Sedimentary alkenone distributions reflect salinity changes in the Baltic Sea over the Holocene

Lisa Warden[1], Marcel T.J. van der Meer[1], Matthias Moros[2], and Jaap S. Sinninghe Damsté[1,3,*]

[1] *NIOZ Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, the Netherlands.*

[2]*The Leibniz Institute for Baltic Sea Research, Department of Marine Geology, Warnemünde, Germany.*

[3]*Utrecht University, Faculty of Geosciences, P.O. Box 80.021, 3508 TA Utrecht, The Netherlands.*

* corresponding author: jaap.damste@nioz.nl

1 **Abstract**

2 The Baltic Sea has had a complex salinity history since the last deglaciation. Here we show

3 how distributions and concentrations of alkenones and their δD values varied with past

4 fluctuations in salinity in the Baltic Sea over the Holocene by examining a Holocene record

5 (11.2 to 0.1 cal kyr BP) from the Arkona Basin. Major changes in the alkenone distribution,

6 i.e. changes in the fractional abundance of the $C_{37:4}$ alkenone, the $C_{38:2}$ Et alkenones and the

7 $C_{36:2}$ alkenone, the latter which has not been reported in the Baltic Sea previously, correlated

8 with known changes in salinity. Both alkenone distributions and hydrogen isotopic

9 composition suggest a shift in haptophyte species composition from lacustrine to brackish

10 type haptophytes around 7.7-7.2 cal kyr BP, corresponding with a salinity change that

11 occurred when the connection between the basin and the North Sea was re-established. A

12 similar salinity change occurred in the Black Sea making it possible to directly compare and

13 use the δD values and alkenone distributions previously published to corroborate the

14 interpretations made about salinity changes from the data presented for the Baltic Sea. Low

15 and variable salinity waters in the Baltic Sea over the Holocene have allowed for alkenones

16 derived from a variable haptophyte community composition, including low salinity adapted

17 species, hindering the use of the unsaturation ratios of long-chain alkenones for sea surface

18 temperature reconstruction. However, these alkenone based indices are potentially useful for

19 studying variations in salinity, regionally as well as in the past.

20

1. Introduction

Long chain alkenones, biolipids composed of predominantly C_{37}- C_{39} n-alkyl chains with di-, tri- or tetra unsaturations and a keto functionality at position C-2 or C-3 (de Leeuw et al., 1980; Rechka and Maxwell, 1988) are produced exclusively by only a few species of haptophyte algae in both open marine (e.g. *Emiliania huxleyi* and *Geophyrocapsa oceanica*; Volkman et al., 1980, 1995) and coastal or lacustrine regions (e.g. *Isochrysis galbana* and *Ruttnera (Chrysotilla) lamellosa;* Marlowe et al., 1984) and the more recently discovered 'Greenland haptophyte' species, so far exclusively found in Greenland and Alaskan lakes (D'Andrea et al., 2006). An unusual C_{36} diunsaturated alkenone was also identified by Xu et al. (2001) in Holocene sediments from the Black Sea and since then Coolen et al. (2009) reported its biological origin in the Black Sea is most likely a specific strain of *E. huxleyi*.

Since field sampling and culture experiments demonstrated a relationship between surface water temperature and the unsaturation ratios of long-chain alkenones, the unsaturation ratios of sedimentary alkenones have been extensively used as a paleotemperature proxy (Prahl and Wakeham, 1987; Jensen, 1995; Müller et al., 1998). Two alkenone unsaturation ratios have been predominantly used in sea surface temperature (SST) reconstructions, the U^K_{37} (which includes the relative abundance of di-, tri- and tetra- unsaturated alkenones; Brassell et al., 1986) and the $U^{K'}_{37}$ (which excludes the tetra-unsaturated alkenone; Prahl and Wakeham, 1987). Even though this proxy has been successfully applied in marine settings, uncertainties still exist due to increasing evidence of non-thermal effects on alkenone distribution patterns such as species and strain composition (Volkman et al., 1995; Conte et al., 1998) and salinity (Chu et al., 2005; Ono et al., 2012; Chivall et al., 2014). For example, Rosell-Melé (1998) demonstrated that the amount of $C_{37:4}$ alkenone compared to the abundance of the other C_{37} alkenones (%$C_{37:4}$) in particulate organic matter from Nordic Seas had a stronger correlation to sea surface salinity (SSS) than SST. Despite these results, the correlation between salinity

48 and %$C_{37:4}$ in surface water and sediment trap samples worldwide varies greatly and so there

49 is no evidence supporting the application of a linear relationship (Sikes and Sicre, 2002),

50 which would make the use of %$C_{37:4}$ as a salinity proxy possible. However, it has been

51 suggested that if the relative abundance of the $C_{37:4}$ alkenone is over 5% this might be an

52 indicator of alkenone contributions from haptophyte populations adapted to lower salinity

53 conditions (Thiel et al., 1997; Rosell-Melé, 1998; Schulz et al., 2000; Bendle et al., 2005).

54 Therefore, this cut-off value of 5% could be applied when determining if alkenone

55 unsaturation ratios can be used for paleotemperature reconstructions.

56 Alkenones are common biomarkers in marine sediments, but occasionally also occur in

57 lake sediments. In lake settings, such as Lake Van in Turkey (Thiel et al., 1997; Randlett et

58 al., 2014) and Chinese lakes (Chu et al., 2005; Song et al., 2016), complications applying

59 long chain alkenone unsaturation patterns as a temperature proxy have often been noted.

60 Previous studies have found that alkenone biosynthesizing haptophyte algae are much more

61 genetically diverse in lacustrine settings than marine and this could affect alkenone

62 composition and have implications for using alkenone distributions for SST reconstructions

63 (Zink et al., 2001; Sun et al., 2007; Randlett et al., 2014). In a study of 37 lakes in China, Chu

64 et al. (2005) demonstrated that the fractional abundance of the $C_{37:4}$ methyl ketone (i.e. 5-96%

65 of the sum of C_{37} alkenones) is much higher than has been observed in marine settings and is

66 highly variable in the different lakes, complicating the use of the U^{K}_{37} SST proxy. Chu et al.

67 (2005) concluded that, although salinity may be an indirect factor affecting %$C_{37:4}$, it is

68 probably not the main factor. A more recent study on lakes in northwestern China (Song et al.,

69 2016) also encountered complications using alkenone unsaturation ratios as a

70 paleotemperature proxy finding that salinity has a large influence on the occurrence,

71 concentration and composition of alkenones. The predominance of the $C_{37:4}$ methyl ketone and

72 its negative relationship with salinity indicated that its production is probably a response to

73   low salinity conditions. Consequently, it was suggested that the long chain alkenone

74   unsaturation ratio omitting $C_{37:4}$ ($U^{K'}_{37}$) yields more accurate SST estimations when used in

75   lakes. However, recently a new index has been proposed for lacustrine, brackish and estuarine

76   settings that includes the $C_{37:4}$ alkenone and instead excludes $C_{37:2}$ ($U^{K''}_{37}$; Zheng et al., 2016).

77   This study suggests that the di-unsaturated alkenones play a less important role than the tri-

78   and tetra- in regards to regulating cell functions in accordance with temperature fluctuations

79   in lower salinity settings. Additionally, an absence of C_{38} methyl ketones has been observed

80   in alkenone containing freshwater lakes in China (Song et al., 2016), indicating that perhaps

81   this could be used as a criterion for identifying alkenone producers coming from freshwater

82   environments.

83   Previous studies in brackish settings have shown that since alkenone distributions co-vary

84   with salinity driven changes in haptophyte species composition, the use of long chain

85   alkenones is difficult in areas with low and/or fluctuating salinity such as in the Black Sea

86   (Coolen et al., 2009), Ace Lake in Antarctica (Coolen et al., 2004), the Baltic Sea (Rosell-

87   Melé, 1998; Schulz et al., 2000; Blanz et al., 2005) and the North Atlantic and Nordic Seas

88   (Rosell-Melé, 1998). In Ace Lake, Coolen et al. (2004) demonstrated that as lake chemistry

89   changed over time, particularly the salinity as it evolved from a freshwater basin to a marine

90   inlet, the alkenone distributions changed reflecting differences in the haptophyte population as

91   evident from palaeogenetic signatures. Similarly, in the Black Sea, Coolen et al. (2009)

92   showed that as salinity increased over the Holocene in the basin that the haptophyte

93   composition changed as well resulting in erroneous alkenone-derived SST estimates at times.

94   For the North Atlantic and Nordic Seas, Rosell-Melé (1998) observed that the %$C_{37:4}$ is

95   related to salinity as well as temperature changes.

96   In addition to these studies, culture experiments have been performed and confirm that

97   alkenone distribution patterns can vary with changing salinity and that this should be taken

98   into account when using the alkenone unsaturation indices for SST reconstructions (Chu et al.,

99   2005; Ono et al., 2012; Chivall et al., 2014). The results from Chivall et al. (2014) indicate

100   salinity has an effect on alkenone distributions, but that other factors such as growth phase

101   and species composition also play a role in whether the long chain alkenone distributions are

102   affected by salinity. This culture study found a positive correlation between $\%C_{37:4}$ and

103   salinity, however, they found growth phase has a larger effect on the $\%C_{37:4}$ than salinity.

104   Further complicating the use of long chain alkenones for SST reconstructions, Ono et al.

105   (2012) established using culture experiments that salinity had an effect on alkenone

106   unsaturation ratios at 20°C, but not at 15°C.

107   In addition to looking at alkenone amounts and distributions to infer salinity changes,

108   culture studies have shown the hydrogen isotopic composition (δD) of long chain alkenones

109   strongly depends on salinity as well (Schouten et al., 2005). M'boule et al. (2014) confirmed

110   in culture experiments involving both *I. galbana* (a coastal species) and *E. huxleyi* (an open

111   ocean species) that a strong linear relationship exists between δD and salinity, suggesting that

112   δD of alkenones might indeed be used to reconstruct relative shifts in paleosalinity. However,

113   coastal species, such as *I. galbana* and *Chrysotila lamellosa,* have been observed to

114   fractionate almost 100‰ less against deuterium than more open marine species, such as *E.*

115   *huleyi* and *Gephyrocapsa oceanica*, in culture (Schouten et al., 2005; Chivall et al., 2014;

116   M'boule et al., 2014). The δD of sedimentary alkenones might therefore also be indicative of

117   specific alkenone producing haptophytes.

118   The present day Baltic Sea has a large range in salinities (~3.5-32 PSU), with fresher

119   water in the northeastern part of the basin (including the Gulf of Bothnia and Gulf of Finland)

120   and saltier water closer to the connection to the North Sea (ICES-CIEM, n.d.) making it an

121   interesting site to study present-day salinity effects on alkenone production. Schulz et al.

