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**The fate of marine lipids: biotic vs. abiotic degradation of  
particulate sterols and alkenones in the Northwestern  
Mediterranean Sea**

J.-F. Rontani<sup>a,\*</sup>, N. Zabeti<sup>a</sup>, S.G. Wakeham<sup>b</sup>

<sup>a</sup> *Laboratoire de Microbiologie de Géochimie et d'Ecologie Marines (UMR 6117), Centre  
d'Océanologie de Marseille, F-13288 Marseille, France.*

<sup>b</sup> *Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411, USA.*

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

25 **Abstract:**

26 Sterol and alkenone compositions in suspended particle and surface sediment samples  
27 collected in the Northwestern Mediterranean Sea during the MEDFLUX program were used  
28 to evaluate the relative importance of biotic and abiotic degradation processes on marine  
29 organic matter. Alkenone concentrations decreased much more rapidly (~500 fold) between 5  
30 and 800 m than  $\Delta^5$ -sterols (~100-fold) or POC (~100-fold). The diverse functional groups  
31 attached to the stable tetracyclic carbon skeleton of  $\Delta^5$ -sterols appeared to be useful for  
32 estimating the relative effects of biotic vs. abiotic (photooxidation and autoxidation)  
33 degradation. Products of abiotic degradation predominated over products of biotic  
34 degradation in suspended particles in the NW Mediterranean. For alkenones, the  $U_{37}^{K'}$  index  
35 increased from 0.43 to 0.55 with increasing water depth, and a good correlation between  
36 variations of  $U_{37}^{K'}$  and concentrations of specific  $\Delta^5$ -sterol autoxidation products points to  
37 selective autoxidation of alkenones in suspended particles. Stereomutated alkenones (with *cis*  
38 double bonds) were detected in the surface sediment, allowing us to estimate that  
39 stereomutation resulted in a +0.05 increase in  $U_{37}^{K'}$ . Therefore, abiotic degradation may be  
40 another factor effect on alkenone-derived paleothermometry.

41  
42 *Keywords:* NW Mediterranean Sea; Suspended particles; Biotic and abiotic degradation;  $\Delta^5$ -  
43 sterols; Alkenones; Autoxidation; Stereomutation.

## 44 1. INTRODUCTION

45

46 Understanding the biogeochemical cycle of carbon in the ocean requires identifying  
47 not only the sources of particulate organic matter (POM) but also those processes responsible  
48 for its alteration. Most studies of the alteration of POM have focussed on biotic degradation,  
49 but the potential impact of abiotic photooxidation and autoxidation is less well understood  
50 owing to the lack of adequate tracers. Nonetheless, photooxidation by photosynthetically  
51 active radiation (PAR) and autoxidation [free radical-mediated oxidation via homolytic  
52 cleavage of photochemically produced hydroperoxides catalyzed by some metal ions  
53 (Pokorny, 1987; Schaich, 1992)] can degrade common marine lipids, including unsaturated  
54 fatty acids, the phytyl side-chain of chlorophyll, sterols and alkenones. Chlorophyll-  
55 sensitized photooxidation can be important within the euphotic layer, whereas autoxidation  
56 may occur throughout the water column and in oxic sediments. Photodegradation and  
57 autoxidation may yield diagnostic products (for a review see Rontani, 2008). In the case of  
58 alkenones, autoxidation may alter significantly their unsaturation ratio and thus constitute a  
59 source of uncertainty during paleotemperature reconstruction (Rontani et al., 2006a, 2007).  
60 Unfortunately, tracers allowing direct estimates of autoxidative alterations of alkenones are  
61 lacking.

62 Significant photooxidative and autoxidative alteration of fast sinking organic matter  
63 collected by sediment traps at the DYFAMED station (Northwestern Mediterranean Sea) have  
64 been documented using newly identified tracers (Marchand et al., 2005; Rontani et al.,  
65 2006a). We now hypothesize that effects of abiotic degradation should be amplified in  
66 suspended particles due to their slower sinking rates and higher residence time in the water  
67 column. To further evaluate the importance of abiotic degradation on marine organic matter,  
68 we have now investigated suspended particles in the NW Mediterranean within the framework

69 of the MEDFLUX program (<http://www.msrc.sunysb.edu/MedFlux/>). Because sterols are  
70 excellent biomarkers for tracing diagenetic transformation of organic matter (Mackenzie et al.,  
71 1982; Volkman, 1986; Wakeham and Beier, 1991), we used their oxidation products to  
72 determine the relative roles of biotic and abiotic degradation. Using our observations of POM  
73 degradation derived from sterols, we then examined how abiotic degradation might affect the  
74 fate of alkenones, and thus the paleotemperature proxy  $U_{37}^{K'}$ , in the Northwestern  
75 Mediterranean.

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## 80 2. MATERIALS AND METHODS

81

### 82 2.1. Collection of the samples

83

84         Suspended particle samples were collected in the Northwestern Mediterranean Sea 52  
85 km off Nice, France at 43°25' N, 07°52'E during MedFlux (R/V *Tethys II*, May, 2006;  
86 cruise). MedFlux was a multidisciplinary investigation designed to determine and model  
87 relationships between organic matter and mineral ballast in particulate matter in the ocean  
88 (Lee et al., 2008a, and associated Special MedFlux issue of Deep-Sea Research II). The  
89 MedFlux site was located at the French Joint Global Ocean Flux Studies DYFAMED time-  
90 series site (Marty 2002, and references therein). Located in the central Ligurian Sea and with  
91 a water depth of ~2300 m, the MedFlux/DYFAMED site is thought to be isolated from  
92 advection of coastal riverine and resuspended sediments (Durrieu de Madron et al., 1990),  
93 although sediment trap experiments suggest there might be some periods of advective flux at  
94 depths of 1000 m or more (Lee et al., 2008b). Plankton succession has been described and  
95 generally consists of a spring diatom bloom followed by oligotrophic summer conditions  
96 (Marty et al., 2002), with corresponding temporal variability in particle flux (Miquel et al.,  
97 1994; Lee et al., 2008b).

98         Particulate matter samples for lipid analyses were collected the 10 May using a large-  
99 volume Challenger in situ filtration system (Cochran et al, 2008). Seawater (500-800 L) from  
100 5, 20, 40, 80, 100, 125, 150, 400 and 800 m was first passed through a 53 µm screen to  
101 remove rapidly sinking (large) particles. Slowly sinking small (“suspended”) particles were  
102 collected on 293 mm combusted (500°C, 6 hr) glass fiber filters (Whatman type A/E) of  
103 nominal pore size of 0.7 µm. GFF filters were cut in half and one-half was analyzed for  
104 lipids; the >53 µm screens were not analyzed. Filters were frozen (-20°C) until analysis.

105 Surface sediments were collected by box coring at the MedFlux site and stored frozen until  
106 analysis.

107 Particulate organic carbon (POC) was measured on HCl-fumed plugs from the filters  
108 using a Carlo Erba 1602 CHN analyzer. Chlorophyll a concentrations were obtained from  
109 fluorescence data obtained by CDT casts during the sampling.

110

## 111 *2.2. Chemical treatments*

112

113 Chemical treatments of the samples involved Soxhlet extraction of lipids and  
114 subsequent NaBH<sub>4</sub>-reduction of the lipid extracts thus obtained in order to reduce labile  
115 hydroperoxides resulting from photooxidative and autoxidative processes to alcohols. During  
116 this treatment, ketones (and notably alkenones) are also reduced to the corresponding alcohols  
117 (or alkenols) and the possibility of some ester cleavage cannot be excluded.

118

### 119 *2.2.1. Lipid extraction*

120

121 Filters or freeze-dried sediments (7.6 gdw) were extracted using  
122 dichloromethane:methanol (DCM:MeOH; 9:1 v:v) in soxhlet extractors for 8 hr. Lipids were  
123 partitioned into DCM with 5% NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Following rotary  
124 evaporation to concentrate the extracts, samples were stored at -20°C until being split for  
125 multiple analyses (Wakeham et al. 2008).

126

### 127 *2.2.2. NaBH<sub>4</sub> reduction*

128

129 Total lipid extracts were reduced (20 min) in Et<sub>2</sub>O/CH<sub>3</sub>OH (3:1, v/v, 5 ml) using  
130 excess NaBH<sub>4</sub> (10 mg/mg of extract). After reduction, a saturated solution of NH<sub>4</sub>Cl (10 ml)  
131 was added cautiously to destroy excess reagent, the pH was adjusted to 1 with dilute HCl (2  
132 N) and the mixture was shaken and extracted with hexane:CHCl<sub>3</sub> (4:1, v/v; x3). The combined  
133 extracts were dried as described above and evaporated to dryness under a stream of nitrogen.

134

### 135 *2.2.3. Alkaline hydrolysis*

136

137 Saponification was carried out on reduced samples. After NaBH<sub>4</sub> reduction, 25 ml of  
138 water and 2.8 g of potassium hydroxide were added and the mixture was directly saponified  
139 by refluxing for 2 h. After cooling, the contents of the flask were acidified with hydrochloric  
140 acid (pH 1) and subsequently extracted three times with dichloromethane. The combined  
141 dichloromethane extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated by  
142 rotary evaporation at 40 °C.

143

### 144 *2.2.4. Derivatisation*

145

146 After solvent evaporation, residues were taken up in 400 µL of a mixture of pyridine  
147 and pure N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco) (3:1, v/v) and silylated  
148 for 1 h at 50°C. After evaporation to dryness under a stream of N<sub>2</sub>, the derivatized residues  
149 were taken up in a mixture of ethyl acetate and BSTFA (to avoid desilylation of fatty acids)  
150 for analysis using gas chromatography-mass spectrometry.

151

## 152 *2.3. Identification and quantification of sterols and alkenones and their degradation products* 153 *by Gas Chromatography - Electron Impact Mass Spectrometry (GC-EIMS)*

154

155 Compounds were identified by comparison of retention times and mass spectra with  
156 those of standards and quantified (calibration with external standards) by GC-EIMS. For low  
157 concentrations, or in the case of co-elutions, quantification was achieved using selected ion  
158 monitoring (SIM). The main characteristic mass fragment ions used to quantify degradation  
159 products of sterols are shown in Figure 1. GC-EIMS was carried out with an Agilent 6890 gas  
160 chromatograph connected to an Agilent 5973 Inert mass spectrometer. The following  
161 conditions were employed: 30 m x 0.25 mm (i.d.) fused silica capillary column coated with  
162 SOLGEL-1 (SGE; 0.25  $\mu\text{m}$  film thickness); oven temperature programmed from 70°C to  
163 130°C at 20°C  $\text{min}^{-1}$ , from 130°C to 250°C at 5°C  $\text{min}^{-1}$  and then from 250°C to 300°C at 3°C  
164  $\text{min}^{-1}$ ; carrier gas (He) maintained at 0.69 bar until the end of the temperature program and  
165 then programmed from 0.69 bar to 1.49 bar at 0.04 bar  $\text{min}^{-1}$ ; injector (on column with  
166 retention gap) temperature, 50°C; electron energy, 70 eV; source temperature, 190°C; cycle  
167 time, 1.99 cycles  $\text{s}^{-1}$ , scan range, 50-800.

