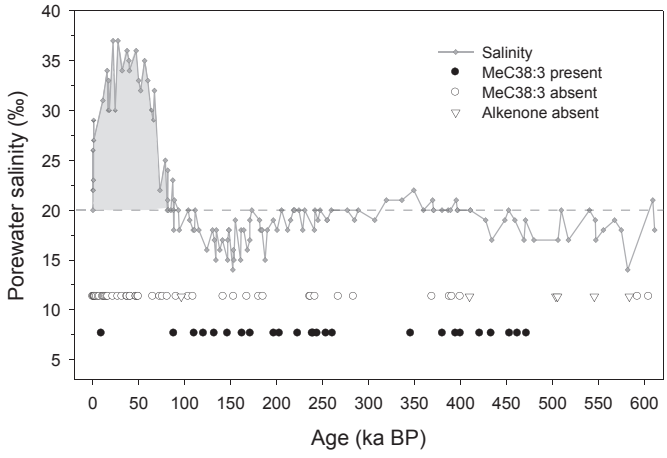

Fig. 7. Salinity (solid line) measured in pore water samples from Lake Van. High salinities between approx. 20 and 100 ka BP represent a high salinity period within which MeC38:3 is absent.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quascirev.2014.07.009.

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