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Sticking properties of transparent exopolymeric particles (TEP) during aging and biodegradation

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Keywords: TEP, Bacterial production, Bacterial respiration, DOC, residence time, carbon cycling.

27 **Abstract**

28

29 Although many studies have addressed the role of bacteria in the degradation of organic  
30 matter, few have examined how bacteria alter the physico-chemical properties of dissolved  
31 and colloidal organic matter in coastal systems. Here we investigate how the sticking  
32 properties of Transparent Exopolymeric Particles (TEP) varied with DOM age in batch  
33 cultures. We show that in two contrasted sites, despite different initial TEP sticking properties  
34 and bulk concentrations, after 48 hours, the sticking properties were similar and increased  
35 (i.e., TEP became stickier) with increasing DOM age. We propose that TEP occurring after 48  
36 hours of incubation are mainly of heterotrophic origin, which is in contrast to the previously  
37 identified TEP of autotrophic origin. These results highlight the potential importance of  
38 bacterial DOM production, particularly in the aphotic zone, and further underline the potential  
39 of bacterial heterotrophs to produce biologically refractory dissolved organic matter that is  
40 physically reactive (i.e. sticky).

## 41 **1. Introduction**

42

43 Dissolved organic carbon (DOC) comprises the largest pool of bioreactive carbon in  
44 the oceans (e.g. Hansell and Carlson, 2002). This large and highly diverse pool of organic  
45 molecules is made up of a wide range of carbon molecules differing dramatically in both  
46 molecular weight and bioavailability. This complexity has rendered the chemical  
47 identification of many of the carbon moieties extremely difficult and up to 80% remains  
48 unidentifiable (Benner, 2002). The main result of this is that DOC concentrations are often  
49 measured as a bulk property and little consideration is given to how concentrations and  
50 properties of different fractions change over time.

51 Whilst DOC is the dominant form of organic carbon in aquatic systems, particulate  
52 and colloidal organic carbon (POC and COC, respectively) can represent a biologically  
53 important, though often small, percentage of the total organic carbon. Moreover, the transfer  
54 of matter between these pools is highly dynamic with colloidal particles forming a dynamic  
55 bridge between the DOC and POC pools (Verdugo et al., 2004). Transparent exopolymeric  
56 particles (TEP) are COC structures, comprised of acidic polysaccharides (Mopper et al., 1995;  
57 Zhou et al., 1998). They are ubiquitous in the aquatic environment and can represent up to  
58 50% of the standing stock of POC in a system (Passow, 2002).

59 Bioreactivity or bioavailability describes the biological remineralisation rates of  
60 organic matter (e.g. Amon and Benner, 1996; Raymond and Bauer, 2000; Rochelle-Newall et  
61 al., 2004). This remineralisation is dependent upon several factors, notably the chemical  
62 structure and molecular size of the organic matter (Amon and Benner, 1996), the availability  
63 of organic or inorganic nutrients and the C:N ratio of the material (e.g. Kroer, 1993) or the  
64 degree of photolysis (e.g. Obernosterer and Herndl, 2000). Although the latter of these is a  
65 physical process, the first two are related either directly or indirectly to the source and age of  
66 the organic matter. For example, it is generally considered that marine organic matter is more  
67 bioavailable than riverine or marsh derived organic matter (del Giorgio and Davis, 2003).  
68 Similarly, it is generally considered that organic matter released during photosynthesis,  
69 constituted mainly of carbohydrates (Biersmith and Benner, 1998), is more bioavailable than  
70 the 'background' organic matter in marine systems although this bioavailability does vary  
71 with the phytoplankton species present (e.g. Renaud et al., 2005). Wild et al.(2004) have also  
72 demonstrated that coral reef mucus is highly bioavailable to the surrounding bacterial  
73 communities, thus providing a potential major source of available organic matter in otherwise  
74 oligotrophic waters. This increased bioavailability of phytoplanktonic and coral reef organic

75 matter may well be one of the reasons why we generally observe a tighter relationship  
76 between bacterial production and primary production in oligotrophic systems than what is  
77 observed in more mesotrophic systems (Morán et al., 2002).

78         Sinking organic matter from the mixed layer of aquatic systems is a major pathway via  
79 which organic carbon is exported to the sediments. However, in order to sink, organic matter  
80 needs to have a density sufficiently high to ensure that it sinks rather than floats. Evidently,  
81 larger particles such as large diatoms will fall through the water column and it has recently  
82 been shown that even picoplankton contribute to this export (Richardson and Jackson, 2007),  
83 however, it is less clear what controls the export of dissolved organic matter to the deeper  
84 layers. Recent work has shown that TEP play an important role in the vertical transport of  
85 dissolved organic matter by coalescing and aggregating with dense particles to form larger,  
86 more dense aggregates that then sink (Azetsu-Scott and Passow, 2004; Engel et al., 2004).  
87 However, the factors controlling the sticking properties of TEP, the process that allows the  
88 formation of large aggregates, have not yet been clearly identified. While it is known that the  
89 presence of metals can alter the sticking properties and hence reduce the formation TEP (Mari  
90 and Robert, 2008), less is known about the impact of aging and bacterial degradation on the  
91 sticking properties of TEP.

92         Recently, it was proposed that residence time of organic matter plays a role in  
93 determining whether or not organic matter is exported from a system (Mari et al., 2007). Here  
94 we present the results from an incubation experiment aimed at testing the impact of aging on  
95 the sticking properties of TEP. The objective of this study was to determine how the sticking  
96 properties of TEP altered with age of the DOM pool at two contrasting sites subject to  
97 different flushing rates.