168

#### 169 *2.4. Standard degradation products*

170  $5\alpha$ - and  $6\alpha/\beta$ -Hydroperoxides were obtained after photosensitized oxidation of the  
171 corresponding  $\Delta^5$ -stenols in pyridine in the presence of haematoporphyrin as sensitizer  
172 (Nickon and Bagli, 1961). Allylic rearrangement of  $5\alpha$ -hydroperoxides to  $7\alpha$ -hydroperoxides  
173 and epimerization of the latter to  $7\beta$ -hydroperoxides was carried out at room temperature in  
174 chloroform (Teng et al., 1973). Subsequent reduction of these different hydroperoxides in  
175 methanol with excess  $\text{NaBH}_4$  afforded the corresponding diols. Treatment of  $\Delta^5$ -stenols with  
176 *meta*-chloroperoxy-benzoic acid in dry methylene chloride yielded a mixture of  $5\alpha,6\alpha$ - and  
177  $5\beta,6\beta$ -epoxides.

178 Tests carried out after NaBH<sub>4</sub> reduction and alkaline hydrolysis showed no significant  
179 differences in the proportions of sterol degradation products. The results discussed below for  
180 free sterols could thus be extended to the composite of free + esterified sterols.

### 181 3. RESULTS AND DISCUSSION

182

#### 183 3.1. $\Delta^5$ -sterol distributions and sterol degradation

184

185 Free sterols identified in suspended particulate matter samples are listed in Table 1 (as  
186 percent relative abundances and total sterol concentrations), along with particulate organic  
187 carbon (POC) concentration. Strong compositional differences were evident between samples.  
188 Samples from 5 and 20 m had high abundances of cholesta-5,24-dien-3 $\beta$ -ol, 27-nor-24-  
189 methylcholesta-5,22*E*-dien-3 $\beta$ -ol and 24-methylcholesta-5,22*E*-dien-3 $\beta$ -ol, whereas the 40 m  
190 sample was enriched in 24-methylcholesta-5,22*E*-dien-3 $\beta$ -ol. In contrast, deeper samples  
191 were dominated by cholest-5-en-3 $\beta$ -ol and were relatively enriched in cholesta-5,22*E*-dien-3 $\beta$ -  
192 ol. This sterol distribution suggests a mix of diatoms (although abundances of 24-  
193 methylcholesta-5,24(28)-dien-3 $\beta$ -ol are low), and haptophytes (Volkman, 1986; 2003; Barrett  
194 et al., 1995), and in the deeper samples, small (<53  $\mu$ m) zooplankton. Sampling for this  
195 investigation was carried out early in May following the regular diatom spring bloom. As  
196 diatom abundances decrease, the phytoplankton community at the MedFlux site typically  
197 shifts to higher contributions to smaller cells, especially cyanobacteria and prymnesiophytes  
198 (Marty et al., 2002). Suspended particles in the mesopelagic zone (generally  $\geq$ 100 m depth)  
199 include labile phytodetrital material whose organic composition has been significantly altered  
200 relative to surface water particles (this study and e.g., Wakeham and Lee, 1989).  
201 Heterotrophic alteration by zooplankton and microbes has been the degradation processes  
202 most frequently discussed in the literature.

203 Total  $\Delta^5$ -sterol concentrations decreased rapidly with increasing depth (~100-fold over  
204 the water column sampled; Table 1), at a similar rate than POC (~100-fold). Due to the  
205 relative stability and diverse functional groups that may be attached to the tetracyclic carbon

206 skeleton of  $\Delta^5$ -sterols, they may be useful for estimating relative effects of biotic and abiotic  
207 (photooxidation and autoxidation) degradation in the marine environment (Christodoulou et  
208 al., 2008).

209 Free radical oxidation (autoxidation) of  $\Delta^5$ -sterols yields mainly  $7\alpha$ - and  $7\beta$ -  
210 hydroperoxides and, to a lesser extent,  $5\alpha/\beta,6\alpha/\beta$ -epoxysterols and  $3\beta,5\alpha,6\beta$ -trihydroxysterols  
211 (Smith, 1981) (Figure 2). Singlet-oxygen mediated photooxidation (type II photoreactions)  
212 produces mainly  $\Delta^6$ - $5\alpha$ -hydroperoxides with low amounts of  $\Delta^4$ - $6\alpha/6\beta$ -hydroperoxides  
213 (Nickon and Bagli, 1961; Kulig and Smith, 1973) (Figure 2).  $\Delta^6$ - $5\alpha$ -hydroperoxysterols are  
214 unstable in seawater as they may undergo: (i) allylic rearrangement to  $\Delta^5$ - $7$ -  
215 hydroperoxysterols (Smith, 1981; Rontani and Marchand, 2000) and (ii) homolytic cleavage  
216 resulting in ring opening (Christodoulou et al., 2008). Characterization by GC/MS of  $\Delta^6$ - $3,5$ -  
217 dihydroxysterols obtained by  $\text{NaBH}_4$  reduction of the corresponding hydroperoxides presents  
218 some difficulties. Derivatization of these diols with pyridine/BSTFA results in the silylation  
219 of only the position 3 and the resulting monosilylated derivatives undergoes quantitative  
220 allylic rearrangement to  $\Delta^5$ - $3,7$ -dihydroxysterols during GC analysis (Rontani and Marchand,  
221 2000). Use of more powerful silylating reagents such as TMSIM/BSA/TMCS (Bortolomeazzi  
222 et al., 1999) or BSTFA/DMSO (Aubert, unpublished results) yield complete silylation of the  
223 diol, allowing GC/MS analyses without allylic rearrangement. However, derivatization with  
224 pyridine/BSTFA is preferred because it is difficult to eliminate DMSO or excess  
225 TMSIM/BSA/TMCS without losses of lipid material. Given these analytical and stability  
226 complications,  $\Delta^5$ - $3\beta,7$ - and  $\Delta^6$ - $3\beta,5$ - unsaturated diols are of little use in the present study.

227 We therefore selected  $5\alpha/\beta,6\alpha/\beta$ -epoxysterols and  $3\beta,5\alpha,6\beta$ -trihydroxysterols as tracers  
228 of free radical oxidation. As for photooxidation, we can use the  $\Delta^4$ - $3,6$ -dihydroxysterols  
229 obtained by  $\text{NaBH}_4$  reduction of  $\Delta^4$ - $6$ -hydroperoxysterols (Figure 2) to estimate the extent of  
230 photooxidation in our samples. This estimation involves the ratios of  $\Delta^4$ - $6$ -

231 hydroperoxysterols/ $\Delta^6$ -5-hydroperoxysterols previously measured in photooxidized senescent  
232 phytoplanktonic cells (0.34; Rontani *et al.*, 1997) and in biological membranes (0.30;  
233 Korytowski *et al.*, 1992). Taking into account that: sterol photooxidation % = ( $\Delta^4$ -3 $\beta$ ,6 $\alpha$ / $\beta$ -  
234 dihydroxysterol %) + ( $\Delta^6$ -3 $\beta$ ,5 $\alpha$ -dihydroxysterol %) (Eq. 1) and that  $\Delta^6$ -3 $\beta$ ,5 $\alpha$ -  
235 dihydroxysterol % = ( $\Delta^4$ -3 $\beta$ ,6 $\alpha$ / $\beta$ -dihydroxysterol %)/0.3 (Eq. 2), we used the following  
236 equation:

237

$$238 \text{ Sterol photooxidation \%} = (\Delta^4\text{-}3\beta,6\alpha/\beta\text{-dihydroxysterol \%}) * (1+1/0.3). \quad (\text{Eq. 3})$$

239

240 Aerobic bacterial degradation of  $\Delta^5$ -sterols may result to a complete mineralization of  
241 the molecule (Owen *et al.*, 1983; Naghibi *et al.*, 2002), probably through a series of  
242 intermediate ster-4-en-3-ones, 5 $\alpha$ (H)-stanones and 5 $\alpha$ (H)-stanols (Gagosian *et al.*, 1982; de  
243 Leeuw and Baas, 1986; Wakeham, 1987; 1989) (Figure 2). NaBH<sub>4</sub> reduction of our samples  
244 that was carried out to reduce hydroperoxides arising from abiotic degradation converted ster-  
245 4-en-3-ones and 5 $\alpha$ (H)-stanones to the corresponding alcohols. We thus selected ster-4-en-3-  
246 ols and 5 $\alpha$ (H)-stanols as tracers of biotic degradation processes. It may be noted that 5 $\alpha$ (H)-  
247 stanols and steroid ketones also are biosynthesized by some dinoflagellates (Robinson *et al.*,  
248 1984; Volkman *et al.*, 1998), but we do not consider this to be a major source here since  
249 dinoflagellate-derived 4 $\alpha$ (H),23,24-trimethylcholest-22-en-3 $\beta$ -ol was insignificant.

250 The use of the selected tracers to estimate the relative importance of the degradation  
251 processes on the parent  $\Delta^5$ -sterols requires that removal rates of the proposed tracers by  
252 further degradation are similar. For the purposes of this discussion we assume that the set of  
253 tracers exhibit similar reactivities towards bacterial mineralization processes. This assumption  
254 is based on the fact that aerobic bacterial mineralization of sterols generally involves initial  
255 degradation of the side chain, which is similar in all the tracers selected for each  $\Delta^5$ -sterol.

256 GC-EIMS analyses allowed the detection of significant amounts of ster-4-en-3-ols,  
257 5 $\alpha$ (H)-stanols, 5 $\alpha$ / $\beta$ ,6 $\alpha$ / $\beta$ -epoxysterols,  $\Delta^4$ -3 $\beta$ ,6- and  $\Delta^5$ -3 $\beta$ ,7- epimeric unsaturated diols  
258 arising from the degradation of each  $\Delta^5$ -sterol in the different samples analyzed (Figure 3).  
259 The estimated percentages of biotic and abiotic degradation (relative to the residual  
260 undegraded sterol) for cholest-5-en-3 $\beta$ -ol and 24-ethylcholest-5-en-3 $\beta$ -ol are shown in Fig 4.  
261 These two sterols were selected since: (i) they were present at significant levels in all the  
262 samples investigated (Table 1), and (ii) the characterization of their autoxidation products is  
263 easier than in the case of diunsaturated sterols (for which there is additional autoxidation of  
264 the side-chain). Figure 4 clearly indicates that products of abiotic degradation predominate  
265 over products of biotic degradation of suspended organic matter in the NW Mediterranean,  
266 not just in the sunlit surface waters but significantly even in the deeper aphotic mesopelagic  
267 zone. Furthermore, the extent of abiotic degradation varied down the water column. Not  
268 surprisingly, particles collected at 20 and 40 m corresponding to the chlorophyll maximum  
269 (centred at 28 m) appeared to the least degraded, either biotically or abiotically, consistent  
270 with healthy cells in good physiological state. We can not adequately explain weakly  
271 degraded material at 150 m, and can not know whether this observation is temporally  
272 significant. Also not surprisingly, photodegradation appeared to be significant at 5 m. If we  
273 extrapolate to other organic components of the particle pool, we would speculate that there  
274 would be intense photobleaching of pigments at 5 m. The elevated abundances of stanols in  
275 surface sediments we analysed are indicative of biotic degradation of sterols following  
276 deposition. On the other hand, lower abundances of stanols in the deep particulate samples  
277 analyzed suggest that the deep particles contain relatively little resuspended sedimentary  
278 organic matter.

