# Biotic and abiotic degradation of alkenones and implications for $U_{37}^{K'}$ paleoproxy applications. A review.

J.-F. Rontani[a], J.K. Volkman[b], F.G. Prahl[c], S.G. Wakeham[d]

[a] *Aix-Marseille University, Mediterranean Institute of Oceanography (M I O), 13288, Marseille, Cedex 9; Université du Sud Toulon-Var, 83957, CNRS-INSU/IRD UM 110, France*

[b] *CSIRO Marine and Atmospheric Research and CSIRO Wealth from Oceans National Research Flagship, GPO Box 1538, Hobart, Tasmania 7001, Australia*

[c] *College of Earth, Ocean and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331-5503, USA*

[d] *Skidaway Institute of Oceanography, Savannah, GA 31411 USA and School of Oceanography, University of Washington, Seattle, WA 98195-7940 USA*

[*]Corresponding author. Tel.: +33-4-91-82-96-51; fax: +33-4-91-82-96-41. *E-mail address*: jean-francois.rontani@univ-amu.fr (J.-F. Rontani).

## ABSTRACT

Lipid biomarkers in sediments are widely used to infer environmental conditions that have occurred in the geological past, but these reconstructions require a careful consideration of the biotic and abiotic processes that degrade and alter the lipid biomarker compositions before and after deposition. In this paper, we use alkenones produced by haptophyte microalgae to explore the range of effects of these degradative processes. Alkenones are now perhaps the best studied of all biomarkers with several hundred papers on their occurrence in organisms, seawater and sediments. Much information has been obtained on their degradation from laboratory incubations and inferences from changes in their distribution in aquatic environments. Although alkenones are often considered as more stable than many other lipid classes, it is now clear that their distributions can be affected by processes such as prolonged oxygen exposure, aerobic bacterial degradation and thiyl radical-induced stereomutation processes which, in some cases, can lead to changes in the proportions of the alkenones used in the $U_{37}^{K'}$ temperature proxy. The same set of chemical and biological processes act on all lipids in aquatic environments and, in cases where there is a marked difference in reactivity, this may lead to significant changes in the biomarker distributions and relative proportions of different lipid classes.



**Keywords:** Lipid tracers; Alkenones; Biotic and abiotic degradation; Photooxidation; Autoxidation; Stereomutation; Aerobic and anaerobic biodegradation; Sulfurization; Paleotemperature estimations.

## 1. Introduction

There is mounting evidence that global climate is changing (IPCC, 2007), notably a warming of the atmosphere and the oceans exacerbated by anthropogenic emissions of greenhouse gases. Yet climate-related temperature changes have occurred throughout Earth's history. In order to put recent global warming trends into the context of natural climate variability, it is essential to be able to reconstruct palaeoenvironments. Lipid biomarkers produced by aquatic organisms and preserved in sediments are particularly useful in this endeavour (e.g. Wakeham et al., 1997; Volkman et al., 1998; Rontani and Volkman, 2005; Bianchi and Canuel, 2011; Castañeda and Schouten, 2011). Distributions of long-chain, $C_{35}$–$C_{40}$ methyl and ethyl alkyl ketones ('alkenones') are especially useful as a paleoceanographic proxy for reconstruction of sea surface temperatures (SST) (e.g. Brassell et al., 1986; Prahl and Wakeham, 1987), partial pressures of $CO_2$ (Jasper and Hayes 1990), and possibly even salinity (Rosell-Melé, 1998, but see also Sikes and Sicre, 2002). Alkenones are synthesized by a subset of haptophyte microalgae (Volkman et al., 1980a, 1995; Marlowe et al., 1984; Conte et al., 1994, 1995; Prahl et al., 2006; Jaraula et al., 2010). *Emiliania huxleyi* appears to be the dominant source of $C_{37}$–$C_{39}$ alkenones in the modern open ocean (Tyrell and Merico, 2004), with additional contributions from *Gephyrocapsa oceanica* and perhaps other species (Conte et al., 1995; Volkman et al., 1995). As with all proxies, however, alkenone-based reconstructions require careful consideration of alteration and preservation during transport through the water column and deposition in sediments. It is necessary to determine the magnitude and relative importance of biotic and/or abiotic causes for change. Here, we explore the range of effects of these degradative processes on alkenone compositions and their implications for the alkenone temperature proxy.

Cellular alkenone composition is directly correlated with growth temperature. Empirical observations of unsaturated $C_{37}$ alkenones in cultures, water column particulate matter and sediment core-tops have shown that the ratio ($[C_{37:2}] - [C_{37:4}]$)/($[C_{37:2}] + [C_{37:3}] + [C_{37:4}]$), referred to as $U^{K}_{37}$ (Brassell et al., 1986), or its simplified version $[C_{37:2}]$/($[C_{37:2}] + [C_{37:3}]$), referred to as $U^{K'}_{37}$ (Prahl and Wakeham, 1987, Prahl et al., 1988), varies with culture temperature or sea surface temperature (SST). Factors other than temperature, including nutrient and light availability and changes in the community of alkenone-producing haptophyte species may also influence unsaturation patterns (Epstein et al., 1998, 2001; Conte et al., 1998, Yamamoto et al., 2000; Prahl et al., 2006). Alkenone production in the upper ocean and export to the sediments is highly seasonal (e.g., Prahl et al., 2000, 2003a, 2005; Wakeham et al., 2002; Popp et al., 2006). Nonetheless, the statistically robust $U^{K'}_{37}$ temperature relationship has become a widely used calibration for reconstruction of SST (Brassell, 1993; Müller et al., 1998).

A recent extensive compilation of $U^{K'}_{37}$ measurements (n = 629) (Conte et al., 2006 and references therein) shows that $U^{K'}_{37}$ values in surface sediments may be systematically higher than the surface water production temperatures. An underlying assumption in alkenone paleothermometry is that the temperature signal established during their initial biosynthesis by the alga is not affected by diagenetic processes (Harvey, 2000; Huguet et al., 2009). However, alkenones are degraded during transport and deposition as indicated by highly attenuated fluxes through the water column (e.g., Prahl et al., 2000; Wakeham et al., 2002). Preferential microbial degradation of $C_{37:3}$ over $C_{37:2}$ alkenones can occur and may increase $U^{K'}_{37}$ values and produce a warm temperature bias (Harvey and Macko, 1997; Rontani et al., 2005, 2008). Abiotic photooxidation, autoxidation and thiyl radical-induced stereomutation (Rontani et al., 2006a,b, 2007a,b) may also act selectively and increase $U^{K'}_{37}$ values. Long-

term exposure of alkenones in sediments to oxygen can lead to large losses of alkenones and small but measurable changes in the $U_{37}^{K'}$ ratio (Huguet et al., 2009). Thus, in addition to seasonality in temperature effects and alkenone production, selective degradation of alkenones may also lead to decoupling of SST and sedimentary $U_{37}^{K'}$ values.

This review summarises the potential effects of diagenetic alteration processes on a range of lipid biomarkers with a particular emphasis on alkenones. This information is expected to benefit the paleoceanographic community that currently embraces the use of alkenones as a key part of their research toolkit because until now there has been no objective way to determine whether alkenone distributions have been altered by degradative processes. Knowledge on selective degradation of alkenones might be useful in reconciling the discrepancies between SSTs derived from different proxies. The information should also be useful to chemical oceanographers and organic geochemists since these same processes act on all lipids in aquatic environments and, in cases where there is a marked difference in reactivity, this may lead to significant changes in the biomarker distributions.

