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Large effect of irradiance on hydrogen isotope fractionation of alkenones in *Emiliania huxleyi*

Marcel T.J. van der Meer [a,*], Albert Benthien [b], Katherine L. French [b,1], Eric Epping [a], Ingrid Zondervan [b], Gert-Jan Reichart [a,c], Jelle Bijma [b], Jaap S. Sinninghe Damsté [a,c], Stefan Schouten [a,c]

[a] *NIOZ Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg, The Netherlands*

[b] *Alfred-Wegener-Institute Helmholtz-Zentrum für Polar- und Meeresforschung, PO Box 12 01 61, D-27515 Bremerhaven, Germany*

[c] *Faculty of Geosciences, Utrecht University, PO Box 80.021, 3508 TA Utrecht, The Netherlands*

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Abstract

The hydrogen isotopic (δD) composition of long-chain alkenones produced by certain haptophyte algae has been suggested as a potential proxy for reconstructing paleo sea surface salinity. However, environmental parameters other than salinity may also affect the δD of alkenones. We investigated the impact of the level of irradiance on hydrogen isotopic fractionation of alkenones versus growth water by cultivating two strains of the cosmopolitan haptophyte *Emiliania huxleyi* at different light intensities. The hydrogen isotope fractionation decreased by approximately 40‰ when irradiance was increased from 15 to 200 μmol photons m^{-2} s^{-1} above which it was relatively constant. The response is likely a direct effect of photosystem I and II activity as the relationship of the fractionation factor α versus light intensity can be described by an Eilers–Peeters photosynthesis model. This irradiance effect is in agreement with published δD data of alkenones derived from suspended particulate matter collected from different depths in the photic zone of the Gulf of California and the eastern tropical North Pacific. However, haptophyte algae tend to bloom at relatively high light intensities (>500 μmol photons m^{-2} s^{-1}) occurring at the sea surface, at which hydrogen isotope fractionation is relatively constant and not affected by changes in light intensity. Alkenones accumulating in the sediment are likely mostly derived from these surface water haptophyte blooms, when the largest amount of biomass is produced. Therefore, the observed irradiance effect is unlikely to affect the applicability of the hydrogen isotopic composition of sedimentary long chain alkenones as a proxy for paleosalinity.
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1. INTRODUCTION

The oxygen and hydrogen isotopic composition of ocean water is strongly correlated with salinity because phase changes between seawater, water vapor and precipitation involves oxygen and hydrogen isotope fractionation. For instance, water vapor is depleted in ^{18}O and D relative to water and evaporation thus results in increased salinity and ^{18}O and D content of seawater in evaporative regions. The isotopically depleted water vapor will condense and precipitate over continents and thus river runoff and precipitation result in both a decrease in salinity and ^{18}O and D content of the seawater. Therefore, for most parts of the ocean–atmosphere interface water isotopes are linearly correlated with salinity (Craig and Gordon, 1965) and thus

* Corresponding author. Tel.: +31 222369568; fax: +31 222319674.

E-mail address: Marcel.van.der.Meer@nioz.nl (M.T.J. van der Meer).

[1] Present address: Woods Hole Oceanographic Institution, Cambridge, MA 02139, United States.

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paleosalinity can be reconstructed from either the oxygen or hydrogen isotopic composition of water using this relation.

