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Large effect of irradiance on hydrogen isotope fractionation of alkenones in

Emiliania huxleyi.

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1 **Abstract**

2   The hydrogen isotopic (δD) composition of long-chain alkenones produced by

3 certain haptophyte algae has been suggested as a potential proxy for reconstructing

4 paleo sea surface salinity. However, environmental parameters other than salinity may

5 also affect the δD of alkenones. We investigated the impact of the level of irradiance on

6 hydrogen isotopic fractionation of alkenones versus growth water by cultivating two

7 strains of the cosmopolitan haptophyte *Emiliania huxleyi* at different light intensities.

8 The hydrogen isotope fractionation decreased by approximately 40‰ when irradiance

9 was increased from 15 to 200 μmol photons m^{-2} s^{-1} above which it was relatively

10 constant. The response is likely a direct effect of photosystem I and II activity as the

11 relationship of the fractionation factor α versus light intensity can be described by an

12 Eilers-Peeters photosynthesis model. This irradiance effect is in agreement with

13 published δD data of alkenones derived from suspended particulate matter collected

14 from different depths in the photic zone of the Gulf of California and the eastern

15 tropical North Pacific. However, haptophyte algae tend to bloom at relatively high light

16 intensities (> 500 μmol photons m^{-2} s^{-1}) occurring at the sea surface, at which hydrogen

17 isotope fractionation is relatively constant and not affected by changes in light intensity.

18 Alkenones accumulating in the sediment are likely mostly derived from these surface

19 water haptophyte blooms, when the largest amount of biomass is produced. Therefore,

20 the observed irradiance effect is unlikely to affect the applicability of the hydrogen

21 isotopic composition of sedimentary long chain alkenones as a proxy for paleosalinity.

22

23   **1. Introduction**

1

24	The oxygen and hydrogen isotopic composition of ocean water is strongly

25	correlated with salinity because phase changes between seawater, water vapor and

26	precipitation involves oxygen and hydrogen isotope fractionation. For instance, water

27	vapor is depleted in ^{18}O and D relative to water and evaporation thus results in increased

28	salinity and ^{18}O and D content of seawater in evaporative regions. The isotopically

29	depleted water vapor will condense and precipitate over continents and thus river runoff

30	and precipitation result in both a decrease in salinity and ^{18}O and D content of the

31	seawater. Therefore, for most parts of the ocean-atmosphere interface water isotopes are

32	linearly correlated with salinity (Craig and Gordon, 1965) and thus paleosalinity can be

33	reconstructed from either the oxygen or hydrogen isotopic composition of water using

34	this relation.

35	The hydrogen isotopic composition of water may be recorded in the non-

36	exchangeable hydrogen in biological organic matter although with a considerable

37	biosynthetic isotopic fractionation effect (Yakir and DeNiro, 1990; Hayes 2001).

38	Nevertheless, as long as this fractionation can be constrained, δD analyses on marine

39	organic matter could provide a means to reconstruct δD of seawater and, thus, if the

40	relation between δD and salinity is known, seawater paleosalinity. Long-chain

41	alkenones produced only by haptophyte algae such as *Emiliania huxleyi* (Volkman et

42	al., 1980; Marlowe et al., 1984; Volkman et al., 1995) possess only covalently bound

43	hydrogen atoms, which are not likely to be exchanged during diagenesis (Sessions et al.,

44	2004), making them excellent candidate compounds for stable hydrogen isotope

45	analysis. Initially the idea was to reconstruct paleo seawater δD directly from the

46	measured alkenone δD assuming a fixed difference between the alkenone and water

47	isotopic composition. This idea was motivated by the relatively constant fractionation of

48 approximately 225‰ between alkenones and water for batch cultures of the haptophyte

49 *Emiliania huxleyi* grown on medium spiked with different levels of deuterated water, at

50 constant salinities (Paul 2002; Englebrecht and Sachs 2005). However, experiments

51 with *E. huxleyi*, and other alkenone-producing haptophytes, i.e. *Gephyrocapsa*

52 *oceanica, Isochrysis galbana* and *Chrysotila Lamellosa*, cultured at different salinities

53 showed that the biological hydrogen isotope fractionation between alkenones and water,

54 expressed as the fractionation factor α, depends on salinity (Schouten et al., 2006;

55 M'Boule et al., 2014; Chivall et al., 2014). Therefore, as salinity increases not only the

56 hydrogen isotopic composition of water increases but α increases as well, both resulting

57 in an increased D content of alkenones with increasing salinity. This indicates the

58 potential of the δD of alkenones as a paleo sea surface salinity proxy. Several studies

59 indicate that salinity dependent hydrogen isotope fractionation might be a general

60 phenomenon in phototrophic organisms. For instance, hydrogen isotope fractionation in

61 cyanobacterial lipids from naturally occurring microbial mats decreases with increasing

62 salinity (Sachse et al., 2008) . The fractionation associated with dinosterol in the

63 Chesapeake Bay estuary (Sachs and Schwab, 2011) and dinosterol and brassicasterol

64 from saline and hypersaline lakes in North America (Nelson and Sachs, 2014) also

65 decreased with increasing salinity.