## 2. Laboratory and field examples of lipid degradation in marine environments

### *2.1. Biotic degradation*

#### *2.1.1. Consumption of energy reserve substances in phytoplankton cells under darkness (auto-metabolism)*

Lipid compositions can change dramatically as algal cells move from exponential to stationary phase growth, particularly when nutrients are limiting (Shifrin and Chisholm, 1981, Bell and Pond, 1996; Brown et al., 1966). Storage fatty acids (mainly as triacylglycerols) are

123 largely accumulated during stationary phase (Dunstan et al., 1993; Brown et al., 1996), where
124 they serve as an energy source for cell auto-metabolism during dark respiration when
125 photosynthetic energy input is unavailable (Voet and Voet, 2011). Storage lipids contain
126 greater proportions of monounsaturated and polyunsaturated fatty acids than saturated fatty
127 acids and these unsaturated fatty acids are preferentially consumed by cell respiration (Lv et
128 al., 2010). Sterols are less abundant among cellular lipids, but play a role as architectural
129 components of microalgal membranes (Nes, 1974). Their distributions tend to be taxon-
130 specific (e.g. Volkman, 1986), but generally they are not affected by environmental
131 conditions. While there are often significant changes in the amount and relative proportions of
132 lipids during different growth phases, it is rare to find the appearance of new compounds not
133 present in other growth phases.

134 Eltgroth et al. (2005) observed the presence of lipid bodies containing biosynthetically
135 related long-chain alkenones, alkenoates and alkenes in *E. huxleyi* cells and showed that these
136 lipids increased in abundance under nutrient limitation but disappeared under prolonged
137 darkness. The authors concluded that these compounds were synthesized in chloroplasts and
138 then exported to cytoplasmic lipid bodies for storage. This observation supports the
139 suggestion by Epstein et al. (2001) of a possible metabolic role for alkenones in *E. huxleyi*
140 cells. The strong decrease of total alkenone concentration often observed during incubation of
141 *E. huxleyi* cells under darkness (Epstein et al., 2001; Prahl et al., 2003b; Pan and Sun, 2011)
142 could be thus attributed to metabolic consumption of alkenones that serve as energy reserve
143 lipids in triacylglycerol-deficient haptophytes cells (e.g. Brown et al., 1993).

144 Alkenone consumption during incubation of different strains of *E. huxleyi* under
145 darkness exhibits various degrees of selectivity (Table 1). Variations in the $U_{37}^{K'}$ index
146 observed during these experiments could result from alkenones being in different locations in
147 the cell (e.g. in membranes vs lipid bodies) depending on the growth state of the cells, which

148 could induce variable fates of cellular alkenones during cell metabolism (Pan and Sun, 2011).
149 Indeed, the highest increase of the $U_{37}^{K'}$ index is observed during incubation of cells during
150 exponential growth phase (Prahl et al., 2003b) (Table 1), while auto-metabolism
151 (consumption of reserve substances) of cells during stationary phase (containing the highest
152 proportion of storage alkenones) induced weak (Epstein et al., 2001) or no changes in the
153 index (Prahl et al., 2003b; Pan and Sun, 2011) (Table 1). Comparisons between culture results
154 and field data (Conte et al., 1998; Prahl et al., 2006) indicate that the average physiological
155 state of alkenone producers in the open ocean seems to be more like cells in the late log or
156 stationary phase of batch cultures. If this is true, the effects of auto-metabolism on the $U_{37}^{K'}$
157 index should be minimal in the natural environment.

158 Most of these dark incubation studies were carried out with non-axenic strains of *E.*
159 *huxleyi* (Table 1). Consequently, the differences observed may also be attributed to bacteria in
160 these cultures. Indeed, the growth of bacteria associated with living *E. huxleyi* cells under
161 darkness may have contributed to the observed selective loss of alkenones in these non-axenic
162 experiments. This hypothesis is supported by: (i) the lack of variation of the $U_{37}^{K'}$ index
163 recently observed during the incubation of an axenic culture of *E. huxleyi* under darkness
164 (Table 1; Pan and Sun, 2011) and (ii) the isolation of bacteria able to degrade alkenones
165 selectively from cells of *E. huxleyi* strain TWP1 (Rontani et al., 2008; Zabeti et al., 2010) (see
166 section 2.1.3).

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168 *2.1.2. Marine invertebrate feeding*

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170 Faecal material from invertebrates contributes to the flux of organic carbon and lipids
171 through the water column of the oceans (Bradshaw and Eglinton, 1993; Steinberg et al.,
172 2008). Zooplankton faecal pellets can sink directly out of surface waters, or be released at

depth by vertical migrators, and lipids in faecal pellets and sinking particulate matter more often than not bear little compositional resemblance to the lipids in the diet ingested by the zooplankton (Volkman et al., 1980b; Prahl et al., 1984a,b; Wakeham and Canuel, 1986; Harvey et al., 1987; Bradshaw et al., 1990; Tiselius et al., 2012). The fraction of primary production that is channelled through zooplankton apparently undergoes substantial transformation. Copepods, like most other crustaceans, are not able to biosynthesize cholesterol which is an important component of all cell membranes and the steroid precursor of many bioactive molecules such as steroidal hormones (Teshima and Kanazawa, 1971). Consequently, they must obtain it directly from diet or indirectly by dealkylating ingested $C_{28}$ and $C_{29}$ phytoplanktonic sterols (Goad, 1981; Grice et al., 1998a). Polyunsaturated fatty acids (PUFAs) in the diet are generally retained by the zooplankton and therefore they are of lower abundance in faecal material compared to saturated and monounsaturated FA, some of which may also derive from the zooplankter itself.

Herbivory by zooplankton results in hydrolysis of the phytyl side-chain of chlorophyll (Shuman and Lorenzen, 1975; Zeigler et al., 1988), yielding faecal pellets enriched in pheopigments and phytol. Several phytol degradation products have been identified after hydrolysis of zooplanktonic faecal material, including pristane, isomeric pristenes, isomeric phytadienes, dihydrophytol and phytanic, pristanic, 4,8,12-trimethyltridecanoic and isomeric phytenic acids (Prahl et al., 1984a; 1984b; 1985). Similarly, algal carotenoids such as fucoxanthin are highly altered by ester hydrolysis during zooplankton herbivory, such that little of the intact dietary molecule is present in sinking particulate material (Repeta and Gagosian, 1982, 1984).

Few laboratory experiments have assessed the effect of invertebrate processing of dietary alkenones. Volkman et al. (1980b) showed that the alkenone distributions in faecal pellets of *Calanus helgolandicus* were unchanged from those measured in *E. huxleyi* provided

198 as food. More recently, feeding experiments using *Isochrysis galbana* fed to the coastal
199 copepod *Temora longicornis* showed insignificant changes in $U_{37}^{K'}$ during zooplankton
200 metabolism (Grice et al., 1998a). Unfortunately, grazing experiments involving alkenone-
201 producing haptophyte diets have focused on arthropods (i.e. copepods primarily). Such
202 organisms are not the primary consumers of nanoplankton like coccolithophorids.
203 Coccolithophorids more typically comprise the diets of ciliates and protists (heterotrophic
204 dinoflagellates, tintinnids) (Olson and Strom, 2002; Antia et al., 2008) and perhaps gelatinous
205 zooplankton (salps, ctenophores) (Silver and Bruland, 1981). To our knowledge, nobody has
206 reported on how the metabolism of such grazers impacts the transformation and degradation
207 of alkenones. Although the range of predator and prey species investigated is very limited, it
208 seems that grazing by zooplankton has little effect on $U_{37}^{K'}$ values and therefore on alkenone-
209 derived temperatures.