98

## 99 **2. Material and Methods**

100

101         Samples were collected from two stations in the Southwest lagoon of New Caledonia (see  
102 Mari et al., 2007 for map). The first station (Stn. Lag) is located close to the coral barrier reef  
103 system and is considered to be isolated from anthropogenic influences. The second station is  
104 located within the Grande Rade Bay (Stn. Ind). This bay is heavily influenced by both  
105 industrial and urban effluents. Furthermore, the average e-flushing times for the two stations  
106 differ, with Stn. Lag having a lower e-flushing time than that of Ind: 0.5 and 45 days,  
107 respectively (Jouon et al., 2006).

108 Sample water (40 L) was collected from each station at a depth of 3 m with a 10 L Niskin  
109 bottle. The sample was transported back to the laboratory in acid cleaned, polycarbonate 20 L  
110 carboys (Nalgene). Upon return to the laboratory, 25 L of water were pre-filtered through a  
111 1.0  $\mu\text{m}$  membrane filter (Whatman Cyclopore) in order to remove larger particles and grazers.  
112 Two acid-washed (10% HCl) and Milli-Q water rinsed, polycarbonate (Nalgene) carboys  
113 were then filled with 20 L of 1.0  $\mu\text{m}$  filtered water. Both before and after filtration, samples  
114 were collected for Chlorophyll *a* (Chl *a*), nutrient and, DOC concentration in order to  
115 determine the efficacy of the filtration and to control for any nutrient contamination arising  
116 from the manipulations. No differences were found between the before and after filtrations for  
117 nutrients and DOC (data not shown).

118 The two carboys were incubated in the dark and at constant temperature (24 °C) during 32  
119 days. Samples were collected at T0 and after 1, 2, 3, 5, 8, 11, 17 and 32 days for analysis of  
120 various biological and chemical parameters, as detailed below.

121 Samples for bacterial abundance (1.8ml) were fixed with glutaraldehyde (final conc.  
122 0.5%) and stored at -80°C until enumeration. Abundance was determined after staining with  
123 SYBR Green I (Molecular Probes; 1: 25,000 dilution of commercial stock) for 10 min at room  
124 temperature using a flow cytometer (FACSCalibur; Becton Dickinson) equipped with a 488-  
125 nm Argon laser (Gasol and Del Giorgio, 2000).

126 Bacterial production (BP) was measured using  $^3\text{H}$ -leucine, following the method of Smith  
127 and Azam (1992). Briefly, triplicate 1.5 mL sub-samples plus one killed control were  
128 incubated for 1 h in the presence of 20 nM (final concentration) high specific activity  $^3\text{H}$ -  
129 leucine (Perkin Elmer) at the incubation temperature. The coefficient of variation (CV) of the  
130 triplicate measurements was always less than 5%. Leucine uptake was converted to carbon  
131 using the conversion factor 1.55 kg C mol<sup>-1</sup> leu (Kirchman, 2001).

132 Bacterial respiration (BR) was measured at each time point using the oxygen  
133 microelectrode technique of Briand et al. (2004). The microprobes (Unisense, Denmark) are  
134 designed with an exterior guard cathode (Revsbech 1989), which results in extremely low  
135 oxygen consumption by the electrodes (4.7- 47 x 10<sup>-7</sup>  $\mu\text{mol O}_2 \text{ h}^{-1}$ ). Probes have a response  
136 time shorter than 1 second and a precision of 0.05%. The precision of oxygen microprobe is  
137 equivalent to highly precise Winkler techniques (Briand et al., 2004).

138 Bacterial Carbon Demand (BCD) was calculated as the sum of BP and BR. Finally,  
139 bacterial growth efficiency (BGE; %) was calculated as BP/BCD \* 100.

140 Chlorophyll *a* was determined fluorometrically (Turner Designs Trilogy) on 50 mL  
141 samples collected on GF/F (Whatman) glass fibre filters using the method of Holm-Hansen et

142 al. (1965). The filters were frozen (-80 °C) until measurement which was always within 72 h  
143 and generally within 24 h after sampling.

144 Nitrate and nitrite concentrations (DIN) were determined according to Raimbault et al.  
145 (1990) on a Bran+Luebbe Autoanalyzer III with an average detection limit and CV of 20  
146 nmol L<sup>-1</sup> and 3% and 2 nmol L<sup>-1</sup> and 8% for eutrophic and oligotrophic waters, respectively.  
147 Phosphate concentrations (DIP) were determined on a Bran+Luebbe Autoanalyzer III with an  
148 detection limit of 10 nmol L<sup>-1</sup> and an average CV of 6 to 11% between replicates (Torréon et  
149 al., 2007).

150 Particulate organic carbon (POC) and particulate nitrogen (PN) were measured on 1 L  
151 subsamples immediately filtered onto 25-mm Whatman GF/F filters pre-combusted at 550°C  
152 for 2 h. After filtration, the filters were dried at 60°C for 24 h and then frozen for later  
153 analysis. Analyses were carried out with a LECO-900 CHN analyzer calibrated with  
154 ethylenediaminetetraacetic acid standards. For the determination of organic carbon,  
155 carbonates were removed with 100 µL of 2 mol L<sup>-1</sup> HCl and renewed until there was no  
156 longer any effervescence. Acidified samples were kept in a drying oven (60–70°C) for 24 h.  
157 Detection limits were 40 and 10 µg for carbon and nitrogen, respectively.