122   (2000) demonstrated that alkenone unsaturation ratios in surface sediments of the Baltic Sea

123   have a low correlation to mean annual SST and instead primarily reflect salinity changes. The

124   authors postulate that lower salinity in parts of the basin causes salinity stress induced

125   changes in alkenone biosynthesis. This, together with the production of alkenones by

126   haptophytes adapted to lower salinities, results in distinct alkenone patterns with lower

127   salinity regions of the basin having patterns more characteristic of freshwater haptophytes or a

128   mixture of freshwater and marine haptophytes, and saltier regions having distributions that

129   resemble more marine haptophyte derived alkenones. Blanz et al. (2005) also reported that in

130   the Baltic Sea salinity-induced stress on *E. huxleyi* could alter the biosynthesis of alkenones,

131   thus affecting the use of the alkenone unsaturation ratios as a proxy for SSTs. Also, the

132   absence of C_{38} methyl ketones observed in lower salinity water masses in the Baltic Sea has

133   been observed in Chinese Lakes as well (Song et al., 2016).

134   Here we determine how alkenone distributions and concentrations, along with the δD of

135   alkenones, varied with past changes in salinity in the Baltic Sea over the Holocene. Not only

136   does salinity vary regionally over the Baltic Sea basin today, but the Baltic Sea also has had a

137   complex salinity history since the last deglaciation and has gone through two fresh water and

138   two brackish water stages (see Section 2.1 for more details). In this study we examined a

139   Holocene record from the Arkona Basin to determine if changes in alkenone amounts and

140   distribution patterns that exist today and correlate with salinity around the basin also existed

141   in the past and co-varied with historical salinity changes.

142

143   **2. Methods**

144   *2.1. Historical setting of the Baltic Sea and description of the study site*

145   The Baltic Sea (Fig. 1) is the world's largest brackish body of water with an area of about

146   377,000 km^2 that is partitioned into multiple sub-basins. The Baltic is almost entirely enclosed

147   by land with a large freshwater contribution (including precipitation) of 660 km^3 yr^{-1} from a

148   drainage basin that is 1.6 million km^2 (Björck, 1995). An inflow of 475 km^3 yr^{-1} of saltwater

149   pours in through the only connection to the North Sea, the narrow Straits of Denmark

150   (Tikkanen and Oksanen, 2002). The Baltic Sea is a fairly shallow basin and on average only

151   about 54 m deep. The salinity varies greatly in the Baltic Sea ranging from ~3.5 PSU in the

152   north to ~8 PSU in the Baltic proper and ~32 PSU in the region where the Baltic connects to

153   the North Sea (ICES-CIEM, n.d.). A permanent halocline exists at about 13-15 m depth,

154   separating a relatively fresh surface and saline bottom waters.

155   The development of the Baltic Sea since the last deglaciation has been the focus of many

156   studies in the last decades (Winterhalter, 1992; Björck, 1995; Jensen, 1995, 1999; Andrén et

157   al., 2000). Reasons for such intense scientific interest include the shifting bathymetry,

158   dynamic hydrology and the resulting fluctuating salinity of the Baltic Sea over the Holocene

159   as the basin went through several different phases. Following deglaciation and before its

160   present state, the Baltic Sea transformed from the freshwater Baltic Ice Lake (c. 12.6-10.3 ka

161   BP) to the slightly brackish Yoldia Sea (c. 10.3-9.5 ka BP) into the freshwater Ancylus Lake

162   (c. 9.5-8.0 ka BP) and then into the brackish Littorina Sea (c. 8.0-3.0 kyr BP) and

163   subsequently into the Post-Littorina Sea / modern Baltic Sea (Winterhalter, 1992; Björck,

164   1995; Andrén et al., 2000). The Baltic Ice Lake formed as large areas of the southern Baltic

165   basin became ice free. Rapid deglaciation resulted in the uplift of the seabed, bringing the

166   connection of the basin with the North Sea above sea level and causing a large influx of fresh

167   melt-water into the system (Björck, 1995). A climatic cooling resulted in less meltwater and

168   the gradually receding ice sheet allowed drainage of the Baltic Ice Lake to occur, lowering the

169   water level and resulting in a short period of seawater ingression, which characterized the

170   very slightly brackish Yolidia Sea (Björck et al., 1995; Jensen, 1995). Continued isostatic

171   rebound caused the basin to be once again cut off from the ocean and resulted in the Ancylus

172   Lake (Jensen et al., 1999). Then, at around 8,000 years ago, eustatic sea level rise re-opened

173   the connection with the North Sea through the Danish Straits allowing salt water to flow into

174   the Ancylus Lake and transforming it into the brackish Littorina Sea (Winterhalter, 1992).

175   The Ancylus Lake/Littorina Sea transition is a complex period characterized by different

176   phases of brackish-water pulses, initially weak and eventually resulting in fully established

177   brackish conditions (15-20‰ in the Baltic proper; Hyvärinen et al., 1988) only after ~2,000

178   years (Andren et al., 2000). The Littorina Sea phase, which lasted from ~8,000-3,000 BP, is

179   characterized by a warmer climate and thought to reflect the most marine-like conditions in

180   the Baltic Sea since deglaciation (Andren et al., 2000). The Post-Littorina Sea/modern Baltic

181   Sea are a continuation of the Littorina Sea, but with a salinity thought to be almost half (7-8‰

182   in the Baltic proper; Hyvärinen et al., 1988) that of the Littorina Sea (Punning et al., 1988).

183   *2.2. Sampling*

184   Two sediment cores were retrieved from the Arkona Basin, which extends from the

185   Bornholm Basin to the Danish Isles of Falster and Zealand (Fig. 1; Table 1). This basin

186   represents a boundary between the Straits of Denmark, where high salinity water flows in, and

187   the lower salinity Baltic Sea basin. The total discharge of brackish water from the basin is on

188   the order of 950 km^3/yr (Björck, 1995). Both sediment cores were 12 m long and collected

189   using a gravity corer on the R/V "Maria S. Merian" in April of 2006. Sediment core 318310

190   was recovered at 46 m water depth at 54°50.34'N and 13°32.03'E and core 318340 was

191   collected nearby at 54°54.77'N and 13°41.44'E at 47 m water depth.

192   Two surface sediment samples from the Skagerrak obtained using a multi-corer

193   provided a marine end member for comparison with our Baltic Sea sediment core samples.

194   The surface sediment samples were collected during R/V "Elisabeth Mann-Borgese" cruise

195   EMB046 in May 2013. The sampling site for EMB046-10 was positioned at 57°49.74´N and

196   07°17.66´E from 457 m water depth. Site EMB046-20 was situated a bit to the east of

197   EMB046-10 at 58°31.60´N and 09°29.09´E from 532 m water depth.

198   *2.3. Loss on ignition (LOI)*

199   The LOI was determined by ashing freeze-dried sediments at 550°C for 3 h. The

200   resulting mass difference was then calculated in wt.%. Previously, it was demonstrated that

201   LOI provides an accurate estimate of the total organic carbon content of the sediments in the

202   Baltic Sea (Leipe et al., 2011). In order to obtain estimates for the total organic carbon (TOC;

203   %) content to normalize the concentration of ketones in the sediments, LOI values were

204   divided by 2.5 (i.e. assuming that the organic matter contains on average 40% C; Dean, 1974)

205   *2.4. X-ray fluorescence (XRF) core scanning*

206   XRF elemental scanning of sediment cores 318310 and 318340 was performed with

207   an Avaatech XRF scanner (Avaatech, n.d.) at a resolution of 0.5 cm.

208   *2.5. Correlation of sediment cores and age model*

209   Sediment cores 318310 and 318340 were correlated to each other on the basis of LOI

210   and XRF-Ca records (Fig. 2). The transition of the Ancylus Lake phase to the Littorina Sea

211   phase is marked by a substantial increase in the TOC content, coinciding with color change of

212   the sediment (e.g. Moros et al., 2002; Rößler et al., 2011). Just after the large increase in TOC

213   (here reflected in the LOI record), there is a maximum in the carbonate content (here reflected

214   in the maximum in the elemental XRF- Ca record) (Fig. 2), which is caused by the occurrence

215   and preservation of benthic foraminifera (Moros et al., 2002; Rößler et al., 2011). The

216   Ancylus Lake regression is also characterized by a clear peak in the LOI (TC) records, which

217   can be used for correlation purposes (Fig. 2). The transitions, Baltic Ice Lake/ Yoldia Sea and

218   Yoldia Sea/Ancylus Lake are revealed by marked changes in the elemental XRF-Ca

219 (carbonate) and bulk density records (Fig. 2; see Moros et al., 2002), and by basin-wide

220 traceable sandy layers (Moros et al., 2002).

221   The age model for sediment core 318310 is based on a previous AMS[14]C date (7.2 cal

222 kyr BP) on *Mytilus edulis* close to the base of the Littorina phase (Rößler et al., 2011) and five

223 additional dates on mollusc shells (Fig. 2). The age model of the section of sediment core

224 318340 that was studied (400-840 cm) is based on the carbonate maximum at 380 cm (7.2 cal

225 kyr BP; Moros et al., 2002; Rößler et al., 2011). The start of the Ancylus Lake/Littorina Sea

226 transitional phase at 7.7 cal. kyr BP (unpublished results) is revealed by the increase in the

227 LOI record at 485 cm, and the Ancylus Lake regression at 10.2 cal kyr BP is denoted by the

228 sharp peak in the LOI record (sandy layer; Moros et al., 2002) at 600 cm. The boundary

229 between the Yoldia Sea phase and the Ancylus Lake phase (10.6 cal kyr BP; Moros et al.,

230 2002) at 768 cm, and the boundary between the Baltic Ice Sea and Yoldia Sea phases (11.6

231 cal. kyr BP; Moros et al., 2002) at 895 cm.

232 *2.6. Lipid extraction and analysis*

233   The sediments were freeze dried and ground and homogenized by mortar and pestle

234 for extraction. In general, 1-3 g of sediment was extracted using a Dionex[TM] accelerated

235 solvent extractor with dichloromethane/methanol (9:1; v/v) as extraction solvent. The total

236 lipid extract was dried over a Na_2SO_4 column and then separated into three fractions using

237 Al_2O_3 column chromatography: apolar (eluted with 9:1 v/v hexane/DCM), ketone (1:1 v/v

238 hexane/DCM), and polar (1:1 v/v DCM/MeOH) fractions. The ketone fraction was then base

239 hydrolyzed by refluxing the dry fraction in a 1 N KOH in MeOH solution for 1 h after which

240 the pH was adjusted using a 2 N HCL/MeOH solution. DCM was added and the solution was

241 washed twice with DCM. The DCM layers were removed and combined to be dried over a

242 Na_2SO_4 column. After the addition of a nonadecan-10-one internal standard, the alkenone

243   fraction was analyzed using gas chromatography (GC) with an Agilent 6890 instrument

244   equipped with an Agilent CP-Sil 5 CB column (50 m x 0.32 i.d.; 0.12 μm film thickness) and

245   a temperature program from 70°C increasing at 20°C/min to 200°C and then at 3°C/min to

246   320°C where it remained stable for 44 min. Alkenones were identified by GC-mass

247   spectrometry (GC-MS), including the $C_{36:2}$ alkenone, using an Agilent 7890A GC instrument

248   equipped with a Agilent 5975C VL mass selective detector (MSD) and by comparing relative

249   retention times with those of known alkenones of a culture of *E. huxleyi*. Peak areas were

250   used to calculate alkenone unsaturation indices and alkenone concentrations were determined

251   based on peak responses relative to the nonadecan-10-one internal standard.

