This is a postprint version of:

[Article begins on next page]

Testing the alkenone D/H ratio as a paleo indicator of sea surface salinity

in a coastal ocean margin (Mozambique Channel)

Sebastian Kasper[a][*], Marcel T.J. van der Meer[a], Isla S. Castañeda[a,b], Rik Tjallingii[c,1], Geert-Jan A. Brummer[c], Jaap S. Sinninghe Damsté[a], Stefan Schouten[a]

[a] *NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, P.O. Box 59, 1790 AB Den Burg (Texel), The Netherlands*

[b] *University of Massachusetts Amherst, Department of Geosciences, Amherst, MA 01003 USA*

[c] *NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Geology and Chemical Oceanography,1790 AB Den Burg (Texel), Netherlands*

.

[*]Corresponding author.Tel.: +31 (0) 222 369 408; fax: +31 (0) 222 319 674.

E mail address: sebastian.kasper@nioz.nl (S. Kasper).

[1]Present address: Geoforschungszentrum Potsdam, Telegrafenberg, C 327, Potsdam D-14473, Germany.

1 ABSTRACT

2 Reconstructing past ocean salinity is important for assessing paleoceanographic change and

3 therefore past climatic dynamics. Commonly, sea water salinity reconstruction is based on

4 planktonic foraminifera oxygen isotope values combined with sea surface temperature

5 reconstruction. However, the approach relies on multiple proxies, resulting in rather large

6 uncertainty and, consequently, relatively low accuracy of salinity estimates. An alternative tool

7 for past ocean salinity reconstruction is the hydrogen isotope composition of long chain (C_{37})

8 alkenones ($\delta D_{alkenone}$). Here, we have applied $\delta D_{alkenone}$ to a 39 ka sedimentary record from the

9 Eastern South African continental shelf in the Mozambique Channel, close to the Zambezi River

10 mouth. Despite changes in global seawater δD related to glacial – interglacial ice volume effects,

11 no clear changes were observed in the $\delta D_{alkenone}$ record throughout the entire 39 ka. The BIT

12 index record from the same core, which provides information on relative contributions of soil

13 organic matter (OM) vs. marine input, indicates high soil OM input during the glacial and low

14 input during the Holocene. This suggests a more pronounced freshwater influence at the core

15 location during the glacial, resulting in alkenones depleted in D during that time, thereby

16 explaining the lack of a clear glacial-interglacial alkenone δD shift. The correlation between the

17 BIT index and $\delta D_{alkenone}$ during the glacial period suggests that increased continental runoff

18 potentially changed the growth conditions of the alkenone-producing haptophytes, promoting

19 coastal haptophyte species with generally more enriched $\delta D_{alkenone}$ values. We therefore suggest

20 that the application of $\delta D_{alkenone}$ for reconstructing past salinity in coastal settings may be

21 complicated by changes in the alkenone-producing haptophyte community.

22

23 **Keywords:** stable hydrogen isotopes, alkenones, salinity, BIT index, coastal environment,

24 Mozambique Channel, Zambezi River, glacial, interglacial.

1. Introduction

The interaction and feedback mechanisms at the interface between oceanic and atmospheric circulation on glacial – interglacial timescales are largely unknown, but are thought to play an important role in the global transmission of climate change (Manabe, 1969; Rahmstorf, 2002). Accurate reconstruction of past oceanic and atmospheric changes is crucial for a better understanding of these mechanisms and should eventually improve prediction of future climate development (Esper et al., 2005). Reconstruction of ocean circulation requires an estimate of seawater density, which is determined by salinity and temperature. Therefore, in order to understand past ocean circulation in relation to climate change it is essential to be able to reconstruct paleosalinity and temperature with reasonable accuracy.

Ocean salinity and the oxygen isotope composition of seawater are linearly correlated (Epstein and Mayeda, 1953) and this can be utilized for reconstruction of past seawater salinity using the $\delta^{18}O$ record of carbonate shells of foraminifera (Duplessy et al., 1991; Rostek et al., 1993) under the assumption of temporal consistency of the relationship between $\delta^{18}O$ and salinity (Rohling and Bigg, 1998; Rohling et al., 2000). However, salinity reconstruction based on $\delta^{18}O$ values of foraminiferal carbonate requires accurate correction for the global ice volume (Rostek et al., 1993) and knowledge of the temperature during calcification (Erez and Luz, 1983). The global ice volume can be estimated from the mean ocean $\delta^{18}O$ record (Waelbroeck et al., 2002), whereas the temperature is mostly obtained from Mg/Ca ratio values of foraminifera(Nürnberg et al., 1996; Lea et al., 1999; Mashiotta et al., 1999) or the alkenone-based $U^{K'}_{37}$ Index (Brassell et al., 1986; Rostek et al., 1993; Nürnberg et al., 1996). However, the correlation between $\delta^{18}O$ and salinity is affected by mixing and evaporation processes (Craig and Gordon, 1965), seasonality (Strain and Tan, 1993) and likely changes over glacial – interglacial periods (Rohling and Bigg,

49   1998). Consequently, salinity reconstruction based on $\delta^{18}O$ values of foraminiferal carbonate is

50   subject to uncertainty andis difficult to calibrate (Rohling and Bigg, 1998; Rohling, 2000).

