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1 **Assimilation of marine extracellular polymeric substances by**  
2 **deep-sea prokaryotes in the NW Mediterranean Sea**

3

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5

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11

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15

16 Running title: EPS assimilation by deep-sea prokaryotes

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18

19 **Abstract**

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21 This study examined total uptake of extracellular polymeric substances (EPS) and  
22 glucose and the percentage of prokaryotic cells (Bacteria, Crenarchaea and  
23 Euryarchaea) consuming these compounds in the major water masses at the DYFAMED  
24 site (NW Mediterranean Sea). The potential assimilation rates of EPS at 10m-depth  
25 were higher but on the same order of magnitude as those at 2000m-depth (from 43.4 to  
26 29.0 pmol L<sup>-1</sup> h<sup>-1</sup>). In contrast, glucose assimilation rates decreased with depth from  
27 49.4 to 0.07 pmol L<sup>-1</sup> h<sup>-1</sup> at 10 and 2000m-depth, respectively. Microautoradiography  
28 analyses indicated similar percentages of active cells assimilating EPS at 10 and 2000  
29 m-depth (13% and 10% of the total-cells). The combination of microautoradiography and  
30 catalyzed reporter deposition fluorescence *in situ* hybridization (MICRO-CARD-FISH)  
31 analyses revealed that the percentages of Bacteria assimilating <sup>3</sup>H-carbohydrates  
32 decreased with depth by 2-fold for EPS. In contrast, the contribution by Euryarchaea to  
33 EPS consumption increased with depth by 6-fold. At 2000m, 50% of active cells  
34 consuming <sup>3</sup>H-carbohydrates were Euryarchaea. These data highlight potential  
35 differences in the roles of Bacteria and Archaea in the deep sea biogeochemical cycles  
36 and shed light on the importance of deep-sea Euryarchaea in the degradation of  
37 dissolved organic matter.

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## 39 1 *Introduction*

40 Over the last decade, research has unequivocally shown that prokaryotes (Bacteria  
41 and Archaea) are the main drivers of marine biogeochemical cycles in the ocean. The  
42 dark ocean, below the euphotic zone, is characterized by the increase of hydrostatic  
43 pressure with depth, the decrease of temperature and the sporadic input of organic  
44 matter. Deep-sea microorganisms display unique metabolic capabilities with respect to  
45 the degradation of complex organic matter (Vezi et al., 2005; DeLong et al., 2006),  
46 molecular architectures (Lauro and Bartlett, 2007) and metabolic activities adapted to *in*  
47 *situ* conditions (Deming et al., 1980; Tabor et al., 1981; Jannasch and Wirsen, 1982;  
48 Tamburini et al., 2003) that allow them to thrive under cold and high-pressure conditions.  
49 Data from fluorescence *in situ* hybridization (FISH) studies indicates that Archaea make  
50 up a larger fraction of total prokaryotic abundance in the dark ocean than in the surface  
51 waters of the North Pacific and North Atlantic Oceans (Karner et al., 2001; Teira et al.,  
52 2004; Herndl et al., 2005) and of the Mediterranean Sea (Tamburini et al., 2009; Winter  
53 et al., 2009). However, little is still known about the metabolic capacity and  
54 biogeochemical roles of both Bacteria and Archaea in the dark ocean. Using  
55 microautoradiography with FISH (or catalyzed reporter deposition-FISH, CARD-FISH), it  
56 is possible to link assimilation of specific radiolabeled compounds to individual members  
57 of the prokaryotic community. Recent studies suggest that some Archaea are  
58 chemoautotrophic, able to fix CO<sub>2</sub> (Herndl et al., 2005) and some are heterotrophic (or  
59 mixotrophic) assimilating for example amino acids in the North Atlantic Ocean (Herndl et

60 al., 2005; Teira et al., 2006b). However, the role of marine Archaea in assimilating other  
61 organic compounds is still not clear.

