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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 μm 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 μm 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 ^3H -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 ^3H -
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⁻¹ 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 10⁻⁷ $\mu\text{mol O}_2 \text{ h}^{-1}$). 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⁻¹ and 3% and 2 nmol L⁻¹ 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⁻¹ 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⁻¹ 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 (H₃PO₄) 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⁻¹. 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⁻¹ 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⁻¹. 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, ϵ , 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 μm 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 (μm) 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

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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 pmol C h^{-1} and between 5 and 25 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.

548

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 μg L ⁻¹ | sd | DIN μM | sd | DON μM | Stn. Ind | | | DOP μM | sd | DOC μM | sd | POC/PON M:M |
|-----|----------------------------|------|-----------|------|-----------|-----------|------|-----------|-----------|------|-----------|------|----------------|
| | | | | | | DIP μM | sd | DON μM | | | | | |
| 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 μg L ⁻¹ | sd | DIN μM | sd | DON μM | Stn. Lag | | | DOP μM | sd | DOC μM | sd | POC/PON M:M |
|-----|----------------------------|------|-----------|------|-----------|-----------|------|-----------|-----------|------|-----------|------|----------------|
| | | | | | | DIP μM | sd | DON μM | | | | | |
| 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 | Intercept | se | p-value | Stn. Lag | | | r ² | 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 | Intercept | se | p-value | Stn. Ind | | | r ² | 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 |