252   *2.7. Compound specific hydrogen isotope compositions*

253   Alkenone hydrogen isotope analyses were carried out on a subset of the samples, i.e.

254   those containing sufficient amounts of alkenones, on a Thermo Scientific DELTA$^+$ xl

255   GC/TC/irMS. The temperature conditions of the GC increased from 70 to 145˚C at 20˚C min$^-$

256   ^{1}, then at 8˚C min^{-1} to 200˚C and to 320˚C at 4˚C min^{-1}, at which it was held isothermal for 20

257   min using an Agilent CP Sil-5 column (25 m x 0.32 mm) with a film thickness of 0.4 μm and

258   helium as carrier gas at 1 ml min^{-1} (constant flow). The high temperature conversion reactor

259   was set at a temperature of 1425°C. The H_3^+ correction factor was determined daily and was

260   constant at 5.6±0.2 before and 3.8±0.1 after a scheduled power outage and retuning of the

261   irm. A set of standard n-alkanes with known isotopic composition (Mixture B prepared by

262   Arndt Schimmelmann, University of Indiana) was analyzed daily prior to analyzing samples

263   in order to monitor the system performance. Samples were only analyzed when the alkanes in

264   Mix B had an average deviation from their off-line determined value of <5‰. Squalane was

265   co-injected as an internal standard with each sample to monitor the accuracy of the alkenone

266   isotope values. The δD of long chain C_{37} alkenones were measured as the combined C_{37}

267   alkenones (δD$_{alkenone}$) (van der Meer et al., 2013) and the same applies to the C_{38} alkenones.

268 The squalane standard yielded an average $\delta D_{alkenone}$ value of -160.7±2.7, which is stable but

269 relatively enriched in D compared to its offline determined δD value of -170 ‰, potentially

270 due to co-eluting compounds in this sample set.

2.8. Calculation of alkenone based proxies

272 %$C_{37:4}$ is the contribution of the tetra-unsaturated 37-carbon methyl alkenone ($C_{37:4}$) to

273 total C_{37} alkenone concentrations and calculated according to Rosell-Melé (1998):

$$\%C_{37:4} = C_{37:4} / (C_{37:2} + C_{37:3} + C_{37:4}) \times 100 \qquad (1)$$

275 The U^{K}_{37} index represents the relative abundance of the diunsaturated ($C_{37:2}$), triunsatured

276 ($C_{37:3}$), and tetraunsaturated ($C_{37:4}$) methyl ketones (Brassell et al., 1986). Later, the

277 tetraunsaturated methyl ketone ($C_{37:4}$) was removed from the equation because this compound

278 was rarely found in open-sea sediments or suspended water column particles and the equation

279 was modified by Prahl and Wakeham (1987):

$$U^{K'}_{37} = C_{37:2} / (C_{37:2} + C_{37:3}) \qquad (2)$$

2.9. Statistical analysis

282 Utilizing the R software package for statistical analysis, principle component analysis

283 (PCA) based on the correlation matrix was executed on the fractional abundances of the eight

284 alkenones quantified in the sediments studied. Four sediment samples from sediment core

285 318340 with no alkenones present were omitted from the PCA.

3. Results

3.1. Phases of the Baltic Sea covered in the Arkona Basin record

288 The XRF (Ca) and LOI (TC) data were used to distinguish different phases captured

289 by each sediment core in this study (Fig. 2) and to correlate the two sediment cores (see

290   methods). The sedimentary record for sediment core 318310 covers the upper section of the

291   freshwater Ancylus Lake stage starting at 10.2 cal kyr BP (642.5 cm), but mostly spans the

292   brackish phase of the basin beginning from 7.1 cal kyr BP (600-20 cm) (Fig. 2a). From

293   sediment core 318310 we studied eight sediment samples representing the brackish phase

294   including the Littorina Sea and Post-Littorina Sea / modern Baltic Sea stage and two samples

295   representing the Ancylus Lake stage (Fig. 2a; Table 1). To obtain more information on

296   alkenone occurrence and distribution during the Ancylus Lake stage, we also studied samples

297   from another sediment core. This core (318340) includes the complete Yoldia Sea stage (11.6-

298   10.6 cal kyr BP; 847.5-780.5 cm), the Ancylus Lake stage (10.6-7.7 cal kyr BP; 750.5-500.5

299   cm), and the Littorina Sea / Post Littorina Sea stage (7.2 -0 cal kyr BP; 400.5-0 cm) (Fig. 2b).

300   We analyzed 14 sediment samples from this core spanning depths 840.5-400.5 cm (Fig. 2b;

301   Table 1).

302   *3.2. Alkenone concentrations and distributions*

303   Total alkenone concentrations were generally higher (i.e. 32±45 µg/g C;

304   average±standard deviation) in the brackish portion of the Arkona Basin record than for the

305   freshwater portion of the record (11±17 µg/g C; Table 1). In the latter case, there were also

306   sediment horizons that did not contain detectable concentrations of alkenones. In the

307   sediments of the Yoldia Sea phase no alkenones were detected (Table 1). Fig. 3 shows some

308   typical alkenone distributions from sediment core 318310. Alkenones are comprised of the

309   more common $C_{37:2,}$ $C_{37:3,}$ and $C_{37:4}$ methyl (Me) ketones, $C_{38:2}$ and $C_{38:3}$ methyl (Me) and

310   ethyl (Et) ketones, and the uncommon $C_{36:2}$ Me ketone. This latter alkenone has not been

311   previously reported in sediments of the Baltic Sea. It is especially relatively abundant in

312   sediments deposited during the Littorina Sea period. Skagerrak surface sediments (Fig. 1)

313   were analyzed as a marine end member for comparison with the results obtained from the

314   Arkona Basin record. We did not detect the presence of the $C_{36:2}$ alkenone in the Skagerrak

315   sediments (Fig. 3a; Table 1). In sediment core 318310 a large difference in the relative

316   abundance of C_{36}, C_{37} and C_{38} alkenones is observed from 600 cm depth (c. 7.1 cal kyr BP;

317   Fig. 3d), which is close to the Ancylus Lake/Littorina Sea transitional phase, to more recent

318   sediments from the brackish phase of the Baltic Sea, i.e. at 100 cm (0.9 cal kyr, BP) and 50

319   cm (0.4 cal kyr BP) depth (Fig. 3b-c). For the Skagerrak surface sediments the alkenone

320   distribution is representative of a more open ocean setting (Fig. 3a). The alkenone distribution

321   at 50 cm depth (Fig. 3b) is more similar to that of the Skagerrak sample than any of the other

322   alkenone distributions shown (Fig. 3d), however, there are still a few differences between the

323   two, such as the absence of the $C_{36:2}$ alkenone and the lower relative abundance of $C_{37:4}$ Me in

324   the Skagerrak sediments.

325   For a statistical evaluation of alkenone distribution changes, PCA was performed on

326   the distributions of C_{36}, C_{37} and C_{38} alkenones in the different sediments studied. Most of the

327   variation is explained by principle component 1 (PC1; expressing 41% of the variance), which

328   is related to the degree of unsaturation of the alkenones with the most unsaturated alkenones

329   scoring negatively on PC1 (Fig. 4a). This is confirmed by the good correlation ($r^2 = 0.86$) of

330   the score on PC1 with $U^{K'}_{37}$ (Fig. 4e). The variation in PC2 (27%) appears to be mostly

331   explained by the fractional abundance of the $C_{36:2}$ alkenone, which scores negatively on PC2

332   (Fig. 4a). Indeed, the score on PC2 significantly ($r^2 = 0.77$) negatively correlates with the

333   fractional abundance of the $C_{36:2}$ alkenone (Fig. 4f). PC3 explains 18% of the variance with

334   the C_{38} Et ketones scoring negatively on PC3 (Fig. 4c). PC3 correlates significantly ($r^2 = 0.77$)

335   negatively with the summed fractional abundance of the $C_{38:3}$ and $C_{38:2}$ Et ketones (Fig. 4g).

336   Most sediments score between -1 and +1 on PC2, however, the Skagerrak sediments

337   plot more positively (ca. 2.0) and sediments from the core 318310 from the Littorina Sea

338   phase (sediment core depths 400, 500 and 600 cm) plot more negatively on PC2 (ca. -3.2)

339   (Fig. 4b). Fig. 5a shows the scores of PC1-3 plotted as a function of age. This reveals that the

340   score on PC1 is mostly negative for sediments older than 7.2 cal kyr BP and is mostly positive

341   during the more recent phases of the Baltic Sea (after 7.2 cal kyr BP) (Fig. 5a). The score on

342   PC2 consistently plots positively throughout the combined record from the Arkona Basin

343   except for the sediment depths that correspond to the Littorina Sea phase (sediment core

344   depths 400-600 cm from core 318310, which spans 7.1-3.7 cal kyr BP) and the end of the

345   Ancylus Lake phase (sediment core depth 400.5 cm in core 318340, which spans 7.1-3.7cal

346   kyr BP; Fig. 5a-b (Fig. 5a). Two other core 318340 samples that plot slightly negatively for

347   PC2 are depths 480.5 cm (7.7 cal kyr BP) and 750.5 cm (10.6 cal kyr BP) (Fig. 5a). PC3

348   scores mostly between -1 and 1 throughout the sediment record and for the Skagerrak

349   samples, however, some samples that fall within the Ancylus Lake phase plot outside of this

350   range as does sediment sample 100 cm from record 318310 (0.9 cal kyr BP; Figs. 4d and 5a).

351

352   *3.3. δD of alkenones*

353   We also determined δD values of alkenones on a subset of the samples from sediment

354   core 318310 (Table 2), which can be an indicator of environmental conditions, mainly salinity

355   and potentially haptophyte species composition (Schouten et al., 2005; van der Meer et al.,

356   2008, 2015; Chivall et al., 2014; M'boule et al., 2014). The surface sediment samples from

357   the Skagerrak have similar δD values for the C_{37} and C_{38} alkenones that fall between -175 and

358   -185‰ (Fig. 6; Table 2). In the Arkona Basin the C_{37} and C_{38} alkenones have lower δD values

359   during the more recent brackish phase going back to about 2.7 cal kyr BP, (-212.2±5.5‰)

360   (Fig. 6; Table 2). However, at the base of the Littorina Sea phase (7.1 cal kyr BP, 600 cm

361   sediment depth from sediment core 318310), the δD values for C_{37} (-182.4‰) and C_{38}

362   alkenones (-170.3‰) are much higher and, in contrast to the other samples, the C_{37} are more

363   depleted in D than the C_{38} alkenones. The obtained δD values of the $C_{36:2}$ alkenone deposited

364   during the brackish portion of the record in the Arkona Basin are enriched in D relative to the

365   C_{37} and C_{38} alkenones from the same samples, but similar to those of the C_{37} and C_{38}

366   alkenones encountered in the modern day Skagerrak (-169.3±3.0‰). Just after the Ancylus

367   Lake/Littorina Sea transition, the δD values of the $C_{36:2}$ alkenone (-168.7‰) is similar to that

368   of the C_{38} alkenones (-170.3‰; Fig. 6; Table 2).