51

52   In addition to the correlation between oxygen isotopes and salinity, there is also a strong

53   correlation between the hydrogen isotope composition of seawater (δD_{sw}) and salinity (Craig,

54   1961; Friedman et al., 1964). δD_{sw} is recorded in the non-exchangeable hydrogen of organic

55   matter (OM; Sessions et al., 1999; Sauer et al., 2001; Sachse et al., 2012) and can potentially be

56   used for reconstructing past δD_{sw}. The hydrogen isotope composition of long chain alkenones, in

57   particular C_{37} alkenones ($\delta D_{alkenone}$) appears well suited for reconstructing past δD values of

58   seawater (Englebrecht and Sachs, 2005; Schouten et al., 2006). The isotopic composition of

59   covalently bound hydrogen is thought to be preserved over long geological timescales (Sessions

60   et al., 2004). Culture studies have shown that $\delta D_{alkenone}$ of two common marine haptophytes,

61   *Emiliania huxleyi* and *Gephyrocapsa oceanica*, depends mainly on salinity and the hydrogen

62   isotope composition of the growth medium and, to a lesser extent, growth rate (Schouten et al.,

63   2006). Attempts to reconstruct past salinity change based on $\delta D_{alkenone}$ has led to reasonable

64   results for the eastern Mediterranean Sea and Black Sea (van der Meer et al., 2007, 2008; Coolen

65   et al., 2013) and the Panama Basin (Pahnke et al., 2007). Recently, the approach has also been

66   applied to open marine sediments to evaluate relative salinity changes in the Agulhas Leakage

67   area at the southern tip of the African continent over two glacial termination periods (Kasper et

68   al., 2014). However, the lack of a clear correlation between $\delta D_{alkenone}$ and seawater salinity in the

69   Chesapeake Bay estuary suggests that the interpretation of this salinity proxy may become

70   problematic for coastal environments (Schwab and Sachs, 2011). A possible explanation for the

71   absence of a clear correlation relates to change in species composition since different haptophytes

72   may have specific D/H fractionation characteristics. Comparison of the coastal haptophyte alga

73   *Isochrysis galbana* with the open ocean species *E. huxleyi* in a culture experiment (M'Boule et

74   al., 2014) revealed a strong fractionation difference between the two species. *I. galbana*

75   fractionated to an extent of ca. 90‰ less than *E. huxleyi*, although the rate of change in D/H

76   fractionation per salinity unit remained similar for both species (M'Boule et al., 2014, Chivall et

77   al., 2014). This raises the question as to which extent $\delta D_{alkenone}$ is applicable as a paleosalinity

78   proxy in coastal settings, where differences in haptophyte communities are to be expected

79   (Marlowe et al., 1990; Noble et al., 2003; Örnólfsdóttir et al., 2004; Schwab and Sachs, 2011).

80

81   To investigate the $\delta D_{alkenone}$-based sea surface salinity proxy in a freshwater runoff-influenced

82   continental margin, we analyzed a 39,000 year (39 ka) long record of $\delta D_{alkenone}$ from a marine

83   sediment core (64PE304-80) from the Mozambique Channel near the Zambezi River mouth

84   inside the Zambezi River fan (Fig. 1; Beiersdorf et al., 1980). The core site is off the continental

85   shelf in the channel, which is traversed by the Mozambique Current, a branch of the South

86   Equatorial Current (SEC; Fig. 1). The Mozambique Current is dominated by large anti-cyclonic

87   eddies passing through the Mozambique Channel, with an annual net flow rate of ca. 15 Sv (de

88   Ruijter et al., 2002; Schouten et al., 2003; Ullgren et al., 2014). However, interannual net flow

89   rate varies substantially by up to 9 Sv in volume transport (Ridderinkhof et al., 2010).

90   Additionally, there is a substantial input of continental freshwater to the Mozambique Channel

91   (Siddorn et al., 2001). A main contributor is the Zambezi River, with a runoff of ca. 224 km^3/yr

92   (7.1 x 10^{-3}Sv; Meybeck, 1982). Due to the location of the core site within the Zambezi River fan

93   and independent reconstruction of high freshwater outflow by the BIT index, we would expect

94   variation in freshwater runoff and therefore in salinity at the core site over time, suggesting it as a

95   promising record for testing $\delta D_{alkenone}$ as a paleo sea surface salinity proxy at a river dominated

96   ocean margin.

97

2. Material and methods

99

2.1. Core and chronology

101

Samples were taken from core 64PE304-80 (18° 14' 26.6274"S; 37° 52' 8.6874" E, 1329 m water

depth), located north of the Zambezi River Delta at the Mozambique shelf (Fig. 1). Thecore was

taken during the RV Pelagia cruise "64PE304, INATEX - GEO" (Brummer et al., 2009). The

chronology was established from ^{14}C dating of 20 samples consisting of ca.10 mg mixed surface-

dwelling planktonic foraminifera (*Globigerinoidess acculifer*, *G.trilobus* and *G. ruber)*. These

sampleswere collected from the washed and sieved > 250 μm sediment fraction by selecting

individual foraminifera specimens under a microscope (Fig. 2). The age model for the upper 6 m,

spanning the last 20 ka, was constrained by 11 ^{14}C dates and by stratigraphic correlation with a

parallel core (GIK16160-3; Wang et al., 2013b; Van der Lubbe et al., 2014). The age model

between 20 and 39.5 ka BP was constrained by 9 additional ^{14}C dating points. The ^{14}C age values

were converted to calendar age using the MARINE09 calibration curve that applies a standard

reservoir correction of ca. 400 yr (Reimer et al., 2011).

114

2.2. Extraction

116

Sediment samples were freeze dried and homogenized with a mortar and pestle. The

homogenized material was extracted using accelerated solvent extraction (ASE) with

dichloromethane (DCM):MeOH 9:1 (v/v) and a pressure of 6895 kPa in 3 extraction cycles. The

120 extract was separated over an Al_2O_3 column into apolar, ketone and polar fractions using

121 hexane:DCM 9:1 (v/v), hexane:DCM 1:1 (v/v) and DCM:MeOH 1:1 (v/v), respectively. Column

122 chromatography was carried out using about 4 cm activated Al_2O_3 (MPO Chemicals; technical

123 quality, basic A) as stationary phase in a Pasteur pipette . Fractions were eluted with 3 column

124 volumes of eluent.