158 Total organic carbon (TOC) analyses were performed on 10 mL sub-samples collected in  
159 pre-combusted (450°C, overnight) 10 mL glass ampoules, preserved with 12 µL 85%  
160 phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) and flame sealed. Samples were stored in the dark until analysis.  
161 TOC concentration was measured on a Shimadzu TOC VCPH analyzer, using potassium  
162 phthalate calibration standards over the measurement range 0 to 250 µmol C L<sup>-1</sup>. Certified  
163 reference materials (CRM; Hansell Laboratory, University of Miami) were also used to assess  
164 the performance of the instrument on and between measurement days. The machine blank was  
165 between 5 and 10 µmol C L<sup>-1</sup> for the measurement days and the CV of the measurement was  
166 always less than 2% of the mean of triplicate injections of duplicate samples. DOC was  
167 calculated by subtraction of the POC measurements from the TOC concentrations.

168 The sticking properties of transparent exopolymeric particles were studied largely  
169 following the method of Mari and Robert (2008). In brief, after filtration through the 1 µm  
170 membranes, 2 L of the filtrate originating from each station that contained sub-micrometer  
171 TEP precursors were put inside a mixing device composed of a grid oscillating vertically  
172 inside two 2 L Plexiglas cylindrical containers, which generates small-scale turbulence inside  
173 the containers under the control of a motor controlling the oscillation frequency. Polybead®  
174 Carboxylate Red Dyed microspheres of 6-µm (Polysciences, Inc.) were added in the filtrate to  
175 yield a theoretical final concentration ( $C_f$ ) of 5000 particles mL<sup>-1</sup>. This value is determined

176 from the manufacturers' product data sheet and the same volume of bead solution was added  
177 to each cylindrical container throughout the experiment. The formation of TEP-bead  
178 aggregates was followed during three successive 1 h periods, under increasing turbulence  
179 intensities. The turbulence kinetic energy dissipation rate,  $\epsilon$ , was set to 0.1, 1 and 10  $\text{cm}^2 \text{s}^{-3}$ ,  
180 during the first, second, and third hour of the experiment, respectively. Samples were  
181 collected every 15 min in order to determine TEP size spectra and the relationship between  
182 the numbers of attached beads vs. TEP size. TEP were stained with Alcian Blue (Alldredge et  
183 al. 1993). The TEP size spectra and the relationships between TEP size and numbers of TEP-  
184 attached beads were determined from 5 mL sub-samples filtered onto 0.2  $\mu\text{m}$  polycarbonate  
185 filters after transfer of the retained particles onto a microscope slide (Passow and Alldredge  
186 1994). TEP size spectra were determined microscopically for each slide by counting and  
187 sizing TEP at two successive magnifications (250x and 400x). Ten images were taken per  
188 slide and for each magnification. The equivalent spherical diameter (ESD) of individual TEP  
189 was calculated by measuring its cross-sectional area with an image-analysis system  
190 (ImagePro Plus, MediaCybernetics) and counts were combined and classified into 20  
191 logarithmic size classes (Mari and Burd 1998). The TEP volume concentration was calculated  
192 from the TEP size spectra assuming a spherical volume for each particle. Estimates of TEP  
193 carbon (TEP-C) concentration were obtained by combining TEP size spectra with the  
194 relationship giving the carbon content of a given TEP particle according to its size (Mari  
195 1999).

196 A relationship between TEP size and the number of attached beads was calculated for  
197 each sample by sizing individual TEP and enumerating its associated beads at 250x  
198 magnification using a compound light microscope. A minimum of 20 mixed aggregates of  
199 TEP-beads were studied for each slide. The number of attached beads was fitted to a linear  
200 relationship,  $n = ad_p + b$ , where  $n$  is the number of associated beads in the mixed aggregate,  $d_p$   
201 ( $\mu\text{m}$ ) is the TEP ESD, and  $a$  and  $b$  are constants for a given sample. Numbers of TEP-attached  
202 beads and TEP size were plotted in linear coordinates and the constants  $a$  and  $b$  were  
203 determined from regression analysis. The constant  $a$ , thereafter called the sticking slope,  
204 describes the ability of TEP to stick to beads (i.e. the higher  $a$  is, the higher the sticking  
205 properties are) and, thus, relates to the propensity of TEP to initiate the formation of large  
206 organic aggregates (Mari and Robert, 2008).

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### 210 3. Results

211

212 The *in situ* Chl *a* concentrations were higher at Stn. Ind (0.44 +/- 0.02  $\mu\text{g L}^{-1}$ ; mean +/-  
213 sd) than in Stn. Lag (0.24 +/- 0.02;  $\mu\text{g L}^{-1}$ ), however, after filtration the concentrations were  
214 the same (0.04  $\mu\text{g L}^{-1}$ , Table 1). *In situ* nutrient concentrations were also low and there was no  
215 difference between the initial concentrations and the concentrations measured immediately  
216 post filtration (data not shown).

217 Nutrient concentrations were low at both stations at the time of sampling (Table 1). In  
218 general, concentrations were higher at Stn. Ind, reflecting the coastal nature of this site and  
219 this general trend of higher nutrients at Stn. Ind continued throughout the 32 days experiment.

220 There was little difference between the initial DOC concentrations at the two sites.  
221 Both stations had initial concentrations of around 70  $\mu\text{M C}$  which decreased during the  
222 experiment. However, the amount of DOC removed during the incubation was higher in the  
223 Stn. Ind incubation than in Stn. Lag. Indeed, at Stn. Ind, 10% of the initial DOC was removed  
224 compared to only 7% at Stn. Lag.