369

370   **4. Discussion**

371   *4.1. Changes in sources of alkenones and its relation to changes in salinity*

372   The observed changes in the relative abundances of the different alkenones through

373   time may be a direct response of alkenone biosynthesis to changing environmental conditions

374   of the Baltic Sea over the Holocene, or alternatively, the changing conditions could result in

375   changing species composition leading to different alkenone distributions. There are many

376   characteristics of alkenones that have been linked to haptophyte species composition and/or

377   environmental conditions. The most important are:

378   (i) The degree of unsaturation of alkenones is commonly interpreted to be

379   predominantly dependent on growth temperature (Brassell et al., 1986; Prahl and Wakeham,

380   1987).

381   (ii) The relative abundance of the $C_{37:4}$ alkenone is generally higher in coastal

382   haptophytes that thrive at lower salinities and this predominance is even more extreme in

383   freshwater systems (Rosell-Mele, 1998; Schulz et al., 2000; Blanz et al., 2005; Liu et al.,

384   2008, 2011).

385   (iii) The ratio of C_{37}/C_{38} alkenones might be indicative of haptophyte species since

386   different C_{37}/C_{38} values were observed for different haptophytes with coastal haptophytes

387 generally showing higher ratios compared to more open ocean species (Prahl et al., 1988;

388 Conte et al., 1998; Schulz et al., 2000). However, it has also been shown that environmental

389 conditions, e.g. temperature, also affect the C_{37}/C_{38} ratio (Conte et al., 1998; Sun et al., 2007).

390   (iv) The presence of the uncommon $C_{36:2}$ alkenone, which only has been reported in

391 the Black Sea (Xu et al., 2001; Prahl et al., 2006), Japan Sea (Fujine et al., 2006), and in an

392 estuary in Florida (Van Soelen et al., 2014). Previous studies suggested it to be an indicator of

393 brackish conditions (Xu et al., 2001; Fujine et al., 2006) and more recently, Coolen et al.

394 (2009) proposed its biological origin in the Black Sea is likely a strain of low salinity-adapted

395 *E. huxleyi*.

396   (v) The δD of alkenones, the values of which are characteristic of certain types of

397 haptophytes, but can also change with changing environmental conditions. Coastal

398 haptophytes tend to fractionate less than more open marine haptophytes (Schouten et al.,

399 2005; Chivall et al., 2014; M'boule et al., 2014), therefore, δD values can aid in assigning

400 biological sources of sedimentary alkenones. However, hydrogen isotope fractionation also

401 depends on environmental factors such as salinity, light intensity and growth rate (Schouten et

402 al., 2005; Prahl et al., 2006; van der Meer et al., 2008, 2015; Wolhowe et al., 2015).

403   Some of these parameters were used to assign potential biological sources of the Baltic

404 Sea sedimentary alkenones. To this end, the Arkona Basin data was compared with that from

405 surface sediments of the Skagerrak (Figs. 4 and 6). Marine haptophytes, such as *E. huxleyi*,

406 living at higher salinities and in more open ocean settings (like the Skagerrak) with a salinity

407 of approximately 34 PSU (Danielssen et al., 1996) will fractionate at approximately 190‰

408 against D. Using a δD of Skagerrak water of ca. 0‰ (Frohlich et al., 1988) the δD values for

409 the C_{37} and C_{38} alkenones are predicted to be ca. -190‰ (Englebrecht and Sachs, 2005;

410 Schouten et al., 2005; M'boule et al., 2014). The δD value of the C_{37} and C_{38} alkenones in the

411   Skagerrak surface sediments is -180±5‰ (Fig. 6; Table 2), indicating the haptophyte species

412   in this region are predominantly of the marine type, most likely derived from *E. huxleyi*.

413   The alkenones in the sedimentary record of the Arkona Basin up to ca. 2.7 cal kyr BP

414   have a distribution that is quite similar to that observed in Skagerrak surface sediments (Figs.

415   3a-c), which is typical of a marine haptophyte such as *E. huxleyi*. The low %$C_{37:4}$ during this

416   time (3.1±2.3%) would also suggest that these alkenones are derived from marine type

417   haptophytes (i.e. *E. huxleyi*) (Fig. 5d). However, the C_{37} and C_{38} alkenones have substantially

418   lower δD values (-212±6‰) than found in the Skagerrak surface sediments for the alkenones

419   (-180±5‰; Fig. 6; Table 2). There are two main factors to consider. Firstly, the present-day

420   δD of surface waters in the Arkona Basin is ca. -40‰ averaged over the photic zone (Frohlich

421   et al., 1988), i.e. 40‰ depleted relative to the Skagerrak waters. This will shift the δD values

422   of alkenones to substantially lower values (e.g., Englebrecht and Sachs, 2005). Secondly,

423   culture studies have shown that hydrogen isotope fractionation is dependent on salinity,

424   among other factors, with increased fractionation at lower salinities (e.g. M'boule et al.,

425   2014). The present-day salinity of surface waters of the Arkona Basin is ~10 PSU (ICES-

426   CIEM, n.d.). If *E. huxleyi* would be able to grow at these low salinities, the alkenone δD value

427   is estimated at ca. -270‰, which is substantially lower than the measured values for the C_{37}

428   and C_{38} alkenones (-212±6‰). This value was arrived upon by extrapolating the isotope

429   fractionation (α)-salinity relationship to these low salinities (M'boule et al., 2014), and using

430   the δD value for surface waters of -40‰ over the photic zone (Frohlich et al., 1988).

431   Consequently, this indicates that an *E. huxleyi* only origin for the C_{37} and C_{38} alkenones in the

432   sedimentary record of the Arkona basin up to ca. 2.7 cal kyr BP, is unlikely. Haptophyte

433   species adapted to lower salinities, such as *I. galbana* or *C. lamellosa*, fractionate less against

434   D (Chivall et al., 2014; M'Boule et al., 2014), and the alkenones produced will have a less

435   negative δD value. For *I. galbana* (M'boule et al., 2014) a δD value of alkenones of ca. -

436   180‰ can be estimated using a salinity of 10 PSU the δD of surface waters of -40. This value

437   is higher than the values observed for the C_{37} and C_{38} alkenones in the Arkona Basin up to ca.

438   2.7 cal kyr BP (i.e. between -205 and -220‰). This suggests that these sedimentary alkenones

439   represent a mixture of alkenones produced by low salinity adapted haptophytes such as *I.*

440   *galbana* and higher salinity adapted haptophytes such as *E. huxleyi*, with a more substantial

441   contribution from the low salinity adapted haptophytes.

442   The $C_{36:2}$ alkenone was detected in the Arkona Basin sediments, but not in the

443   Skagerrak surface sediments (Fig. 3; Table 1). This supports the premise that the $C_{36:2}$

444   alkenone is exclusively produced by a low-salinity adapted haptophyte (Coolen et al., 2009).

445   PCA revealed that the fractional abundance of the $C_{36:2}$ alkenone is an important factor in the

446   changing alkenone distributions in the Baltic Sea (Figs. 4a and f); i.e. PC2, explaining 27% of

447   the total variance, is predominantly determined by the fractional abundance of the $C_{36:2}$

448   alkenone (Fig. 4a). In the sedimentary record of the Arkona Basin up to ca. 2.7 cal kyr BP, the

449   fractional abundance of the $C_{36:2}$ alkenone amounts to 0.10±0.03 (Figs. 5c; Table 1). From

450   7.1-3.7 cal kyr BP in the sediment record the fractional abundance of the $C_{36:2}$ alkenone

451   increases to 0.51±0.11 and it dominates the alkenone distribution (Figs. 3d and 5c; Table 1).

452   For the entire period of 7.1-0.1 cal kyr BP the δD values for the $C_{36:2}$ alkenone show only

453   minor variation and are similar to the δD values for the C_{37} and C_{38} alkenones from the

454   modern day Skagerrak (-170±3‰; Fig. 6; Table 2). However, for most of the record the δD

455   value of the $C_{36:2}$ alkenone is significantly higher than those of the C_{37} and C_{38} alkenones.

456   Since the δD value of the $C_{36:2}$ alkenone is close to that (-180‰) calculated for *I. galbana*

457   using a salinity of 10 PSU and a δD of surface waters of -40‰ (see above) this suggests that it

458   is derived from a single low-salinity adapted haptophyte species. Previous studies (Coolen et

459   al., 2009; Van Soelen et al., 2014) have also reported a substantial offset in δD values for C_{37}

460   and $C_{36:2}$ alkenones with that of the $C_{36:2}$ alkenone being significantly higher. Van Soelen et al.

461   (2014) concluded that the offset in δD values for C_{37} and $C_{36:2}$ alkenones found in an estuary

462   in Florida is evidence that different haptophytes, yet still unknown, are producing the $C_{36:2}$

463   alkenone. Interestingly, close to the Ancylus Lake/Littorina Sea transition (7.1 cal kyr BP,

464   600 cm sediment depth from core 318310), which falls within the period characterized by the

465   high fractional abundance of the $C_{36:2}$ alkenone, the δD values for C_{37} (-182.4‰) and C_{38} (-

466   170.3‰) alkenones are much higher than in the other Arkona Basin samples and similar to

467   the δD values of the $C_{36:2}$ alkenone (Fig. 6; Table 2). This suggests a similar origin for most of

468   the C_{36}, C_{37} and C_{38} alkenones at this time, most likely a low salinity adapted haptophyte

469   species. This is strongly supported by the deviating alkenone distribution at this time (Fig. 3d)

470   dominated by the $C_{36:2}$ alkenone. The observed trends in δD values of alkenones over the

471   period between 7.1 and 0.1 cal kyr BP, thus, corroborate the idea that during this period in the

472   Baltic Sea there is more than one alkenone producing haptophyte species.

473   From circa 7.1-3.7 cal kyr BP, during the Littorina Sea phase in the Baltic Sea, the

474     $C_{36:2}$ ratio is highest demonstrating the greatest contribution from these low-salinity adapted

475   haptophytes and the %$C_{37:4}$ is more variable during this period ranging from 0.1-13.6%. This

476   suggests mutable input from non-marine type haptophytes and therefore potentially

477   fluctuating salinities (Fig. 5d; Table 2). The enrichment in the δD of the C_{37} and C_{38} alkenones

478   corroborates the contribution from non-marine haptophytes as well (Fig 6). Why the %$C_{37:4}$

479   and the fractional abundance of the $C_{36:2}$ alkenone is higher and the C_{37} and C_{38} alkenones are

480   more enriched in D during the Littorina Sea phase than after is not clear since they are both

481   brackish water periods. Possibly this is related to the period after the Ancylus Lake/Littorina

482   Sea transition being a time of not only low, but also variable salinity. Perhaps the haptophytes

483   producing the $C_{36:2}$ alkenone had a competitive advantage over other haptophytes at this time

484   because they were better adapted to changing salinities, or alternatively, certain haptophyte

485   species biosynthesize this compound in response to changing salinities or marine haptophytes

486　brought in from the North Sea were not yet established. These possibilities suggest that

487　variable salinity was a characteristic of the Littorina Sea phase.