279 The observation of significant abiotic degradation for the abundant slowly sinking  
280 suspended particles that dominate the standing stock of POC (McCave, 1984; Wakeham,

281 1989) contrasts with previous conclusions that the rare, rapidly sinking particles that dominate  
282 the vertical flux of POC but are collected in sediment traps (or on the 53  $\mu\text{m}$  screens used  
283 here) were more affected by biotic degradation than by abiotic degradation (Christodoulou et  
284 al., 2008). Steroidal ketones formed via bacterial degradation of sterols were a significant part  
285 of the lipids in the sinking particles in the Pacific Ocean, but not in suspended particles  
286 (Wakeham, 1987; Wakeham and Lee, 1989). The most straight-forward explanation for these  
287 differences is that slowly sinking suspended particles spend much more time in the photic  
288 layer where photooxidation can occur. Eventually, even the suspended particles sink, albeit  
289 slowly, during which time they are subject to further non-photolytic abiotic degradation  
290 and/or particles at depth simply reflect processes that occurred in surface waters sometime in  
291 the past.

292         The present results also contrast with analyses of suspended particles from the Black Sea  
293 (Rontani and Wakeham, 2008). In that case, biotic degradation appeared to be much more  
294 important (70% of the residual sterol) than abiotic degradation (5% of the residual sterol).  
295 There are, however, important differences between the NW Mediterranean and the Black Sea  
296 that would impact the relative importance of abiotic vs biotic degradation. Although the  
297 entire water column of the Mediterranean site is oxygenated ( $\sim 6$  ml/l  $\text{O}_2$  in the mixed layer to  
298 25 m depth,  $\sim 4$  ml/l from 100 – 800 m) which might support aerobic microbial degradation,  
299 bacterial cell numbers decrease significantly with depth ( $25 \times 10^4$  cells/ ml at 20 m,  $45 \times 10^4$   
300 cells/ ml at 60 m,  $2 \times 10^4$  cells/ ml at 1000 m; Tamburini et al., 2008). This would imply  
301 significant microbial degradation “potential”, recognizing that cell numbers do not readily  
302 extrapolate to microbial decomposition rates. On the other hand, the Black Sea is anoxic  
303 below  $\sim 100$  m, but cell numbers change to a lesser degree between the oxic surface waters ( $22$   
304  $\times 10^4$  cells/ ml at 10 m), the intermediate suboxic zone ( $27 \times 10^4$  cells/ ml at 20 m), and the  
305 deep anoxic zone ( $10 \times 10^4$  cells/ ml at 800 m). In fact, it is because of the intense microbial

306 activity that the suboxic and anoxic zones are sustained, so that a higher proportion of biotic  
307 degradation in the Black Sea is logical.

308 Comparison of apparent degradation states of cholest-5-en-3 $\beta$ -ol and 24-ethylcholest-5-  
309 en-3 $\beta$ -ol suggests that 24-ethylcholest-5-en-3 $\beta$ -ol might be more affected by abiotic  
310 degradation than cholest-5-en-3 $\beta$ -ol., even in surface waters, with relatively little difference  
311 for biotic degradation (Fig. 4). It is intriguing to speculate that this difference might result  
312 from the origins and transport mechanisms for the two sterols. 24-Ethylcholest-5-en-3 $\beta$ -ol is  
313 present in some marine algae, as is cholest-5-en-3 $\beta$ -ol (Volkman, 1986, 2003), but a vascular  
314 plant origin may dominate in areas influenced by terrigenous sources. Indeed, terrestrial  
315 inputs resulting from eolian transport of dust from Africa are frequent in Northwestern  
316 Mediterranean Sea (e.g. Migon et al., 2002; Guieu et al., 2002; Bartoli et al., 2005) and were  
317 common features during the MedFlux program (Lee et al., 2008). Atmospheric inputs could  
318 thus constitute a potential allochthonous source of “predegraded” material to the NW  
319 Mediterranean. Cholest-5-en-3 $\beta$ -ol, on the other hand, is likely derived primarily from  
320 autochthonous (marine) sources. It is interesting to note that the presence of an additional  
321 double bond in the side-chain of  $\Delta^5$  sterols does not induce systematically higher degradation  
322 rates.

323

### 324 *3.2. Alkenone degradation in the NW Mediterranean*

325

326 Results discussed above show that both biotic and abiotic degradation can have a  
327 significant affect on sterol compositions and by inference other components of the particulate  
328 organic matter pool. Biotic and abiotic degradation can also affect alkenones (Rontani et al.,  
329 2006a; 2008). Treatment of the lipid extracts in this study (NaBH<sub>4</sub> reduction carried out to  
330 reduce photochemically or autoxidatively-produced hydroperoxides and subsequent

331 derivatisation), reduced alkenones to alkenols which were then silylated. The silylated  
332 alkenols thus formed display better chromatographic characteristics than the corresponding  
333 alkenones and have diagnostic EI mass spectra, (strong fragment ions at  $m/z$  117 and 131 due  
334 to cleavage  $\alpha$  to the functional group, allowing methyl and ethyl alkenols (and hence the  
335 parent alkenones) to be readily differentiated by selected ion monitoring (SIM), even at low  
336 abundances (Rontani et al., 2001). For our particle samples here, C<sub>36</sub>-C<sub>39</sub> alkenols (and hence  
337 the parent alkenones) are readily detected (Figure 5). The diunsaturated C<sub>36</sub> alkenone  
338 exhibiting an unusual double bond spacing of three methylene groups instead of five (Figure  
339 6) has also been reported in DYFAMED sediment trap samples (Rontani et al., 2001), and  
340 particulate matter and sediments from the Black Sea. (Xu et al., 2001; Rontani and Wakeham,  
341 2008).

342 The shape of the alkenone concentration profile is similar to that of  $\Delta^5$ -sterols (Table 2);  
343 alkenone concentrations decrease much more rapidly (~500 fold) than sterols (~100-fold) or  
344 POC (~100-fold). This might be related to the fact that the haptophyte source of alkenones is  
345 restricted to surface waters, or that alkenones are very labile. Previous studies (Prahl et al.,  
346 2000; Wakeham et al., 2002) have shown that only a small fraction of alkenones present in the  
347 upper water column reaches the underlying sediments, yet the unsaturation index remains  
348 relatively unchanged, a requirement from a palaeothermometer perspective. There is,  
349 however, evidence that selective degradation might affect alkenone unsaturation and the  
350 resulting  $U_{37}^{K'}$  (Freeman and Wakeham, 1992; Hoefs et al., 1998; Gong and Hollander, 1999;  
351 Rontani and Wakeham, 2008). For our samples,  $U_{37}^{K'}$  values increase significantly (from  
352 0.43 to 0.55) with increasing water depth (Table 2).

353 Selective degradation of the 37:3 alkenone compared to the 37:2 alkenone would cause  
354 the  $U_{37}^{K'}$  to increase. On the basis of the results obtained above for  $\Delta^5$ -sterols, this increase  
355 might be attributed to a selective autoxidative degradation of alkenones (Rontani et al., 2006a,

2007). In order to check on the relevance of this process for our NW Mediterranean particles, we plotted variations of  $U_{37}^{K'}$  index against percentages of sterol tracers of autoxidation ( $5\alpha/\beta,6\alpha/\beta$ -epoxycholestan- $3\beta$ -ol and  $5\alpha/\beta,6\alpha/\beta$ -epoxy-24-ethylcholestan- $3\beta$ -ol; Fig. 7). Although comparison of the  $U_{37}^{K'}$  values at deeper and shallower depths is difficult, since these samples represent well distinct time integration of temperature signal, the correspondence observed between the profiles well supports our suggestion. Thus, in the NW Mediterranean, autoxidation of alkenones associated with suspended particles can yield a non-trivial increase in  $U_{37}^{K'}$  values with increasing depth in the water column, which could translate into a  $+3.2^{\circ}\text{C}$  change in the inferred temperature when interpreted using the Ternois et al. (1997)  $U_{37}^{K'}$ -temperature calibration equation ( $U_{37}^{K'} = 0.041T - 0.21$ ) that is appropriate to the NW Mediterranean. It must be stressed that the suspended particle pool sinks only slowly through the water column and may be uncoupled from the fast sinking particles that constitute most of the flux to sediments (Wakeham and Lee, 1989), and thus contribute to the sediment temperature record. Autoxidative alteration of  $U_{37}^{K'}$  has been observed for sinking particulate matter collected during DYFAMED sediment trap deployments (Rontani et al., 2006a; Christodoulou et al., 2008).

Aerobic microbial degradation also has the potential to alter  $U_{37}^{K'}$  values and thus introduce further uncertainty in the paleotemperature reconstruction, particularly for oxic sediments environments (Rontani et al., 2008). Bacterial epoxidation of alkenone double bonds produces epoxyketones that are easy to detect after  $\text{NaBH}_4$  reduction to the corresponding diols and subsequent silylation and thus might be used as potential indicators of *in situ* aerobic bacterial alteration of the alkenone unsaturation ratio. We did not find any epoxyketones in the present particle samples. Thus in the absence of measurable biotic degradation, autoxidation must predominate.

380 Double bonds of alkenones can be stereomutated by thiyl radical-dependent mechanisms  
381 (Rontani et al., 2006b), with differences in reactivity of the C<sub>37:2</sub> and C<sub>37:3</sub> alkenones affecting  
382  $U_{37}^{K'}$ . The  $U_{37}^{K'}$  value measured in the surface sediment (0-0.5 cm) we analysed was 0.61  
383 (Table 2), agreeing with values of 0.54 to 0.65 reported by Ternois et al. (1996) for other NW  
384 Mediterranean surface sediments. Careful GC-EIMS examination of trimethylsilylated alkenol  
385 peaks for the sediment sample (Fig. 8) allowed us to detect stereomutated compounds (i.e.  
386 with *cis* double bonds). Changes in measured  $U_{37}^{K'}$  values might result from co-elution  
387 problems as well as from differential rates of stereomutation of C<sub>37:2</sub> and C<sub>37:3</sub> alkenones.  
388 Using a relationship between  $U_{37}^{K'}$  and the percentage of stereomutated MeC<sub>37:2</sub> alkenone that  
389 we developed during a study in the Black Sea (Rontani and Wakeham, 2008), we estimate  
390 that the increase in  $U_{37}^{K'}$  index resulting from stereomutation recorded in our surface sediment  
391 would be +0.05 (equal to a +1.3°C change in the inferred temperature). Perhaps  
392 coincidentally, the corrected  $U_{37}^{K'}$  index value thus obtained (0.56) is very close to the  
393 measured values in suspended particulate matter samples collected at 400 and 800 m (0.54  
394 and 0.55, respectively) (Table 2), but markedly different from surface values (0.43).  
395

396 **4. CONCLUSIONS**

397

398 The degradation of  $\Delta^5$ -sterols and alkenones was studied in suspended particulate matter  
399 and sediment samples collected in Northwestern Mediterranean Sea. Total  $\Delta^5$ -sterol and  
400 alkenone concentrations strongly decrease with increasing depth, suggesting significant  
401 degradation in the water column. The relative stability and diverse functional groups of the  
402 steroidal tetracycle are useful for estimating the relative effects of biotic (bacterial  
403 hydrogenation) and abiotic (photooxidation and autoxidation) degradation processes. We  
404 propose that  $5\alpha/\beta,6\alpha/\beta$ -epoxysterols and  $3\beta,5\alpha,6\beta$ -trihydroxysterols are good tracers of  
405 estimating the relative importance of free radical (auto)oxidation,  $\Delta^4$ -3,6-dihydroxysterols  
406 obtained by  $\text{NaBH}_4$  reduction of  $\Delta^4$ -6-hydroperoxysterols are useful for photooxidation, and  
407 ster-4-en-3-ols and  $5\alpha(\text{H})$ -stanols can be used for biotic degradation. The results obtained  
408 showed that abiotic degradation strongly predominates in suspended particles in the NW  
409 Mediterranean.