210 Many studies have demonstrated that benthic macrofauna greatly affect degradation
211 and hence preservation of organic matter in sediments (e.g., Aller, 1982; Kristensen et al.,
212 1992; Green et al., 2002). However, fewer studies have addressed the influence of
213 macrofaunal activities on the fates of specific lipid biomarkers in marine sediments. For
214 example, Sun et al. (1999) examined the effect of *Yoldia limatula*, a protobranch bivalve
215 common in shallow marine estuarine and shelf muds, on $^{13}$C-labelled algal fatty acids and
216 phytol. Ding and Sun (2006) incubated *E. huxleyi* cells in microcosms in the presence of the
217 grass shrimp *Palaemonetes pugio* and observed that fatty acids were much more efficiently
218 degraded than were alkenones when the shrimp were present. Importantly, there was no
219 preferential degradation of the triunsaturated relative to the diunsaturated $C_{37}$ alkenone.
220 Although surface sediment deposits are often processed extensively by polychaete feeding
221 and thereby exposed to redox cycling through bioturbation and bioirrigation, the effects of
222 such benthic infaunal activity on lipid biomarkers have not been investigated to any

223 significant extent (e.g., Sun et al., 2002 for effects of redox oscillation on sedimentary lipids).
224 Nonetheless, the few data available suggest that sedimentary $U_{37}^{K'}$ values are unaffected by
225 macrobenthic feeding processes.
226
227 *2.1.3. Aerobic bacterial degradation*
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229 The rate and extent of degradation of organic compounds in sediments depend on the
230 molecular structure of the substrate, protective effects offered by association of organic matter
231 with particle matrices and sedimentary redox conditions that affect the activity of benthic
232 animals and microorganisms (reviewed by Wakeham and Canuel, 2006). Exposure to
233 molecular oxygen in sediments and the effect this would have on the respiration state of
234 benthic microorganisms are important in determining the fate of sedimentary organic matter
235 (Aller, 1994; Hartnett et al., 1998; Hedges et al., 1999). Due to their powerful and diverse
236 complement of enzymes, aerobic bacteria thus play a key role in the degradation of
237 phytodetritus, the major source of particulate marine organic matter to the seafloor. The
238 common consensus among microbiologists is that a suitable microbe and biochemical
239 pathway exists for the degradation of every natural organic component and xenobiotic, given
240 adequate time (Schink, 1988). This 'principle of biological infallibility' is generally
241 considered to be true only under aerobic conditions, when oxygen acts simultaneously as a
242 terminal acceptor of electrons released during oxidation of organic carbon and as a reactant in
243 a primary attack on the substrate molecules themselves.
244 Only a few laboratory studies have investigated the effects of bacteria on differential
245 degradation of alkenones. Teece et al. (1998) studied microbial degradation of *E. huxleyi*
246 lipids under oxic and anoxic conditions and observed extensive degradation of the $C_{37}$ methyl
247 alkenone under all the conditions examined, with up to 85% degraded under oxic conditions,

but $U_{37}^{K'}$ values remained constant. Contrasting results by Rontani et al. (2005) showed that heterotrophic bacteria enriched from microbial mats very rich in these biomarkers efficiently and selectively degraded $C_{37}$ alkenones under aerobic conditions, leading to variations in the $U_{37}^{K'}$ index ranging from 0 to +0.10 that correspond to an inferred temperature difference of 0 to +3°C (based on the established calibration equation of Prahl et al., 1988). This variability was attributed to the heterogeneity of the inoculum (microbial mats) and to the diversity of bacteria that could attack the alkenone molecules *via* different pathways. A subsequent study examined the *in vitro* degradation of alkenones by four bacterial communities enriched from *E. huxleyi* strain TWP1 cultures after different antibiotic treatments (Rontani et al., 2008). Extensive degradation of alkenones and significant selectivity resulted in increases in $U_{37}^{K'}$ values equivalent to +2°C and +3.2°C changes in the inferred temperature, whereas the error of mean annual SST (maSST) estimation for a given $U_{37}^{K'}$ measurement is generally considered to be ±1.4°C (Prahl et al., 2010).

More recently, several bacterial strains isolated from cultures of *E. huxleyi* strain TWP1 efficiently degraded alkenones suggesting that many marine bacteria may have the ability to degrade these biomarkers (Zabeti et al., 2010). Although the degradation of $C_{37}$ alkenones by *Sphingomonas* sp. AG6, *Nocardioides* sp. S3, *Marinobacter* sp. S2 and *Micrococcus* sp. AG10 appeared to be non-selective, the strain *Dietzia maris* sp. S1 did selectively degrade triunsaturated alkenones leading to a +0.05 to +0.10 unit increase in values of the $U_{37}^{K'}$. This aerobic degradation is thought to be carried out by a monooxygenase that epoxidizes the alkenone double bonds with a preference for attack on the ω29 double bond (Pathway IV in Fig. 1). These collective results show that aerobic bacteria capable of selectively degrading alkenones are not limited to particular environments such as microbial mats; they can be epiphytic, i.e. actually associated with living *E. huxleyi* cells. Metabolic pathways involving

attack of the terminal groups of the molecule (Pathways I-III in Fig. 1) should be essentially non-selective, while those acting on alkenone double bonds (Pathway IV in Fig. 1) should be selective (Zabeti et al., 2010). Inconsistencies observed between previous studies of the aerobic microbial degradation of alkenones (Teece et al., 1998; Rontani et al., 2005; 2008) may simply reflect the type of bacterial species present. However, the localization of alkenones in the cells does seem to play a role in the selectivity of their degradation by bacteria. During *E. huxleyi* cell degradation experiments, Pan and Sun (2011) observed a selective degradation of alkenones only in the case of cells collected in the late stationary phase; i.e. when cellular storage alkenones (in the form of cytoplasmic vesicles, Elgroth et al., 2005) were abundant.

Several field studies have addressed bacterial degradation of alkenones. The presence of $C_{35:2}$, $C_{35:3}$ and $C_{35:4}$ alken-1-ols in alkenone-rich microbial mats in the Camargue, France, (Rontani and Volkman, 2005), provides support for the involvement of non-selective degradation of alkenones *via* a bacterial-mediated Baeyer-Villiger sequence (Britton et al., 1974) (Pathway I in Fig. 1) in nature. The presence of epoxyalkenones in sediments from the Black Sea (Rontani and Wakeham, 2008), the SE Alaskan coastal margin (Prahl et al., 2010) (Fig. 2) and the upwelling region off Peru (Rontani and Prahl, unpublished results) as well as in particulate matter samples from the Black Sea (Rontani and Wakeham, 2008) and Pacific Ocean (Rontani et al., 2011a) attests to the wide distribution of aerobic bacteria capable of attacking alkenone double bonds. In each case, $U_{37}^{K'}$ values were affected and introduced a warm-bias to reconstructed temperatures.