488　　　Prior to 7.1 cal kyr BP in the Arkona Basin sediment record we do not have δD values

489　of alkenones to report, however, some remarkable changes in the alkenone distributions are

490　observed. Firstly, the $U^{K'}_{37}$ is lower prior to the Ancylus Lake/Littorina Sea transition (Fig.

491　5e) potentially due to a change in the composition of the haptophyte community as indicated

492　by the higher fractional abundance of the $C_{37:4}$ at this time (Fig. 5d; Table 1). Secondly, the

493　fractional abundance of the $C_{36:2}$ alkenone is relatively low from 10.7 cal kyr BP up to the

494　transition (0.02±0.03; Fig. 5c; Table 1). Thirdly, during the transitional phases of this time

495　period, both the Yoldia Sea phase to Ancylus Lake transition (c. 10.7-10.6 cal kyr BP) and at

496　the Ancylus Lake/Littorina Sea transition (c. 7.3 cal kyr BP), the alkenone distributions are

497　dominated by C_{38} ethyl alkenones (i.e. a low score on PC3; Figs. 4c-d and i.e. summed

498　average fractional abundance of 0.55±0.03; Fig. 5b; Table 1). The lower fractional abundance

499　of the $C_{36:2}$ alkenone during the Ancylus Lake phase, (Fig. 5c; Table 1) suggests that most

500　likely a change in haptophyte species composition occurred related to salinity. Additionally,

501　the $C_{36:2}$ alkenone is absent in the Arkona Basin sedimentary record from 10.2-8.0 cal kyr BP

502　(Fig. 5c; Table 1). Since it is a potential indicator for the low salinity adapted, but not

503　freshwater haptophyte species, the presence of the $C_{36:2}$ alkenone prior to the Anyclus Lake

504　phase ending suggests marine influxes had already begun in the basin at that time. A diatom

505　study by Witkowski et al. (2005) reported that the first brackish water inflows began just

506　before this time period, i.e. between 8.9-8.4 kcal yr BP. The presence of the $C_{36:2}$ alkenone

507　from 10.6-10.2 cal kyr BP aligns with the ending of the slightly brackish Yoldia Sea phase.

508　Lastly, the higher %$C_{37:4}$ during the Ancylus Lake phase verifies that there was an increase in

509　freshwater haptophytes during this time.

510　*5.2. Comparison with the Holocene alkenone record of the Black Sea*

511   A previous study of alkenones in the Black Sea (Coolen et al., 2009) reported similar

512   trends with respect to the fractional abundance of the $C_{36:2}$ alkenone to those reported here for

513   the Baltic Sea. The Black Sea experienced a somewhat comparable geological history to the

514   Baltic Sea. In the early Holocene it was a freshwater lake until a connection was established

515   with the Aegean and Mediterranean Seas due to the global transgression allowing the influx

516   of more saline waters (Ryan et al., 1997). The permanent establishment of this connection is

517   dated at c. 7.2 cal kyr BP (Ryan et al., 1997; Ballard et al., 2000). The resultant increase in

518   salinity is reflected by the sedimentary sequence revealing a transition from banded clay with

519   graded sand and silt layers (Unit III) to sapropel mud (Unit II) (Ross et al., 1970). As the

520   influx of salty Mediterranean waters continued it caused an increase in the surface salinity of

521   the Black Sea allowing a massive growth of *E. huxleyi* (Jones and Gagnon, 1994) in the basin

522   c. 2.7 cal kyr BP, resulting in deposition of a coccolith ooze (Jones and Gagnon, 1994). The

523   abundance in *E. huxleyi* at this time has been attributed to a surface water salinity increasing

524   above 11 PSU and the base of Unit I is generally defined as the horizon that reveals the first

525   invasion of *E. huxleyi*, ca. 700 yr earlier (Fig. 7) (Arthur and Dean, 1998; Hay, 1988).

526   Since the salinity changes in the Baltic Sea and Black Sea occurred around the same

527   time (~7.2 cal kyr BP), we compared the relative abundance of the $C_{36:2}$ alkenone from the

528   Baltic Sea directly to that in the Black Sea reported by Coolen et al. (2009) (Fig. 7a). In both

529   the Baltic Sea and Black Sea the fractional abundance of the $C_{36:2}$ alkenone is rapidly

530   increasing to values of 40-70% just after the inflow of more saline waters started.

531   Subsequently, a period of sustained high fractional abundances follows in both basins up to

532   ca. 2.6 cal kyr BP. The higher resolution record of the Black Sea shows that the period

533   between 7.0-5.4 cal kyr BP is characterized by the highest values (up to 75%), followed by a

534   drop to a fractional abundance of ca. 25% for the period 5.0-2.6 cal kyr BP. This latter period

535   is interrupted by the horizon of the first invasion of *E. huxleyi* at 3.5 cal kyr BP when the

536 fractional abundance drops to 5%. For the Baltic Sea the fractional abundance of the $C_{36:2}$

537 alkenone is high throughout the 7.0-2.6 cal kyr BP period. In both basins the fractional

538 abundance of the $C_{36:2}$ alkenone is substantially reduced in the most recent period (2.6-0.0 cal

539 kyr BP) although for the Baltic Sea it does not drop to the low values seen in the Black Sea

540 (i.e. 1%) and it increases towards the present day situation (Fig. 7a). In conclusion, we note a

541 quite similar behavior for the fractional abundance of the $C_{36:2}$ alkenone in both enclosed

542 basins with limited connection to the open ocean. This may relate to a somewhat comparable

543 response to the global sea level transgression during the Holocene. For the Black Sea

544 substantial additional data is available for the interpretation of this trend and this may help, by

545 analogy, to provide a more detailed interpretation of the Baltic Sea record.

546 Coolen et al. (2006, 2009) provided through ancient DNA analysis clues on the

547 biological origin of the sedimentary alkenone in the Black Sea. The most extensive record

548 comes from a site in the western Black Sea. It reveals that during deposition of the base of

549 Unit II *Isochrysis*-related haptophytes thrived (Fig. 7c). This fits with the time of the newly

550 established connection with the Mediterranean since these type of haptophytes are adapted to

551 low salinity. Subsequently, there is a short period (5.7-4.8 cal kyr BP) where Coolen et al.

552 (2009) detected both *Isochrysis*-related haptophytes and *E. huxleyi*, followed by a period

553 where only ancient DNA of *E. huxleyi* was found. When this information is combined with

554 the record of the fractional abundance of the $C_{36:2}$ alkenone (Fig. 7a), it is evident that this

555 alkenone must have been produced by *Isochrysis*-related haptophytes since the period of

556 highest fractional abundance (up to 75%) falls in the period where only ancient DNA of

557 *Isochrysis*-related haptophytes is detected (Fig. 7). However, the $C_{36:2}$ alkenone also occurs

558 (albeit at a substantially reduced fractional abundance) in more recent periods when only

559 ancient DNA of *E. huxleyi* is detected, suggesting that this haptophyte may also produce this

560 alkenone. However, this latter conclusion is at odds with the large difference (90-100‰) in

561   δD composition of the $C_{36:2}$ and C_{37} alkenones as reported by Giosan et al. (2012) for this

562   section, which indicates clearly distinct biological sources for these alkenones. In fact, when

563   the δD record of the $C_{36:2}$ alkenone is combined with the recent determination of the isotopic

564   fractionation factor α for *Isochrysis galbana* (M'boule et al., 2014) to estimate palaeosalinity

565   of the surface waters of the Black Sea over the Holocene, we obtain a record (Fig. 7c; blue

566   line) that is in good agreement with our general concept of the development of surface salinity

567   of the Black Sea. In the lowermost part of Unit II the estimated palaeosalinity is only a few

568   PSU, it subsequently rises to 15 PSU at the Unit I/II transition, reaches a maximum of ca. 26

569   PSU at 2.0 cal kyr BP and then declines to 17 PSU for the most recent period. These data are

570   in good agreement with salinity calculations (Fig. 7c) based on δD data of C_{37} alkenones in

571   cores from both the western and eastern Black Sea (van der Meer et al., 2008; Giosan et al.,

572   2012) in combination with the isotopic fractionation factor α for *E. huxleyi* (M'boule et al.,

573   2014). For the period where the fractional abundance of the $C_{36:2}$ alkenone is still elevated (i.e.

574   up to 2.6 cal kyr BP) these estimations of paleaosalinity are on the high end (except for the

575   horizon reflecting the first invasion of *E. huxleyi* in the Eastern Basin). This is most likely

576   caused by the fact that the $C_{36:2}$ alkenone-producing haptophytes are also contributing D-

577   enriched C_{37} alkenones to the total pool of C_{37} alkenones, influencing the palaeosalinity

578   calculation that is based on a 100% origin from *E. huxleyi*. Hence, the δD data of the $C_{36:2}$

579   alkenone in combination with the salinity calculations strongly suggest that the $C_{36:2}$ alkenone

580   has been produced by an *Isochrysis*-related haptophyte and not by a lower salinity adapted

581   strain of *E. huxleyi* as suggested previously (Coolen et al., 2009). It remains unclear why

582   ancient DNA of this haptophyte is only detected for the period 7.4-4.8 cal kyr BP. However, it

583   is known that Denaturing Gradient Gel Electrophoresis (DGGE), the method used by Coolen

584   et al. (2009) to detect ancient DNA is only able to quantify the predominant DNA sequences.

585   Combining the fractional abundance record of the $C_{36:2}$ alkenone (Fig. 7a) with the

586   palaeosalinity record (Fig. 7c) now makes it possible to determine the optimal salinity for the

587   *Isochrysis*-related haptophyte producing the $C_{36:2}$ alkenone. In the Black Sea at salinities from

588   2-8 PSU, the $C_{36:2}$ alkenone dominates the alkenone distribution. At a salinity of up to ca. 19

589   PSU the $C_{36:2}$ alkenone can still contribute substantially (25%) and above this level it becomes

590   a minor alkenone. It is clear that salinity is not the only environmental control on the $C_{36:2}$

591   alkenone-producing haptophyte since when in recent times salinities drop to ca. 17 PSU, the

592    $C_{36:2}$ alkenone still remains a minor alkenone (Fig. 7).