125

126 *2.3. Branched isoprenoid tetraether (BIT) index*

127

128 The polar fraction was analyzed for the BIT index, a proxy originally thought to reflect the input

129 of soil OM (Hopmans et al., 2004) but which has recently been shown to be affected by in situ

130 production of branched glycerol dibiphytanyl glycerol tetraethers (br GDGTs) in rivers (Zell et

131 al., 2013; De Jonge et al., 2014). Analysis was performed using high performance liquid

132 chromatography-mass spectrometry (HPLC-MS) with an Agilent 1100 series instrument

133 equipped with an auto-injector. Separation was achieved with an Alltech Prevail Cyano column

134 (2.1 x 150 mm, 3μm), maintained at 30 °C. GDGTs were eluted with 99% hexane and 1%

135 propanol for 5 min, followed by a linear gradient to 1.8% propanol in 45 min, followed by back-

136 flushing with hexane/propanol (9:1, v/v) at 0.2ml/min for 10 min. Detection was achieved using

137 atmospheric pressure positive ion chemical ionization MS (APCI-MS). APCI-MS settings were:

138 nebulizer 414 kPa, vaporizer 400 °C, drying gas (N_2) at 6 l/min and 200 °C, capillary voltage -3

139 kV and a corona 5 μA (ca. 3.2 kV). GDGTs were detected using selected ion monitoring (SIM) of

140 the $[M+H]^+$ ions (*m/z* 1292 for crenarchaeol and *m/z* 1050, 1036 and 1022 for br GDGTs,

141 respectively, dwell time 234 ms; Schouten et al., 2007) and integrated peak areas were used for

142 quantification. BIT values were calculated according to the formula of Hopmans et al. (2004).

143

144 *2.4. C_{37}/C_{38} alkenone ratio*

145

146 The ketone fraction, containing long chain alkenones, was analyzed using an Agilent 6890 gas

147 chromatography (GC) instrument with a flame ionization detection (FID) and an Agilent CP Sil-5

148 fused silica column (50 m x 0.32 mm, film thickness 0.12 μm) with He as carrier gas. The GC

149 oven was programmed from 70 to 200 °C at 20 °C/min and then at 3°C/min to 320 °C (held 25

150 min). Peak integration for the calculation of ratio values for the total abundance of the C_{37}/C_{38}

151 alkenones was performed using the Atlas 8.2 Chromatography Data System software from

152 Thermo Electron Cooperation.

153

154 *2.5. Alkenone δD analysis*

155

156 The ketone fraction was analyzed using GC-high temperature conversion-isotope ratio MS (GC-

157 TC-irMS) to determine the combined hydrogen isotope composition of the di- and triunsaturated

158 C_{37} alkenones (van der Meer et al., 2013). Isotope analyses were carried out with a Thermo –

159 Scientific DELTA V GC-TC-irMS instrument. The GC temperature conditions were: 70 to 145 °C

160 at 20 °C/min, then heated at 8 °C/min to 200 °C and to 320 °C (held 20 min) at 4°C/min. An

161 Agilent CP Sil-5 column (25 m x 0.32 mm) with a film thickness of 0.4 μm was used with He as

162 carrier gas at 1 ml/min (constant flow). The high temperature conversion reactor was at 1420 °C.

163 The H_3^+ correction factor was determined daily and was constant at 5.6 ± 0.5. The $\delta D_{alkenone}$

164 values were calculated with the Isodat software relative to pulses of H_2 reference gas. A set of

165 standard *n*-alkanes with known isotopic composition (Mix B prepared by A.Schimmelmann,

166 University of Indiana) was analyzed daily prior to analyzing samples in order to monitor system

167 performance. Samples were only analyzed when the alkanes in Mix B had an average deviation

168 from their off-line determined value of < 5‰. Squalane was co-injected as internal standard with

169 each sample to monitor the accuracy and precision of alkenone isotope values. The standard had

170 an average δD value of -165 ± 3.6‰, which compared favorably with that of -170‰ determined

171 offline. The alkenone fraction was analyzed in duplicate if sufficient amount of material was

172 available. Standard deviation of replicate analyses varied from ± 0.1‰ to ± 5.9‰.

173

174 **3. Results**

175

176 *3.1. BIT index*

177 To trace the relative importance of the river outflow at the core location we determined the BIT

178 index, indicating riverine transported soil OM (Hopmans et al., 2004; Kim et al., 2007; Walsh et

179 al., 2008; Schouten et al., 2013) as well as in situ produced crenarcheol from the river system

180 (Zell et al., 2013; De Jonge et al., 2014). Values ranged between 0 and 1- a value near 0 indicates

181 marine dominated OM and values near 1 a dominance of soil/river OM. Values were relatively

182 high at ca. 0.50 between 39 and 38 ka and decreased to ca. 0.3 at 37 ka (Fig. 2). Between 37 and

183 16 ka they increased continuously to a maximum of ca. 0.7 during Heinrich Event 1 (HE1).

184 Subsequently, they decreased to a plateau of ca. 0.25 between 15 and 12 ka in the Younger Dryas

185 (YD). At ca. 12 ka they decreased to the Holocene level of ca. 0.1.

186

187 *3.2. $\delta D_{alkenone}$*

188 The δDalkenone values were measured as the combined signal from $C_{37:2}$ and $C_{37:3}$, as this has

189 been suggested to yield a more robust water δD and salinity signal and to reduce additional

190 biosynthetic effects related to the synthesis of $C_{37:3}$ alkenone from the $C_{37:2}$ alkenone (van der

191   Meer et al., 2013). The total variability in the $\delta D_{alkenone}$ record was ca. 23‰, with absolute values

192   ranging between -181 and -204‰. They varied around a mean of -191‰ ± 5‰ (1σ standard

193   deviation) over the entire record (Fig. 2). No trend from glacial Marine Isotope Stage (MIS) 3,

194   through the Last Glacial Maximum (LGM) to the Holocene, was observed. Instead, the record

195   was highly variable, showing a minor tendency of ca. 5‰ to increasingly D-depleted values with

196   decreasing age during the Holocene. The record showed several periods of relatively D-enriched

197   values of ca. -185‰ to -180‰ at 31 ka, 23 ka and during HE1, and more D-depleted values of ca.

198   -200‰ at 37 ka, 27 ka and the LGM.

199

200   *3.3. C_{37}/C_{38} alkenone ratio*

201   The ratio ranged between 0.8 and 1.7, with an average of 1.3 ± 0.2 over the entire record (Fig. 2).

202   During the period between 39– 21ka, it was 1.3 ± 0.1, and after 21 ka it increased to a maximum

203   of 1.7 at 19 ka, followed by a decrease to 1.3 at 10 ka. After this, it increased slightly to 1.4 at 9

204   ka and then rapidly decreased to the minimum value of ca. 0.8 at 8 ka. Itwas generally lower at

205   1.1 ± 0.1 during the Holocene comparedwith the glacial period.