The hydrogen isotopic composition of water may be recorded in the non-exchangeable hydrogen in biological organic matter although with a considerable biosynthetic isotopic fractionation effect (Yakir and DeNiro, 1990; Hayes, 2001). Nevertheless, as long as this fractionation can be constrained, δD analyses on marine organic matter could provide a means to reconstruct δD of seawater and, thus, if the relation between δD and salinity is known, seawater paleosalinity. Long-chain alkenones produced only by haptophyte algae such as *Emiliania huxleyi* (Volkman et al., 1980, 1995; Marlowe et al., 1984) possess only covalently bound hydrogen atoms, which are not likely to be exchanged during diagenesis (Sessions et al., 2004), making them excellent candidate compounds for stable hydrogen isotope analysis. Initially the idea was to reconstruct paleo seawater δD directly from the measured alkenone δD assuming a fixed difference between the alkenone and water isotopic composition. This idea was motivated by the relatively constant fractionation of approximately 225‰ between alkenones and water for batch cultures of the haptophyte *E. huxleyi* grown on medium spiked with different levels of deuterated water, at constant salinities (Paul, 2002; Englebrecht and Sachs, 2005). However, experiments with *E. huxleyi*, and other alkenone-producing haptophytes, i.e. *Gephyrocapsa oceanica*, *Isochrysis galbana* and *Chrysotila Lamellosa*, cultured at different salinities showed that the biological hydrogen isotope fractionation between alkenones and water, expressed as the fractionation factor α, depends on salinity (Schouten et al., 2006; M'Boule et al., 2014; Chivall et al., 2014). Therefore, as salinity increases not only the hydrogen isotopic composition of water increases but α increases as well, both resulting in an increased D content of alkenones with increasing salinity. This indicates the potential of the δD of alkenones as a paleo sea surface salinity proxy. Several studies indicate that salinity dependent hydrogen isotope fractionation might be a general phenomenon in phototrophic organisms. For instance, hydrogen isotope fractionation in cyanobacterial lipids from naturally occurring microbial mats decreases with increasing salinity (Sachse and Sachs, 2008). The fractionation associated with dinosterol in the Chesapeake Bay estuary (Sachs and Schwab, 2011) and dinosterol and brassicasterol from saline and hypersaline lakes in North America (Nelson and Sachs, 2014a) also decreased with increasing salinity.

Consequently, the hydrogen isotopic composition of C_{37} alkenones has been used to estimate paleo sea surface salinity (SSS) changes in the Aegean Sea at the time of sapropel S5 deposition (van der Meer et al., 2007). Here the δD record of combined C_{37} alkenones ($C_{37:2}$ and $C_{37:3}$) showed a large and abrupt shift to lower δD values at the onset of sapropel deposition similar to the shift observed for foraminiferal $\delta^{18}O$ values measured on the carbonate tests of surface dwelling foraminifera (Marino et al., 2007). This shift towards more D depleted alkenones suggests that this proxy does indeed record the drop in SSS caused by the significantly increased input of freshwater from the continent at the onset of sapropel formation. The δD alkenone proxy has subsequently been used to assess paleo SSS changes in the Black Sea (van der Meer et al., 2008; Giosan et al., 2012; Coolen et al., 2013) and glacial-interglacial salinity changes in the Agulhas leakage area (Kasper et al., 2014) and Mozambique channel (Kasper et al., 2015).

Despite these successful applications of the δD alkenone proxy for the reconstruction of paleo SSS, several complications exist. Firstly, the δD of alkenones in the Chesapeake Bay estuary and from saline and hypersaline lakes in continental North America shows a correlation with the δD of the water, but does not reveal a relation of the fractionation factor α between alkenones and growth water with salinity as observed in for cultures (Schwab and Sachs, 2011; Nelson and Sachs, 2014b). Secondly, factors other than salinity have been shown to also affect the fractionation factor α between alkenones and growth water. For example, *E. huxleyi*, *G. oceanica*, *I. galbana* and *C. lamellosa* all show differences in α at the same salinity (Schouten et al., 2006; M'Boule et al., 2014; Chivall et al., 2014) and the relationships between α and salinity are different for cultures harvested during different growth phases (Wolhowe et al., 2009; Chivall et al., 2014). Additionally, it has been suggested that growth rate also affects α (Schouten et al., 2006). A yet unexplored factor in determining hydrogen isotope fractionation is light intensity, which might have an effect because the production of NADPH, the major source of hydrogen in biosynthesis (Zhang et al., 2009), is directly linked to photosynthetic activity (Allen, 2002). Here, we examined the impact of irradiance on the hydrogen isotope fractionation in *E. huxleyi* and discuss the implication of our findings for hydrogen isotopic fractionation in natural settings.