*2.1.4. Anaerobic bacterial degradation*

296 In the absence of oxygen, the function of terminal acceptor of electrons may be
297 transferred to other substrates, such as nitrate, metal ions, sulfate or carbon dioxide, but with
298 smaller energy gains (Schink, 1988). In contrast, there are only relatively few compounds,
299 including water (Rontani et al., 2002; Grossi et al., 2011), carbon dioxide (Platen and Schink,
300 1989; Hirschler et al., 1998) and fumarate (Rabus et al., 2001; Wilkes et al., 2002), which can
301 fulfil the function of a reactant in substrate activation. Thus, the range of reactions possible
302 under anaerobic conditions is largely restricted to hydrogenations, dehydrogenations,
303 hydrations, dehydrations, hydrolyses, carboxylations, decarboxylations, condensations and
304 lyase reactions. Despite these limitations, anaerobic bacteria are able to degrade efficiently *n*-
305 alkanes (Wilkes et al., 2002), pristane (Bregnard et al., 1997; Grossi et al., 2000), *n*-alkenes
306 (Grossi et al., 2011), squalene (Rontani et al., 2002), phytol (Grossi et al., 1998; Rontani et
307 al., 1999), sterols (Harder and Probian, 1997) and numerous other lipids.
308 Alkenones degrade non-selectively under *in vitro* methanogenic, sulfate-reducing and
309 denitrifying conditions (Teece et al., 1998; Rontani et al., 2005). Although the existence of
310 anaerobes able to hydrate alkenone double bonds cannot be totally excluded, it is possible that
311 anaerobic degradation of alkenones involves carboxylation at the $\alpha$ position relative to the
312 carbonyl group (Platen and Schink, 1989) or hydration of the enol forms of this functionality
313 (Lukins and Foster, 1963) (Fig. 3) and consequently occurs non-selectively (Rontani et al.,
314 2008).
315 Enzymatic hydrogenation reactions, which play an integral role in the metabolism of
316 fatty acids, generally occur on the two carbon atoms adjacent to a carbonyl group.
317 Hydrogenation can involve either reduced flavin coenzyme or NADPH as the direct source of
318 hydride (Hunter et al., 1976; Blehert et al., 1999). In the marine environment, anaerobic
319 bacterial hydrogenation (biohydrogenation) of $\Delta^5$-sterols to the corresponding stanols has long
320 been recognized (Wakeham, 1989 and references therein). This process involves initial

conversion of $\Delta^5$-sterols to $\Delta^4$-ster-3-one and subsequent hydrogenation of these conjugated ketones to the corresponding stanones. While most double bond hydrogenations involve reaction at the α-β position relative to a carbonyl group, there are examples of enzymatic hydrogenation of isolated (non-conjugated) double bonds. This is the case notably of oleic and vaccenic acids, which may be hydrogenated by rumen bacteria (Van de Vossenberg and Joblin, 2003; Laverroux et al., 2011) and sedimentary bacteria (Rhead et al., 1971) to stearic acid. Geranylgeranyl reductase, a multifunctional enzyme, can also reduce the double bonds of the geranylgeranyl side chain of chlorophyll (Gomez Maqueo Chew et al., 2008) and archaeal 2,3-di-O-geranylgeranylglycerylphosphate (Sasaki et al., 2011).

Similarly, hydrogenation of alkenone double bonds by bacteria has been inferred based on the detection of monounsaturated alkenones generated *via* the partial hydrogenation of di-unsaturated alkenones during bacterial incubation of *E. huxleyi* cells under oxic conditions (Rontani et al., 2008). However, contrasting results were obtained after different incubations under oxic (Zabeti et al., 2010) and anoxic (Rontani et al., 2013) conditions suggesting that only very specific bacteria are able to hydrogenate these compounds. The rare detection of monounsaturated alkenones in sediments also argues against the quantitative significance of biohydrogenation of alkenones in nature.

### *2.2. Abiotic degradation*

#### *2.2.1. Photosensitized oxidation*

Visible light-induced photosensitized processes act intensively during the senescence of phototrophic organisms due to the presence of chlorophyll, a very efficient photosensitizer (Foote, 1976; Knox and Dodge, 1985). During senescence, the fast reactions of

346 photosynthesis clearly do not operate, so an accelerated rate of formation of triplet
347 chlorophyll ($^3$Chl) and singlet oxygen ($^1O_2$) (Nelson, 1993) can exceed the quenching capacity
348 of the photoprotective system, leading to photodegradation (so called photodynamic effect;
349 Merzlyak and Hendry, 1994). In phytodetritus, when the ordered structure of the thylakoid
350 membranes has been disrupted, pigments tend to remain associated with other hydrophobic
351 cellular components such as membrane lipids (Nelson, 1993). As a result, the photooxidative
352 effect of chlorophyll sensitization might be strongly amplified within such a hydrophobic
353 microenvironment. Moreover, the lifetime of $^1O_2$ produced from sensitizers in a lipid-rich
354 hydrophobic environment could be longer, and its potential diffusive distance greater, than its
355 behaviour in aqueous solution (Suwa et al., 1977). It is not surprising, therefore, that
356 photodegradation acts intensively on unsaturated lipids (including $\Delta^5$-sterols, unsaturated fatty
357 acids, the phytyl side chain of chlorophyll, carotenoids, *n*-alkenes and highly branched
358 isoprenoid alkenes) during the senescence of phytoplankton (for reviews see Rontani, 2008,
359 2012), producing allylic hydroperoxides.

360 The photosensitized oxidation of alkenones does not appreciably modify $U_{37}^{K'}$ values
361 during algal senescence (Rontani et al., 1997; Mouzdahir et al., 2001). In solution, $^1O_2$-
362 mediated photooxidation of alkenones was significantly slower than for fatty acids with the
363 same degree of unsaturation. This difference of reactivity was attributed to: (i) the *trans* (*E*)
364 geometry of the alkenone double bonds (Rechka and Maxwell, 1988) that is 7 to 10 times less
365 sensitive to $^1O_2$-mediated oxidation than the *cis* (*Z*) configuration of fatty acids (Hurst et al.,
366 1985) and (ii) the separation of the double bonds by five carbon atoms in the alkenone
367 structure instead of one in the case of fatty acids. Photochemical degradation of alkenones is
368 not fast enough in killed cells of *E. huxleyi* to induce strong modifications of the $U_{37}^{K'}$ ratio
369 before the photodestruction of the photosensitizing substances (the increase in observed $U_{37}^{K'}$
370 ranges from 0 to +0.04; Rontani et al., 1997; Mouzdahir et al., 2001). This poor

photoreactivity was attributed not only to the *trans* geometry of the double bonds of alkenones, but also to the occurrence of these compounds in less accessible parts of the cell compared to cell membranes. This hypothesis is consistent with alkenones being mainly localized in chloroplasts or cytoplasmic vesicles, the relative importance depending on the physiological growth stage of the cell (Eltgroth et al., 2005), although there are indications that $^1O_2$ should easily diffuse into such microenvironments (Christodoulou et al., 2010). These authors further showed that alkenones were the sole unsaturated lipid components of *E. huxleyi* unaffected by photodegradation after irradiation by visible and UV radiation.