206

207   **4. Discussion**

208

209   The transition from glacial to interglacial periods is marked by a significant decrease in the stable

210   oxygen isotope ratio ($\delta^{18}O_{sw}$) and the stable hydrogen isotope ratio (δD_{sw}) of seawater as a

211   consequence of melting of a large ice volume accumulated during the glacial (Shackelton, 1987).

212   Global change in $\delta^{18}O_{sw}$ was obtained from a mean global benthic isotope record (Waelbroeck et

213   al., 2002) and indicates a gradual increase in $\delta^{18}O_{sw}$ values from 0.53 to 1.02‰ between 39 and

214   20 ka BP (Fig. 2e). The values decrease linearly between 18 and 8 ka by almost 1‰. Based on a

215   meteoric water line for the Indian Ocean (Srivastava et al., 2010), this record suggests an increase

216   in the mean global δD_{sw} of ca. 4‰ during the last glacial and a decrease of nearly 8‰ during the

217   deglaciation. In contrast, the $\delta D_{alkenone}$ record from the upstream Mozambique slope showed

218   fairly constant average values of -191 ± 5‰, with no trend that could be attributed to the inferred

219   change related to the glacial – interglacial transition (Fig. 2b). The absence of a clear glacial-

220   interglacial change related to the changing global ice volume is unlike that for a $\delta D_{alkenone}$ record

221   obtained for open ocean sediments in the Agulhas Leakage area (Kasper et al., 2014). This

222   suggests that additional environmental factors affect the δD values of alkenones in the

223   Mozambique Channel. This could be related to the change in salinity, in combination with the

224   input of relatively D-depleted freshwater via the Zambezi River outflow (Schefuß et al., 2011).

225   An impact of temperature induced change in the $U^{K'}_{37}$ ratio on the fractionation of hydrogen

226   isotopes seems unlikely as this effect has not been observed in marine haptophyte algae

227   (Schouten et al., 2006; van der Meer et al., 2013). Furthermore, the temperature change in this

228   region has found to be relatively small (3.5 °C; Wang et al., 2013b) and we did not find any

229   correlation between $\delta D_{alkenone}$ and local $U^{K'}_{37}$ sea surface temperature (SST; data not shown).

230

231   In order to assess changes in freshwater input, we examined the BIT index for the same core

232   (64PE304-80). The values indicate a relative increase in continental OM input during MIS2/3,

233   with a relative maximum at ca. 17 ka during HE1 and at ca. 12 ka during the Younger Dryas,

234   suggesting an increased influence of river outflow (Fig. 2; Van der Lubbe et al., 2014). These

235   events were followed by a rapid decrease in the index during the deglaciation phase between 15-

236   12ka and the period between11-8ka, showing reduced input of continent-derived OM. This could

237   indicate a strongly reduced river outflow, consistent with the rising sea level at the time and the

238 subsequent transgression of the shelf (Walford et al., 2005; März et al., 2008), moving the

239 Zambezi River mouth further away from the core site. Based on δD values of leaf wax alkanes,

240 Schefuß et al. (2011) demonstrated that, during northern hemisphere cold events, such as the

241 Younger Dryas and HE1, rainfall was enhanced in the Zambezi catchment area in response to a

242 southwards forcing of the intertropical convergence zone (ITCZ). In contrast, a northward shift of

243 the ITCZ in the Holocene would have resulted in reduced rainfall and decreasing river discharge

244 as compared with glacial conditions (Schefuß et al., 2011; Wang et al., 2013a).The BIT record

245 covering the last 17 ka from the Zambezi River mouth presented by Schefuß et al. (2011) is in

246 good agreement with the BIT record here. The constant high BIT values during the glacial

247 indicate an increased impact of freshwater input at the study site compared with the Holocene

248 when BIT values were low. Increased freshwater input, either via closer proximity to the coast or

249 increased precipitation or a combination of both, would lead to a relative depletion in δD_{sw} at the

250 core site and subsequently to a decrease in $\delta D_{alkenone}$ during the glacial. The combination of lower

251 salinity, resulting in increased biosynthetic hydrogen isotope fractionation (Schouten et al., 2006)

252 and the more negative δD_{sw} values from the freshwater runoff may have resulted in production of

253 relatively D-depleted alkenones during the glacial; this may have counteractedthe global ice

254 volume-induced increase in δD_{sw}, potentially leading to relatively constant values of alkenone δD

255 during the glacial-interglacial transition.

256

257 During MIS 2/3, positive excursions in $\delta D_{alkenone}$ of up to 10‰ correspond with elevated BIT

258 values (Fig. 2), such as between 34 and 31ka, between 26 and 24 ka, and during HE1 (19-16 ka)

259 (Fig. 2). In fact, a crossplot of BIT values vs. $\delta D_{alkenone}$ showed (Fig. 3) that, during the glacial

260 period (39.4 to 16.2 ka), BIT index and $\delta D_{alkenone}$correlated significantly (R^2 0.209, p value

261 0.0001), whilst during the Holocene no statistically significant relationship was observed (R^2

262 0.0186, p value 0.5249). This suggests that variation in freshwater input had a significant impact

263 on the variability in $\delta D_{alkenone}$ during the glacial. However, a negative correlation between BIT

264 and $\delta D_{alkenone}$ would be expected, as an increasing freshwater input would lead to an input of D-

265 depleted water, as well as increasing fractionation due to a decrease in salinity. Thus, the trend

266 cannot be explained by a salinity effect or by an increasing influx of D-depleted water.

267 Alternative factors, which could lead to the apparent positive correlation between BIT values and

268 δD of alkenones during the glacial period, are changes in growth rate or in haptophyte species

269 composition (Schouten et al., 2006; Wolhowe et al., 2009; M'Boule et al., 2014). The reason why

270 this effect is apparent in the glacial and not in the interglacial, may be because the core site was in

271 much closer proximity to the coast during glacial sea level low stands (Walford et al., 2005; März

272 et al., 2008; van der Lubbe et al., 2014), thereby allowing a potential proliferation of coastal

273 haptophyte species as well as an increased impact of river-transported nutrients on growth rate.