66   Consequently, the hydrogen isotopic composition of C_{37} alkenones has been

67 used to estimate paleo sea surface salinity (SSS) changes in the Aegean Sea at the time

68 of sapropel S5 deposition (van der Meer et al., 2007). Here the δD record of combined

69 C_{37} alkenones ($C_{37:2}$ and $C_{37:3}$) showed a large and abrupt shift to lower δD values at the

70 onset of sapropel deposition similar to the shift observed for forminiferal $\delta^{18}O$ values

71 measured on the carbonate tests of surface dwelling foraminifera (Marino et al., 2007).

72   This shift towards more D depleted alkenones suggests that this proxy does indeed

73   record the drop in SSS caused by the significantly increased input of freshwater from

74   the continent at the onset of sapropel formation. The δD alkenone proxy has

75   subsequently been used to assess paleo SSS changes in the Black Sea (van der Meer et

76   al., 2008; Giosan et al., 2012; Coolen et al., 2013) and glacial-interglacial salinity

77   changes in the Agulhas leakage area (Kasper et al., 2014) and Mozambique channel

78   (Kasper et al., 2015).

79   Despite these successful applications of the δD alkenone proxy for the

80   reconstruction of paleo SSS, several complications exist. Firstly, the δD of alkenones in

81   the Chesapeake Bay estuary and from saline and hypersaline lakes in continental North

82   America shows a correlation with the δD of the water, but does not reveal a relation of

83   the fractionation factor α between alkenones and growth water with salinity as observed

84   in for cultures (Schwab and Sachs, 2011; Nelson and Sachs, 2014). Secondly, factors

85   other than salinity have been shown to also affect the fractionation factor α between

86   alkenones and growth water. For example, *E. huxleyi*, *G. oceanica* , *I. galbana* and *C.*

87   *lamellosa* all show differences in α at the same salinity (Schouten et al., 2006; M'Boule

88   et al., 2014; Chivall et al., 2014) and the relationships between α and salinity are

89   different for cultures harvested during different growth phases (Wolhowe et al., 2009;

90   Chivall et al., 2014). Additionally, it has been suggested that growth rate also affects α

91   (Schouten et al., 2006). A yet unexplored factor in determining hydrogen isotope

92   fractionation is light intensity, which might have an effect because the production of

93   NADPH, the major source of hydrogen in biosynthesis (Zhang et al., 2009), is directly

94   linked to photosynthetic activity (Allen 2002). Here, we examined the impact of

95   irradiance on the hydrogen isotope fractionation in *E. huxleyi* and discuss the

96   implication of our findings for hydrogen isotopic fractionation in natural settings.

97

98   **2. Materials and methods**

99   *2.1 Incubation experiments*

100   Two sets of light experiments were carried out. Monospecific cultures of the

101   haptophyte algae *E. huxleyi* (strain PML B92/11) were grown at a constant temperature

102   of 15°C, at a constant salinity of 32.5, and varying light intensities of 15, 30, 50, 100

103   and 200 µmol photons m^{-2} s^{-1}. In a second experiment, batch cultures of *E. huxleyi*

104   (strain RCC1238) were grown in triplicate at four different light intensities (100, 200,

105   400, and 600 µmol photons m^{-2} s^{-1}) in autoclaved 1 L bottles at a constant temperature

106   and salinity of 15°C and 32.2, respectively. The two strains show similar growth

107   responses relative to nutrients, temperature, light etc. The main difference between the

108   two strains has to do with differences in their carbonate chemistry (Langer et al., 2009).

109   All cultures were grown in Rumed cabinets, providing cool-white fluorescent light with

110   a 16:8 h light:dark cycle. The seawater medium prepared according to F/2 (Guillard,

111   1975) for the first and F/2R for the second experiment, respectively. The enriched

112   medium was sterile filtered using a 0.45µm filter cartridge in the first experiment and

113   0.2µm filter cartridge in the second experiment. All cultures were allowed to acclimate

114   to the experimental conditions in a pre-culture before being used to inoculate the main

115   batch cultures to provide an initial cell density between $0.5-7 \times 10^{3}$ cell ml^{-1} for the first

116   experiment and a target initial cell density of approximately 0.9×10^{2} cell ml^{-1} for the

117   second experiment. Cultivation took place in bottles that were closed and incubated for

118   4 to 12 days depending on algal growth rate.