62 Semi-labile dissolved organic matter (DOM) is defined as the fraction of the total DOM  
63 pool that escapes rapid degradation by marine heterotrophs in surface waters and is  
64 available for export to the dark ocean (Carlson et al., 1994). Semi-labile DOM is made  
65 up of a variety of compounds including extracellular polymeric substances (EPS) which  
66 includes complex organic macromolecules such as lipids, nucleic acids, proteins and  
67 carbohydrates. Carbohydrates are the most abundant component, generally  
68 representing 40 to 95% of the EPS in marine environments (Hoagland et al., 1993;  
69 Bhaskar et al., 2005). Chemically, EPS are rich in high molecular weight  
70 polysaccharides, thus, EPS are considered as a part of high molecular weight dissolved  
71 organic matter (HMW-DOM > 1kDa) (Aluwihare et al., 1999; Bhaskar et al., 2005).  
72 Within high molecular weight (HMW)-DOM, carbohydrates represents 40 to 50% in  
73 surface waters of DOM and 10 to 30% in the deep-sea waters (Benner et al., 1992;  
74 Aluwihare et al., 1997). Lastly, the deep Mediterranean water masses may represent a  
75 peculiar case since they are regularly enriched by organic carbon input during deep  
76 water formation events (open-sea convection as well as dense shelf water cascading)  
77 (Canals et al., 2006; Santinelli et al., 2010). The aim of this study was to determine the  
78 capacity of deep-sea prokaryotes to degrade and assimilate complex carbohydrates (<sup>3</sup>H-  
79 EPS) by measuring both bulk and single-cell activity measurements. We used a simple  
80 carbohydrate (<sup>3</sup>H-glucose) as an indicator of the assimilation of labile compounds.  
81 Experiments were conducted in triplicate at three depths in the water column at the

82 DYFAMED site (NW Mediterranean Sea). To accurately measure prokaryotic activities in  
83 the deep-sea waters, we performed our sampling, retrieval and incubation under *in situ*  
84 temperature and pressure conditions (see Supporting Information for more details) using  
85 our high-pressure sampler (Bianchi et al., 1999; Tamburini et al., 2003).

86

## 87 *2 Results and discussion*

### 88 *Microbial assimilation rates*

89 The potential assimilation rates of EPS ranged from 43.4 pmol L<sup>-1</sup> h<sup>-1</sup> at 10m to 29.0  
90 pmol L<sup>-1</sup> h<sup>-1</sup> at 2000m-depth (Table 1). In contrast, glucose assimilation rates (glucose  
91 being added at “trace” concentration) diminished by three orders of magnitude from the  
92 surface to the deep waters (from 49.4 to 0.07 pmol L<sup>-1</sup> h<sup>-1</sup>). In order to determine the  
93 contribution of the total prokaryotic community to <sup>3</sup>H-EPS and <sup>3</sup>H-glucose assimilation,  
94 we divided the number of DAPI-positive cells that were silver grain positive as revealed  
95 by microautoradiography by the total-cells counts determined by DAPI staining (Table  
96 1). The percentage of the prokaryotic community actively assimilating <sup>3</sup>H-EPS was  
97 similar at 10 and 2000m-depth (13 ± 6% and 10 ± 1%). In contrast, the percentage of  
98 total prokaryotes actively assimilating <sup>3</sup>H-glucose decreased with depth from 34% at 10  
99 m to 5% at 2000m-depth (Table 1). In bathypelagic waters of North Atlantic Ocean,  
100 microautoradiography analyses (Herndl et al., 2005; Teira et al., 2006b) indicated that  
101 about 5% and 16% of the heterotrophic prokaryotic community take up D- and L-aspartic  
102 acid and leucine. The potential assimilation of <sup>3</sup>H-EPS and <sup>3</sup>H-glucose has been studied  
103 only in the surface waters of the Delaware Estuary (Elifantz et al., 2005) and of the