274 Regarding the latter hypothesis, although high freshwater input might have provided nutrients to

275 the core site, thereby promoting algal growth (Verity et al., 1993; Pedersen and Borum, 1996;

276 Gillanders and Kingsford, 2002), increased growth rate would be expected to lead to a depletion

277 in $\delta D_{alkenone}$ (Schouten et al., 2006) and so cannot explain the positive excursions in $\delta D_{alkenone}$.

278 Since coastal haptophytes produce alkenones 90‰ enriched in D compared with open ocean

279 haptophytes (M'Boule et al., 2014; Chivall et al., 2014), changes in marine vs. coastal

280 haptophytes in the alkenone producing community could explain the positive excursions of ca.

281 10‰ in hydrogen isotopic composition during the glacial period. The relative amount of the $C_{37:4}$

282 alkenone has been suggested as a marker for low salinity haptophyte algae (Schulz et al., 2000;

283 Liu et al., 2008; 2011), but its abundance was very low in the sediment record (data not shown).

284 However, the hypothesis of a changing contribution to the alkenone pool is supported by the

285 observed change in the ratio between C_{37} and C_{38} alkenones (Fig. 2). This ratio has been reported

286   to be more elevated for coastal haptophytes than for open ocean species (Marlowe et al., 1984;

287   Prahl et al., 1988; Schulz et al., 2000; Liu et al., 2011). No significant positive anomalies in the

288   C_{37}/C_{38} ratio were apparent between 39 and 22 ka. Nevertheless, C_{37}/C_{38} ratio indicated elevated

289   values during the glacial, with the highest at ca. 18.5 ka (Fig. 2), which coincides with relatively

290   more enriched $\delta D_{alkenone}$ values and elevated BIT values (Fig. 2). After 18.5 ka the ratio generally

291   decrease to values of ca. 1.1 throughout the Holocene. This suggests that, during these periods of

292   elevated C_{37}/C_{38} alkenone ratio, coastal haptophyte species may have contributed more to the

293   alkenone pool and consequently shifted $\delta D_{alkenone}$ towards more positive values at times of

294   increased freshwater input. Changing haptophyte assemblages in coastal settings in response to

295   changing environmental conditions appear, therefore, to have a strong effect on $\delta D_{alkenone}$. This

296   highlights the care that has to be taken when using the $\delta D_{alkenone}$ as a proxy for freshwater input in

297   coastal settings or other environments where changes in the dominant alkenone producer(s) ares

298   likely to have occurred.

299

300   **5. Conclusion**

301   Despite changes in global seawater δD related to glacial – interglacial ice volume effects, no long

302   term change in the 39 ka record of $\delta D_{alkenone}$ off the Mozambique shelf was observed. We suggest

303   that glacial – interglacial changes in the $\delta D_{alkenone}$ were masked by changing freshwater input to

304   the core site, as evidenced by the BIT index. During the glacial, the site was in closer proximity

305   to the coast, when there was likely also larger river outflow. These conditions probably led to

306   locally more D-depleted water and increased fractionation of hydrogen isotopes during alkenone

307   biosynthesis due to decreased salinity. The correlation between periods of enriched $\delta D_{alkenone}$ and

308   elevated BIT values during the glacial is possibly related to an increased contribution from

309   coastal alkenone-producing haptophyte species coinciding with elevated river outflow. This

310   complicates the application of $\delta D_{alkenone}$ as a proxy for freshwater input in coastal settings.

311   However, the technique appears promising in more open ocean settings.

312

313

324   *Associate Editor –* **S. Wakeham**

325

326   **References**

327 Beal, L.M., De Ruijter, W.P.M., Biastoch, A., and Zahn, R., 2011. On the role of the Agulhas
328   system in ocean circulation and climate. Nature 472, 429-436.
329 Beiersdorf, H., Kudrass, H.R., Stackelbert, U.v., 1980. Placer deposits of Ilmenite and Zircon on
330   the Zambezi Shelf, Geologisches Jahrbuch Reihe D. Schweizbardt, Stuttgart.
331 Brassell, S.C., Eglinton, G., Marlowe, I.T., Pflaumann, U., Sarnthein, M., 1986. Molecular
332   stratigraphy: a new tool for climatic assessment. Nature 320, 129-133.
333 Brummer, G.-J.A., Jung, S. and Shipboard Scientific Party, 2009. RV Pelagiacruise report: Cruise
334   64PE304, INATEX-GEO, SE African margin, 10 March - 2 April. NIOZ, Royal
335   Netherlands Institute for Sea Research, Texel, The Netherlands.
336 Chivall, D., M'Boule, D., Sinke-Schoen, D., Sinninghe Damsté, J.S., Schouten, S., van der Meer,
337   M.T.J., 2014. The effects of growth phase and salinity on the hydrogen isotopic
338   composition of alkenones produced by coastal haptophyte algae. Geochimica et
339   Cosmochimica Acta 140, 381-390.