5

119   Cells were counted daily using a Beckman Coulter Multisizer 3 particle counter.

120   Cell numbers were log transformed and plotted versus time, growth rate μ (d^{-1}) was

121   estimated by linear regression. The cultures were harvested by filtration over ashed 0.7

122   μm GF/F filters (Whatman) when the cultures were in exponential growth phase and

123   had achieved cell densities within the range of $0.55\text{-}1.5\times10^5$ cells ml^{-1}. Filters and

124   aliquots of the culture medium were frozen immediately and stored at < -25°C until

125   analysis. The culture waters were stored with no headspace in 12 mL exetainers (Labco)

126   in the dark at ~5°C until analysis.

127

128   *2.2 Alkenone preparation*

129   Filters from the first experiment were extracted ultrasonically using first

130   methanol, followed by methanol:dichloromethane (DCM) 1:1 (v:v) and finally DCM. A

131   ketone fraction was obtained by purifying the total lipid extracts by passing them over a

132   silica gel cartridge (Varian Bond Elut; 1 cm^3/100 mg), followed by saponification in 0.3

133   mL of 0.1 M KOH in methanol : water 9:1 (v/v) at 80 °C in a capped vial for 2 hours.

134   The alkenone containing fraction was subsequently obtained by partitioning in hexane

135   (Benthien et al., 2002). The alkenone fractions were analyzed by gas chromatography

136   (GC) and GC/mass spectrometry (GC/MS) (van der Meer et al., 2007). The alkenone

137   hydrogen isotopic composition was determined by GC thermal conversion isotope ratio

138   monitoring MS (GC/TC/irMS).

139   Filters from the second experiment were freeze dried for 24 h prior to automated

140   solvent extraction by a Dionex ASE using a 9:1 (v:v) DCM:methanol mixture. Total

141   lipid extracts (TLEs) were dried down using a rotary evaporator. The TLEs were

142   subsequently saponified by adding methanol and 1 ml 0.1 M KOH and heating at 80°C

143 for 2 h. The saponified alkenone fraction was analyzed by gas chromatography with

144 flame ionization detection (GC-FID).

145

146 *2.3 Instrumental analysis*

147 The algal culture media δD water values were determined by Elemental Analysis

148 (EA)/Thermal Conversion (TC)/irmMS using a Thermo Electron EA/TC coupled to a

149 Thermo Electron DELTA^{Plus} XL mass spectrometer for the first experiment according to

150 Schouten et al., 2006. In short, about 1 μl of water was injected into a glassy carbon

151 filled ceramic tube at a temperature of 1425 ˚C. The H_3^+ -factor was determined daily

152 and was approximately 8.0 ± 0.3 ppm mV^{-1}. Waters were analyzed with at least ten

153 replicate analyses. Hydrogen gas with a predetermined isotopic composition was used

154 as reference and the water isotope values were calibrated against in-house lab standards

155 (North Sea water: +5‰ and bidistilled water: −76‰ that were calibrated by using

156 Vienna Standard Mean Ocean Water (VSMOW) and Greenland Ice Sheet Precipitation

157 (GISP) standards). The hydrogen isotopic composition of the medium used in the

158 second experiment was determined by the hydrogen gas-water equilibrium method

159 using a gas bench coupled to a Thermo Electron DELTA^{Plus} XP (Wong and Clarke,

160 2012) at the University of Utrecht.

161 Compound-specific hydrogen isotopic compositions for the combined C_{37}

162 alkenones (cf. van der Meer et al., 2013) from the first experiment were measured by

163 GC/TC/irmMS using a Thermo Electron DELTA^{Plus} XL mass spectrometer using a

164 CPSil 5 GC column with a 0.4 μm film thickness and a constant flow of He of 1 ml

165 min^{-1}. Compounds were converted to hydrogen gas and graphite at 1425 °C in an empty

166 ceramic tube which was pre-conditioned by injecting 0.2 μl of hexane several times (~5)

7

167   in the first week after installing a new reactor tube. Hydrogen gas with a predetermined

168   isotopic composition was used as reference gas at the beginning and end of each

169   analytical run and a C_{16}-C_{32} *n*-alkanes mixture with offline determined isotopic

170   compositions (ranging from −42‰ to −256‰ vs. VSMOW, Schimmelmann MixB)

171   was used to monitor the system performance daily. The average offsets between the

172   measured δD values of the C_{16}-C_{32} *n*-alkanes and their offline determined values were

173   generally 5‰ or less. Samples were analyzed at least in duplicate and the

174   reproducibility was typically better than 5‰ (Table 1). As additional control, squalane

175   was co-injected with every analysis and the average squalane value typically was -166 ±

176   3 ‰, while the offline determined value was -170‰.