410  $\text{C}_{36}$ - $\text{C}_{39}$  alkenones (measured as alkenols obtained by  $\text{NaBH}_4$  reduction of parent  
411 alkenones) in water column particle and sediment samples investigated yielded  $U_{37}^{K'}$  indices  
412 that increase significantly (from 0.43 to 0.55) with increasing water depth. Biotic degradation  
413 of alkenones appeared unimportant in this water column environment. However, a good  
414 correlation between variations of  $U_{37}^{K'}$  index and concentrations of  $\Delta^5$ -sterol autoxidation  
415 products supports autoxidation of alkenones as a key degradative process. We also detected  
416 stereomutated alkenones (with *cis* double bonds) in the surface sediment and estimate that  
417 stereomutation increases  $U_{37}^{K'}$  index in this sediment by +0.05. Degradation of alkenones in  
418 the marine water column may therefore have a significant effect on paleotemperature  
419 reconstructions.

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435

436 **References**

437

438 Barrett, S.M., Volkman, J.K., Dunstan, G.A., Leroi, J.-M., 1995. Sterols of 14 species of  
439 marine diatoms (Bacillariophyla). *Journal of Phycology* 31, 360-369.

440 Bartoli, G., Migon, C., Losno, R., 2005. Atmospheric input of dissolved inorganic phosphorus  
441 and silicon to the coastal northwestern Mediterranean Sea: fluxes, variability and  
442 possible impact on phytoplankton dynamics. *Deep-Sea Research I* 52, 2005-2016

443 Bortolomeazzi, R., De Zan, M., Pizzale, L., Conte, L.S., 1999. Mass spectrometry  
444 characterization of the 5 $\alpha$ -, 7 $\alpha$ , and 7 $\beta$ -hydroxy derivatives of  $\beta$ -sitosterol, campesterol,  
445 stigmasterol and brassicasterol. *Journal of the Agricultural Food Chemistry* 47: 3069-  
446 3074.

447 Christodoulou, S., Marty, J.-C., Miquel, J.-C., Volkman, J.K., Rontani, J.-F. 2008. Use of  
448 lipids and their degradation products as biomarkers for carbon cycling in the  
449 northwestern Mediterranean Sea. *Marine Chemistry* (submitted).

450 Cochran, J.K., Miquel, J.-C., Fowler, S., Gasser, B., Hirschberg, D., Szlosek, J., Rodriguez y  
451 Baena, A.M., Armstrong, R., Stewart, G., Masqué, P. 2008. Time-series measurements  
452 of <sup>234</sup>Th in water column and sediment trap samples from the Northwestern  
453 Mediterranean. *Deep-Sea Research II* (In press).

454 Durrieu de Madron, X., Nyffeler, F., Godet, C. H., 1990. Hydrographic structure and  
455 nepheloid spatial distribution in the Gulf of Lions continental margin. *Continental*  
456 *Shelf Research* 10, 915-929.

457 Freeman, K.H., Wakeham, S.G., 1992. Variations in the distributions and isotopic  
458 compositions of alkenones in Black Sea particles and sediments. *Organic*  
459 *Geochemistry* 19, 277-285.

460 Gagosian, R.B., Smith, S.O., Nigrelli, G.E., 1982. Vertical transport of steroid alcohols and  
461 ketones measured in a sediment trap experiment in the equatorial Atlantic Ocean.  
462 *Geochimica et Cosmochimica Acta* 46, 1163-1172.

463 Gong, C., Hollander, D.J., 1999. Evidence for differential degradation of alkenones under  
464 contrasting bottom water oxygen conditions: Implication for paleotemperature  
465 reconstruction. *Geochimica et Cosmochimica Acta* 63, 405-411.

466 Guieu, C., Bozec, Y., Blain, S., Ridame, C., Sarthou, G., Leblond, N., 2002. Impact of high  
467 Saharan dust inputs on dissolved iron concentrations in the Mediterranean Sea.  
468 *Geophysical Research Letters*, 10.1029/2001GL014454.

469 Hoefs, M.J.L., Versteegh, G.J.M., Rijpstra, W.I.C., de Leeuw, J.W., Sinninghe Damsté, J.S.,  
470 1998. Postdepositional oxic degradation of alkenones: Implications for the measurement  
471 of palaeo sea surface temperatures. *Paleoceanography* 13, 42-49.

472 Korytowski, W., Bachowski, G.J., Girotti, A.W., 1992. Photoperoxidation of cholesterol in  
473 homogeneous solution, isolated membranes, and cells: comparison of the 5 $\alpha$ - and 6 $\beta$ -  
474 hydroperoxides as indicators of singlet oxygen intermediacy. *Photochemistry and*  
475 *Photobiology* 56, 1-8.

476 Kulig, M.J., Smith, L.L., 1973. Sterol metabolism. XXV. Cholesterol oxidation by singlet  
477 molecular oxygen. *Journal of Organic Chemistry* 38, 3639-3642.

478 Lee, C., Armstrong, R.A., Cochran, J.K., Engel, A. Fowler, S., Goutx, M., Masqué, P.,  
479 Miquel, J.-C., Peterson, M.L., Tamburini, C., Wakeham, S.G., 2008a. MedFlux:  
480 Investigations of particle flux in the Twilight Zone. *Deep-Sea Research II*, in press.

481 Lee, C., Armstrong, R.A., Wakeham, S.G., Peterson, M.L., Miquel, J.-C., Cochran, J.K.,  
482 Fowler, S.W., Hirschberg, D., Beck, A. Xue, J., 2008b. Particulate matter fluxes in  
483 time-series and settling velocity sediment traps in the northwestern Mediterranean Sea,  
484 *Deep-Sea Research II*, in press.

485 de Leeuw, J.W., Baas, M., 1986. Early stage diagenesis of steroids. In: Johns, R.B. (Ed.),  
486 Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, pp. 101-123.

487 Mackenzie, A.S., Brassell, S.C., Eglinton, G., Maxwell, J.R., 1982. Chemical fossils: the  
488 geological fate of steroids. *Science* 217, 491-504.

489 McCave, I.N., 1984. Size spectra and aggregation of suspended particles in the ocean. *Deep-*  
490 *Sea Research* 31, 329-352.

491 Marchand, D., Marty, J.-C., Miquel, J.-C., Rontani, J.-F., 2005. Lipids and their oxidation  
492 products as biomarkers for carbon cycling in the northwestern Mediterranean Sea:  
493 results from a sediment trap study. *Marine Chemistry* 95, 129-147.

494 Marty, J.-C., 2002. The DYFAMED time-series program (French JGOFS). *Deep-Sea*  
495 *Research II* 49, 1963-1964.

496 Marty, J.-C., Chiaverini, J., Pizay, M.-D., Avril, B., 2002. Seasonal and interannual dynamics  
497 of nutrients and phytoplankton pigments in the western Mediterranean sea at the  
498 DYFAMED time-series station (1991-1999). *Deep-Sea Research II* 49, 1965-1985.

499 Migon, C., Sandroni, V., Marty, J.-C., Gasser, B., Miquel, J.-C., 2002. Transfer of  
500 atmospheric matter through the euphotic layer in the northwestern Mediterranean:  
501 seasonal pattern and driving forces. *Deep-Sea Research II* 49, 2125-2141.

502 Miquel J.-C., Fowler, S.W., La Rosa, J., Buat-Menard, P., 1994. Dynamics of the downward  
503 flux of particles and carbon in the open NW Mediterranean Sea. *Deep-Sea Research*  
504 41, 242-261.

505 Naghibi, F., Tabatabai Yazdi, M., Sahebgharani, M., Noori Dalooi, M.R., 2002. Microbial  
506 Transformation of Cholesterol by *Mycobacterium smegmatis*. *Journal of Sciences* 13,  
507 103-106.

508 Nickon, A., Bagli, J.F., 1961. Reactivity and geochemistry in allylic systems. I.  
509 Stereochemistry of photosensitized oxygenation of monoolefins. *Journal of the*  
510 *American Chemical Society* 83, 1498-1508.

511 Owen, R.W., Mason, A.N., Bilton, R.F., 1983. The degradation of cholesterol by  
512 *Pseudomonas* sp. NCIB 10590 under aerobic conditions. *Journal of Lipid Research* 24,  
513 1500-1511.

514 Pokorny, J., 1987. Major factors affecting the autoxidation of lipids. In: Chan, H.W.-S. (Ed.),  
515 *Autoxidation of Unsaturated Lipids*. Academic Press, London, pp. 141–206.

516 Prahl, F.G., Dymond, J., Sparrow, M.A., 2000. Annual biomarker record for export  
517 production in the central Arabian Sea. *Deep-Sea Research II* 47, 1581-1604.

518 Robinson, N., Eglinton, G., Brassell, S.C., Cranwell, P.A., 1984. Dinoflagellate origin for  
519 sedimentary 4 $\alpha$ -methylsteroids and 5 $\alpha$ (H) stanols. *Nature* 308, 439–442.

520 Rontani, J.-F., Cuny, P., Aubert, C., 1997. Rates and mechanism of light-dependent  
521 degradation of sterols in senescing cells of phytoplankton. *Journal of Photochemistry*  
522 *and Photobiology* 111A, 139-144.

523 Rontani, J.-F., Marchand, D., 2000. Photoproducts of phytoplanktonic sterols: a potential  
524 source of hydroperoxides in marine sediments? *Organic Geochemistry* 31, 169-180.

525 Rontani, J.-F., Marchand, D., Volkman, J.K., 2001. NaBH<sub>4</sub> reduction of alkenones to the  
526 corresponding alkenols: a useful tool for their characterisation in natural samples.  
527 *Organic Geochemistry* 32, 1329-1341.

528 Rontani, J.-F., Marty, J.-C., Miquel, J.-C., Volkman, J.K., 2006a. Free radical oxidation  
529 (autoxidation) of alkenones and other microalgal lipids in seawater. *Organic*  
530 *Geochemistry* 37, 354-368.

531 Rontani, J.-F., Bonin, P., Prahl, F.G., Jameson, I., Volkman, J.K., 2006b. Experimental and  
532 field evidence for thiyl radical-induced stereomutation of alkenones and other lipids in  
533 sediments and seawater. *Organic Geochemistry* 37, 1489-1504.

534 Rontani, J.-F., Jameson, I., Christodoulou, S., Volkman, J.K., 2007. Free radical oxidation  
535 (autoxidation) of alkenones and other lipids in cells of *Emiliana huxleyi*.  
536 *Phytochemistry* 68, 913-924.

537 Rontani J.-F., 2008. Photooxidative and Autoxidative Degradation of Lipid Components  
538 during the Senescence of Phototrophic Organisms. In: Matsumoto, T. (Ed),  
539 *Phytochemistry Research Progress*, 2008. Nova Science Publishers, pp. 115-144.