340   Coolen, M.J.L., Orsi, W.D., Balkema, C., Quince, C., Harris, K., Sylva, S.P., Filipova-Marinova,
341   M., Giosan, L., 2013. Evolution of the plankton paleome in the Black Sea from the
342   Deglacial to Anthropocene. Proceedings of the National Academy of SciencesUSA 110,
343   8609-8614.
344   Craig, H., 1961. Isotopic variations in meteoric waters. Science 133, 1702-1703.
345   Craig, H., Gordon, L.I., 1965. Deuterium and oxygen 18 variations in the ocean and marine
346   atmosphere, Proceedings of a Conference on Stable Isotopes in Oceanographic Studies
347   and Paleotemperatures, Pisa:.V. Lischi & Figli., Spoleto, Italy, pp. 9 - 130.
348   De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Sinninghe Damsté,
349   J.S., 2014. In situ produced branched glycerol dialkyl glycerol tetraethers in suspended
350   particulate matter from the Yenisei River, Eastern Siberia. Geochimica et
351   CosmochimicaActa 125, 476-491.
352   de Ruijter, W.P.M., Ridderinkhof, H., Lutjeharms, J.R.E., Schouten, M.W., Veth, C., 2002.
353   Observations of the flow in the Mozambique Channel. Geophysical Research Letters 29,
354   140-141.
355   Duplessy, J.C., Labeyrie, L., Juilletleclerc, A., Maitre, F., Duprat, J., Sarnthein, M., 1991. Surface
356   salinity reconstruction of the north-Atlantic ocean during the last glacial maximum.
357   Oceanologica Acta 14, 311-324.
358   Englebrecht, A.C., Sachs, J.P., 2005. Determination of sediment provenance at drift sites using
359   hydrogen isotopes and unsaturation ratios in alkenones. Geochimica et Cosmochimica
360   Acta 69, 4253-4265.
361   Epstein, S., Mayeda, T., 1953. Variation of ^{18}O content of waters from natural sources.
362   Geochimica et Cosmochimica Acta 4, 213-224.
363   Erez, J., Luz, B., 1983. Experimental paleotemperature equation for planktonic foraminifera.
364   Geochimica et Cosmochimica Acta 47, 1025-1031.
365   Esper, J., Wilson, R.J.S., Frank, D.C., Moberg, A., Wanner, H., Luterbacher, J., 2005. Climate:
366   past ranges and future changes. Quaternary Science Reviews 24, 2164-2166.
367   Friedman, I., Redfield, A.C., Schoen, B., Harris, J., 1964. The variation of the deuterium content
368   of natural waters in the hydrologic cycle. Reviews of Geophysics 2, 177-224.
369   Gillanders, B.M., Kingsford, M.J., 2002. Impact of changes in flow of freshwater on estuarine
370   and open coastal habitats and the associated organisms. CRC Press, Boca Raton, FL,
371   USA.
372   Greenland Ice-core Project, M., 1993. Climate instability during the last interglacial period
373   recorded in the GRIP ice core. Nature 364, 203-207.
374   Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S., Schouten, S.,
375   2004. A novel proxy for terrestrial organic matter in sediments based on branched and
376   isoprenoid tetraether lipids. Earth and Planetary Science Letters 224, 107-116.
377   Kasper, S., van der Meer, M.T.J., Mets, A., Zahn, R., Sinninghe Damsté, J.S., Schouten, S., 2014.
378   Salinity changes in the Agulhas leakage area recorded by stable hydrogen isotopes of C_{37}
379   alkenones during Termination I and II. Climate of the Past 10, 251-260.
380   Kim, J.-H., Ludwig, W., Schouten, S., Kerhervé, P., Herfort, L., Bonnin, J., Sinninghe Damsté,
381   J.S., 2007. Impact of flood events on the transport of terrestrial organic matter to the
382   ocean: A study of the Têt River (SW France) using the BIT index. Organic Geochemistry
383   38, 1593-1606.
384   Lea, D.W., Mashiotta, T.A., Spero, H.J., 1999. Controls on magnesium and strontium uptake in
385   planktonic foraminifera determined by live culturing. Geochimica et Cosmochimica Acta
386   63, 2369-2379.

387 Liu, W., Liu, Z., Fu, M. and An, Z., 2008. Distribution of the C_{37} tetra-unsaturated alkenone in
388   Lake Qinghai, China: A potential lake salinity indicator. Geochimica et Cosmochimica
389   Acta 72, 988-997.
390 Liu, W., Liu, Z., Wang, H., He, Y., Wang, Z., Xu, L., 2011. Salinity control on long-chain
391   alkenone distributions in lake surface waters and sediments of the northern Qinghai-
392   Tibetan Plateau, China. Geochimica et Cosmochimica Acta 75, 1693-1703.
393 M'Boule, D., Chivall, D., Sinke-Schoen, D., Sinninghe Damsté, J.S., Schouten, S., van der Meer,
394   M.T.J., 2014. Salinity dependent hydrogen isotope fractionation in alkenones produced by
395   coastal and open ocean haptophyte algae. Geochimica et Cosmochimica Acta 130, 126-
396   135.
397 Manabe, S., 1969. Climate and the ocean circulation 1. Monthly Weather Review 97, 739-774.
398 Marlowe, I.T., Brassell, S.C., Eglinton, G., Green, J.C., 1990. Long-chain alkenones and alkyl
399   alkenoates and the fossil coccolith record of marine sediments. Chemical Geology 88,
400   349-375.
401 Marlowe, I.T., Green, J.C., Neal, A.C., Brassell, S.C., Eglinton, G., Course, P.A., 1984. Long
402   chain (n-C_{37}-C_{39}) alkenones in the Prymnesiophyceae. Distribution of alkenones and other
403   lipids and their taxonomic significance. British Phycological Journal 19, 203 - 216.
404 März, C., Hoffmann, J., Bleil, U., de Lange, G.J., Kasten, S., 2008. Diagenetic changes of
405   magnetic and geochemical signals by anaerobic methane oxidation in sediments of the
406   Zambezi deep-sea fan (SW Indian Ocean). Marine Geology 255, 118-130.
407 Mashiotta, T.A., Lea, D.W., Spero, H.J., 1999. Glacial-interglacial changes in Subantarctic sea
408   surface temperature and d^{18}O-water using foraminiferal Mg. Earth and Planetary Science
409   Letters, 170, 417-432.
410 Meybeck, M., 1982. Carbon, nitrogen, and phosphorus transport by world rivers. American
411   Journal of Science 282, 401-450.
412 Noble, P.A., Tymowski, R.G., Fletcher, M., Morris, J.T., Lewitus, A.J., 2003. Contrasting patterns
413   of phytoplankton community pigment composition in two salt marsh estuaries in
414   Southeastern United States. Applied and Environmental Microbiology 69, 4129-4143.
415 Nürnberg, D., Bijma, J., Hemleben, C., 1996. Assessing the reliability of magnesium in
416   foraminiferal calcite as a proxy for water mass temperatures.Geochimica et
417   Cosmochimica Acta 60, 2483-2483.
418 Örnólfsdóttir, E.B., Lumsden, S.E., Pinckney, J.L., 2004. Phytoplankton community growth-rate
419   response to nutrient pulses in a shallow turbid estuary, Galveston Bay, Texas. Journal of
420   Plankton Research 26, 325-339.
421 Pahnke, K., Sachs, J.P., Keigwin, L., Timmermann, A., Xie, S.-P., 2007. Eastern tropical Pacific
422   hydrologic changes during the past 27,000 years from D/H ratios in alkenones.
423   Paleoceanography 22, PA4214.
424 Pedersen, M.F., Borum, J., 1996. Nutrient control of algal growth in estuarine waters. Nutrient
425   limitation and the importance of nitrogen requirements and nitrogen storage among
426   phytoplankton and species of macroalgae. Marine Ecology Progress Series, Oldendorf
427   142, 261-272.
428 Prahl, F.G., Muehlhausen, L.A., Zahnle, D.L., 1988. Further evaluation of long-chain alkenones
429   as indicators of paleoceanographic conditions. Geochimica et CosmochimicaActa 52,
430   2303-2310.
431 Rahmstorf, S., 2002. Ocean circulation and climate during the past 120,000 years. Nature 419,
432   207-214.