177   Compound-specific hydrogen isotope values for the alkenones from the second

178   experiment were determined by GC/TC/irmMS with a Thermo Electron DELTA[Plus] XP

179   mass spectrometer using high temperature conversion at the University of Utrecht.

180   Compounds were converted to hydrogen gas and graphite in an empty ceramic tube

181   heated to 1400 °C. The hydrogen isotopic composition of the combined C_{37} alkenones

182   was corrected using the Schimmelmann *n*-alkane mix, Mix A. A squalane standard was

183   co-injected with every sample and its average value was -166.3 ± 5.1 ‰, which

184   compared well with its offline determined value of -169‰.

185

186   *2.4 Modelling*

187   A modified Eilers-Peeters formulation (Eilers and Peeters, 1988) was used to

188   describe both growth rate μ and fractionation factor α in response to irradiance. This

189   model can be applied directly to describe growth rate μ:

$$\mu = \mu_{max} * \frac{2*(1+\beta)*{}^I/_{Iopt}}{\left({}^I/_{Iopt}\right)^2 + 2*\beta*{}^I/_{Iopt} + 1} \qquad (Eq.\,1)$$

190   where β is a shape factor and μ_{max} represents the maximum growth rate. Growth rate μ

191   attains a maximum value at optimal irradiance (I_{opt}). The shape factor β determines the

192   'peakedness' or rounding of the production curve (e.g. Soetaert et al., 1994).

193   The model cannot be applied directly to describe hydrogen isotope fractionation,

194   as the α value does not equal zero in the dark. Therefore the basic equation was

195   extended with an offset value, α_0, which defines the fractionation at zero light intensity:

$$\alpha = \alpha_0 + \gamma * \frac{2*(1+\beta)*{}^I/_{I_{opt}}}{\left({}^I/_{I_{opt}}\right)^2 + 2*\beta*{}^I/_{I_{opt}} + 1} \qquad (Eq.\,2)$$

196   where α attains a maximum value at I_{opt} equal to $\alpha_{max} = \alpha_0 + \gamma$. Parameter values

197   μ_{max}, α_0, α_{max}, and I_{opt} were estimated by minimizing the sum of squared differences

198   between the model and experimental data using the Excel Solver routine.

199

200   **3. Results**

201   We analyzed the δD values of alkenones produced by *E. huxleyi* grown in batch

202   cultures at different irradiance levels. For the first experiment, where *E. huxleyi* strain

203   PML B92/11 was grown with light intensities ranging from 15 to 200 μmol photons m^{-2}

204   s^{-1}, the relationship between the growth rate and irradiance indicates that *E. huxleyi* is

205   growing under light limitation at light intensities < 100 μmol photons m^{-2} s^{-1}. Growth

206   rate is approximately 0.5 d^{-1} at the lowest irradiance and increases to approximately 1.0

207   d^{-1} at 50 μmol photons m^{-2} s^{-1}. The growth rates level off at approximately 1.1 d^{-1} for

208   irradiances exceeding 100 μmol photons m^{-2} s^{-1} (Table 1; Fig. 1). For the second

209   experiment with *E. huxleyi* strain RCC1238 and irradiance levels ranging from 100 to

210   600 μmol photons m^{-2} s^{-1}, a relatively constant growth rate of approximately 1.3 d^{-1} was

211   observed (Table 1; Fig. 1). The growth rates for *E. huxleyi* strain RCC1238 in

212   experiment 2 are slightly higher than for strain PML B92/11 in experiment 1 at the

213   corresponding irradiances of 100 and 200 μmol photons m^{-2} s^{-1}.

214   The hydrogen isotopic composition of the combined $C_{37:2}$ and $C_{37:3}$ alkenones

215   ranged from approximately -230 ‰ at the lowest level of irradiance to

216   approximately -189 ‰ at an irradiance of 200 μmol photons m^{-2} s^{-1} for the first

217   experiment (Table 1). For the second experiment the isotopic composition of the

218   combined $C_{37:2}$ and $C_{37:3}$ alkenones ranged from approximately -212 ‰ at 100 μmol

219   photons m^{-2} s^{-1} to -188 ‰ at 200 μmol photons m^{-2} s^{-1}. At irradiance levels > 200 μmol

220   photons m^{-2} s^{-1} the δD alkenones was approximately -193 ‰.

