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▶ **To cite this version:**

Emma Rochelle-Newall, Xavier Mari, Olivier Pringault. Sticking properties of transparent exopolymeric particles (TEP) during aging and biodegradation. Journal of Plankton Research, 2010, 32 (10), pp.1433-1442. 10.1093/plankt/fbq060 . bioemco-00529280

HAL Id: bioemco-00529280

https://hal-bioemco.ccsd.cnrs.fr/bioemco-00529280v1

Submitted on 27 Oct 2010

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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.

Abstract

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

1. Introduction

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

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

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

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

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

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

2. Material and Methods

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

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

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

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

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

Bacterial respiration (BR) was measured at each time point using the oxygen microelectrode technique of Briand et al. (2004). The microprobes (Unisense, Denmark) are designed with an exterior guard cathode (Revsbech 1989), which results in extremely low oxygen consumption by the electrodes (4.7- 47 x 10^{-7} µmol O_2 h^{-1}). Probes have a response time shorter than 1 second and a precision of 0.05%. The precision of oxygen microprobe is equivalent to highly precise Winkler techniques (Briand et al., 2004).

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

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

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

Nitrate and nitrite concentrations (DIN) were determined according to Raimbault et al. (1990) on a Bran+Luebbe Autoanalyzer III with an average detection limit and CV of 20 nmol L^{-1} and 3% and 2 nmol L^{-1} and 8% for eutrophic and oligotrophic waters, respectively. Phosphate concentrations (DIP) were determined on a Bran+Luebbe Autoanalyzer III with an detection limit of 10 nmol L^{-1} and an average CV of 6 to 11% between replicates (Torréton et al., 2007).

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

Total organic carbon (TOC) analyses were performed on 10 mL sub-samples collected in pre-combusted (450°C, overnight) 10 mL glass ampoules, preserved with 12 µL 85% phosphoric acid (H_3PO_4) and flame sealed. Samples were stored in the dark until analysis. TOC concentration was measured on a Shimadzu TOC VCPH analyzer, using potassium phthalate calibration standards over the measurement range 0 to 250 µmol C L^{-1}. Certified reference materials (CRM; Hansell Laboratory, University of Miami) were also used to assess the performance of the instrument on and between measurement days. The machine blank was between 5 and 10 µmol C L^{-1} for the measurement days and the CV of the measurement was always less than 2% of the mean of triplicate injections of duplicate samples. DOC was calculated by subtraction of the POC measurements from the TOC concentrations.

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

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

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

3. Results

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

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

There was little difference between the initial DOC concentrations at the two sites. Both stations had initial concentrations of around 70 μM C which decreased during the experiment. However, the amount of DOC removed during the incubation was higher in the Stn. Ind incubation than in Stn. Lag. Indeed, at Stn. Ind, 10% of the initial DOC was removed compared to only 7% at Stn. Lag.

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

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

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

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

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

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

4. Discussion

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

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

Renewal rates, or flushing times, also play a role in determining the age and concentrations the DOM (Mari et al., 2007). The flushing time of Stn. Lag is on the order of 0.5 days and it is unlikely that large stocks of terrestrial organic matter are present in this 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 amol C $cell^{-1}$ 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

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

Acknowledgements. This research was financed by the PNETOX program of the French Ministry for Ecology and Sustainable Development (Ministère de l'Ecologie et du Développement Durable), the French National Research Agency (ANR-BLANC program: MAORY project), the Ministry of Overseas Territories (Ministère de l'Outre Mer) and the French Research Institute for Development (IRD).