2. MATERIALS AND METHODS

2.1. Incubation experiments

Two sets of light experiments were carried out. Monospecific cultures of the haptophyte algae *E. huxleyi* (strain PML B92/11) were grown at a constant temperature of 15 °C, at a constant salinity of 32.5, and varying light intensities of 15, 30, 50, 100 and 200 μmol photons m^{-2} s^{-1}. In a second experiment, batch cultures of *E. huxleyi* (strain RCC1238) were grown in triplicate at four different light intensities (100, 200, 400, and 600 μmol photons m^{-2} s^{-1}) in autoclaved 1 L bottles at a constant temperature and salinity of 15 °C and 32.2, respectively. The two strains show similar growth responses relative to nutrients, temperature, light etc. The main difference between the two strains has to do with differences in their carbonate chemistry (Langer et al., 2009). All cultures were grown in Rumed cabinets, providing cool-white fluorescent light with a 16:8 h light:dark cycle. The seawater medium prepared according to F/2 (Guillard, 1975) for the first and F/2R for the second experiment, respectively. The enriched medium was sterile filtered using a 0.45 μm filter cartridge in the first experiment and 0.2 μm filter cartridge in the second experiment. All cultures were allowed to acclimate to the experimental conditions in a pre-culture before being used to inoculate the main batch cultures to provide an initial

cell density between 0.5–7 × 10^3 cell ml^{-1} for the first experiment and a target initial cell density of approximately 0.9 × 10^2 cell ml^{-1} for the second experiment. Cultivation took place in bottles that were closed and incubated for 4–12 days depending on algal growth rate.

Cells were counted daily using a Beckman Coulter Multisizer 3 particle counter. Cell numbers were log transformed and plotted versus time, growth rate μ (d^{-1}) was estimated by linear regression. The cultures were harvested by filtration over ashed 0.7 μm GF/F filters (Whatman) when the cultures were in exponential growth phase and had achieved cell densities within the range of 0.55–1.5 × 10^5 cells ml^{-1}. Filters and aliquots of the culture medium were frozen immediately and stored at <−25 °C until analysis. The culture waters were stored with no headspace in 12 mL exetainers (Labco) in the dark at ~5 °C until analysis.

2.2. Alkenone preparation

Filters from the first experiment were extracted ultrasonically using first methanol, followed by methanol:dichloromethane (DCM) 1:1 (v:v) and finally DCM. A ketone fraction was obtained by purifying the total lipid extracts by passing them over a silica gel cartridge (Varian Bond Elut; 1 cm^3/100 mg), followed by saponification in 0.3 mL of 0.1 M KOH in methanol: water 9:1 (v/v) at 80 °C in a capped vial for 2 h. The alkenone containing fraction was subsequently obtained by partitioning in hexane (Benthien et al., 2002). The alkenone fractions were analyzed by gas chromatography (GC) and GC/mass spectrometry (GC/MS) (van der Meer et al., 2007). The alkenone hydrogen isotopic composition was determined by GC thermal conversion isotope ratio monitoring MS (GC/TC/irMS).

Filters from the second experiment were freeze dried for 24 h prior to automated solvent extraction by a Dionex ASE using a 9:1 (v:v) DCM:methanol mixture. Total lipid extracts (TLEs) were dried down using a rotary evaporator. The TLEs were subsequently saponified by adding methanol and 1 ml 0.1 M KOH and heating at 80 °C for 2 h. The saponified alkenone fraction was analyzed by gas chromatography with flame ionization detection (GC-FID).