221   The fractionation factor α between the hydrogen isotopic composition of the

222   alkenones and the culture medium ranged from approximately 0.77 at the lowest level

223   of irradiance to approximately 0.82 at an irradiance >200 μmol photons m^{-2} s^{-1} (Table

224   1). A strong and positive linear relationship between the fractionation factor α and

225   irradiance is observed for the first set of experiments up to an irradiance level of 200

226   μmol photons m^{-2} s^{-1} (Fig. 2). For the second experiment, the fractionation factor shows

227   values similar to those of experiment 1 at corresponding irradiance levels of 100 and

228   200 μmol photons m^{-2} s^{-1} (Fig. 2). This suggests that the two strains fractionate similarly

229   at similar irradiance levels. At light intensities exceeding 200 μmol photons m^{-2} s^{-1} α

230   decreases slightly from approximately 0.815 to approximately 0.805 (Table 1).

231

232   **4. Discussion**

233   *4.1 Influence of the level of irradiance on the hydrogen isotopic fractionation.*

234 Our culture results demonstrate that the level of irradiance affects both the growth

235 rate of *E. huxleyi* (Fig. 1) and the hydrogen isotope fractionation between the alkenones

236 produced and the water (Fig. 2). The growth rate increased linearly with irradiance up to

237 between 50 and 100 μmol photons m^{-2} s^{-1}, and leveled off at irradiances above 100

238 μmol photons m^{-2} s^{-1}. Even though the data are from experiments using two different *E.*

239 *huxleyi* strains, the combined data of growth rates versus irradiance can be described by

240 a single fit with the Eilers-Peeters model (Eq. 1) (R^2= 0.89; Fig. 1). Based on these

241 results, it seems growth of *E. huxleyi* is not inhibited by irradiance levels of up to 600

242 μmol photons m^{-2} s^{-1}.

243 The decrease in α at higher irradiance levels (> 200 μmol photons m^{-2} s^{-1}; Fig. 2) is

244 similar to what is typically observed in Photosynthesis-Irradiance curves and is typically

245 attributed to light inhibition (e.g. Eilers and Peeters, 1988). A modified Eilers-Peeters

246 type of equation (Eq. 2) was used to describe the observed relationship of irradiance

247 with α, yielding a good fit (R^2 = 0.94; Fig. 2). This fit predicts a maximum fractionation

248 factor of 0.814 at an optimum irradiance (I_{opt}) of approximately 310 μmol photons m^{-2} s^{-}

249 ^{1}, which is in the range of saturation irradiance (I$_{sat}$) values (200-400 μmol photons m^{-2}

250 s^{-1}) reported for photosynthesis in *E. huxleyi* strains (Flameling and Kromkamp, 1998;

251 Feng et al., 2008; Harris et al., 2005). However, higher and lower I$_{sat}$ values have also

252 been reported (Nanninga and Tyrrell 1996, and references therein). Because the

253 modified Eilers-Peeters equation describes our data well, we suggest that irradiance is a

254 major factor influencing the fractionation factor α between the alkenones and growth

255 water of the haptophytes grown in our culture experiments.

256 Schouten et al. (2006) showed that α decreases with increasing growth rate (Fig. 3)

257 potentially suggesting that our observed correlation may be due to changing growth

258   rates controlled by the level of irradiance. However, plotting the growth rate against the

259   fractionation factor α for all irradiances from both experiments performed here shows

260   no clear correlation between α and growth rate (Fig. 3). Fractionation factor α increases

261   from growth rates of 0.4 to approximately 1.2 d^{-1} after which it decreases a little,

262   although there is some scatter at these higher growth rates. These results suggest that in

263   our experiments α does not change because of changing growth rates, but that both α

264   and growth rate are a function of irradiance. These findings are different from the

265   results of Schouten et al. (2006; Fig. 3), where *E. huxleyi* was grown at constant

266   irradiance but different salinities and temperatures, suggesting that hydrogen isotope

267   fractionation in alkenone biosynthesis in these experiments is more likely controlled by

268   downstream biosynthetic effects.