104 Arctic Ocean (Elifantz et al., 2007; Kirchman et al., 2007). In the Arctic, a higher fraction  
105 of cells assimilated EPS (35 to 50%) than glucose (10 to 30 %) while most of the surface  
106 bacterial groups in the Delaware were more active in assimilating glucose than  $^3\text{H}$ -EPS  
107 (Kirchman et al., 2007). Different trophic conditions in surface waters of Delaware  
108 Estuary and Arctic Ocean could explain differences found in glucose and EPS  
109 assimilation by prokaryotic communities (Elifantz et al., 2007; Kirchman et al., 2007). In  
110 the present study, we estimated that the percentage of cells assimilating glucose is 3  
111 times higher than the percentage of cells assimilating  $^3\text{H}$ -EPS in surface waters while in  
112 deep waters the percentage of cells assimilating  $^3\text{H}$ -EPS is 2 times higher than for  $^3\text{H}$ -  
113 glucose (see Table 1).

114

#### 115 *$^3\text{H}$ -compounds specific-cell activities*

116 In order to determine the specific activity per cell according to the depth and the  
117 substrate assimilated, we divided the  $^3\text{H}$ -EPS or  $^3\text{H}$ -glucose assimilation rates by the  
118 number of silver grain-positive cells (consuming tritiated substrates), as revealed by  
119 microautoradiography. Deep-sea prokaryotes assimilated more EPS than surface  
120 prokaryotes ; the cell-specific assimilation rate increased (more than 6-fold) from 0.7 at  
121 10m to 4.5 amol assimilating cell<sup>-1</sup> h<sup>-1</sup> at 2000m-depth (Figure 1A). For the glucose, the  
122 opposite trend was observed with a decrease of one order of magnitude from 0.2 at 10m  
123 to 0.01 amol cell<sup>-1</sup> h<sup>-1</sup> at 2000m-depth (Figure 1B). Growth of deep-sea prokaryotes is C-  
124 limited and deep-sea prokaryotes deployed high levels of ectoenzymatic activities to  
125 recover carbon and energy coming from complex organic matter (Nagata et al., 2000;

126 Hoppe et al., 2002; Tamburini et al., 2009; Baltar et al., 2010). Also, EPS being a  
127 complex mixture of compounds including mono- and polysaccharides, lipids, proteins  
128 and non-sugar moieties (Bhaskar et al., 2005), they provide a large spectrum of  
129 macromolecules to deep-sea prokaryotic assemblage. When faced with a lack of  
130 nutrient sources, heterotrophic prokaryotes could be better adapted to a “mixed  
131 substrate growth” as suggested by Egli (2010).

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### 133 *Contribution of phylogenetic groups assimilating $^3\text{H}$ -compounds*

134 Using microautoradiography coupled with CARD-FISH, we compared throughout the  
135 water column the contribution of Bacteria, Crenarchaea and Euryarchaea to the  
136 degradation and assimilation of HMW and LMW compounds ( $^3\text{H}$ -EPS and  $^3\text{H}$ -glucose).  
137 We calculated for each phylogenetic group and substrate the number of active cells that  
138 were probe and silver grain-positive. We normalized the contribution of each group to  
139 the number of total-cells that were silver grain-positive for  $^3\text{H}$ -EPS and  $^3\text{H}$ -glucose  
140 (Figure 2).

141 In the upper layer, Bacteria dominated the assimilation of  $^3\text{H}$ -EPS and  $^3\text{H}$ -glucose,  
142 representing 74% and 88% of cells consuming  $^3\text{H}$ -compounds, respectively (Figure 2A  
143 and B), in concordance with data obtained in surface waters of the Arctic Ocean (Elifantz  
144 et al., 2005; Kirchman et al., 2007). Crenarchaea and Euryarchaea contributed less than  
145 20% to the  $^3\text{H}$ -EPS and 10% to the  $^3\text{H}$ -glucose assimilation (Figure 2A and B).