404 **References**

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406 Amon, R.M.W. and Benner, R. (1996). Bacterial utilization of different size classes of
407 dissolved organic matter. *Limnology and Oceanography*. **41**, 41-51.
408 Azetsu-Scott, K. and Passow, U. (2004). Ascending marine particles: Significance of
409 transparent exopolymer particles (TEP) in the upper ocean. *Limnology and*
410 *Oceanography*. **49**, 741-748.
411 Benner, R. 2002. Chemical composition and reactivity. in: D. Hansell and C.A. Carlson
412 (Editors), *Biogeochemistry of marine dissolved organic matter*. Academic Press, San
413 Diego, pp. 59-90.
414 Biersmith, A. and Benner, R. (1998). Carbohydrates in phytoplankton and freshly produced
415 dissolved organic matter. *Marine Chemistry*. **63**, 131-144.
416 Briand, E., Pringault, O., Jacquet, S. and Torréton, J.P. (2004). The use of oxygen
417 microprobes to measure bacterial respiration for determining bacterioplankton growth
418 efficiency. *Limnology and Oceanography: Methods*. **2**, 406-416.
419 del Giorgio, P.A. and Davis, J. 2003. Patterns in dissolved organic matter lability and
420 consumption across aquatic ecosystems. in: S. Findlay and R. Sinsbaugh (Editors),
421 *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*. Academic Press, pp.
422 399-424.
423 Engel, A. (2000). The role of transparent exopolymer particles (TEP) in the increase in
424 apparent particle stickiness (alpha) during the decline of a diatom bloom. *Journal of*
425 *Plankton Research*. **22**, 485-497.
426 Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E. and Zondervan, I. (2004).
427 Polysaccharide aggregation as a potential sink of marine dissolved organic carbon.
428 *Nature*. **428**, 929-932.
429 Gasol, J.M. and Del Giorgio, P.A. (2000). Using flow cytometry for counting natural
430 planktonic bacteria and understanding the structure of planktonic bacterial
431 communities. *Scientia Marina*. **64**, 197-224.
432 Grossart, H.P. and Ploug, H. (2000). Bacterial production and growth efficiencies: Direct
433 measurements on riverine aggregates. *Limnology and Oceanography*. **45**, 436-445.
434 Hansell, D.A. and Carlson, C.A. 2002. Preface. in: D.A. Hansell and C.A. Carlson (Editors),
435 *Biogeochemistry of marine dissolved organic matter*. Academic Press, San Diego,
436 California, pp. XXI-XXII.
437 Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W. and Strickland, J.D.H. (1965). Fluorimetric
438 determination of chlorophyll. *Rapports et procès-verbaux des réunions - Conseil*
439 *international pour l'exploration de la mer* **30**, 3-15.
440 Jacquet, S., Delesalle, B., Torreton, J.-P. and Blanchot, J. (2006). Responses of the
441 phytoplankton communities to increased anthropogenic influences (Southwestern
442 Lagoon, New Caledonia). *Marine Ecology Progress Series*. **320**, 65-78.
443 Jouon, A., Douillet, P., Ouillon, S. and Fraunié, P. (2006). Calculations of hydrodynamic time
444 parameters in a semi-opened coastal zone using a 3D hydrodynamic model.
445 *Continental Shelf Research*. **26**, 1395-1415.
446 Kirchman, D. (2001). Measuring bacterial biomass production and growth rates from leucine
447 incorporation in natural aquatic environments. *Methods in Microbiology*. **30**, 227-237.
448 Kroer, N. (1993). Bacterial growth efficiency on natural dissolved organic matter. *Limnology*
449 *and Oceanography*. **38**, 1282-1290.
450 Kroer, N. (1994). Relationships between biovolume and carbon and nitrogen content of
451 bacterioplankton. *FEMS Microbiology Ecology*. **13**, 217-224.