225 The POC:PON molar ratios changed during the incubations. The C:N ratio in the Stn.  
226 Ind incubation was initially higher than that of Stn. Lag (19.5 and 4.7, respectively; Table 1).  
227 After 24 hours, the C:N ratio of Stn. Ind decreased to around 5 whilst that of Stn. Lag  
228 increased slightly to 8.6. Thereafter, the C:N molar ratio remained relatively constant for both  
229 incubations. At the end of the experiment the ratio was close to that of Redfield (7 and 6.2 for  
230 Stn. Ind and Stn. Lag, respectively). The only exceptions to this were on days 8 and 17 in Stn.  
231 Lag, where exceptionally high values of over 30 were observed.

232 Initial bacterial abundance was higher at Stn. Ind than Stn. Lag. In both incubation BA  
233 increased during the first 24 hrs and thereafter declined towards the end of the incubation  
234 (Fig. 1A). Initial bacterial production rates were low in the incubations (Fig. 1B). BP  
235 increased thereafter in the two incubations, with the increases in Stn. Ind being up to a factor  
236 of 5 higher than those of Stn. Lag. However, this peak in bacterial activity was transient and  
237 by day 5, the values had returned to low levels for both incubations. Respiration was initially  
238 low in Stn. Lag, peaked on day 3 and thereafter fell to near initial values (Fig. 1C). In  
239 contrast, in Stn. Ind, respiration was initially higher than in Stn. Lag and after a slight  
240 decrease on day 5, increased towards a maximum at day 32. The high bacterial production  
241 rates and relatively low bacterial respiration rates meant that bacterial growth efficiency  
242 (BGE) reached maximum values on day 1 in Stn. Ind (Fig. 1D). This was in contrast to that  
243 observed in the Stn. Lag incubation where BGE remained very low (<10%), with only a slight

244 increase on day 1; BGE then decreased in both incubations to attain the same, low levels  
245 towards the end of the incubation.

246 TEP-C concentration was very similar at Stn. Ind and Stn. Lag at the start of the  
247 experiment (3.1 and 2.7  $\mu\text{M C}$ , respectively; Fig. 2A). The TEP-C concentration then  
248 increased rapidly in Stn. Lag on day 1 to represent 14% of total DOC concentration and  
249 thereafter decreased to levels lower than the initial concentrations of 4%. TEP-C  
250 concentration in Stn. Ind also initially increased however, the increase was much less  
251 dramatic than in Stn. Lag, reaching only 6% of total DOC as opposed to 4% at the start of the  
252 experiment. By the end of the experiment, TEP-C concentration represented only 0.1% of  
253 total DOC.

254 The number of beads that stick to TEP is a measure of the sticking properties of the  
255 particles (Fig. 3, Table 2). Despite similar initial TEP-C concentrations, the number of beads  
256 that were stuck to each TEP particle was 4 times higher in Stn. Lag than in Stn. Ind (Figs. 2B,  
257 3, Table 2). However, this difference in sticking properties was transient and by day 2, there  
258 was no difference between the two stations. After day 2, the number of beads stuck to each  
259 TEP then increased towards the end of the experiment in both incubations to reach the same  
260 concentrations as was initially observed at Stn. Lag (Fig. 2B). Similarly, the sticking slope ( $a$ )  
261 which describes ‘the slope of the sticking properties curve’ (i.e. Fig. 3, Table 2) and is a  
262 measure of the ability of TEP to stick to the beads, was highest in Stn. Lag at the start of the  
263 experiment (Fig. 2C). By day 2,  $a$  had decreased to the same level as in Stn. Ind and both  
264 values then followed the same increasing trend towards the end of the experiment.

265 In order to assess the bioavailability of the organic matter and TEP-C for the two sites  
266 sampled, we examined the relationship between TEP-C concentration and BGE (Fig. 4). We  
267 observed an increasing linear trend between TEP-C concentration and BGE for Stn. Ind. The  
268 trend was less apparent for Stn. Lag. In other words, increases in TEP-C were related to  
269 higher increases in BGE in Stn. Ind than in Stn. Lag. If we consider that BGE can be used as  
270 proxy for bioavailability (Rochelle-Newall et al., 2004), the higher BGE per unit TEP  
271 observed in Stn. Ind suggests that TEP in this incubation was more bioavailable than that in  
272 Stn. Lag, despite there being a higher concentration of TEP in Stn. Lag.

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#### 278 **4. Discussion**

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280 Previous work from the southwest lagoon of New Caledonia has shown that marked  
281 gradients in inorganic and organic matter concentrations exist between the outer lagoon and  
282 the inner bays (Mari et al., 2007; Rochelle-Newall et al., 2008) and this has been shown to be  
283 related to the water residence times in this system (Migon et al., 2007; Torr eton et al., 2007).  
284 In this work, we failed to see similar marked gradients despite large differences in the average  
285 flushing time at the two sites, 0.5 and 45 days for Stn. Lag and Stn. Ind, respectively (Jouon et  
286 al., 2006). Indeed, the only bulk parameters that demonstrated a large difference were  
287 silicates, where we observed a factor of 6 difference in concentration between Stn. Ind and  
288 Stn. Lag (data not shown). The reason for the lack of difference in the other nutrients is  
289 probably due to the wind regime prior to sampling. The days preceding sampling were  
290 characterized by north westerly winds as opposed to the south easterly trade winds usually  
291 experienced in this site and such conditions generally favor an enhanced mixing of the water  
292 masses (Pinazo et al., 2004). Nevertheless, even despite the relatively small differences in  
293 bulk parameters, particularly in organic carbon concentrations, differences were observed  
294 when we examined the characteristics of the organic matter in terms of sticking properties and  
295 in terms of bacterial growth efficiency, at least during the first hours of the incubation.