593   The $C_{36:2}$ alkenone data of the Black Sea allow the interpretation of the $C_{36:2}$ alkenone

594   record of the Baltic Sea in term of changes in salinity. This should be done cautiously since it

595   is clear that other environmental factors also may have an effect. Nevertheless, the sudden

596   increase of the fractional abundance of the $C_{36:2}$ alkenone record at the Ancylus Lake/Littorina

597   Sea transition is highly comparable to what happened in the Black Sea at the Unit III/II

598   transition and indicates an incursion of marine waters into the freshwater lakes most probably

599   by the worldwide sea level transgression, resulting in a modest increase in surface water

600   salinity to ca. 2 PSU. In the Baltic Sea the fractional abundance of the $C_{36:2}$ alkenone remains

601   high until ca. 3.0 cal kyr BP, suggesting that the salinity of the surface waters of the Arkona

602   Basin increased at a lower rate than in the Black Sea. The lowest fractional abundance of the

603    $C_{36:2}$ alkenone is recorded in the Arkona Basin at 0.9 cal kyr BP, suggesting that the salinity

604   was highest at that time, which corresponds to the medieval climate anomaly (MCA, which

605   occurred between 950-1,250 BP). This trend is similar to salinity records for the whole Baltic

606   Sea based on combined proxies and modelling (Gustafsson and Westman, 2002) although the

607   maximum salinity is thought to be earlier even when we correct for the different age models.

608   Generally, it is believed that the Littorina phase of the Baltic Sea was more saline than the

609   post-Littorina phase, however, other studies do not reveal this difference (Andren et al., 2000;

610   Westman and Sohlenius, 1999; Andrén et al, 2002; Witkowski et al., 2005) or show the

611   opposite (Emeis et al., 2003).

612

613   *5.3 Potential uses of alkenones as environmental indicators for SST*

614   The indices and ratios we have presented in this study all corroborate that a haptophyte

615   species composition change, most likely driven by a salinity shift, occurred during the Yoldia

616   Regression (10.6 cal kyr BP), the Ancylus Lake/Littorina Sea transition (7.7-7.2 cal kyr BP),

617   and at the MCA (0.9 cal kyr BP). The results also indicate that the haptophyte species

618   composition since 7.2 cal kyr BP in the Baltic Sea basin is a combination of marine (*E.*

619   *huxleyi* type) and low-salinity adapted haptophytes. This designates that higher salinity

620   conditions have prevailed since the Ancylus Lake/Littorina Sea transition.

621   To determine how shifts in haptophyte species composition in the Baltic Sea could

622   affect paleoclimate reconstructions using long chain alkenones, we examined the $U^{K'}_{37}$ index

623   over the Holocene. $U^{K'}_{37}$ values changed across the Ancylus Lake/Littorina Sea transition

624   with lower values (0.24±0.04) during the Ancylus Lake phase and an increase in the $U^{K'}_{37}$

625   index after 7.2 cal kyr BP (0.42±0.15; Fig. 5e; Table 2). This resulted in an increase in

626   average estimated SSTs based on the $U^{K'}_{37}$ index from ~6°C during the Ancylus Lake phase

627   to ~13°C during the brackish phase. We believe that variations in haptophyte community

628   composition resulting from fluctuating salinity is most likely responsible for this change in

629     $U^{K'}_{37}$ values and the corresponding unrealistic increase in SST over the Ancylus

630   Lake/Littorina Sea transition. The highest contribution of the $C_{36:2}$ alkenone occurred during

631   the Littorina Sea phase, which indicates salinity was relatively low at that time. The presence

632   of this alkenone even in the more recent phase of the Baltic Sea is evidence of the continued

633   contribution from low salinity adapted haptophytes, which are most likely complicating the

634 use of alkenone unsaturation ratios for SST reconstructions in this region. Schulz et al. (2000)

635 demonstrated in a study performed in the Baltic Sea that $U^{K'}_{37}$ varied regionally depending on

636 salinity and that higher salinity areas in the Baltic had higher $U^{K'}_{37}$ values and vice versa.

637 Since previous studies have also shown that alkenone distributions co-vary not only with

638 temperature changes, but also with salinity driven changes in haptophyte species composition

639 (Coolen et al., 2004, 2009) we cannot apply the $U^{K'}_{37}$ index for SST reconstructions in the

640 Baltic Sea basin over the Holocene.

641   Interestingly, we observed that during the brackish phase the alkenone distribution at

642 0.9 cal kyr BP (100 cm depth) is unique compared to the other sediment samples (Fig. 3) from

643 the brackish phase. This sediment horizon has the lowest contribution of the $C_{36:2}$ alkenone,

644 the lowest $\%C_{37:4}$ and the highest fractional abundance of the C_{38} Et alkenone (Fig. 5b-d;

645 Tables 1-2), all indicating the increased presence of marine type haptophyte species and

646 therefore that more marine conditions prevailed in the Baltic Sea at this time. This sample

647 falls within the MCA, also known as the Medieval Warm Period. The lower contribution of

648 the $C_{37:3}$ alkenone compared with $C_{37:2}$ corroborate that warmer temperatures (Fig. 3c)

649 occurred during this time, although the minor contribution of *Isochrysis*-related haptophytes

650 do not allow absolute SST determination.

651

652 **Conclusions**

653 This research demonstrates the usefulness of alkenone distributions along with the δD of the

654 alkenones for paleosalinity studies in the Baltic Sea and other environments as well. Both

655 alkenone distributions and hydrogen isotopic composition indicate a shift in haptophyte

656 species composition in the Arkona Basin of the Baltic Sea from the Ancylus Lake to the

657 Littorina Sea phase, c. 7.2 cal kyr BP, from lacustrine to brackish type haptophytes,

658 corresponding to the incursion of marine waters that occurred in the Baltic Sea at that time as

659 a consequence of the global sea level rise. During the Littorina Sea Phase the fractional

660 abundance of the $C_{36:2}$ alkenone remains high, suggesting that salinity did not rise above 8

661 PSU. From ca. 3.0 cal kyr BP onwards the fractional abundance of the $C_{36:2}$ alkenone is lower,

662 suggesting a slightly higher salinity. During this phase there is a substantial offset in δD

663 values with the $C_{36:2}$ alkenone substantially more enriched than the C_{37} alkenones. The

664 presence of the $C_{36:2}$ alkenone in the Baltic Sea as well as the δD record suggest it is produced

665 by a different species of haptophyte adapted to lower salinity conditions that is not

666 contributing much to the production of C_{37} and C_{38} alkenones. The contribution of alkenones

667 from lower salinity adapted species in the Baltic Sea hinders the use of the $U^{K'}_{37}$ index for

668 SST reconstructions.

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876 **Figure legends**

877 **Figure 1** A map of the Baltic Sea region and sampling sites. The sediment coring sites

878 318310 and 318340 in the Arkona Basin are designated by blue circles and the two stations in

879 the Skagerrak where surface sediment samples were collected are indicated by red squares.

880 **Figure 2** Correlation of sediment cores 318310 (left panel) and 318340 (right panel) using Ca

881 data obtained from XRF analysis (cps; designated by the gold line) and LOI (wt%; designated

882 by the black line). The red numbers indicate the radiocarbon dates (in cal yr BP) of carbonate

883 fossils from specific horizons in core 318310. The blue lines indicate tie points used for

884 correlating both cores (see text). The closed circles along the depth axes indicate the depths of

885 the sediments analyzed for alkenones in this study.

886 **Figure 3** Partial GC-FID chromatograms displaying alkenone distribution from various

887 sediment horizons. (**a**) Sample EMB0461-10-MUC from the Skagerrak shows a typical

888 marine distribution, (**b**) a sediment interval from 0.4 cal kyr BP (50 cm depth from core

889 318310) is from a brackish period and displays a distribution similar to the marine distribution

890 except with the additional presence of the $C_{36:2}$ alkenone, (**c**) the sediment interval from 0.9

891 cal kyr BP (100 cm depth from core 318310) has a different distribution from the other depths

892 in both cores with no $C_{37:4}$ alkenone, $C_{37:3} < C_{37:2}$, and a lower contribution of the $C_{36:2}$

893 alkenone relative to the C_{37} alkenones, (**d**) the sediment interval from 7.1 cal kyr BP (600 cm

894 depth from core 318310) is from the period immediately following the Ancylus

895 Lake/Littorina Sea transition and has an alkenone distribution characteristic of lower salinity

896 haptophytes. Note the high relative abundance of the $C_{36:2}$ alkenone at this time. The

897 alkenones are color coded according to the legend with circles designating the C_{38} alkenones,

898 triangles signifying the $C_{36:2}$ alkenone, and squares indicating the C_{37} alkenones.

899   **Figure 4** Principal component analysis based on the standardized fractional abundances of the

900   eight alkenones found consistently in the sediments from the Baltic Sea used in this study.

901   Samples where alkenones were not detected were left out of the PCA. (a) Plot showing the

902   scores of the alkenones and scores of the different sites on PC1 (41.0%) and PC2 (25.6%). (b)

903   Displays the scores of the alkenones on PC1 ac PC3 (18.1%) as well as the scores of the

904   different sites. Scatter plots displaying the correlation of (c) PC1 with $U^{K'}_{37}$ (R^2=0.86) (d)

905   negative correlation of PC2 and $C_{36:2}$ Et (R^2=0.77) (e) and negative correlation with PC3 and

906   the sum of the C_{38} Et (R^2=0.74).

907   **Figure 5** Plots of the combined Arkona Basin record (sediment core 318310 designated by

908   closed symbols and 318340 by open symbols) with age (cal kyr BP) for (a) PC1-PC3 (b)

909   summed fractional abundance of the C_{38} Et alkenones, (c) fractional abundance of the $C_{36:2}$

910   alkenone (%) (d) %$C_{37:4}$, and (e) $U^{K'}_{37}$.

911   **Figure 6** δD values of the $C_{36:2}$, C_{37} and C_{38} alkenones plotted against age (cal kyr BP) from

912   the record 318310 and the two Skagerrak surface sediment samples. In the Arkona Basin

913   conditions were fresh until 7.8 cal kyr BP and brackish from 7.1 cal kyr BP onwards. The

914   sample points in black are for sediments from the Arkona Basin record (sediment core

915   318310) and the red sample points are for the Skagerrak surface sediments. The circles denote

916   the δD of the $C_{36:2}$ alkenones, the squares signify the δD values C_{37} alkenones and the

917   diamonds represent the δD of the C_{38} alkenones.