2.3. Instrumental analysis

The algal culture media δD water values were determined by Elemental Analysis (EA)/Thermal Conversion (TC)/irmMS using a Thermo Electron EA/TC coupled to a Thermo Electron $DELTA^{Plus}$ XL mass spectrometer for the first experiment according to Schouten et al., 2006. In short, about 1 μl of water was injected into a glassy carbon filled ceramic tube at a temperature of 1425 °C. The H_3^+ -factor was determined daily and was approximately 8.0 ± 0.3 ppm mV^{-1}. Waters were analyzed with at least ten replicate analyses. Hydrogen gas with a predetermined isotopic composition was used as reference and the water isotope values were calibrated against in-house lab standards (North Sea water: +5‰ and bidistilled water: −76‰ that were calibrated by using Vienna Standard Mean Ocean Water (VSMOW) and Greenland Ice Sheet Precipitation (GISP) standards). The hydrogen isotopic composition of the medium used in the second experiment was determined by the hydrogen gas–water equilibrium method using a gas bench coupled to a Thermo Electron $DELTA^{Plus}$ XP (Wong and Clarke, 2012) at the University of Utrecht.

Compound-specific hydrogen isotopic compositions for the combined C_{37} alkenones (cf. van der Meer et al., 2013) from the first experiment were measured by GC/TC/irmMS using a Thermo Electron $DELTA^{Plus}$ XL mass spectrometer using a CPSil 5 GC column with a 0.4 μm film thickness and a constant flow of He of 1 ml min^{-1}. Compounds were converted to hydrogen gas and graphite at 1425 °C in an empty ceramic tube which was pre-conditioned by injecting 0.2 μl of hexane several times (~5) in the first week after installing a new reactor tube. Hydrogen gas with a predetermined isotopic composition was used as reference gas at the beginning and end of each analytical run and a C_{16}-C_{32} *n*-alkanes mixture with offline determined isotopic compositions (ranging from −42‰ to −256‰ vs. VSMOW, Schimmelmann MixB) was used to monitor the system performance daily. The average offsets between the measured δD values of the C_{16}–C_{32} *n*-alkanes and their offline determined values were generally 5‰ or less. Samples were analyzed at least in duplicate and the reproducibility was typically better than 5‰ (Table 1). As additional control, squalane was co-injected with every analysis and the average squalane value typically was −166‰ ± 3‰, while the offline determined value was −170‰.

Compound-specific hydrogen isotope values for the alkenones from the second experiment were determined by GC/TC/irmMS with a Thermo Electron $DELTA^{Plus}$ XP mass spectrometer using high temperature conversion at the University of Utrecht. Compounds were converted to hydrogen gas and graphite in an empty ceramic tube heated to 1400 °C. The hydrogen isotopic composition of the combined C_{37} alkenones was corrected using the Schimmelmann *n*-alkane mix, Mix A. A squalane standard was co-injected with every sample and its average value was −166.3‰ ± 5.1 ‰, which compared well with its offline determined value of −169‰.

2.4. Modelling

A modified Eilers–Peeters formulation (Eilers and Peeters, 1988) was used to describe both growth rate μ and fractionation factor α in response to irradiance. This model can be applied directly to describe growth rate μ:

$$\mu = \mu_{max} * \frac{2 * (1+\beta) * I/I_{opt}}{(I/I_{opt})^2 + 2 * \beta * I/I_{opt} + 1} \tag{1}$$

where β is a shape factor and μ_{max} represents the maximum growth rate. Growth rate μ attains a maximum value at optimal irradiance (I_{opt}). The shape factor β determines the 'peakedness' or rounding of the production curve (e.g. Soetaert et al., 1994).

The model cannot be applied directly to describe hydrogen isotope fractionation, as the α value does not equal zero

Table 1
Results from two culture experiments in which two strains of *E. huxleyi* (PML B92/11 and RCC1238) were cultured at different light intensities to study the effect of light intensity on hydrogen isotope fractionation. All cultures were harvested in the exponential growth phase after 4–12 days depending on the cell numbers.

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

in the dark. Therefore the basic equation was extended with an offset value, α_0, which defines the fractionation at zero light intensity:

$$\alpha = \alpha_0 + \gamma * \frac{2 * (1+\beta) * I/I_{opt}}{(I/I_{opt})^2 + 2 * \beta * I/I_{opt} + 1} \tag{2}$$

where α attains a maximum value at I_{opt} equal to $\alpha_{max} = \alpha_0 + \gamma$. Parameter values μ_{max}, α_0, α_{max}, and I_{opt} were estimated by minimizing the sum of squared differences between the model and experimental data using the Excel Solver routine.