Visible and UV-induced photolysis is not expected to increase $U_{37}^{K'}$ values as senescent cells of *E. huxleyi* settle through the euphotic layer of the oceans. Indeed, as noted above, the *trans* geometry of alkenone double bonds appears to limit the effects of $^1O_2$-mediated photolysis. Great differences of photoreactivities were previously observed in *E. huxleyi* killed cells between minor $C_{31}$ and $C_{33}$ *n*-alkenes (with *cis* double bonds) and the major $C_{37}$ and $C_{38}$ *n*-alkenes (with *trans* double bonds) (Mouzdahir et al., 2001). It may be expected that if the geometry of their double bonds had been *cis*, then selective photolysis of di- and triunsaturated alkenones would occur intensively during the senescence of haptophytes, thus confounding the use of the alkenone unsaturation index for paleotemperature estimation.

*2.2.2. Free radical oxidation (autoxidation)*

Free radical-induced oxidation (autoxidation) in the marine environment has largely been ignored. Autoxidation is generally defined as the spontaneous reaction of molecular oxygen with organic compounds. Oxygen acts as a biradical with two unpaired electrons in the ground state and so it is said to be in a triplet state (Fossey et al., 1995). Autoxidation proceeds by a radical chain mechanism and thus includes three steps: initiation, propagation

396 and termination. Autoxidation acts mainly on compounds possessing double bonds or
397 hydrogen atoms whose bond energies are relatively low (e.g. allylic, tertiary, α to oxygen,
398 etc) (Fossey et al., 1995) and on *cis* and *trans* disubstituted double bonds without notable
399 selectivity (Stephani et al., 1970).

400 The detection of *Z* allylic hydroperoxyacids, stera-3β,5α,6β-triols and 3,7,11,15-
401 tetramethylhexadec-2(*Z/E*)-1,4-diols, in several marine particulate matter samples (Marchand
402 et al., 2005; Christodoulou et al., 2009; Rontani et al., 2009) attests to the involvement of
403 autoxidation of algal fatty acids, sterols, and chlorophyll, respectively, in nature. These
404 products are not easily produced in laboratory cultures of actively growing microalgae.
405 Indeed, radical oxidation of lipids seems to be initiated by the homolytic cleavage of
406 photochemically produced hydroperoxides in phytodetritus rather than in living cells (Rontani
407 et al., 2003). Redox-active metal ions are generally considered as the initiators of greatest
408 importance for lipid oxidation in biological systems (Pokorny, 1987; Schaich, 2005). They
409 may direct the cleavage of hydroperoxides either through alkoxyl or peroxyl radicals. In
410 culture media such as f/2, the high amounts of the metal-chelator EDTA tightly binds free
411 catalytic metal ions and thus renders them unavailable. EDTA thus acts in the culture media
412 as an antioxidant and strongly limits radical oxidation processes.

413 The autoxidative reactivity of alkenones has been studied in the laboratory (Rontani et
414 al., 2006a). Alkenones appeared to be more sensitive towards oxidative free radical processes
415 than analogues of other common marine lipids such as phytyl acetate (model for the
416 chlorophyll phytyl side-chain), methyl oleate (model for esterified fatty acids) and cholesteryl
417 acetate (model for esterified sterols), and their oxidation rates increase in proportion with their
418 number of double bonds. As the result of this increasing reactivity with degree of
419 unsaturation, $U_{37}^{K'}$ values increased significantly (up to 0.20) during the incubation.

Free radical oxidation of isolated 1,2-disubstituted double bonds generally involves allylic hydrogen abstraction. The addition of peroxyl or alkoxyl radicals to the double bond becomes competitive only in the case of conjugated, terminal, or trisubstituted double bonds (Schaich, 2005). Effectively, the autoxidation of alkenones appears to mainly involve allylic hydrogen abstraction and subsequent oxidation of the allylic radical thus formed (Fig. 4). Accordingly, *in vitro* oxidation of each double bond in the alkenones and subsequent $NaBH_4$ reduction affords four positional isomeric alkenediols, which are amenable to GC-MS analysis and thus could be useful indicators of autoxidation of alkenones. Unfortunately, due to the presence of additional reactive double bonds, hydroperoxyalkenones may undergo subsequent oxidation reactions affording di-, tri- and tetrahydroperoxyalkenones according to the degree of unsaturation of the starting alkenone. In seawater, these different hydroperoxides may undergo two main degradative processes: (i) homolysis of the O-O bond leading to carbonyl (dehydration), alcoholic (reduction) and fragmentation (β-scission) products (Fig. 4) and (ii) heterolysis of the O-O bond leading to the formation of two carbonyl fragments (Hock cleavage); this proton-catalysed cleavage being initiated by migration of groups to positively charged oxygen (Frimer, 1979). Dimeric and oligomeric compounds cross-linked through either peroxide or ether linkages (Frankel, 1998) may also be formed during autoxidation of alkenones (Fig. 4). These or similar autoxidative cross-linking reactions could play a role in sequestering otherwise reactive lipids in immature kerogen (Sinninghe Damsté et al., 1990; Koopmans et al., 1997).

Alkenone autoxidation, measured indirectly as alkenediols produced from post-extraction $NaBH_4$-reduction of the corresponding hydroperoxyalkenones (Fig. 5), has been observed in cultures of *E. huxleyi* strain CS-57 that exhibited anomalously high $U_{37}^{K'}$ values (Rontani et al., 2007a) and strain *E. huxleyi* TWP1 maintained under darkness (Rontani, unpublished results). How well do laboratory simulations translate to natural marine

conditions? Sediment trap samples collected in the northwestern Mediterranean Sea and covering the transition from a spring bloom to summer oligotrophic conditions (Rontani et al., 2006a, 2007b) contained autoxidation products of monounsaturated fatty acids and the chlorophyll phytyl side-chain during the post-bloom period but not during active phytoplankton growth. The anomalously high $U_{37}^{K'}$ values (equivalent to an inferred temperature change of +2°C) observed during the post-bloom period and the detection of products resulting from the cleavage of hydroperoxyalkenones in the corresponding samples provides strong support for the involvement of alkenone autoxidation.

Isomeric $C_{37}$ and $C_{38}$ diols have been detected in surface sediments from the SE Alaska (Rontani, unpublished results) (Fig. 6). These diols were generated during analysis by $NaBH_4$ reduction of allylic hydroperoxyalkenones that had been produced *via* free radical oxidation of the ω15 double bond of $C_{37}$ alkenones initially in the sediments. These results constitute the first direct evidence of alkenone autoxidation *in situ*. Reduction of lipid extracts with $NaBD_4$ instead of $NaBH_4$ showed that isomeric hydroperoxyketones arising from the oxidation of alkenone allylic carbons are partly converted to the corresponding diketones in sediments (Fig. 6).

Autoxidation of alkenones may thus be significant in some marine settings and could explain some discrepancies observed between SSTs and alkenone-based temperature estimates in marine particles (Freeman and Wakeham, 1992) and oxic sediments (Hoefs et al., 1998; Gong and Hollander, 1999; Prahl et al., 2003a). Selective autoxidative degradation of alkenones would lead to observations that $U_{37}^{K'}$ values in deposited sediments are often higher than those in the particles settling through the water column (Prahl et al., 1993; Ternois et al., 1996; Cacho et al., 1999; Conte et al., 2006). However, it may be noted that these shifts in $U_{37}^{K'}$ values may also result from: (i) a non-uniform, seasonal flux-dependant burial efficiency (Sawada et al., 1998; Harada et al., 2001; Lee et al., 2011), (ii) different ages for the

alkenones in sediments implying that they were not produced at the same time (Mollenhauer and Eglinton, 2007) and (iii) preferential adsorption of $C_{37:3}$ alkenone on gas chromatographic column at low alkenone concentrations (Grimalt et al., 2000; 2001).