540 Rontani, J.-F., Wakeham, S.G., 2008. Alteration of alkenone unsaturation ratio with depth in  
541 Black Sea: potential roles of stereomutation and aerobic biodegradation. *Organic*  
542 *Geochemistry* 39, 1259-1268.

543 Rontani, J.-F., Harji, R., Guasco, S., Prahl, F.G., Volkman, J.K., Bhosle, N.B., Bonin, P.,  
544 2008. Degradation of alkenones by aerobic heterotrophic bacteria: Selective or not?  
545 *Organic Geochemistry* 39, 34-51.

546 Schaich, K.M., 1992. Metals and lipid oxidation. *Contemporary issues. Lipids* 27, 209–218.

547 Smith, L.L., 1981. *The Autoxidation of Cholesterol*. Plenum Press, New York.

548 Tamburini, C., Goutx, M., Guigue, C., Garel, M., Lefèvre, D., Charrière, B., Sempéré, R.,  
549 Pepa, S., Peterson, M.L., Wakeham, S.G., Lee, C., 2008. Microbial alteration of sinking  
550 fecal pellets: Effects of a continuous increase in pressure that simulates descent in the  
551 water column. *Deep-Sea Res. II* (In press).

552 Teng, J.I., Kulig, M.J., Smith, L.L., Kan, G., van Lier, J.E., 1973. Sterol metabolism. XX.  
553 Cholesterol 7-hydroperoxide. *Journal of Organic Chemistry*, 38: 119-123.

554 Ternois, Y., Sicre, M.-A., Boireau, A., Marty, J.-C., Miquel, J.-C., 1996. Production pattern of  
555 alkenones in the Mediterranean Sea. *Geophysical Research Letters* 23, 3171-3174.

556 Ternois, Y., Sicre, M.-A., Boireau, A., Conte, M.H., Eglinton, G., 1997. Evaluation of long-  
557 chain alkenones as paleotemperature indicators in the Mediterranean Sea. *Deep-Sea*  
558 *Research I* 44, 271-286.

559 Volkman, J.K., 1986. A review of sterol markers for marine and terrigenous organic matter.  
560 *Organic Geochemistry* 9, 83-99.

561 Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998.  
562 Microbial biomarkers: A review of recent research developments. *Organic*  
563 *Geochemistry* 29, 1163-1179.

564 Volkman, J.K., 2003. Sterols in microorganisms. *Applied Microbiology and Biotechnology*  
565 60, 495-506.

566 Wakeham, S.G., 1987. Steroid geochemistry in the oxygen minimum zone of the eastern  
567 tropical North Pacific Ocean. *Geochimica et Cosmochimica Acta* 51, 3051-3069.

568 Wakeham, S.G., 1989. Reduction of stenols to stanols in particulate matter at oxic-anoxic  
569 boundaries in sea water. *Nature* 342, 787-790.

570 Wakeham, S.G., Lee, C., 1989. Organic geochemistry of particulate matter in the ocean: The  
571 role of particles in oceanic sedimentary cycles. *Organic Geochemistry* 14, 83-96.

572 Wakeham, S.G., Beier, J.A., 1991. Fatty acid and sterol biomarkers as indicators of particulate  
573 organic matter source and alteration processes in the water column of the Black Sea.  
574 *Deep-Sea Research* 38 (Suppl. 2), S943-S968.

575 Wakeham, S.G., Peterson, M.L., Hedges, J.I., Lee, C., 2002. Lipid biomarker fluxes in the  
576 Arabian Sea: with a comparison to the Equatorial Pacific Ocean. *Deep-Sea Research II*.  
577 49: 2265-2301.

578 Wakeham, S.G., Lee, C., Peterson, M.L., Liu, Z., Szlosek, J., Putnam, I.F., J. Xue, J., 2008.  
579 Organic compound composition and fluxes in the twilight zone – Time series and  
580 settling velocity sediment traps during MedFlux. *Deep-Sea Res. II*, in press.

581 Xu, L., Reddy, C.M., Farrington, J.W., Frysinger, G.S., Gaines, R.B., Johnson, C.G., Nelson,  
582 R.K., Eglinton, T.I., 2001. Identification of a novel alkenone in Black Sea sediments.  
583 *Organic Geochemistry* 32, 633–645.

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589 **FIGURE CAPTIONS**

590

591 Figure 1. Mass spectrometric characterization of sterols and their degradation products (the  
592 example given is cholesterol).

593

594 Figure 2. Pathways of proposed tracers for biotic and abiotic degradation of  $\Delta^5$  sterols.

595

596 Figure 3. Partial  $m/z$  458, 456, 403, 474, 460 mass fragmentograms of total lipid extracts  
597 showing the presence of cholesterol degradation products in suspended particulate matter  
598 collected at 100 m.

599

600 Figure 4. Chlorophyll concentration and percentages of biotic and abiotic degradation  
601 products for cholest-5-en-3 $\beta$ -ol (A) and 24-ethylcholest-5-en-3 $\beta$ -ol (B) in the samples  
602 investigated. (Abiotic degradation products include photo- and autoxidation products).

603

604 Figure 5. Partial  $m/z$  117 and 131 mass fragmentograms of silylated NaBH<sub>4</sub>-reduced total lipid  
605 extracts showing the presence of alkenols in suspended particulate matter collected at 5 m.

606

607 Figure 6. Partial  $m/z$  276, 278, 290, 304 mass fragmentograms of 3-hydroxyhexatriaconta-  
608 14,21-diene (obtained after NaBH<sub>4</sub> reduction of the alkenone fraction of the *E. huxleyi* strain  
609 CCMP 1742 now producing strong proportion of shorter alkenones; Prahl et al., 2006) (A), 3-  
610 hydroxyhexatriaconta-16,21-diene (obtained after NaBH<sub>4</sub> reduction of the alkenone fraction  
611 of Unit II Black Sea sediments) (B) and silylated NaBH<sub>4</sub>-reduced EtC<sub>36:2</sub> alkenone present in  
612 the sample collected at 5 m (C).

613

614 Figure 7.  $U_{37}^{K'}$  index and percentages of 5,6-epoxycholestan-3 $\beta$ -ol and 5,6-epoxy-24-  
615 ethylcholestan-3 $\beta$ -ol measured in the samples investigated.

616

617 Figure 8. Partial  $m/z$  512.5, 514.5, 526.5, 528.5 mass fragmentograms showing the presence  
618 of stereomutated silylated alkenols in the top layer (0-0.5 cm) of sediments.

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Table 1. Concentration of free  $\Delta^5$  sterols and POC in the suspended particulate matter samples investigated.

	Depth (m)								
	5	20	40	80	100	125	150	400	800
24-Norcholesta-5,22 <i>E</i> -dien-3 $\beta$ -ol	4.1	4.2	5.8	2.8	2.5	5.8	2.0	2.5	-
27-Nor-24-methylcholesta-5,22 <i>E</i> -dien-3-ol	18.1	17.8	9.9	10.0	11.7	8.5	8.7	7.2	4.5
Cholesta-5,22 <i>E</i> -dien-3 $\beta$ -ol	5.3	5.1	12.6	11.9	11.7	9.6	12.2	10.2	3.3
Cholest-5-en-3 $\beta$ -ol	5.4	6.6	14.1	24.1	27.0	38.5	27.0	31.7	65.0
Cholesta-5,24-dien-3 $\beta$ -ol	27.5	25.6	2.8	3.0	3.2	4.9	2.6	-	-
24-Methylcholesta-5,22 <i>E</i> -dien-3 $\beta$ -ol	17.8	17.8	31.9	15.9	16.3	11.1	13.3	14.6	11.6
24-Methylcholesta-5,24(28)-dien-3 $\beta$ -ol	7.5	8.2	3.8	5.1	4.1	3.3	3.6	5.2	-
24-Methylcholest-5-en-3 $\beta$ -ol	-	-	1.2	4.1	3.2	-	3.4	3.6	-
24-Ethylcholesta-5,22 <i>E</i> -dien-3 $\beta$ -ol	4.4	4.3	4.8	6.2	4.3	3.6	7.2	7.2	5.9
24-Ethylcholest-5-en-3 $\beta$ -ol	3.1	3.7	7.2	8.8	8.7	11.5	11.6	12.7	9.7
24-Ethylcholesta-5,24(28) <i>Z</i> -dien-3 $\beta$ -ol	2.9	2.6	2.0	2.8	2.5	3.1	3.1	5.1	-
24- <i>n</i> -propylcholesta-5,24(28) <i>E</i> -dien-3 $\beta$ -ol	1.7	2.0	1.0	1.8	1.5	-	2.4	-	-
24- <i>n</i> -propylcholesta-5,24(28) <i>Z</i> -dien-3 $\beta$ -ol	2.2	2.5	2.9	3.6	3.3	-	2.9	-	-
Total sterol concentration ( $\mu\text{g l}^{-1}$ )	3.17	1.57	0.35	0.13	0.10	0.10	0.09	0.04	0.03
POC ( $\mu\text{g l}^{-1}$ )	126.3	103.5	16.6	9.3	4.8	5.9	4.0	4.0	1.2



Table 2. Alkenone concentration and unsaturation ratio in the suspended particulate matter samples investigated.

Depth (m)	$\Sigma\text{alk}^{\text{a}}$ ( $\text{ng l}^{-1}$ )	$\text{MeC}_{37}^{\text{b}}$ (%)	$\text{MeC}_{38}^{\text{b}}$ (%)	$\text{EtC}_{36:2}^{\text{b}}$ (%)	$\text{EtC}_{38}^{\text{b}}$ (%)	$U_{37}^{\text{k}}$	Estimated temperature <sup>f</sup>
5	28.8	51.8	19.6	7.0	21.6	0.43	15.6
20	13.3	55.0	16.5	6.3	22.2	0.43	15.6
40	1.3	54.4	14.5	4.0	27.1	0.43	15.6
80	1.8	56.7	18.3	0.5	24.5	0.45	16.1
100	1.0	52.6	17.5	0.9	28.8	0.49	17.1
125	0.4	57.9	18.1	0.8	23.2	0.46	16.3
150	0.5	55.3	17.0	2.1	25.6	0.45	16.1
400	0.1	64.7	16.7	tr <sup>c</sup>	18.6	0.54	18.3
800	0.06	71.7	13.9	tr	15.9	0.55	18.5
	<u>(<math>\text{ng g}^{-1}</math> dry weight)</u>						
Sediment (0-0.5 cm)	8.8	65.7	8.0	tr	26.3	0.61 <sup>d</sup> 0.56 <sup>e</sup>	20 <sup>d</sup> 18.7 <sup>e</sup>

<sup>a</sup> Total alkenone concentration.