269   A possible explanation for this effect of irradiance on the hydrogen isotopic

270   fractionation of *E. huxleyi* could be the central role NADPH has as hydrogen source for

271   biosynthesis (Yakir and DeNiro, 1990; Hayes, 2001), i.e. approximately 50% of non-

272   exchangeable hydrogen in lipids is derived from NADPH (Zhang et al., 2009). The

273   initial biosynthetic isotopic fractionation effect from water to the primary photosynthate

274   is considerable, ca. 171 ‰, suggested to be largely due to the reduction of NADP^{+} to

275   NADPH (Yakir and DeNiro, 1990; reviewed by Hayes, 2001). The reduction of

276   NADP^{+} to NADPH in photosynthetic organisms is directly linked to photosystem

277   activity (Allen 2002 and references therein) and therefore potentially light intensity.

278   This probably explains the link between irradiance level and hydrogen isotopic

279   fractionation, although the exact biochemical mechanisms responsible for this irradiance

280   depended hydrogen isotope fractionation effect is unclear and subject for future

281   research.

282

4.2 Potential implications for the natural environment.

The magnitude of the change in $\delta D_{alkenones}$ between cultures grown at 15 and 200

μmol photons m^{-2} s^{-1} (~40 ‰ or 0.2 ‰ per μmol photons m^{-2} s^{-1}) is relatively large and

comparable in magnitude to the change observed for cultures grown in salinities varying

by ~20 salinity units (i.e. 1-3 ‰ change per salinity unit observed in cultures; Schouten

et al., 2006; M'Boule et al., 2014; Chivall et al., 2014). This suggests that an irradiance

effect could be large enough to limit the applicability of $\delta D_{alkenones}$ as a proxy for paleo

salinity. An important constraint will be the overall *in situ* irradiance level during

biomass formation and alkenone synthesis (Wolhowe et al., 2015), as well as how much

variability in irradiance, which is related to seasonal variability and water depth, is

captured by sedimentary alkenones, especially when averaged over geological time

scales.

Depending on season, latitude and depth, photosynthetically available radiation in

the ocean will range from 0 to approximately 810 μmol photons m^{-2} s^{-1} (Frouin and

Murakami 2007), a range almost entirely covered by our irradiance experiments. Our

results show that irradiance has the strongest effect on the hydrogen isotopic

fractionation at light intensities from 15 to 200 μmol photons m^{-2} s^{-1}. This irradiance

effect is in agreement with $\alpha_{alkenones/water}$ in suspended particulate matter from the photic

zone of the Gulf of California and the eastern tropical North Pacific which show

decreasing values with increasing depth and thus decreasing light levels (Wolhowe et

al., 2015).

Algae, including alkenone producing haptophytes, tend to form large blooms when

the growth conditions, specifically nutrient levels, temperature and irradiance, are

13

306   optimal. *E. huxleyi*, for instance, is thought to thrive under high light conditions, at

307   mixed layer depths generally <30 meter (Tyrrell and Merico, 2004; Harris et al., 2005).

308   They outcompete other algal species that suffer from photoinhibition under these

309   conditions, a process that is apparently absent in *E. huxleyi* (Nanninga and Tyrrell,

310   1996). In fact, based on field data collected during *E. huxleyi* blooms, mesocosm studies

311   and culture experiments, *E. huxleyi* is thought to only form large blooms at light

312   intensities >530 μmol photons m^{-2} s^{-1} (Nanninga and Tyrrell, 1996 and references

313   therein; Harris et al., 2005). This is in the range of irradiance levels in our experiments

314   where α is relatively constant (Fig. 2), indicating that the δD of alkenones synthesized

315   during blooming would show only minor variation due to variations in the level of

316   irradiance. If the majority of alkenones in the sediment are derived from haptophytes

317   blooming at the surface, this indicates that variations in the level of irradiance would

318   only have a minor effect on the δD of sedimentary alkenones . Indeed, it has been

319   shown often that the degree of unsaturation of alkenones, the $U^{K'}_{37}$, which is used as a

320   paleo sea surface temperature proxy, correlates on a global scale best with annual mean

321   sea surface temperatures rather than deeper water temperatures, i.e. at the bottom of the

322   photic zone (e.g. Müller et al., 1998). Furthermore, during bloom conditions when

323   growth becomes limited by nutrient limitation, but photosynthesis continues as long as

324   there is enough light, the haptophyte algae produce more alkenones per cell to store the

325   reducing equivalents (i.e. NADPH) produced during photosynthesis (Eltgroth et al.,

326   2005). High cell densities during bloom conditions might also promote grazing and

327   packaging of cells and alkenones in fecal pellets, cell aggregation and increase the

328   possibility of cell material attaching to sinking particles, increasing the transport

329   efficiency of haptophyte cell material, including alkenones, to the underlying sediment.

330   Therefore it seems likely that the majority of alkenones in the sediment are derived from

331   haptophyte blooms and reflect high light conditions.