Reimer, P.J., Baillie, M.G.L., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Ramsey, C.B., Buck, C.E., Burr, G.S., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Hajdas, I., Heaton, T.J., Hogg, A.G., Hughen, K.A., Kaiser, K.F., Kromer, B., McCormac, F.G., Manning, S.W., Reimer, R.W., Richards, D.A., Southon, J.R., Talamo, S., Turney, C.S.M., van der Plicht, J., Weyhenmeyer, C.E., 2011. IntCal09 and Marine09 Radiocarbon Age Calibration Curves, 0-50,000 Years cal BP. Radiocarbon 51, 1111-1150.

Ridderinkhof, H., van der Werf, P.M., Ullgren, J.E., van Aken, H.M., van Leeuwen, P.J., de Ruijter, W.P.M., 2010. Seasonal and interannual variability in the Mozambique Channel from moored current observations. Journal of Geophysical Research: Oceans 115, C06010.

Rohling, E.J., 2000. Paleosalinity: confidence limits and future applications. Marine Geology 163, 1-11.

Rohling, E.J., Bigg, G.R., 1998. Paleosalinity and $\delta^{18}O$: A critical assessment. Journal of Geophysical Research-Oceans 103, 1307-1318.

Rostek, F., Ruhlandt, G., Bassinot, F.C., Muller, P.J., Labeyrie, L.D., Lancelot, Y., Bard, E., 1993. Reconstructing sea surface temperature and salinity using . $\delta^{18}O$ and alkenone records. Nature 364, 319-321.

Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J., Freeman, K.H., Magill, C.R., McInerney, F.A., van der Meer, M.T.J., Polissar, P., Robins, R.J., Sachs, J.P., Schmidt, H.-L., Sessions, A.L., White, J.W.C., West, J.B., Kahmen, A., 2012. Molecular Paleohydrology: Interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. Annual Review of Earth and Planetary Sciences 40, 221-249.

Sætre, R., Da Silva, A.J., 1984. The circulation of the Mozambique channel. Deep Sea Research Part A. Oceanographic Research Papers 31, 485-508.

Sauer, P.E., Eglinton, T.I., Hayes, J.M., Schimmelmann, A., Sessions, A.L., 2001. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental andclimatic conditions. Geochimica et Cosmochimica Acta 65, 213-222.

Schefuß, E., Kuhlmann, H., Mollenhauer, G., Prange, M., Patzold, J., 2011. Forcing of wet phases in southeast Africa over the past 17,000 years. Nature 480, 509-512.

Schouten, M.W., de Ruijter, W.P.M., van Leeuwen, P.J., Ridderinkhof, H., 2003. Eddies and variability in the Mozambique Channel. Deep Sea Research Part II: Topical Studies in Oceanography 50, 1987-2003.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. Organic Geochemistry 54, 19-61.

Schouten, S., Huguet, C., Hopmans, E.C., Kienhuis, M.V.M., Sinninghe Damsté, J.S., 2007. Analytical methodology for TEX$_{86}$paleothermometry by high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry.Analytical Chemistry 79, 2940-2944.

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

Schulz, H.-M., Schöner, A., Emeis, K.-C., 2000. Long-chain alkenone patterns in the Baltic sea--an ocean-freshwater transition. Geochimica et Cosmochimica Acta 64, 469-477.