296 BGE is a measure of both substrate properties and community capacity. Therefore,  
297 while it is clear that substrate quality is important, bacterial genetic functional diversity is also  
298 intrinsically linked with bioavailability (Rochelle-Newall et al., 2004). The differences  
299 observed in our experiments in BGE could therefore be due to available nutrient  
300 concentrations (Kroer, 1993), the age and the origin of the organic matter (Mari et al., 2007),  
301 or even the bacterial community composition present (Weinbauer et al., in press). Inorganic  
302 and organic nutrient limitation can have an impact on DOM bioavailability and BGE (Kroer,  
303 1993; Kroer, 1994) particularly in sites that are nutrient limited, such as oligotrophic water.  
304 Indeed, recently Jacquet et al. (2006) showed that at some periods of the year it is probable  
305 that at the Lag station, nitrogen is the most limiting nutrient. However, given the small  
306 differences in inorganic nutrient concentrations observed here, it is unlikely that there was a  
307 large difference in limitation between the two sites.

308 Renewal rates, or flushing times, also play a role in determining the age and  
309 concentrations the DOM (Mari et al., 2007). The flushing time of Stn. Lag is on the order of  
310 0.5 days and it is unlikely that large stocks of terrestrial organic matter are present in this  
311 water. Moreover, this site is adjacent to the coral reef barrier and it is probable that a

312 proportion of the DOC measured was of coral origin. Corals are known to produce large  
313 amounts of mucus and this mucus is bioavailable to bacteria (Moriarty et al., 1985; Vacelet  
314 and Thomassin, 1991; Wild et al., 2004). Moriarty et al (1985) found high glucosidase  
315 activities in coral mucus suggesting high carbohydrate concentrations. This may well explain  
316 the higher stickiness observed at the beginning of the incubation in the offshore station as the  
317 mucus is known to form aggregates and to enhance particle export (Wild et al., 2004). In  
318 contrast, Stn. Ind is located in a coastal bay that has a much longer flushing time (up to 45  
319 days). The longer flushing time leads to the accumulation of older organic matter originating  
320 from a wider range of sources (terrestrial, aquatic as well as industrial and urban) than that of  
321 the lagoon site. Moreover, this inshore station is subject to industrial inputs from the nearby  
322 nickel smelt as well as from untreated urban wastewater and runoff. Recent work (Mari and  
323 Robert, 2008) has shown that the presence of metal can reduce the stickiness of TEP and it is  
324 known that in the Stn. Ind metal concentrations are higher than those of the lagoon site  
325 (Migon et al., 2007).

326         Despite the initial differences in TEP stickiness, it is interesting to note that after day  
327 2, there were no significant differences between the number of beads stuck to TEP or the  
328 sticking slopes (Figs. 2, 3 and Table 2). This seems to indicate that whatever differences  
329 existed in TEP sticking potential between the two sites disappeared rapidly and were no  
330 longer measurable after 24 hours suggesting a bacterially-mediated homogenization of the  
331 DOC composition of the available DOC fraction. Moreover, from day 2 onwards sticking  
332 properties increased in the incubations towards the end of the experiment. This seems to  
333 indicate that the sticking properties of TEP increase with the age of organic matter. This poses  
334 an interesting question as recent work from this system, based on *in situ* measurements has  
335 proposed that freshly produced organic matter was more sticky than older organic matter  
336 (Mari et al., 2007). This is in contrast to what we observed in these incubations, perhaps  
337 pointing towards an alternative source of organic matter such as bacterial recycling of organic  
338 matter. Bacteria are known to release dissolved organic compounds which can represent up to  
339 25% of bacterial respiration in terms of carbon (Stoderegger and Herndl, 1998). This carbon  
340 is considered to be refractory and so it is probable that during batch incubations, such as those  
341 we performed, considerable accumulations of bacterially derived DOM occur, particularly in  
342 situations of low nutrient concentrations. Using the values of  $15.2 \text{ amol C cell}^{-1} \text{ h}^{-1}$  of  
343 Stoderegger and Herndl (1998), we calculated that the average rates of C release in the  
344 incubations varied between 4 and 19  $\text{pmol C h}^{-1}$  and between 5 and 25  $\text{pmol C h}^{-1}$ , for Stn Lag  
345 and Ind, respectively. These are just rough estimations of bacterial carbon release as our

346 incubation set up differed from that of Stoderegger and Herndl (1998), nevertheless, it gives  
347 us an estimate of the carbon transformation rates in the incubations. Over the period of the  
348 incubation, and if we consider that each C is used only once by the bacteria, we consider that  
349 approximately 5 to 6 nmol C were released by the bacteria. Considering that the final TEP-C  
350 content was 50 and 179nM C for Stn. Lag and Ind, respectively, we calculate that the  
351 potential contribution of bacterial C to TEP-C is of the order of 3 to 10 % of the total TEP-C  
352 pool.

353 We propose that this bacterially released DOM plays a role in the increased stickiness  
354 in the incubations. This reposes on the hypothesis of the existence of at least two types of  
355 “sticky-TEP”. The first type is a by-product of primary production as proposed by Mari et al.  
356 (2007) and by Engel (2000) for *in situ* and algal cultures, and by Wild et al (2004) for coral  
357 reef mucus, respectively. This autotrophically produced sticky TEP is probably highly  
358 bioavailable and has a relatively short half life (Engel, 2000; Grossart and Ploug, 2000; Wild  
359 et al., 2004), and as is evidenced by the rapid decrease in TEP sticking properties in Stn. Lag  
360 during the first 24 h of the incubation. This autotrophically produced sticky-TEP is subject to  
361 various factors that can alter its stickiness, such as the presence of heavy metals and changes  
362 in pH (Mari, 2008; Mari and Robert, 2008) and as pointed out by Mari et al. (2007) this  
363 potentially explains why the sticking properties of TEP in Stn. Ind are lower than that of Stn.  
364 Lag, despite higher slightly initial nutrient and chlorophyll *a* concentrations.