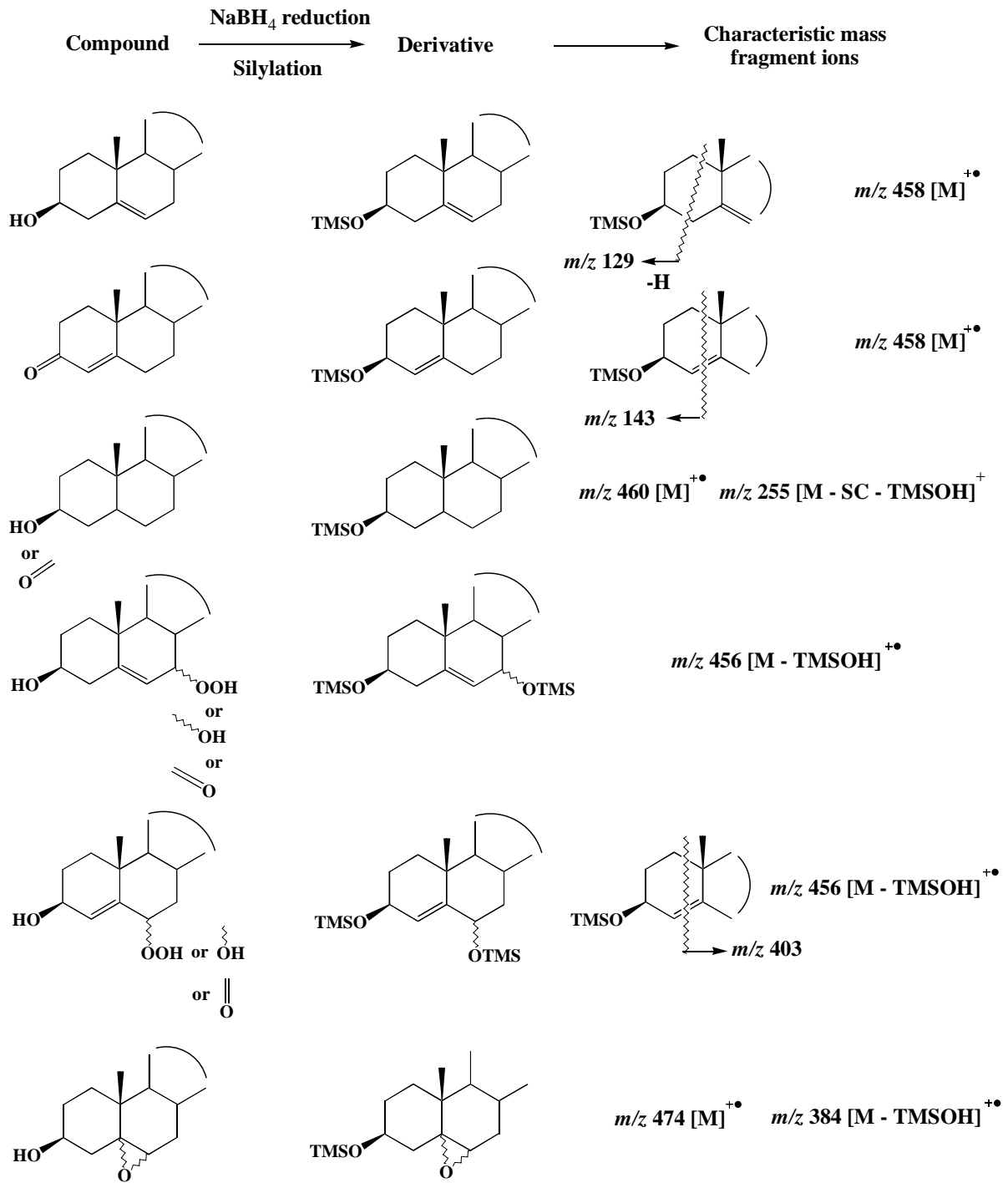
<sup>b</sup> Concentration of C<sub>37</sub> methyl, C<sub>38</sub> methyl, C<sub>36:2</sub> ethyl and C<sub>38</sub> ethyl ketones as a percentage of total alkenone concentration, respectively.

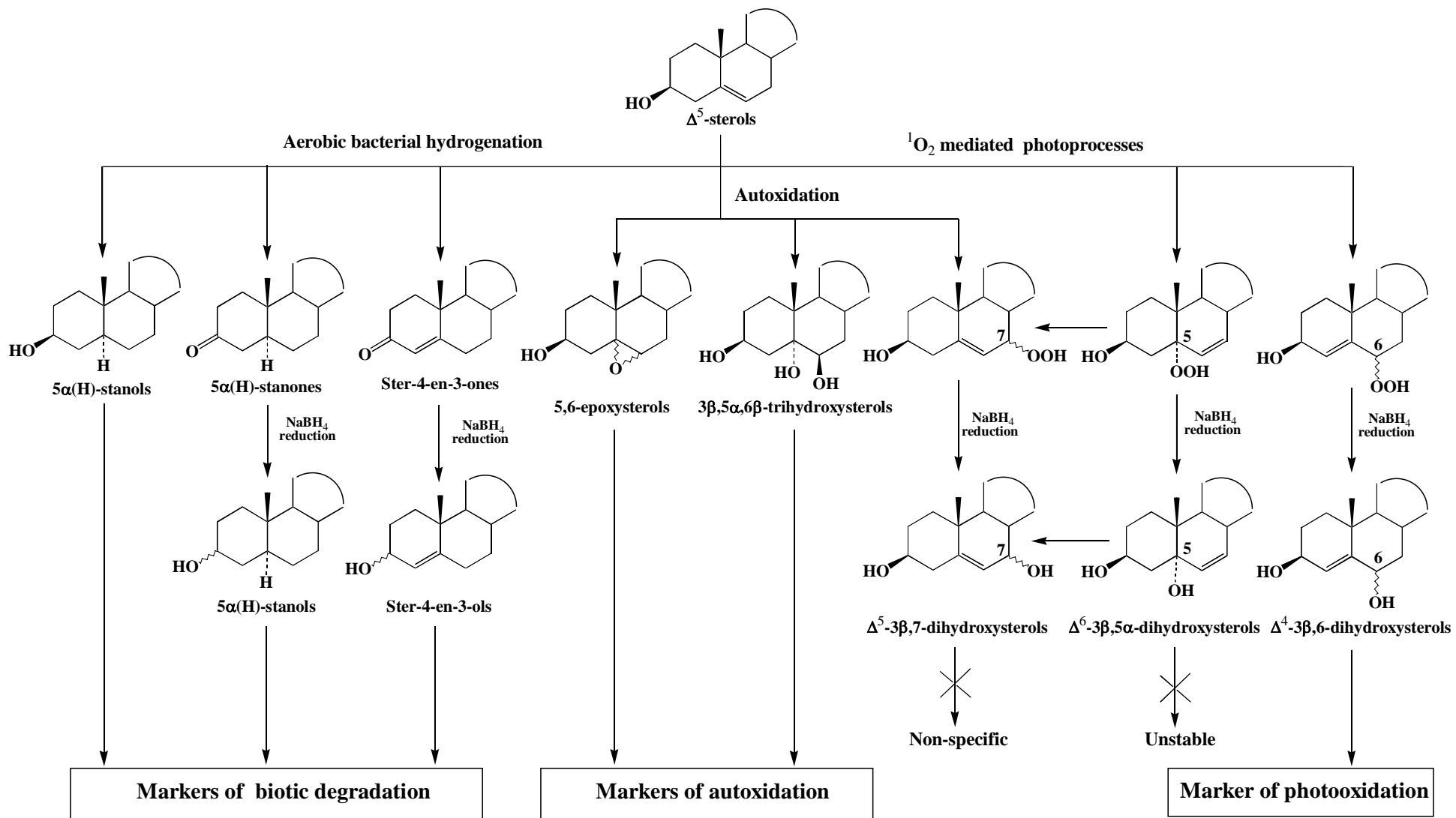
<sup>c</sup> Trace amounts (< 0.1%).

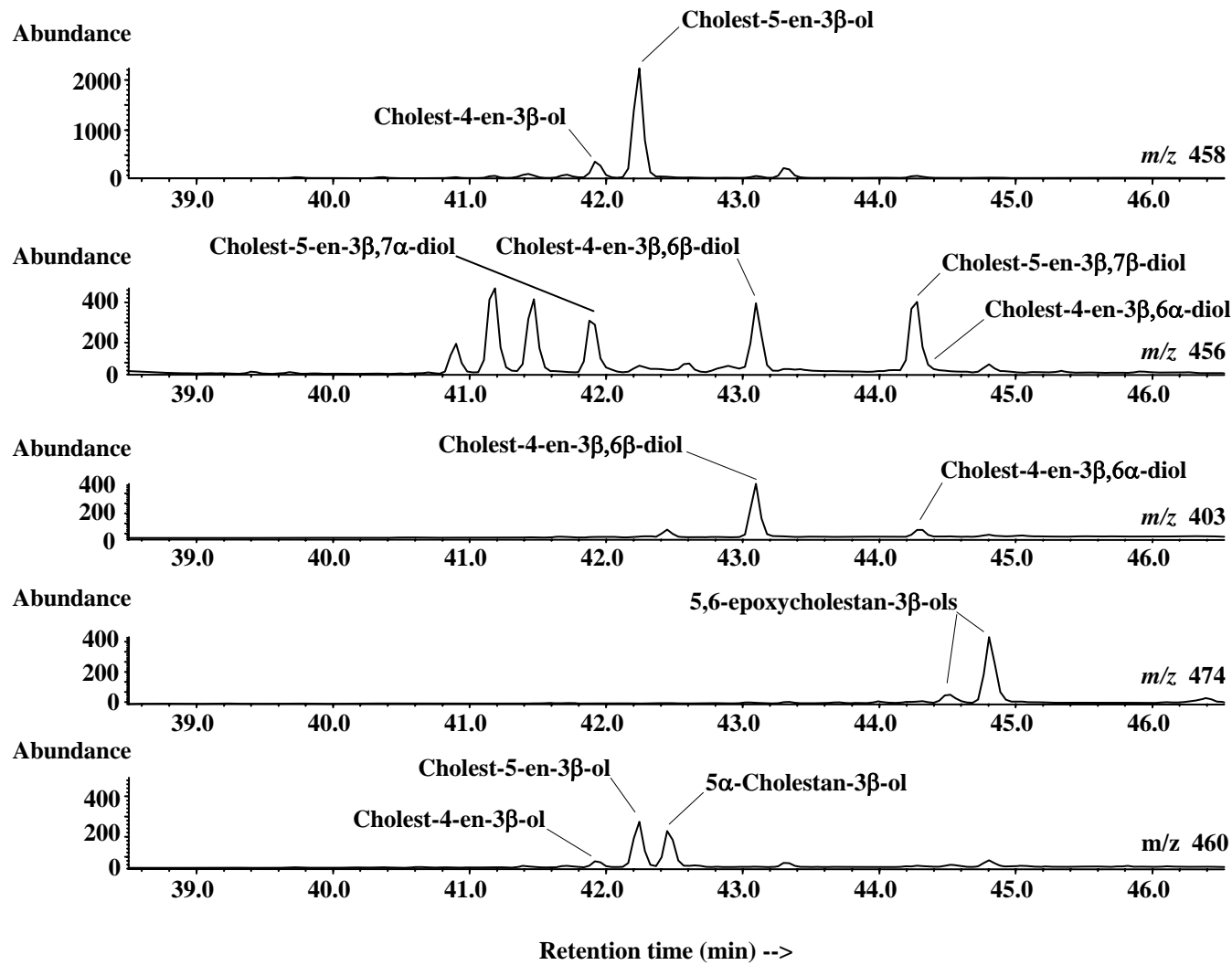
<sup>d</sup> Without correction of stereomutation