3. RESULTS

We analyzed the δD values of alkenones produced by *E. huxleyi* grown in batch cultures at different irradiance levels. For the first experiment, where *E. huxleyi* strain PML B92/11 was grown with light intensities ranging from 15 to 200 μmol photons m^{-2} s^{-1}, the relationship between the growth rate and irradiance indicates that *E. huxleyi* is growing under light limitation at light intensities <100 μmol photons m^{-2} s^{-1}. Growth rate is approximately 0.5 d^{-1} at the lowest irradiance and increases to approximately 1.0 d^{-1} at 50 μmol photons m^{-2} s^{-1}. The growth rates level off at approximately 1.1 d^{-1} for irradiances exceeding 100 μmol photons m^{-2} s^{-1} (Table 1; Fig. 1). For the second experiment with *E. huxleyi* strain RCC1238 and irradiance levels ranging from 100 to 600 μmol photons m^{-2} s^{-1}, a relatively constant growth rate of approximately 1.3 d^{-1} was observed (Table 1; Fig. 1). The growth rates for *E. huxleyi* strain RCC1238 in experiment 2 are slightly higher than for strain PML B92/11 in experiment 1 at the corresponding irradiances of 100 and 200 μmol photons m^{-2} s^{-1}.

The hydrogen isotopic composition of the combined $C_{37:2}$ and $C_{37:3}$ alkenones ranged from approximately −230‰ at the lowest level of irradiance to approximately −189‰ at an irradiance of 200 μmol photons m^{-2} s^{-1} for the first experiment (Table 1). For the second experiment the isotopic composition of the combined $C_{37:2}$ and $C_{37:3}$ alkenones ranged from approximately −212‰ at 100 μmol photons m^{-2} s^{-1} to −188‰ at 200 μmol photons m^{-2} s^{-1}. At irradiance levels >200 μmol photons m^{-2} s^{-1} the δD alkenones was approximately −193‰.

The fractionation factor α between the hydrogen isotopic composition of the alkenones and the culture medium ranged from approximately 0.77 at the lowest level of irradiance to approximately 0.82 at an irradiance >200 μmol photons m^{-2} s^{-1} (Table 1). A strong and positive linear relationship between the fractionation factor α and irradiance is observed for the first set of experiments up to an irradiance level of 200 μmol photons m^{-2} s^{-1} (Fig. 2). For the second experiment, the fractionation factor shows values similar to those of experiment 1 at corresponding irradiance levels of 100 and 200 μmol photons m^{-2} s^{-1} (Fig. 2). This suggests that the two strains