Alkenones can be sequestered in proto-kerogens by oxygen bonds. Hydrous pyrolysis of a sedimentary rock has yielded O-bound *n*-$C_{37}$ and *n*-$C_{38}$ alkanes and saturated *n*-$C_{37}$ and *n*-$C_{38}$ mid-chain ketones with the carbonyl group predominantly at C-15 and C-16 corresponding to the position of one of the double bonds in alkenones (Sinninghe Damsté et al., 1990; Koopmans et al., 1997). These cross-linking reactions should not alter the $U_{37}^{K'}$ index (Koopmans et al., 1997). However, if free radical oxidation plays a role in the formation of these cross-linked polymers (Frankel, 1998) (Fig. 3), then the strong selectivity of these processes relative to di- and tri-unsaturated alkenones may indeed alter the $U_{37}^{K'}$ index.

*2.2.3. Stereomutation (cis/trans isomerization)*

Unsaturated fatty acids with *trans* double bonds are often detected in marine sediments (Perry et al., 1979; Navarrete et al., 2000; Rontani et al., 2012). Their formation from the corresponding *cis* configurations may result from: (i) photosensitized isomerization processes induced by UVR (Christodoulou et al., 2010) generally involving ketonic triplet energy sensitizers (Testa, 1964; Horspool and Armesto, 1992), (ii) *cis-trans* isomerase activity enabling Gram-negative bacteria belonging to the genera *Pseudomonas* and *Vibrio* to adapt to several forms of environmental stress (Heipieper et al., 2003), or (iii) the formation of thiyl radicals (catalyzing double bond isomerization; Ferreri et al., 2004) during the antioxidant reactions of biologically relevant thiols (e.g. glutathione) (Chatgilialoglu et al., 2002), or after methanethiol homolytic cleavage (Ferchichi et al., 1986; Lomans et al., 2002) or thiolate oxidation (Wlodek, 2002).

The *trans* (or *E*) geometry of alkenone double bonds is distinct from most non-conjugated polyunsaturated lipid natural products, which as a rule have double bonds with *cis* (or *Z*) geometry. During incubations of *E. huxleyi* cells with non-denitrifying anaerobic bacterial communities (isolated from Camargue microbial mats), the production of *cis/trans* alkenones was previously observed (Rontani et al., 2005). This stereomutation (i.e. *cis/trans* isomerization without double bond migration), was observed when sulfate-reducing conditions were well established as shown by a strong production of sulfides. *In vitro* experiments have demonstrated that alkenone stereomutation may be induced by thiyl radicals (Rontani et al., 2006b). Thus the *cis/trans* alkenone isomerization previously observed during bacterial incubations of *E. huxleyi* cells under sulfate-reducing conditions (Rontani et al., 2005) is attributed to the formation of thiyl radicals either from methanethiol produced by bacterial degradation of DMSP (produced by *E. huxleyi*) or from oxidation of thiolate ions by transition metals (Fig. 7). The difference in reactivity of the $MeC_{37:2}$ and $MeC_{37:3}$ alkenones during stereomutation can result in a significant increase in $U_{37}^{K'}$ values. Alkenones possessing *cis* double bonds exhibit similar EI mass spectra to, but shorter retention times than, the corresponding all-*trans* alkenones, so changes in measured $U_{37}^{K'}$ values might also result from poor gas chromatographic resolution of *cis/trans* $C_{37:2}$ and all-*trans* $C_{37:3}$ alkenones. Thus stereomutation, which acts on all the acyclic, unsaturated lipid components of *E. huxleyi* in addition to alkenones, may cause a significant increase (+0.06) in $U_{37}^{K'}$ values (Rontani and Wakeham, 2008). Moreover, it is noteworthy that the dominant bacterial species associated with *E. huxleyi* blooms are related to the *Roseobacter* group (Zubkov et al., 2002) which are known methanethiol producers (Yoch, 2002). It is possible that methanethiol-initiated stereomutation of alkenones occurs in the oxygenated water column of the oceans (Fig. 7). This assumption is consistent with the detection of stereomutated

519 alkenones in some aerobic bacterial incubations of *E. huxleyi* cells (Rontani et al., 2005) as
520 well as in a culture of the strain *E. huxleyi* TWP1 (Rontani, unpublished results) (Fig. 8).
521 UV-induced photosensitized *cis/trans* isomerization of double bonds is also well known
522 (Horspool and Armesto, 1992). This process generally involves ketonic triplet energy
523 sensitizers (Testa, 1964) and could potentially affect alkenones in well-lit surface waters.
524 However, Christodoulou et al. (2010) failed to detect alkenone stereomutation after UV-light
525 irradiation of senescent cells of *E. huxleyi*, although monounsaturated fatty acids and the
526 chlorophyll phytyl side-chain appeared to be significantly stereomutated. The absence of
527 alkenone stereomutation during UV exposure was attributed to their localization in cells, and
528 hence protection from irradiation. Indeed, the likelihood of interaction between the triplet
529 state ketonic sensitizers needed for *cis/trans* photosensitized isomerization (located in the
530 membranes of chloroplasts) and the alkenones (located in cytoplasmic vesicles) would be
531 expected to be very weak.
532 Stereomutated alkenones, probably derived from thiyl radical-induced isomerisation,
533 have been detected in Unit II Black Sea sediments (Rontani et al., 2006b). Careful gas
534 chromatography-mass spectrometry analysis in selected ion monitoring mode was required
535 for detection of "stereomutated" (i.e. with *cis* double bonds) alkenones in suspended
536 particulate matter samples from the water column of the Black Sea (Rontani and Wakeham,
537 2008). Interestingly, thiyl radical-induced stereomutation occurs throughout the Black Sea's
538 water column, including oxic, suboxic and anoxic layers. Stereomutated alkenones have also
539 been detected in sediments from the Ligurian Sea (Rontani et al., 2009) (Fig. 9).
540 Stereomutation may thus be another potential cause for a "warm bias" in alkenone
541 paleothermometry. However, as this process has yet to be demonstrated in other marine
542 areas, its effect, if any, remains to be better quantified.
543

### *2.2.4. Sulfurization*

Sulfur incorporation into sedimentary organic matter may occur during diagenesis in Recent and immature sediments (Sinninghe Damsté et al., 1989; Kohnen et al., 1990; Wakeham et al., 1995; Kok et al., 2000). Hydrogen sulfide (Sinninghe Damsté et al., 1989), polysulfides (Kohnen et al., 1990), elemental sulfur (Rowland et al., 1993) and hydrogen polysulfide ions (de Graaf et al., 1992) have all been suggested as inorganic S species that can cross-link with functionalized lipids. Sulfurization may remove functionalized carbon skeletons ("sequester them") from the "analytical window" of gas-chromatographically amenable low molecular-weight-compounds typically employed in organic geochemistry and thus lead to incomplete assessments of depositional environments (Koopmans et al., 1997). Carbon-carbon double bonds or carbonyl groups react with inorganic S species to form organic sulfur compounds. Compounds possessing carbon-carbon double bonds cross-link sulfur *via* an addition reaction that apparently follows the Markovnikov rule (Schouten et al., 1994). The oxo-groups of ketones and aldehydes are substituted by sulfur yielding polysulfide-linked dimers (Schouten et al., 1994). Laboratory sulfurization studies (Schouten et al., 1993, 1994; de Graaf et al., 1995) have shown that ketones are much more reactive than alkenes.