146 At 2000m-depth, Bacteria represented 38% of the assimilation of  $^3\text{H}$ -EPS and 32% of  
147 the assimilation of  $^3\text{H}$ -glucose (Figure 2A and B). Crenarchaea contributed weakly to  $^3\text{H}$ -



148 EPS and  $^3\text{H}$ -glucose assimilation. Several authors have shown that Crenarchaea in the  
149 dark ocean are mixotrophic or chemoautotrophic (Herndl et al., 2005; Ingalls et al., 2006;  
150 Kirchman et al., 2007) and are ammonium-oxidizers as revealed by the presence of the  
151 *amoA* gene (Francis et al., 2005; Konneke et al., 2005; Hallam et al., 2006). Ingalls *et al.*  
152 (2006) suggested that the use of organic carbon through mixotrophy or heterotrophy  
153 might be associated with the need to derive energy. Herndl *et al.* (2005) showed that  
154 Euryarchaea also assimilate inorganic carbon, but their contribution decreased with  
155 depth. Their heterotrophy capacity has also been highlighted (Herndl et al., 2005; Teira  
156 et al., 2006a; Teira et al., 2006b), since this archaeal group is also able to assimilate  
157 amino acids and leucine. In this experiment, Euryarchaea were the main contributors to  
158  $^3\text{H}$ -EPS and  $^3\text{H}$ -glucose assimilation at 2000m-depth, representing respectively 58%  
159 and 53% of the total cells actively assimilating  $^3\text{H}$ -EPS and  $^3\text{H}$ -glucose (Figure 2A and  
160 B). This result, provided from a snapshot view performed in deep-sea waters of the  
161 Mediterranean Sea at the DYFAMED Site, highlights a potential major role of  
162 Euryarchaea in the deep-sea waters. Consequently, more experiments are required to  
163 generalize the role of Euryarchaea in the carbon cycle.

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### 165 *3 Concluding remarks*

166 Our study supplies new clues about the interactions between deep-sea prokaryotes  
167 and the degradation of different size classes of DOM. In this preliminary work, we have  
168 performed an original experiment taking into account *in situ* conditions (of pressure and

169 temperature). These conditions are potentially crucial to correctly estimating the deep-  
170 sea prokaryotic capacity to degrade complex organic matter (Vezi et al., 2005).

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184 **Figure captions**

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186 Figure 1: The specific-cell activity assimilating **(A)**  $^3\text{H}$ -EPS and **(B)**  $^3\text{H}$ -Glucose ( $\text{amol cell}^{-1} \text{ h}^{-1}$ ) at 10m,  
187 500m and 2000m-depth. **Note the different scale between figure A and B.** Errors bars indicate standard  
188 deviation ( $n=3$ )

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190 Figure 2: Contribution of Bacteria, Crenarchaea and Euryarchaea to  $^3\text{H}$ -EPS degradation **(A)** and  $^3\text{H}$ -  
191 glucose assimilation **(B)** at 10, 500 and 2000m-depth, respectively, at DYFAMED site (NW Med. Sea).  
192 Contribution expressed as percentage of total silver grain and DAPI-positive cells as determine by  
193 microautoradiography. Errors bars indicate standard deviation ( $n=3$ )

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206 Table 1: Assimilation rates ( $\text{pmol L}^{-1} \text{h}^{-1}$ ) and percentage of cells consuming  $^3\text{H-EPS}$  and  $^3\text{H-Glucose}$  by  
207 the total prokaryotic community at 10, 500 and 2000m-depth at the DYFAMED site in April 2008.  
208 Percentages assayed by microradiography (DAPI silver grain-positive) and were calculated relative to  
209 the total prokaryotic community (DAPI total counts). Mean values  $\pm$  SD are given (n=3).

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		Consumption rates (pmol L <sup>-1</sup> h <sup>-1</sup> )	% of cell consuming <sup>3</sup> H-compounds (% from total DAPI-cells)
	10m	43.3 ± 4.2	13.1 ± 6.2
EPS	500m	33.9 ± 4.2	2.8 ± 1.7
	2000m	27.0 ± 3.4	10.6 ± 1.4
	10m	49.3 ± 0.84	34.1 ± 4.5
Glucose	500m	0.1 ± 0.01	2.6 ± 0.9
	2000m	0.1 ± 0.02	4.8 ± 1.1

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