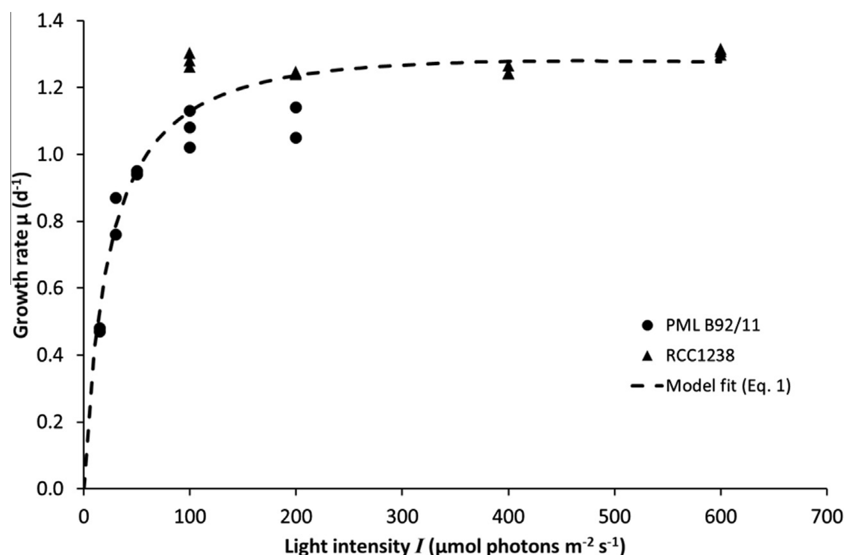

Fig. 1. Growth rate μ (d^{-1}) plotted against irradiance I (μmol photons m^{-2} s^{-1}) for both the first experiment with *E. huxleyi* strain PML B92/11 (●) and second experiment with strain RCC1238 (▲) and the model fit using the Eilers–Peeters equation (Eq. (1)) (---)(Eilers and Peeters, 1988) .

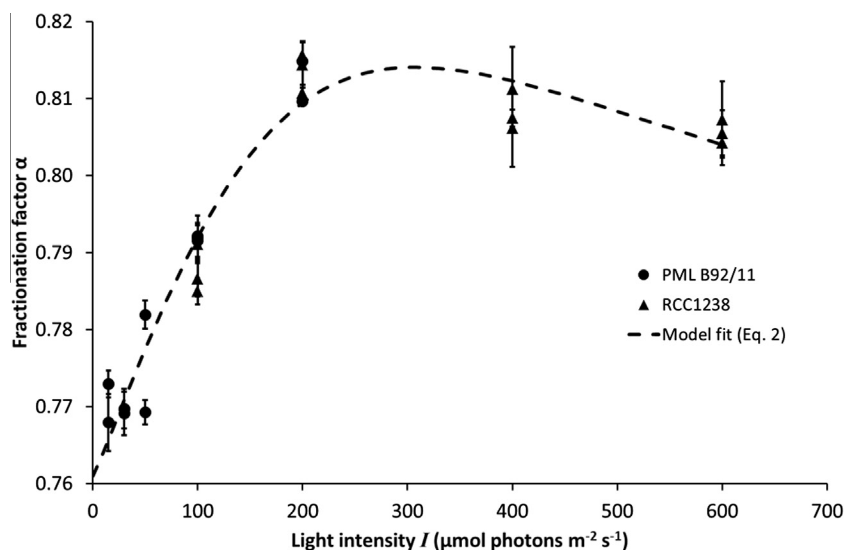

Fig. 2. Fractionation factor α alkenones versus medium water plotted against irradiance I (μmol photons m^{-2} s^{-1}) for both the first experiment with *E. huxleyi* strain PML B92/11 (●) and second experiment with strain RCC1238 (▲) and the model fit using a modified Eilers–Peeters equation (Eq. (2)) (---)(Eilers and Peeters, 1988).

fractionate similarly at similar irradiance levels. At light intensities exceeding 200 μmol photons m^{-2} s^{-1} α decreases slightly from approximately 0.815 to approximately 0.805 (Table 1).

4. DISCUSSION

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

Our culture results demonstrate that the level of irradiance affects both the growth rate of *E. huxleyi* (Fig. 1) and the hydrogen isotope fractionation between the alkenones produced and the water (Fig. 2). The growth rate increased linearly with irradiance up to between 50 and 100 μmol photons m^{-2} s^{-1}, and leveled off at irradiances above 100 μmol photons m^{-2} s^{-1}. Even though the data are from experiments using two different *E. huxleyi* strains, the combined data of growth rates versus irradiance can be described by a single fit with the Eilers–Peeters model (Eq. (1)) ($R^2 = 0.89$; Fig. 1). Based on these results, it seems growth of *E. huxleyi* is not inhibited by irradiance levels of up to 600 μmol photons m^{-2} s^{-1}.