However, sulfurization is selective for reasons that are not well understood. Within Holocene Antarctic Ace Lake sediments, only the $C_{27}$–$C_{29}$ steroids appeared to be extensively sulfurized (Kok et al., 2000), whereas in Black Sea sediments only highly branched isoprenoid alkenes were sulfurized but long-chain alkenones and alkenones were not (Wakeham et al., 1995). Artificial maturation by hydrous pyrolysis of immature sedimentary rocks showed that alkenones may indeed be incorporated into kerogen by sulfur cross-linking (Sinninghe Damsté et al., 1990; Schaeffer et al., 1995; Koopmans et al., 1997) (Fig. 10).

569 Despite these results and the occurrence of S-bound and S-containing *n*-$C_{37}$ and *n*-$C_{38}$
570 skeletons in sediments (Sinninghe Damsté et al., 1988; 1989; Rullkötter et al., 1990) not much
571 attention has been paid to the effect of sulfurization on values of the $U_{37}^{K'}$ index. Preferential
572 sequestration of the carbon skeletons of either the di- or tri-unsaturated ketones into high-
573 molecular-weight fractions might directly affect the $U_{37}^{K'}$ index of the residual alkenones.
574 Since ketones react faster with reduced inorganic sulfur than mid-chain double bonds
575 (Schouten et al., 1993) (Fig. 10), partial sulfurization of di- and tri-unsaturated alkenones
576 would not be expected to change the $U_{37}^{K'}$ index significantly (Koopmans et al., 1997).
577 However, until now this lack of selectivity has not been clearly demonstrated.

578 It is difficult to apply alkenones as paleoceanographic indicators in sediments that are
579 thermally mature, because functionalized compounds disappear with increasing thermal
580 maturity (Marlowe et al., 1990; Simoneit et al., 1994). There are, however, reports of S-bound
581 and S-containing *n*-$C_{37}$ and *n*-$C_{38}$ skeletons in sediments (Sinninghe Damsté et al., 1988;
582 1989; 1990; Rullkötter et al., 1990; Rullkötter and Michaelis, 1990; Kohnen et al., 1990).
583 High proportions of *n*-$C_{37}$ and *n*-$C_{38}$ alkanes have been found in crude oils (Marlowe et al.,
584 1990), Late Miocene evaporitic marls (Schaeffer et al., 1995), Middle Miocene calcareous
585 claystones (McEvoy et al., 1981), early Eocene lacustrine mudstones (Grice et al., 1998b) and
586 Middle to Late Miocene siliceous mudstones (Sampei et al., 2003). The formation of these
587 compounds is attributed to a sequestration of the corresponding alkenones or alkenes in
588 kerogen during diagenesis involving C-S bonding followed by a subsequent release of alkanes
589 under comparatively low maturity conditions (Schouten et al., 1994; Sampei et al., 2003).
590 These observations strongly suggest that sulfurization processes can play an important role in
591 the sequestration of the carbon-skeletons of alkenones in proto-kerogen of sediments.
592

593 **3. Potential effects of these degradation processes on paleotemperature estimations**

The potential effects of each of the degradative processes discussed above to modify $U_{37}^{K'}$ index values are summarized in Table 2. It appears that autoxidation and aerobic microbial degradation have the potential to introduce a significant 'warm' bias in any paleotemperature reconstruction. Such a bias can be significant and might explain apparent anomalies in paleotemperatures inferred from alkenone distributions in strongly oxidized sediments (Hoefs et al., 1998; Gong and Hollander, 1999; Kim et al., 2009). It is important to recognize that multiple degradation processes may act simultaneously on alkenones and in such cases their effects on $U_{37}^{K'}$ index may be cumulative. For example, hydroperoxyalkenones and epoxyalkenones could both be detected in stressed *E. huxleyi* cells that exhibit anomalously high alkenone unsaturation ratios when incubated under darkness (Rontani, unpublished results), suggesting simultaneous involvement of autoxidation and aerobic bacterial degradation, respectively.

Figure 11 summarizes the various biotic and abiotic degradation processes that may act on lipids in suspended and sinking particles and in sediments. This cartoon also illustrates interactions between the several processes which, although very complex, need to be included in any organic geochemical assessment. For example, a synergy between senescing phytoplankton cells and attached bacteria and between photooxidation and biodegradation helps to account for the relative importance of biotic and abiotic processes for suspended vs. sinking POM (Rontani et al., 2011a). Light-induced, $^1O_2$-producing photoprocesses in sunlit surface waters may be more efficient for suspended (or slowly sinking) POM than for fast-sinking POM, probably due to longer residence times of the suspended material in the photic zone. At the same time, photodegradation of suspended phytodetritus in the photic zone produces $^1O_2$ that may limit alkenone degradation by inhibiting bacterial growth. Due to these different interactions, degradative alteration of the alkenone unsaturation index in the ocean

should thus mainly result from: (i) autoxidation in suspended particles and in oxic sediments, (ii) aerobic bacterial degradation in sinking particles and in oxic sediments, or (iii) stereomutation in anoxic sediments.

## 4. Detecting potential biases resulting from selective degradation of alkenones in natural samples

### *4.1. Biases resulting from autoxidation*

It is important to identify factors, either directly or indirectly, other than growth temperature that affect alkenone compositions and thus $U_{37}^{K'}$ values. Products of alkenone autoxidation are too unstable to be used as direct tracers. In their place, a "pool" of surrogate autoxidation products have been identified; some autoxidation products (such as oleic acid oxidation products) are very sensitive but labile indicators and others (such as chlorophyll phytyl side-chain and sterol oxidation products) are less sensitive but more refractory (Rontani et al., 2006a; Rontani, 2008) . These compounds could be used to identify cases where autoxidation of organic matter, and thus alkenones by extension, has been significant and thus where an overestimation of $U_{37}^{K'}$ values would be expected. For example, suspended particle samples in the Ligurian Sea (Lee et al., 2009) showed an increase of the $U_{37}^{K'}$ index from 0.43 to 0.55 with increasing water depth (Rontani et al., 2009). In the absence of direct measurements of alkenone autoxidation products, a strong correlation between variations in $U_{37}^{K'}$ and concentrations of $\Delta^5$-sterol autoxidation products (5,6-epoxycholesterol and 5,6-epoxysitosterol) (Fig. 12) is a good indication of autoxidation of alkenones in the suspended particles. Assigning a magnitude for a temperature bias due to autoxidation is more problematic.

644

645 *4.2. Biases resulting from aerobic bacterial degradation*

646

647 Epoxy ketones resulting from bacterial epoxidation of alkenone double bonds may
648 prove useful as indicators of *in situ* aerobic bacterial alteration of the alkenone unsaturation
649 ratio, although again the quantitative magnitude may be elusive at present. Because
650 epoxyalkenones display mass spectra with very weak molecular peaks and are thus difficult to
651 detect in natural samples, $NaBH_4$-reduction to the corresponding diols followed by silylation,
652 as discussed above, is recommended. The silylated alkenols thus formed display better
653 chromatographic characteristics than the corresponding alkenones. Silylated diols resulting
654 from $NaBH_4$-reduction of $C_{37}$ epoxyalkenones elute near to the $C_{38}$ alkenols but have
655 diagnostic EI mass spectra exhibiting strong fragments ions at *m/z* 117 and 131 (cleavage α to
656 the functional group), allowing methyl and ethyl alkenols (and hence the parent alkenones) to
657 be readily differentiated by selected ion monitoring (SIM), even at low abundances (Fig. 2).
658 The difference between the unsaturation ratio of reduced epoxyalkenones ($ep U_{37}^{K'}$) and the
659 $U_{37}^{K'}$ value of the residual reduced alkenones may prove useful in evaluating the selectivity of
660 bacterial attack (Prahl et al., 2010).