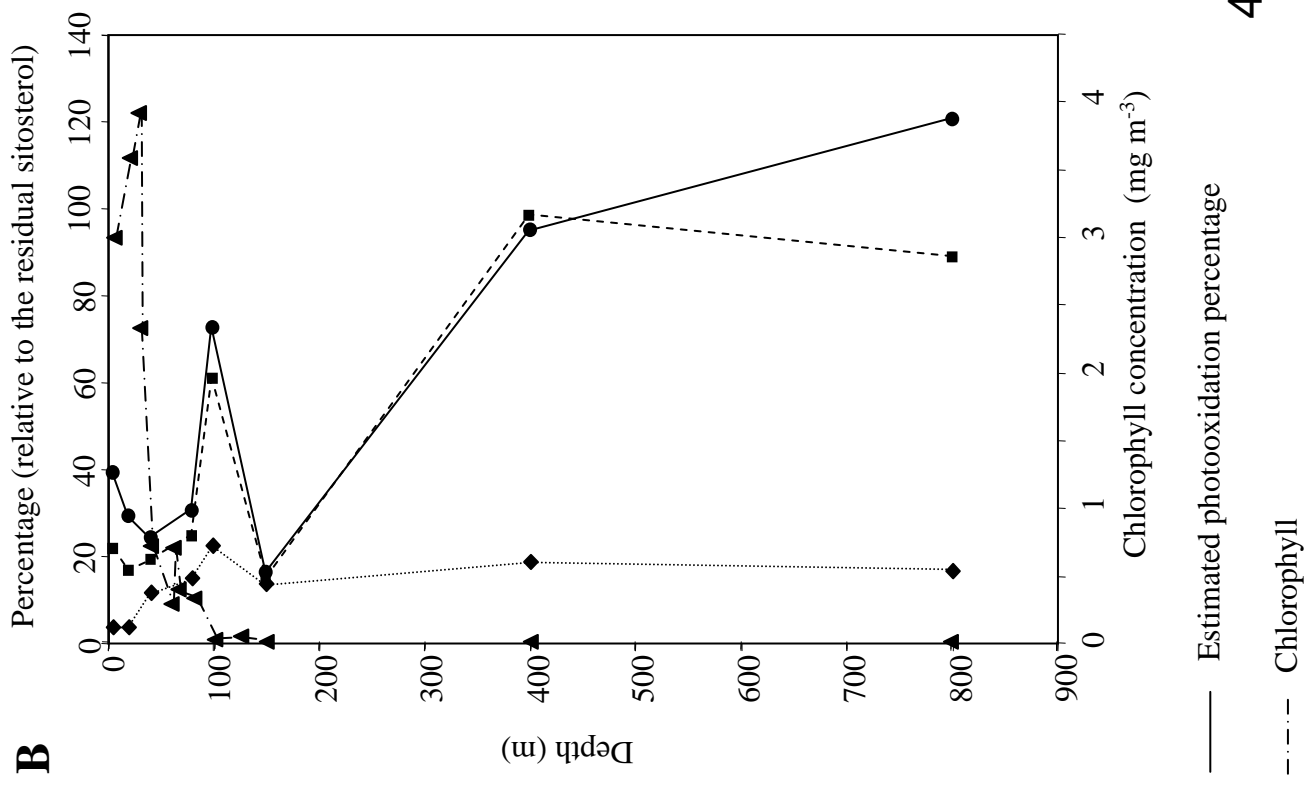
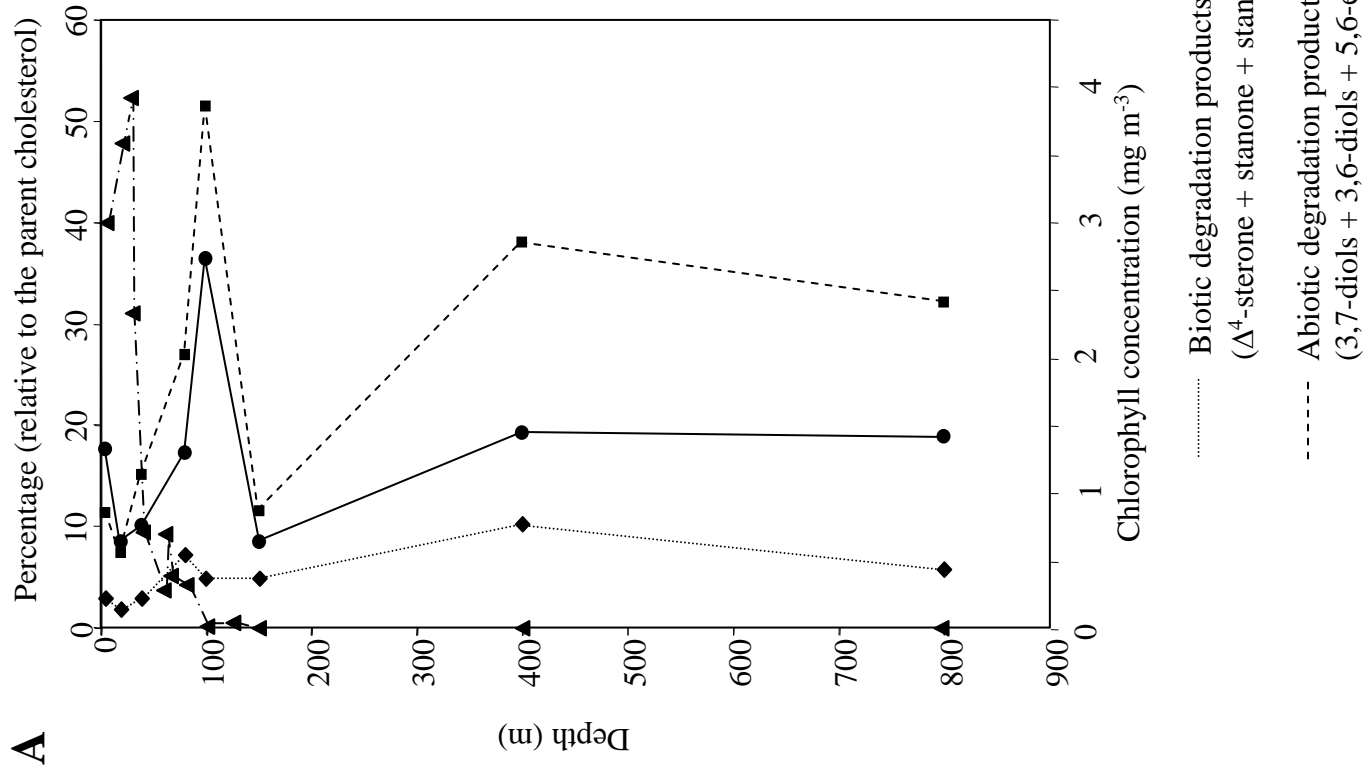
<sup>e</sup> With correction of stereomutation

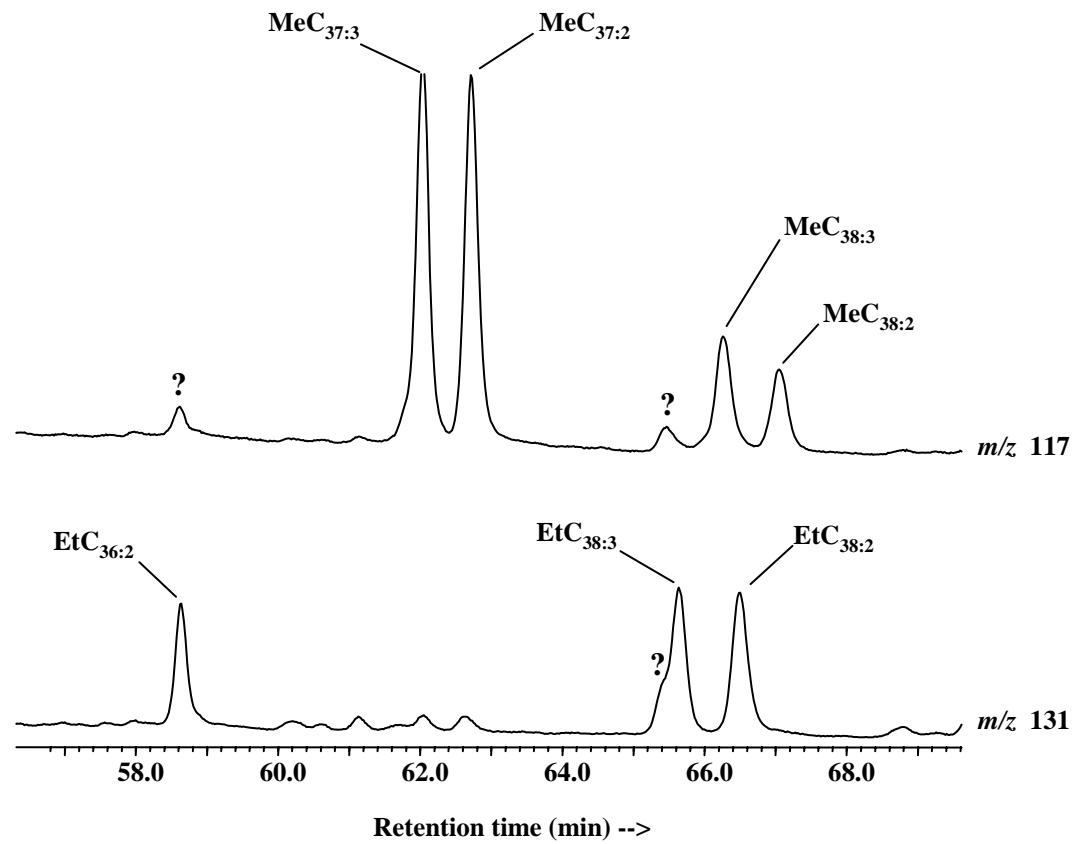
<sup>f</sup> Based on the Ternois et al. (1997) equation