332   Nevertheless, the conditions under which the majority of the sedimentary alkenones

333   are produced together with the environmental significance of irradiance on the hydrogen

334   isotope fractionation should be further tested in nature by sampling suspended

335   particulate matter from different water depths (c.f. Wolhowe et al., 2015) and bloom

336   and non-bloom derived alkenones using sediment traps and analyzing core tops from

337   close to the equator to high latitudes to capture seasonal variability in irradiance.

338

339   **5. Conclusion**

340   Cultivation of two *E. huxleyi* strains show that when growth rate is irradiance-

341   limited, increasing growth results in decreased hydrogen isotope fractionation, the

342   opposite response to temperature/salinity-limited growth rate. Rather, our results

343   suggest that irradiance is directly affecting the hydrogen isotopic fractionation of *E.*

344   *huxleyi* up to levels of 200 μmol photons m^{-2} s^{-1} after which it remains relatively

345   constant. *E. huxleyi* usually thrives under relatively high light conditions and is thought

346   to bloom at light intensities > 500 μmol photons m^{-2} s^{-1}. Therefore, it seems unlikely

347   that light affects the use of the hydrogen isotopic composition of sedimentary long chain

348   alkenones as a proxy for paleosalinity, assuming that the majority of sedimentary

349   alkenones are derived from surface water haptophyte blooms. The actual conditions

350   under which most of the sedimentary alkenones are produced, together with the

351   significance of irradiance on the hydrogen isotopic composition of long chain alkenones

352   in natural settings should be further investigated.

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502

503   **Figure legends**

504   Figure 1: Growth rate μ (d^{-1}) plotted against irradiance I (μmol photons m^{-2} s^{-1}) for both

505   the first experiment with *E. huxleyi* strain PML B92/11 ($\bullet$) and second experiment

506   with strain RCC1238 ($\blacktriangle$) and the model fit using the Eilers-Peeters equation (Eq.

507   1) (---)(Eilers and Peeters, 1988) .

508   Figure 2: Fractionation factor α alkenones versus medium water plotted against

509   irradiance I (μmol photons m^{-2} s^{-1}) for both the first experiment with *E. huxleyi*

510   strain PML B92/11 ($\bullet$) and second experiment with strain RCC1238 ($\blacktriangle$) and the

511   model fit using a modified Eilers-Peeters equation (Eq. 2) (---)(Eilers and Peeters,

512   1988).

513   Figure 3: Fractionation factor α for alkenones versus medium water plotted against

514   growth rate μ (d^{-1}) for both the first with *E. huxleyi* strain PML B92/11 ($\bullet$) and

515   second experiment with strain RCC1238 ($\blacktriangle$) in which both α and μ were

516   controlled by light intensity. Included are also the α and μ data from Schouten et

517   al., 2006 ($\times$) for *E. huxleyi* grown at different salinities and temperatures at a single

518   light intensity.

519   **Table 1**: Results from two culture experiments in which two strains of *E. huxleyi* (PML

520   B92/11 and RCC1238) were cultured at different light intensities to study the effect of

521   light intensity on hydrogen isotope fractionation. All cultures were harvested in the

522   exponential growth phase after 4 to 12 days depending on the cell numbers.