661

662 *4.3. Biases resulting from stereomutation*

663

664 It is unlikely that alkenone stereomutation would have been previously detected
665 without particular care taken to identify the presence of weak molecular ions in EI mass
666 spectra. It is strongly recommended that alkenones be analysed by GC-MS with adjustments
667 made to enhance the higher masses in order to check the relative abundances of the peaks at
668 *m/z* 528, 529 and 530 in the mass spectrum of the $C_{37:3}$ alkenone. Measured relative

abundances should be very close to the theoretical values calculated from the elemental composition $C_{37}H_{68}O$ (i.e. 100% for *m/z* 528, 41% for *m/z* 529 and 8% for *m/z* 530) if stereomutated isomers are not present. Any enhanced abundance of the *m/z* 530 ion would suggest coelution with a *cis/trans* $C_{37:2}$ alkenone produced by stereomutation. Furthermore, stereomutated alkenones do not perfectly coelute with the more unsaturated homologues, thus leading to an apparent deterioration of the alkenone chromatographic resolution when present in significant amounts. Thus, the importance of stereomutation could also be recognized by this diagnostic chromatographic feature.

## 5. Conclusions

Diverse biotic and abiotic processes alter the composition of marine organic matter from its algal source, through the water column, and into the sediments. Photooxidation, aerobic microbial degradation and zooplankton metabolism begin this alteration, modifying algal lipids in the upper sunlit waters. As phytodetritus sinks and eventually accumulates in sediments, it is further altered by additional food-web processing and bacterial degradation, along with abiotic autoxidation, stereomutation and, eventually, sulfurisation in euxinic sediments. The relative importance of these several processes depends on a variety of factors including the organisms present, water column and sediment oxicity/anoxicity, residence times and the chemical structure of the lipid biomarkers.

Alkenones are considered more recalcitrant towards degradation than other lipids but indeed, diverse marine bacteria do degrade them. Aerobic bacterial metabolism of alkenones may be initiated either by the same mechanisms known to be employed in alkanone metabolism or by epoxidation of the double bonds. Thus, alkenone biodegradation may be

693 selective or not according to the bacteria implicated in the process. Alkenones are strongly
694 degraded by anaerobes but without any evidence for molecular selectivity.

695 Photodegradation may be the most important abiotic degradation process for most
696 marine lipids, as it is strongly favored in phytodetritus when present in the photic zone of the
697 ocean. Alkenones appear to be relatively protected from photodegradation by the *trans*
698 geometry of their double bonds, but a small change in $U_{37}^{K'}$ values may still occur. All
699 unsaturated lipids, including alkenones, are susceptible to autoxidation (free radical oxidation)
700 in oxic environments. These processes act very intensively and selectively on alkenones and
701 thus have the potential to induce a significant increase in $U_{37}^{K'}$ values. Stereomutation (*cis-*
702 *trans* isomerization) of alkenones may be induced by thiyl radicals and can occur under both
703 oxic and anoxic (e.g., sulfate-reducing) conditions. These processes can result in a significant
704 increase in $U_{37}^{K'}$ values. Partial sulfurization of di- and tri-unsaturated alkenones should not
705 change the $U_{37}^{K'}$ index of residual unsulfurized alkenones significantly.

706 Given that the alkenone-based $U_{37}^{K'}$ index is now universally accepted as a robust
707 proxy for reconstructing environmental temperatures, it is important that the potential biases
708 resulting from differential degradation of alkenones be kept in mind when using $U_{37}^{K'}$ -
709 temperature proxy. Alkenones, like all lipids, are subject to a diversity of biotic and abiotic
710 processes in aquatic and sedimentary environments. Our tabulations of these reactions and
711 their effects on the $U_{37}^{K'}$ index should provide helpful guidance in evaluating the validity of
712 paleotemperature studies in the future. As an illustration, some examples of previous
713 overestimations of SST, which may be in part attributed to degradative alterations of
714 alkenones are given in Table 3.

715

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**Figure captions**

**Fig. 1**. Metabolic pathways which may be potentially involved during aerobic bacterial degradation of methyl alkenones. (In order to simplify the scheme only the degradation of the $C_{37:3}$ alkenone is shown)

**Fig. 2**. Partial *m/z* 117 and 131 mass fragmentograms showing the presence of diols (as silylated derivatives) produced from $NaBH_4$ reduction of epoxyalkenones in a lipid fraction isolated from surface sediments from southeast Alaska. (Adapted from Prahl et al., 2010)

**Fig. 3**. Metabolic pathways which may be potentially involved during anaerobic bacterial degradation of methyl alkenones. (In order to simplify the scheme only the degradation of the $C_{37:3}$ alkenone is shown)

**Fig. 4**. Proposed pathways for the free radical oxidation of alkenones. (In order to simplify the scheme only the degradation of the $C_{37:3}$ alkenone is shown)

**Fig. 5**. Partial mass fragmentograms of *m/z* 311 and 325 revealing the presence of silylated $C_{37}$ and $C_{38}$ alkenediols after $NaBH_4$-reduction and silylation of standard autoxidized alkenones (a) and of the total lipid fraction of *E. huxleyi* strain TWP1 incubated under darkness (b).

**Fig. 6**. Partial mass fragmentograms of *m/z* 118, 311, 312, 325 and 326 revealing the presence of deuterated and non-deuterated silylated $C_{37}$ alkenediols after $NaBD_4$-reduction and silylation of the total lipid fraction of surface sediments from southeast Alaska.

**Fig. 7**. Proposed processes for thiyl radical-induced stereomutation of alkenones under oxic and anoxic conditions. $Me^n$ = transition metal ion (e.g. $Fe^{+3}$).

**Fig. 8**. Total ion chromatogram showing the presence of stereomutated alkenones in the total lipid extract of *E. huxleyi* strain TWP1 grown at 20 °C.

**Fig. 9**. Partial *m/z* 512.5 and 514.5 mass fragmentograms showing the presence of stereomutated silylated alkenols after $NaBH_4$-reduction and silylation of the total lipid extract of surface sediments from Ligurian Sea. (Adapted from Rontani et al., 2009)

**Fig. 10**. Proposed pathways for the sulfurization-desulfurization of alkenones in sediments. (Adapted from Schouten et al., 1993). Polysulfides are taken as representative of the several sulfur species that might be involved in these processes.

**Fig. 11**. Conceptual model showing the relative importance of photooxidation (hν), autoxidation ($RO^{\bullet}$) and bacterial biodegradation on autochthonous phytodetritus lipids in sinking and suspended particles and in sediments. Sizes of open arrows indicate relative importance of each degradative process. (ZP = Zooplankton) (Adapted from Rontani et al., 2011a)

**Fig. 12**. $U_{37}^{K'}$ values and percentages of 5,6-epoxycholestan-3β-ol and 5,6-epoxy-24-ethylcholestan-3β-ol measured in suspended particulate matter samples collected in the Ligurian Sea. (Adapted from Rontani et al., 2009)