365 In contrast to the autotrophic production of sticky TEP, the second type of sticky TEP  
366 is heterotrophically produced through bacterial transformation of existing DOM. This TEP  
367 fraction accumulates within the bulk TEP pool with time and increases the overall sticking  
368 properties of the TEP pool, similar to the accumulations of bacterially produced CDOM in  
369 batch cultures (Rochelle-Newall and Fisher, 2002). Active bacteria produce muco-  
370 polysaccharidic capsular material (Luft, 1971; Stoderegger and Herndl, 1998) and this  
371 material forms aggregates and due to its polysaccharide content, is stained by Alcian Blue and  
372 is therefore included in the TEP pool. Moreover, even in the absence of freshly produced  
373 photosynthetic products, bacteria continue to produce this capsular material which  
374 accumulates in the water column due to its relatively recalcitrant nature (Stoderegger and  
375 Herndl, 1998). Thus effectively forming a shunt of semi-labile organic matter towards the  
376 refractory and hence weakly bioavailable, DOM pool.

377 While it is clear that we cannot rule out abiotic production of sticky TEP in our  
378 incubations, it is clear that despite differing initial DOM physico-chemical qualities, the final  
379 stickiness was similar. It is already known that metals can alter TEP stickiness (Mari and

380 Robert, 2008) and that sticky TEP is produced by autotrophic processes (e.g. Engel et al.,  
381 2004; Mari et al., 2007; Wild et al., 2004). Here we suggest that the hypothesis of a  
382 heterotrophic source of sticky TEP clearly merits further investigation. This potential pathway  
383 of organic carbon transformation further highlights the fact that bacteria should not just be  
384 considered as remineralisers of DOM but also as transformers of DOM in aquatic systems.  
385 The biogeochemical impacts of these transformations in terms of vertical transport of  
386 particles, particularly below the euphotic zone, away from autotrophic DOM production, need  
387 to be considered.

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542 **Figure legends**

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544 Figure 1. Bacterial abundance, respiration, production and growth efficiency in the  
545 incubations. A: bacterial abundance (BA,  $10^5$  cells/ml); B: bacterial production (BP,  
546  $\mu\text{mol C l}^{-1} \text{ h}^{-1}$ ); C: respiration (R,  $\mu\text{mol C l}^{-1} \text{ h}^{-1}$ ) and D: bacterial growth efficiency  
547 (BGE, %). Open circles: Stn. Lag; Filled circles: Stn. Ind. Note log scale on x-axis.

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549 Figure 2. Relationship between BGE (%) and BP ( $\mu\text{mol C l}^{-1} \text{ h}^{-1}$ ). A: Stn. Lag (open circles,  $r^2$   
550  $= 0.961$ ,  $p=0.006$ ); B: Stn. Ind (filled circles,  $r^2 = 0.99$ ,  $p< 0.0001$ ). Note the log scale  
551 on the x-axis. Figure deleted

552

553 Figure 2. Concentration and sticking properties of TEP (transparent exopolymeric particles).  
554 A: concentration of TEP ( $\mu\text{mol L}^{-1} \text{ C}$ ); B: number of beads per TEP ( $10^{-3} \mu\text{m}^{-3}$ ); C:  
555 sticking slope (a). Open circles: Stn. Lag; Filled circles: Stn. Ind. Note log scale on x-  
556 axis.

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558 Figure 3. Relationship between the number of beads attached to each TEP and the equivalent  
559 spherical diameter of TEP for the duration of the experiment. Statistical details of the  
560 regressions are given in Table 2. Left side column: Stn. Ind, right side column Stn.  
561 Lag.

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564 Figure 4. Relationship between TEP-C concentration and BGE. Open circles: Stn. Lag; Filled  
565 circles: Stn. Ind.

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569 **Table 1.** Organic and inorganic nutrient and dissolved organic carbon concentrations (DOC)  
 570 and the C:N molar ratio of the dissolved organic fractions for both sample sites. Chla :  
 571 extracted chlorophyll a; DIN : dissolved inorganic nitrogen; DON : dissolved organic  
 572 nitrogen; DIP : dissolved inorganic phosphorus; DOP : Dissolved organic phosphorus; sd :  
 573 standard deviation of the analytical replicates.  
 574

Day	Chla $\mu\text{g L}^{-1}$	sd	DIN $\mu\text{M}$	sd	DON $\mu\text{M}$	Stn. Ind			DOP $\mu\text{M}$	sd	DOC $\mu\text{M}$	sd	POC/PON M:M
						DIP $\mu\text{M}$	sd	DIP $\mu\text{M}$					
1	0.04	0.00	0.02	0.00	6.99	0.10	0.04	0.00	0.41	0.06	69.68	0.00	19.4
2	0.05	0.00	0.03	0.00	7.07	0.40	0.01	0.01	0.53	0.07	70.17	0.00	5.4
3	0.04	0.00	0.02	0.00	7.30	0.18	0.01	0.00	0.47	0.04	72.82	0.00	6.4
5	0.01	0.00	0.02	0.00	7.31	1.06	0.04	0.00	0.50	0.02	69.90	0.81	5.9
8	0.01	0.00	0.02	0.00	7.38	0.99	0.05	0.00	0.57	0.00	70.84	0.00	7.5
11	0.00	0.00	0.03	0.01	6.98	0.82	0.06	0.02	0.49	0.04	67.19	0.53	16.4
17	0.00	0.00	0.02	0.00	7.39	0.87	0.05	0.00	0.43	0.09	68.85	0.38	5.0
32	0.00	0.00	0.07	0.00	8.18	1.17	0.07	0.01	0.34	0.11	62.24	0.30	7.0