523

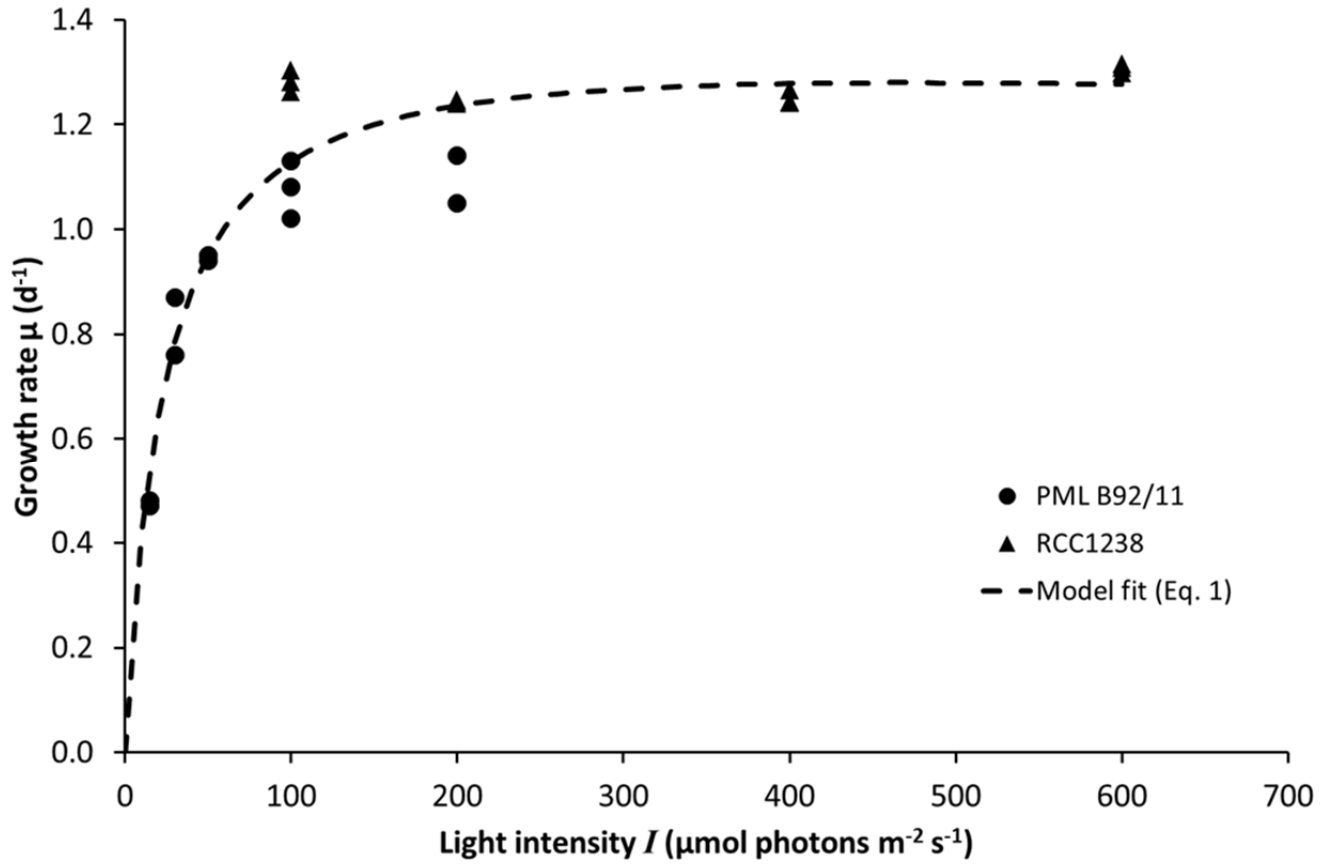| Irradiance I (μmol photons $m^{-2} s^{-1}$)	Growth rate μ (d^{-1})	δD_{water} (‰ vs. VSMOW)	Stdev	$\delta D_{alkenones}$ (‰ vs. VSMOW)	Stdev	α	Error
Experiment 1 Strain PML B92/11							
15	0.47	-1.5	2.5	-233.2	3.2	0.768	0.004
15	0.48	-2.9	2.2	-229.3	0.1	0.773	0.002
30	0.76	-0.7	1.5	-231.4	0.1	0.769	0.001
30	0.87	-2.0	2.3	-231.8	2.2	0.770	0.003
50	0.94	-1.1	1.8	-231.6	2.3	0.769	0.003
50	0.95	-0.7	1.4	-218.6	2.3	0.782	0.003
100	1.02	-1.7	1.7	-209.2	0.8	0.792	0.002
100	1.13	-1.3	2.0	-209.5	0.8	0.792	0.002
100	1.08	-2.3	1.3	-209.9	1.5	0.792	0.002
200	1.05	-2.0	1.4	-186.8	2.4	0.815	0.003
200	1.14	-0.9	1.8	-191.1	1.8	0.810	0.002
Experiment 2 Strain RCC1238							
100	1.26	-0.3	1.3	-213.6	2.1	0.787	0.002
100	1.30	0.2	1.6	-214.8	1.1	0.785	0.002
100	1.28	-1.1	2.1	-209.8	1.8	0.791	0.002
200	1.24	-2.4	1.9	-186.3	1.0	0.816	0.002
200	1.25	-0.7	0.0	-189.8	1.1	0.811	0.001
200	1.24	-1.8	2.6	-187.1	2.0	0.814	0.003
400	1.24	1.2	1.3	-192.9	5.0	0.806	0.005
400	1.27	0.4	1.2	-192.3	0.5	0.807	0.001
400	1.24	0.3	1.2	-188.5	5.4	0.811	0.006
600	1.30	-2.8	1.5	-196.8	2.7	0.805	0.003
600	1.32	-0.9	0.2	-196.4	2.9	0.804	0.003
600	1.31	0.1	4.8	-192.6	3.1	0.807	0.005

524
525

Figure 1

Figure 2

Figure 3