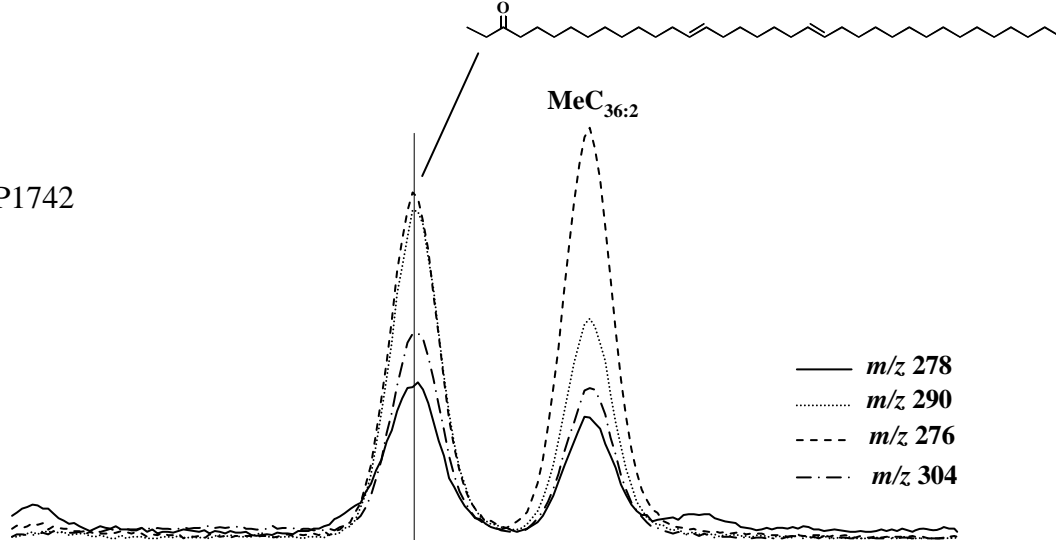




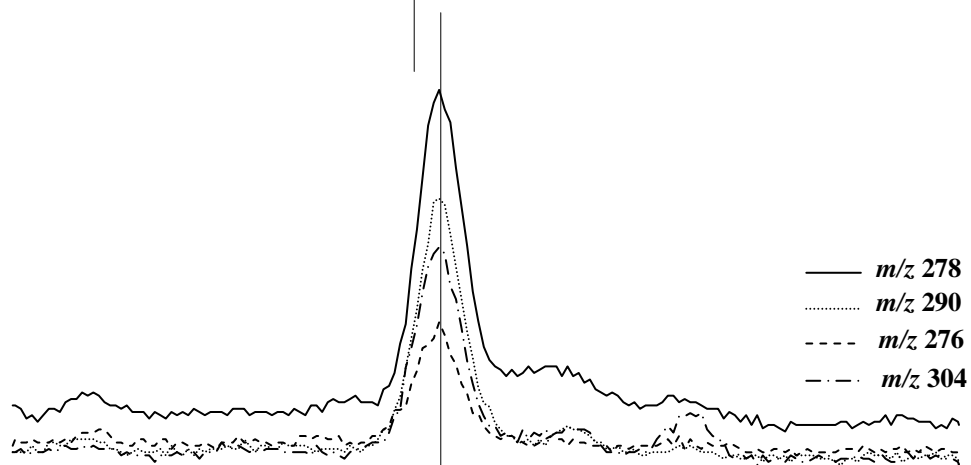




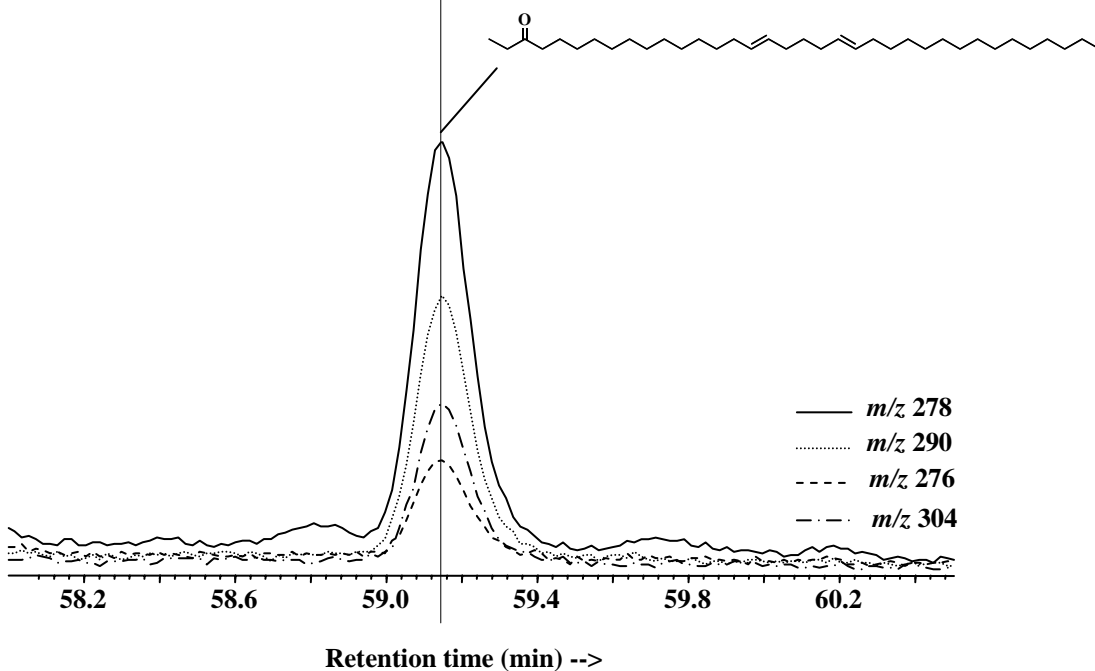
**A**  
*E. Hux* CCMP1742

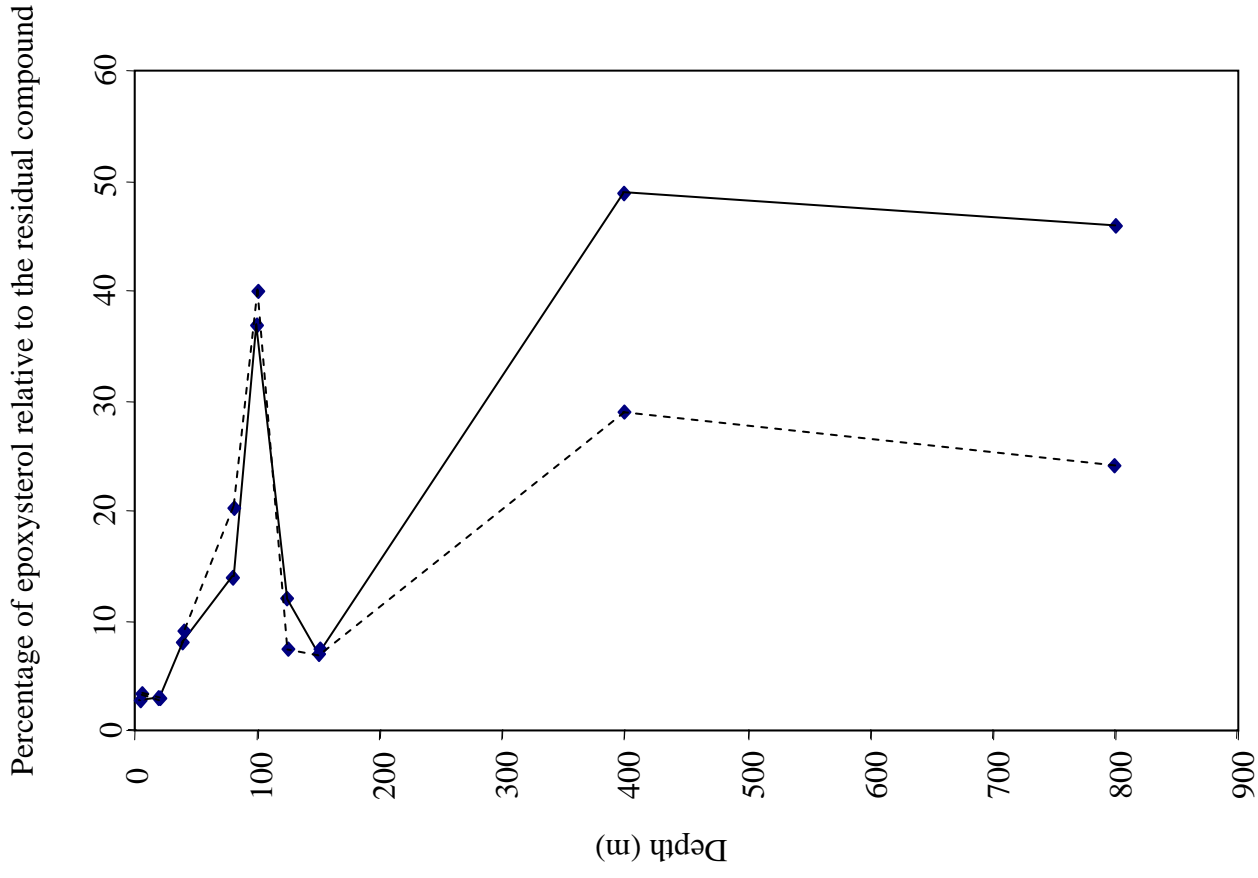
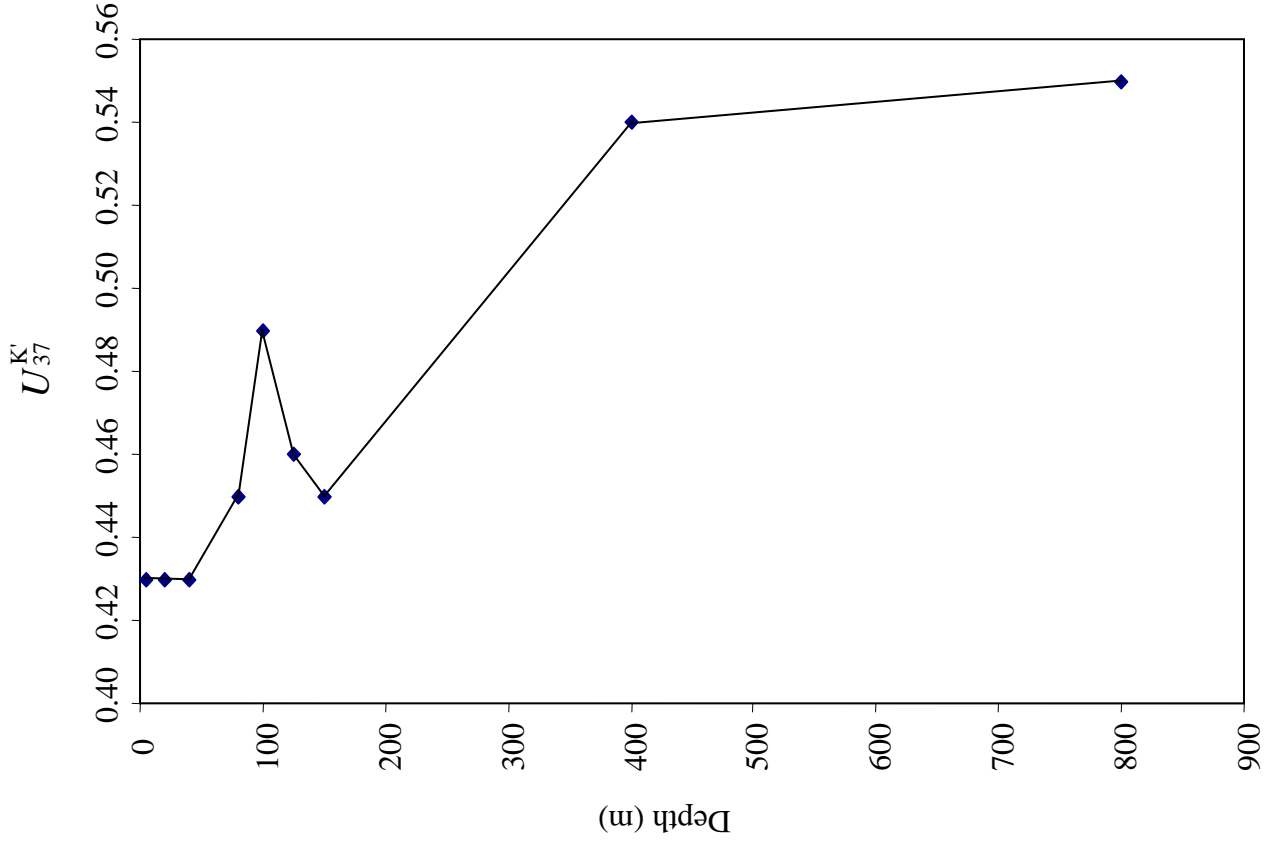


**B**  
Medflux



**C**  
Black Sea





----- 5,6-Epoxycholestan-3 $\beta$ -ol

— 5,6-Epoxy-24-ethylcholestan-3 $\beta$ -ol