The decrease in α at higher irradiance levels (>200 μmol photons m^{-2} s^{-1}; Fig. 2) is similar to what is typically observed in Photosynthesis-Irradiance curves

and is typically attributed to light inhibition (e.g. Eilers and Peeters, 1988). A modified Eilers–Peeters type of equation (Eq. (2)) was used to describe the observed relationship of irradiance with α, yielding a good fit ($R^2 = 0.94$; Fig. 2). This fit predicts a maximum fractionation factor of 0.814 at an optimum irradiance (I_{opt}) of approximately 310 μmol photons $m^{-2}\,s^{-1}$, which is in the range of saturation irradiance (I_{sat}) values (200–400 μmol photons $m^{-2}\,s^{-1}$) reported for photosynthesis in *E. huxleyi* strains (Flameling and Kromkamp, 1998; Harris et al., 2005; Feng et al., 2008). However, higher and lower I_{sat} values have also been reported (Nanninga and Tyrrell, 1996, and references therein). Because the modified Eilers–Peeters equation describes our data well, we suggest that irradiance is a major factor influencing the fractionation factor α between the alkenones and growth water of the haptophytes grown in our culture experiments.

Schouten et al. (2006) showed that α decreases with increasing growth rate (Fig. 3) potentially suggesting that our observed correlation may be due to changing growth rates controlled by the level of irradiance. However, plotting the growth rate against the fractionation factor α for all irradiances from both experiments performed here shows no clear correlation between α and growth rate (Fig. 3). Fractionation factor α increases from growth rates of 0.4 to approximately 1.2 d^{-1} after which it decreases a little, although there is some scatter at these higher growth rates. These results suggest that in our experiments α does not change because of changing growth rates, but that both α and growth rate are a function of irradiance. These findings are different from the results of (Schouten et al., 2006; Fig. 3), where *E. huxleyi* was grown at constant irradiance but different salinities and temperatures, suggesting that hydrogen isotope fractionation in alkenone biosynthesis in these experiments is more likely controlled by downstream biosynthetic effects.

A possible explanation for this effect of irradiance on the hydrogen isotopic fractionation of *E. huxleyi* could be the central role NADPH has as hydrogen source for biosynthesis (Yakir and DeNiro, 1990; Hayes, 2001), i.e. approximately 50% of non-exchangeable hydrogen in lipids is derived from NADPH (Zhang et al., 2009). The initial biosynthetic isotopic fractionation effect from water to the primary photosynthate is considerable, ca. 171‰, suggested to be largely due to the reduction of $NADP^+$ to NADPH (Yakir and DeNiro, 1990; reviewed by Hayes, 2001). The reduction of $NADP^+$ to NADPH in photosynthetic organisms is directly linked to photosystem activity (Allen, 2002 and references therein) and therefore potentially light intensity. This probably explains the link between irradiance level and hydrogen isotopic fractionation, although the exact biochemical mechanisms responsible for this irradiance depended hydrogen isotope fractionation effect is unclear and subject for future research.