Day	Chla $\mu\text{g L}^{-1}$	sd	DIN $\mu\text{M}$	sd	DON $\mu\text{M}$	Stn. Lag			DOP $\mu\text{M}$	sd	DOC $\mu\text{M}$	sd	POC/PON M:M
						DIP $\mu\text{M}$	sd	DIP $\mu\text{M}$					
1	0.04	0.00	0.01	0.00	7.17	1.17	0.01	0.00	0.42	0.05	70.49	0.29	4.7
2	0.04	0.00	0.03	0.00	6.22	0.64	0.00	0.00	0.42	0.01	70.22	0.07	8.6
3	0.04	0.00	0.02	0.00	5.85	0.30	0.01	0.02	0.46	0.02	70.76	1.68	6.9
5	0.01	0.00	0.01	0.00	5.82	0.11	0.01	0.00	0.40	0.01	70.22	0.23	11.2
8	0.02	0.00	0.02	0.00	5.59	1.51	0.04	0.02	0.51	0.02	68.96	2.21	34
11	0.00	0.00	0.02	0.00	5.69	0.15	0.02	0.01	0.48	0.02	69.64	0.00	8.4
17	0.00	0.00	0.02	0.00	6.08	0.16	0.02	0.00	0.42	0.07	66.08	0.36	31.6
32	0.00	0.00	0.02	0.00	5.09	0.04	0.02	0.00	0.39	0.04	65.84	0.67	8.7

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**Table 2.** Statistical parameters of the stickiness regression calculations.

Day				Stn. Lag			r <sup>2</sup>	p-value
	Intercept	se	p-value	Slope	se	p-value		
1	17.61	1.69	<0.0001	2.26	0.14	0.302	0.77	<0.0001
2	2.23	0.57	<0.0001	0.34	0.03	<0.0001	0.40	<0.0001
3	5.78	1.17	<0.0001	0.72	0.07	<0.0001	0.42	<0.0001
5	10.60	0.78	<0.0001	1.13	0.04	<0.0001	0.79	<0.0001
11	10.08	1.05	<0.0001	1.26	0.07	<0.0001	0.72	<0.0001
32	16.28	2.11	<0.0001	2.19	0.16	<0.0001	0.71	<0.0001

Day				Stn. Ind			r <sup>2</sup>	p-value
	Intercept	se	p-value	Slope	se	p-value		
1	0.77	0.75	<0.0001	0.28	0.05	<0.0001	0.25	<0.0001
2	4.27	0.49	<0.0001	0.40	0.02	<0.0001	0.64	<0.0001
3	3.56	0.72	<0.0001	0.55	0.05	<0.0001	0.52	<0.0001
5	10.17	1.34	<0.0001	1.07	0.07	<0.0001	0.56	<0.0001
11	7.42	1.25	<0.0001	1.09	0.09	<0.0001	0.63	<0.0001
32	12.07	2.70	<0.0001	1.96	0.21	<0.0001	0.80	<0.0001