918   **Figure 7** Comparison of $C_{36:2}$ alkenone abundance data for the Baltic Sea and the Black Sea

919   over the Holocene. Top panel (**a**) shows the fractional abundance of the $C_{36:2}$ alkenone

920   relative to the C_{36}-C_{38} alkenones for the Black Sea (blue circles; data from Coolen et al., 2009)

921   and Baltic Sea (green triangles; this study). The middle panel (**b**) shows the haptophyte

922   community composition in the Black Sea as reconstructed based on DGGE analysis of partial

923   18S rRNA genes amplified with a specific haptophyte primer set with *Isochrysis*-related

924   haptophytes in red and *E. huxleyi* in blue (data modified from Coolen et al., 2009; note that in

925   their Fig. 3 relative abundance data is shown based on the relative abundance of all DGGE

926   bands not only those related to alkenone-producing haptophytes, Coolen, personal

927   communication). These data are in agreement with earlier haptophyte 18S rRNA gene work

928   on a box core just penetrating Unit 2 from a deep water site in the eastern Black Sea (Coolen

929   et al., 2006). The bottom panel (**c**) shows reconstructed salinities for the Black Sea based on

930   the hydrogen isotopic compositions of the $C_{36:2}$ and C_{37} alkenones. For all data the

931   fractionation factor α was calculated using a water hydrogen isotopic composition of -20

932   permille (Swart, 1991). Salinities were reconstructed based on the α-salinity relationship for

933   *Isochrysis galbana* (α=0.0019*S+0.836; M'Boule et al., 2014) for the $C_{36:2}$ alkenone and *E.*

934   *huxleyi* (α=0.0021*S+0.740; M'Boule et al., 2014) for the C_{37} alkenones. Original alkenone

935   hydrogen isotope data are for the eastern Black Sea from van der Meer et al. (2008) and for

936   the western Black Sea from Giosan et al. (2012). The stratigraphy for the Baltic Sea (top) and

937   Black Sea (bottom) is indicated. TS denotes transition sapropel. Note that the stratigraphy

938   described for the Black Sea core in the study of Coolen et al. (2009) and Giosan et al. (2012)

939   has been adjusted to fit the commonly applied stratigraphy for the Black Sea (see lowermost

940   part of the figure), i.e. the layer of the first invasion of *E. huxleyi* (grey bar in the other panels)

941   is taken as the start of Unit 1 deposition (Hay et al., 1992; Arthur et al. 1994; Jones and

942   Gagnon, 1994). In the sediment core used in the work of Coolen et al. (2009) this layer is not

943   clearly revealed by an increase of the carbonate content but the alkenone distribution shows a

944   distinct change at ca. 3,350 cal yr BP (see sudden decrease of the relative abundance of the

945   $C_{36:2}$ alkenone in panel **a**; in addition this horizon is also characterized by a 3-4 fold increase

946   in total alkenone concentration and the detection of C_{39} alkenones; Coolen et al., 2009)

947   towards a composition highly comparable to the upper part of Unit I that is composed of

948   coccolithic ooze. Their reported radiocarbon date for this section (3,360±68 cal yr BP) is by

949   this adjustment in good agreement with the reported age of the base of Unit I at other