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

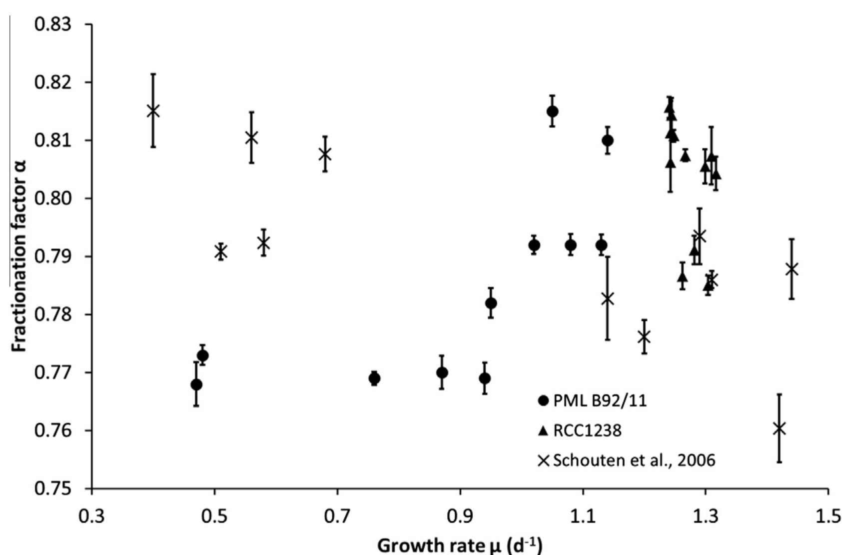

Fig. 3. Fractionation factor α for alkenones versus medium water plotted against growth rate μ (d^{-1}) for both the first with *E. huxleyi* strain PML B92/11 (●) and second experiment with strain RCC1238 (▲) in which both α and μ were controlled by light intensity. Included are also the α and μ data from Schouten et al., 2006 (×) for *E. huxleyi* grown at different salinities and temperatures at a single light intensity.

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 optimal. *E. huxleyi*, for instance, is thought to thrive under high light conditions, at mixed layer depths generally <30 meter (Tyrrell and Merico, 2004; Harris et al., 2005). They outcompete other algal species that suffer from photoinhibition under these conditions, a process that is apparently absent in *E. huxleyi* (Nanninga and Tyrrell, 1996). In fact, based on field data collected during *E. huxleyi* blooms, mesocosm studies and culture experiments, *E. huxleyi* is thought to only form large blooms at light intensities >530 μmol photons m^{-2} s^{-1} (Nanninga and Tyrrell, 1996 and references therein; Harris et al., 2005). This is in the range of irradiance levels in our experiments where α is relatively constant (Fig. 2), indicating that the δD of alkenones synthesized during blooming would show only minor variation due to variations in the level of irradiance. If the majority of alkenones in the sediment are derived from haptophytes blooming at the surface, this indicates that variations in the level of irradiance would only have a minor effect on the δD of sedimentary alkenones. Indeed, it has been shown often that the degree of unsaturation of alkenones, the $U_{37}^{K'}$, which is used as a paleo sea surface temperature proxy, correlates on a global scale best with annual mean sea surface temperatures rather than deeper water temperatures, i.e. at the bottom of the photic zone (e.g. Müller et al., 1998). Furthermore, during bloom conditions when growth becomes limited by nutrient limitation, but photosynthesis continues as long as there is enough light, the haptophyte algae produce more alkenones per cell to store the reducing equivalents (i.e. NADPH) produced during photosynthesis (Eltgroth et al., 2005). High cell densities during bloom conditions might also promote grazing and packaging of cells and alkenones in fecal pellets, cell aggregation and increase the possibility of cell material attaching to sinking particles, increasing the transport efficiency of haptophyte cell material, including alkenones, to the underlying sediment. Therefore it seems likely that the majority of alkenones in the sediment are derived from haptophyte blooms and reflect high light conditions.

Nevertheless, the conditions under which the majority of the sedimentary alkenones are produced together with the environmental significance of irradiance on the hydrogen isotope fractionation should be further tested in nature by sampling suspended particulate matter from different water depths (c.f. Wolhowe et al., 2015) and bloom and non-bloom derived alkenones using sediment traps and analyzing core tops from close to the equator to high latitudes to capture seasonal variability in irradiance.

5. CONCLUSION

Cultivation of two *E. huxleyi* strains show that when growth rate is irradiance-limited, increasing growth results in decreased hydrogen isotope fractionation, the opposite response to temperature/salinity-limited growth rate. Rather, our results suggest that irradiance is directly affecting the hydrogen isotopic fractionation of *E. huxleyi* up to levels of 200 μmol photons m^{-2} s^{-1} after which it remains relatively constant. *E. huxleyi* usually thrives under relatively high light conditions and is thought to bloom at light intensities >500 μmol photons m^{-2} s^{-1}. Therefore, it seems unlikely that light affects the use of the hydrogen isotopic composition of sedimentary long chain alkenones as a proxy for paleosalinity, assuming that the majority of sedimentary alkenones are derived from surface water haptophyte blooms. The actual conditions under which most of the sedimentary alkenones are produced, together with the significance of irradiance on the hydrogen isotopic composition of long chain alkenones in natural settings should be further investigated.

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