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

480 Sessions, A.L., Burgoyne, T.W., Schimmelmann, A., Hayes, J.M., 1999. Fractionation of
481   hydrogen isotopes in lipid biosynthesis. Organic Geochemistry 30, 1193-1200.
482 Sessions, A.L., Sylva, S.P., Summons, R.E., Hayes, J.M., 2004. Isotopic exchange of carbon-
483   bound hydrogen over geologic timescales. Geochimica et Cosmochimica Acta 68, 1545-
484   1559.
485 Shackleton, N.J., 1987. Oxygen isotopes, ice volume and sea level. Quaternary Science Reviews
486   6, 183-190.
487 Siddorn, J.R., Bowers, D.G., Hoguane, A.M., 2001. Detecting the Zambezi River Plume using
488   observed optical properties. Marine Pollution Bulletin 42, 942-950.
489 Srivastava, R., Ramesh, R., Jani, R.A., Anilkumar, N., Sudhakar, M., 2010. Stable oxygen,
490   hydrogen isotope ratios and salinity variations of the surface Southern Indian Ocean
491   waters. Current Science 99, 1395-1399.
492 Strain, P.M., Tan, F.C., 1993. Seasonal evolution of oxygen isotope-salinity relationships in high-
493   latitude surface waters. Journal of Geophysical Research: Oceans 98, 14589-14598.
494 Ullgren, J.E., van Aken, H.M., Ridderinkhof, H., de Ruijter, W.P.M., 2012. The hydrography of
495   the Mozambique Channel from six years of continuous temperature, salinity, and velocity
496   observations. Deep Sea Research Part 1: Oceanographic Research Papers 69, 36-50.
497 Van der Lubbe, J.J.L., Tjallingii, R., Prins, M.A., Brummer, G.-J.A., Jung, S.J.A., Kroon, D.,
498   Schneider, R.R., 2014. Sedimentation patterns off the Zambezi River over the last 20,000
499   years. Marine Geology 355, 189-201.
500 van der Meer, M.T.J., Baas, M., Rijpstra, W.I.C., Marino, G., Rohling, E.J., Sinninghe Damsté,
501   J.S., Schouten, S., 2007. Hydrogen isotopic compositions of long-chain alkenones record
502   freshwater flooding of the Eastern Mediterranean at the onset of sapropel deposition.
503   Earth and Planetary Science Letters 262, 594-600.
504 van der Meer, M.T.J., Benthien, A., Bijma, J., Schouten, S., Sinninghe Damsté, J.S., 2013.
505   Alkenone distribution impacts the hydrogen isotopic composition of the $C_{37:2}$ and $C_{37:3}$
506   alkan-2-ones in *Emiliania huxleyi*. Geochimica et CosmochimicaActa 111, 162-166.
507 van der Meer, M.T.J., Sangiorgi, F., Baas, M., Brinkhuis, H., Sinninghe Damsté, J.S., Schouten,
508   S., 2008. Molecular isotopic and dinoflagellate evidence for Late Holocene freshening of
509   the Black Sea. Earth and Planetary Science Letters 267, 426-434.
510 Verity, P.G., Yoder, J.A., Stephen Bishop, S., Nelson, J.R., Craven, D.B., Blanton, J.O.,
511   Robertson, C.Y., Tronzo, C.R., 1993. Composition, productivity and nutrient chemistry of
512   a coastal ocean planktonic food web. Continental Shelf Research 13, 741-776.
513 Waelbroeck, C., Labeyrie, L., Michel, E., Duplessy, J.C., McManus, J.F., Lambeck, K., Balbon,
514   E., Labracherie, M., 2002. Sea-level and deep water temperature changes derived from
515   benthic foraminifera isotopic records. Quaternary Science Reviews 21, 295-305.
516 Walford, H.L., White, N.J., Sydow, J.C., 2005. Solid sediment load history of the Zambezi Delta.
517   Earth and Planetary Science Letters 238, 49-63.
518 Walsh, E.M., Ingalls, A.E., Keil, R.G., 2008. Sources and transport of terrestrial organic matter in
519   Vancouver Island fjords and the Vancouver-Washington Margin: A multiproxy approach
520   using $\delta^{13}C_{org}$, lignin phenols, and the ether lipid BIT index. Limnology and Oceanography
521   53, 1054-1063.
522 Wang, Y.V., Larsen, T., Leduc, G., Andersen, N., Blanz, T., Schneider, R.R., 2013a. What does
523   leaf wax δD from a mixed C_3/C_4 vegetation region tell us? Geochimica et Cosmochimica
524   Acta 111, 128-139.

525 Wang, Y.V., Leduc, G., Regenberg, M., Andersen, N., Larsen, T., Blanz, T., Schneider, R.R.,
526   2013b. Northern and southern hemisphere controls on seasonal sea surface temperatures
527   in the Indian Ocean during the last deglaciation. Paleoceanography 28, 2013PA002458.
528 Wolhowe, M.D., Prahl, F.G., Probert, I., Maldonado, M., 2009. Growth phase dependent
529   hydrogen isotopic fractionation in alkenone-producing haptophytes. Biogeosciences 6,
530   1681-1694.
531 Zell, C., Kim, J.-H., Moreira-Turcq, P., Abril, G., Hopmans, E.C., Bonnet, M.-P., Sobrinho, R.L.,
532   Damsté, J.S.S., 2013. Disentangling the origins of branched tetraether lipids and
533   crenarchaeol in the lower Amazon River: Implications for GDGT-based proxies.
534   Limnology and Oceanography 58, 343-353.

535

536
537
538
539
540
541
542
543
544

545

546 **Fig. 1.** Map of oceanic currents in the study region and core location. (a) Main ocean current

547 systems in the Western Indian Ocean (white arrows), showing the South Equatorial Current

548 (SEC), the Eastern Madagascar Current (EMC), the Mozambique Current (MC) and the greater

549 Agulhas Current (Beal et al., 2011). The location of core 64PE304-80 is given by the red circle in

550 the Mozambique Channel. Fig. (b) Shows an enlargement of the Mozambique Channel and the

551 location of the Zambezi River mouth; the large gray arrow indicates the general current direction

552 of the Mozambique Current, small gray arrows coastal circulation cells, and white shaded area

553 represents the sediment distribution of Zambezi River fan (Sætre and Da Silva, 1984).

554

555   **Fig. 2.**(a) Temperature variation over Greenland indicated by the $\delta^{18}O$ record of GRIP ice core

556   (blue; Greenland Ice-core Project, 1993); (b) $\delta D_{alkenone}$ record (red line) with analytical error (1σ),

557   (c) C_{37}/C_{38} alkenone ratio (black line) and (d) BIT index (green) for core 64PE304-80; (e) mean

558   global benthic $\delta^{18}O_{sw}$ curve (Waelbroeck et al., 2002). Gray shaded bars indicate Younger Dryas

559   (YD) (ca. 12.5-11 ka), Heinrich Event 1 (HE1) (ca. 19-14.6 ka), Last Glacial Maximum (LGM;

560   ca. 23-19 ka) and Heinrich Event 2 (HE2; ca. 24 ka). Radiocarbon samples are indicated as black

561   triangles.

562

563   **Fig. 3.** Cross plot of $\delta D_{alkenone}$ vs. BIT index. Values for Holocene (ca.12.7 – 0.5 ka) are indicated

564   by closed gray circles, regression II (dashed line). Values for pre-LGM (ca. 39.4 – 16.1 ka) are

565   indicated by open squares, regression I (solid line).

566