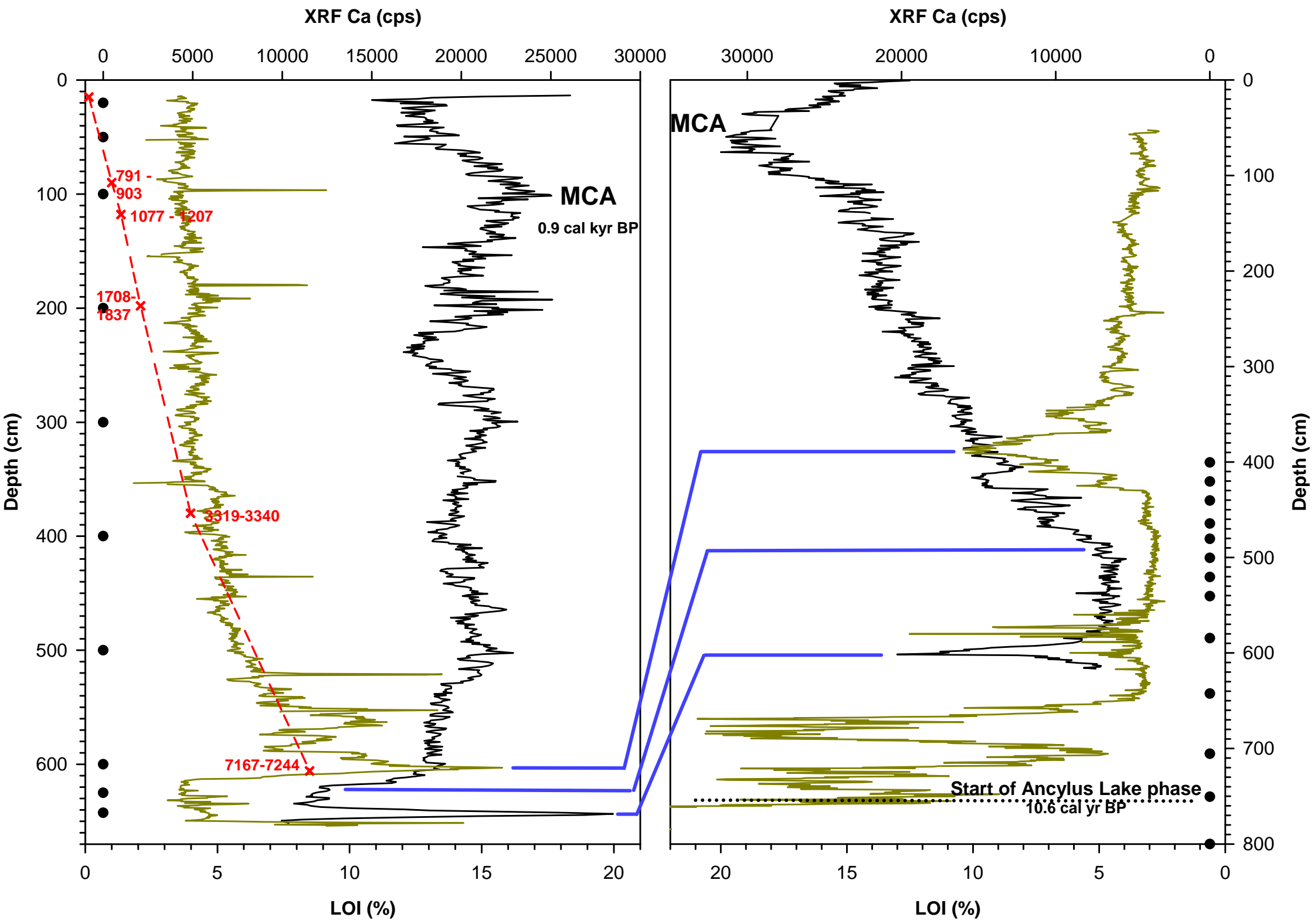950   locations in the Black Sea (Hay et al., 1992; Arthur et al. 1994; Jones and Gagnon, 1994).
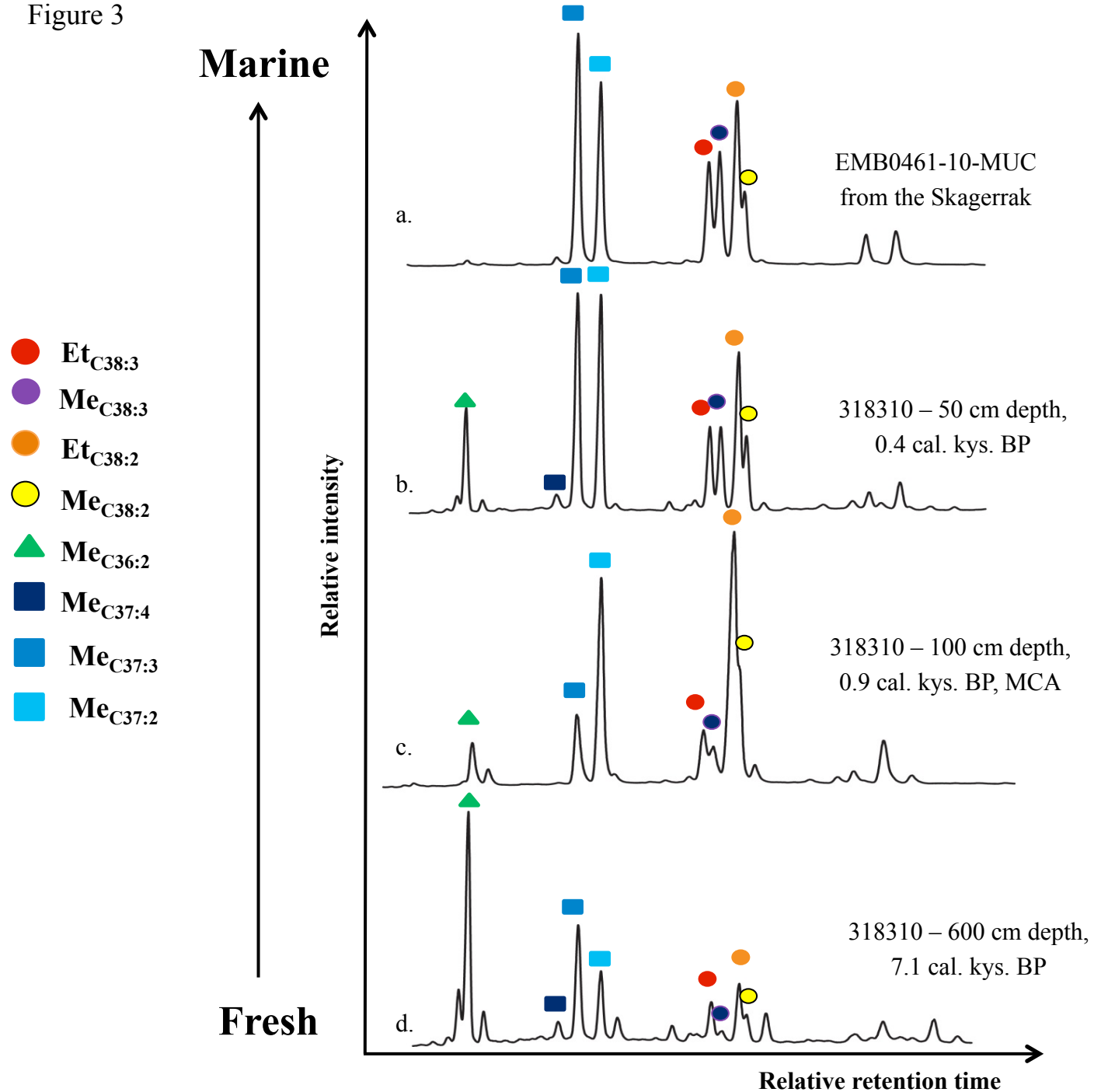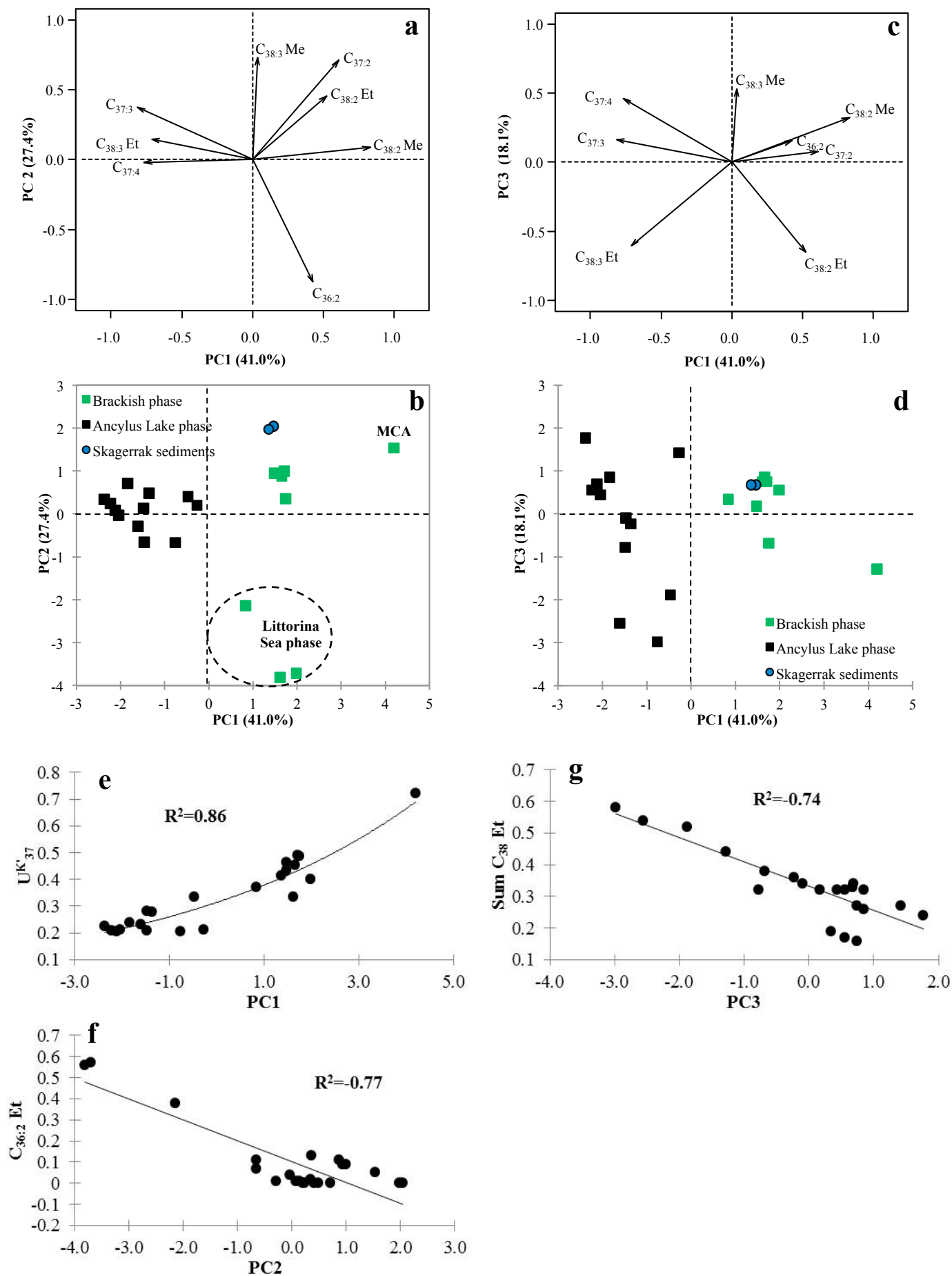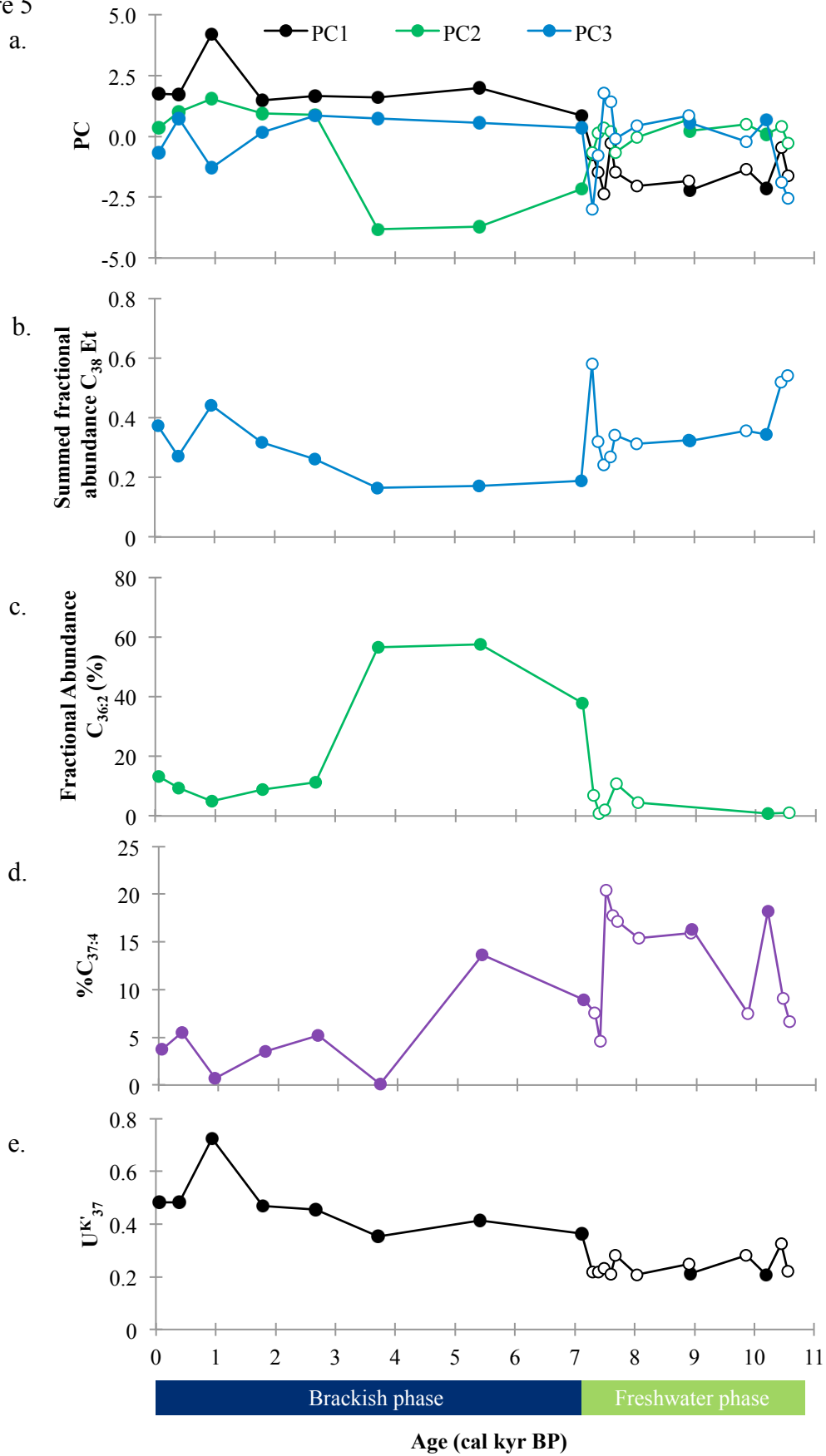
Figure 1
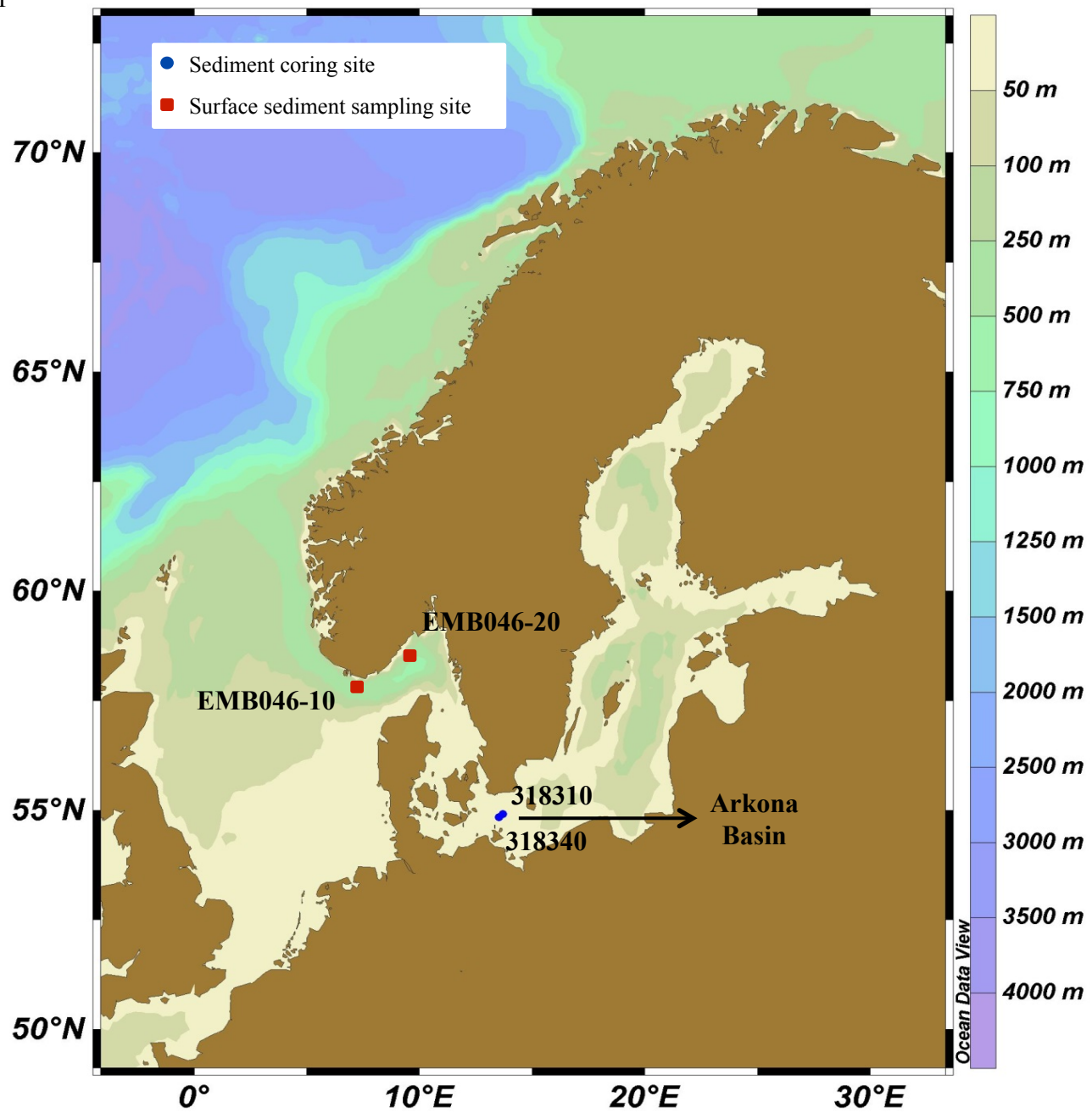
Figure 3

Figure 4

Figure 5

Figure 6:

Baltic Sea