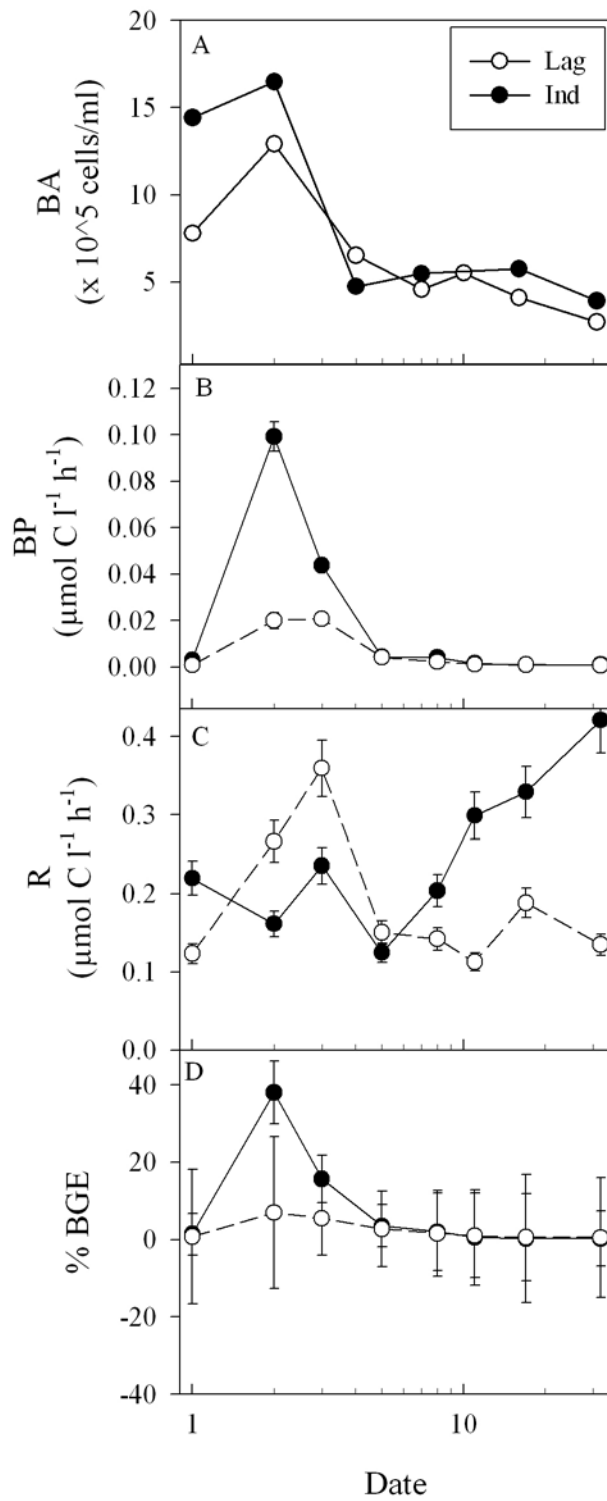


Figure 1

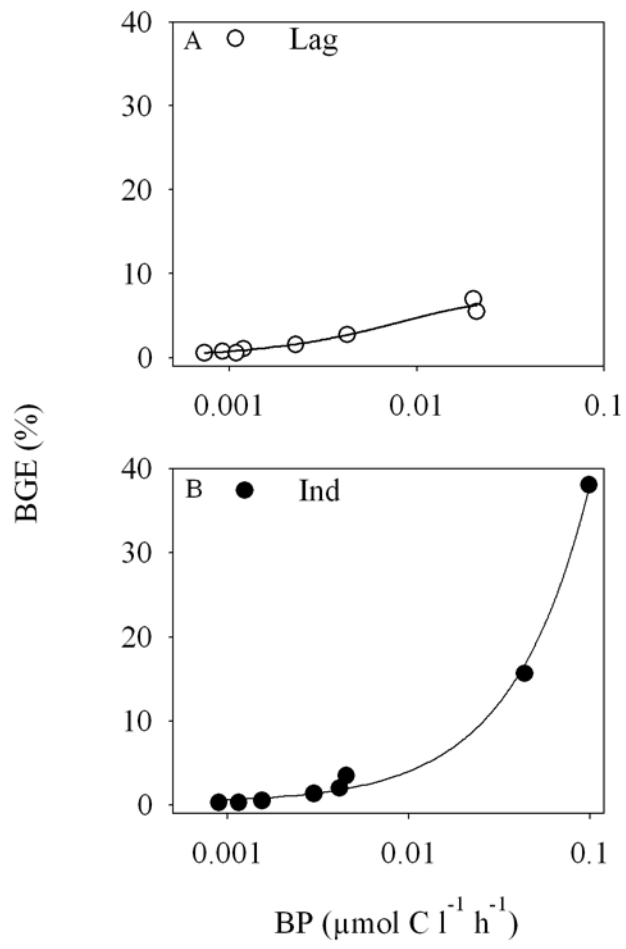


Figure 2.

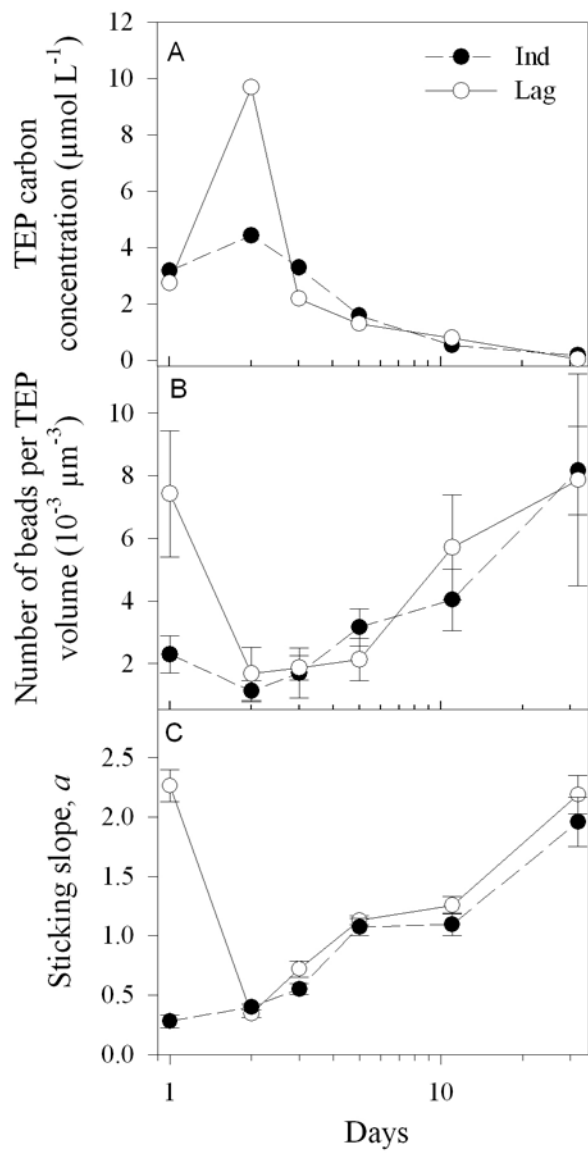


Figure 3.

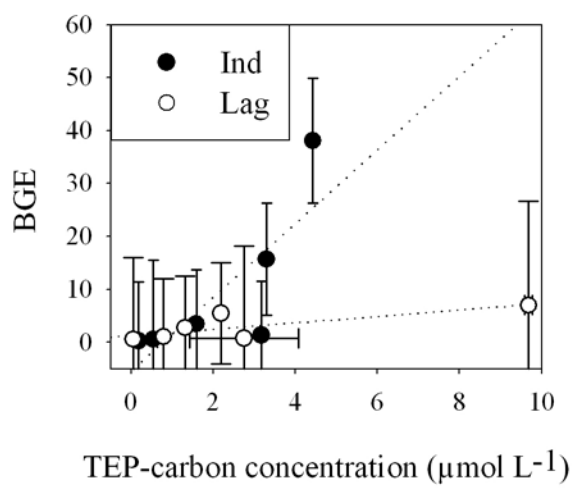


Figure 4