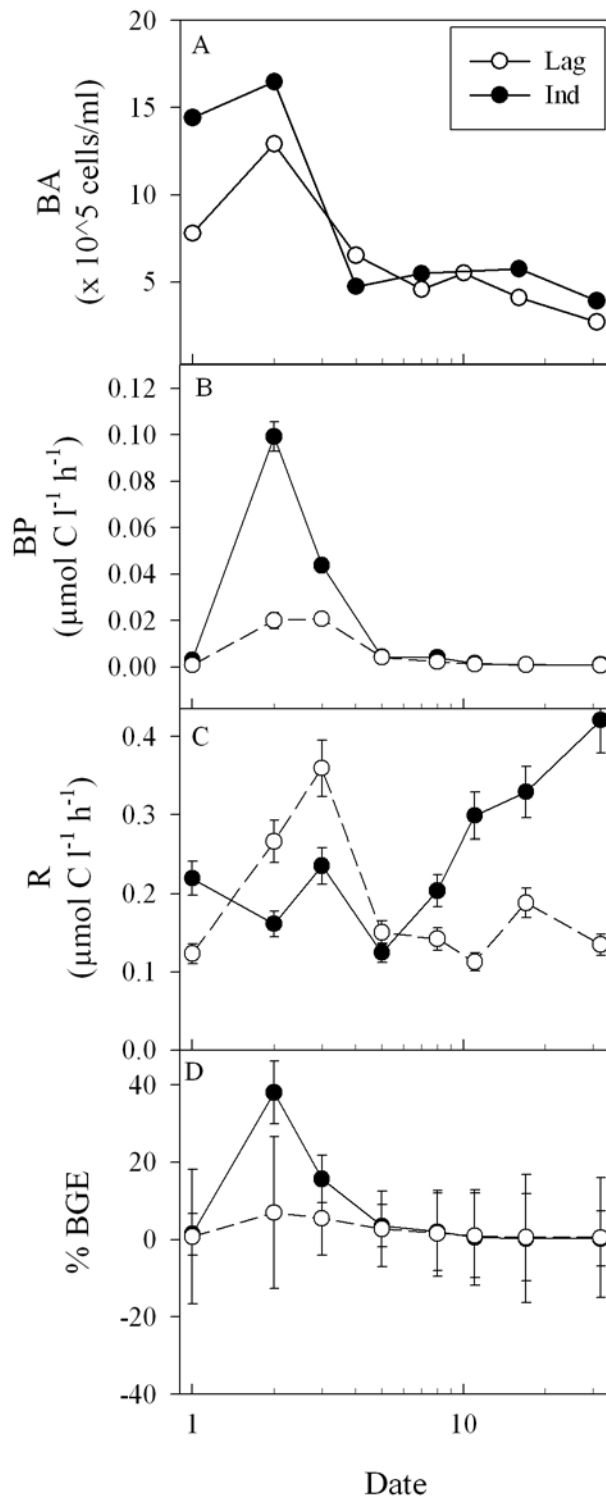


Figure 1

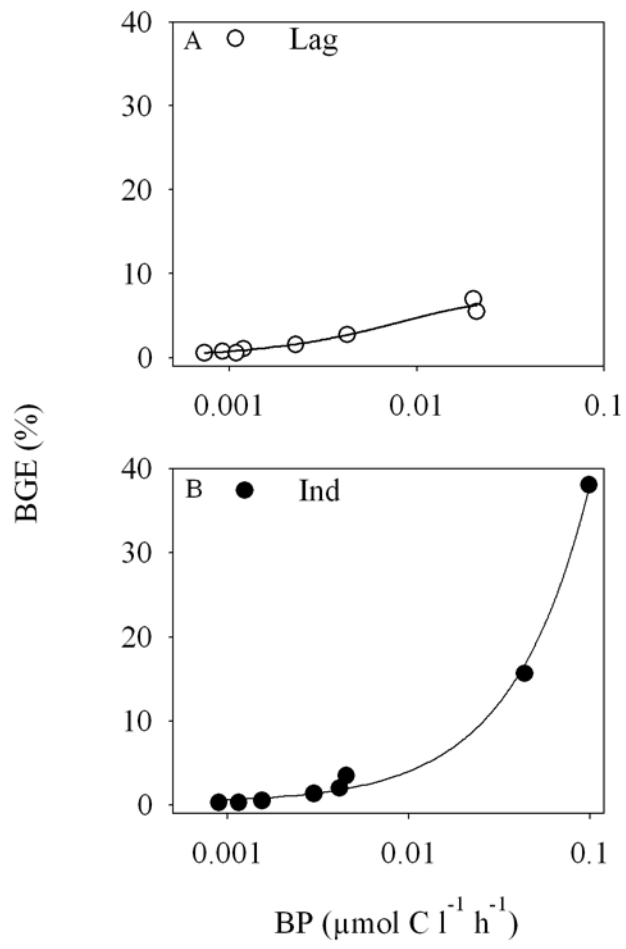


Figure 2.

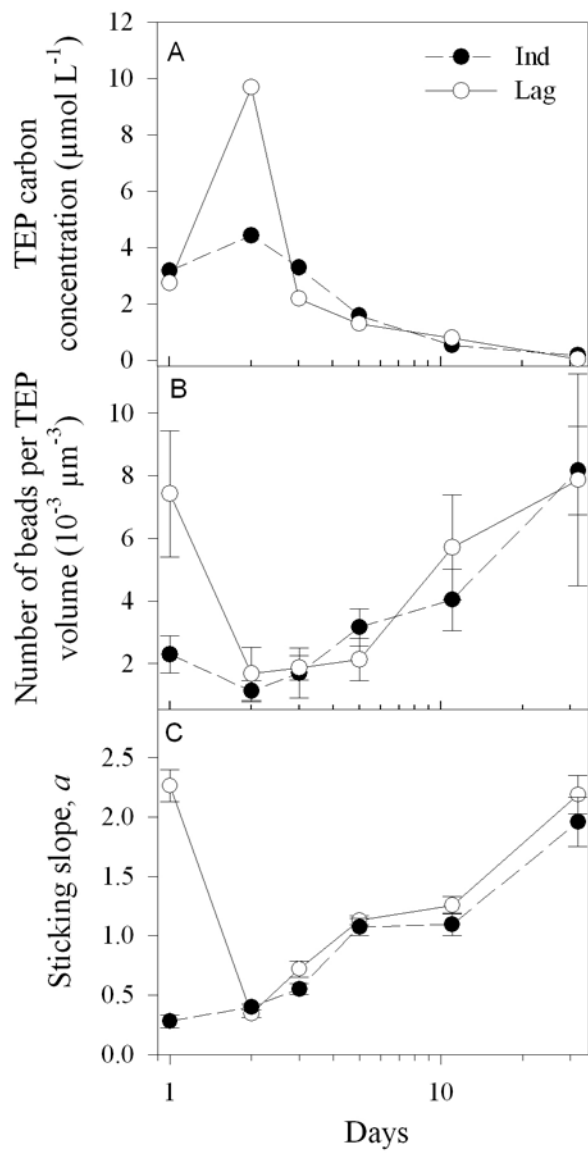


Figure 3.

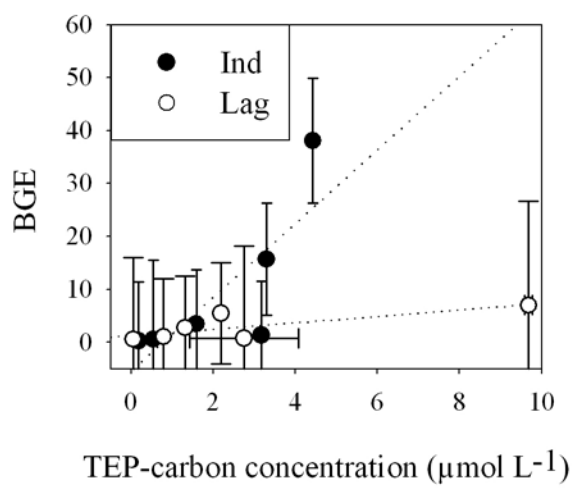


Figure 4