452 Luft, J.H. (1971). Ruthenium Red and Violet .1. Chemistry, Purification, Methods of Use for
453 Electron Microscopy and Mechanism of Action. *Anatomical Record*. **171**, 347-&.
454 Mari, X. (2008). Does ocean acidification induce an upward flux of marine aggregates?
455 *Biogeosciences*. **5**, 1023-1031.
456 Mari, X. and Robert, M. (2008). Metal induced variations of TEP sticking properties in the
457 southwestern lagoon of New Caledonia. *Marine Chemistry*. **110**, 98-108.
458 Mari, X. et al. (2007). Water residence time: A regulatory factor of the DOM to POM transfer
459 efficiency. *Limnology and Oceanography*. **52**, 808-819.
460 Migon, C., Ouillon, S., Mari, X. and Nicolas, E. (2007). Geochemical and hydrodynamic
461 constraints on the distribution of trace metal concentrations in the lagoon of Noumea,
462 New Caledonia. *Estuarine Coastal and Shelf Science*. **74**, 756-765.
463 Mopper, K. et al. (1995). The Role of Surface-Active Carbohydrates in the Flocculation of a
464 Diatom Bloom in a Mesocosm. *Deep-Sea Research Part Ii-Topical Studies in*
465 *Oceanography*. **42**, 47-73.
466 Morán, X.A.G., Estrada, M., Gasol, J.M. and Pedrós-Alió, C. (2002). Dissolved primary
467 production and the strength of phytoplankton bacterioplankton coupling in contrasting
468 marine regions. *Microbial Ecology*. **44**, 217-223.
469 Moriarty, D.J.W., Pollard, P.C. and Hunt, W.G. (1985). Temporal and spatial variation in
470 bacterial production in the water column over a coral reef. *Marine Biology*. **85**, 285-
471 292.
472 Obernosterer, I. and Herndl, G.J. (2000). Differences in the optical and biological reactivity of
473 the humic and nonhumic dissolved organic carbon component in two contrasting
474 coastal marine environments. *Limnology and Oceanography*. **45**, 1120–1129.
475 Passow, U. (2002). Transparent exopolymer particles (TEP) in aquatic environments.
476 *Progress in Oceanography*. **55**, 287-333.
477 Pinazo, C. et al. (2004). Impact of wind and freshwater inputs on phytoplankton biomass in
478 the coral reef lagoon of New Caledonia during the summer cyclonic period: a coupled
479 three-dimensional biogeochemical modeling approach. *Coral Reefs*. **23**, 281-296.
480 Raimbault, P., Slawyk, G., Coste, B. and Fry, J. (1990). Feasibility of using an automated
481 colorimetric procedure for the determination of seawater nitrate in the 0 to 100 nM
482 range: examples from field and culture. *Marine Biology*. **104**, 347-351.
483 Raymond, P.A. and Bauer, J.E. (2000). Bacterial consumption of DOC during transport
484 through a temperate estuary. *Aquatic Microbial Ecology*. **22**, 1-12.
485 Renaud, F., Pringault, O. and Rochelle-Newall, E. (2005). Effects of the colonial
486 cyanobacterium *Trichodesmium spp*. on bacterial activity. *Aquatic Microbial Ecology*.
487 **41**, 261-270.
488 Richardson, T.L. and Jackson, G.A. (2007). Small phytoplankton and carbon export from the
489 surface ocean. *Science*. **315**, 838-840.
490 Rochelle-Newall, E.J. and Fisher, T.R. (2002). Production of chromophoric dissolved organic
491 matter fluorescence in marine and estuarine environments: an investigation into the
492 role of phytoplankton. *Marine Chemistry*. **77**, 7-21.
493 Rochelle-Newall, E.J., Pizay, M.D., Middelburg, J.J., Boschker, H.T.S. and Gattuso, J.-P.
494 (2004). Degradation of riverine dissolved organic matter by seawater bacteria. *Aquat.*
495 *Microb. Ecol.* **37**, 9-22.
496 Rochelle-Newall, E.J., Torréton, J.-P., Mari, X. and Pringault, O. (2008). Phytoplankton-
497 bacterioplankton coupling in a sub-tropical South Pacific coral reef lagoon. *Aquat.*
498 *Microb. Ecol.* **50**, 221-229.
499 Smith, D.C. and Azam, F. (1992). A simple, economical method for measuring bacterial
500 protein synthesis rates in seawater using ^{3}H-leucine. *Marine Microbial Food Webs*. **6**,
501 107-114.

Stoderegger, K. and Herndl, G.J. (1998). Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. *Limnology and Oceanography*. **43**, 877-884.

Torréton, J.-P. et al. (2007). Correspondence between the distribution of hydrodynamic time parameters and the distribution of biological and chemical variables in a semi-enclosed coral reef lagoon. *Estuarine and Coastal Shelf Science*. **74**, 766-776.

Vacelet, E. and Thomassin, B. (1991). Microbial utilization of coral mucus in long term in situ incubation over a coral reef. *Hydrobiologia*. **211**, 19-32.

Verdugo, P. et al. (2004). The oceanic gel phase: a bridge in the DOM-POM continuum. *Marine Chemistry*. **92**, 67-85.

Weinbauer, M. et al. (in press). Bacterial community composition and potential controlling mechanisms along a trophic gradient in a barrier reef system. *Aquatic microbial ecology*.

Wild, C. et al. (2004). Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature*. **428**, 66-70.

Zhou, J., Mopper, K. and Passow, U. (1998). The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnology and Oceanography*. **43**, 1860-1871.

Figure legends

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

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

Figure 2. Concentration and sticking properties of TEP (transparent exopolymeric particles). A: concentration of TEP (µmol L^{-1} C); B: number of beads per TEP (10^{-3} μm^{-3}); C: sticking slope (a). Open circles: Stn. Lag; Filled circles: Stn. Ind. Note log scale on x-axis.

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

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

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

						Stn. Ind							
Day	Chla $\mu g\ L^{-1}$	sd	DIN µM	sd	DON µM	sd	DIP µM	sd	DOP µM	sd	DOC µM	sd	POC/PON M: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
						Stn. Lag							
Day	Chla $\mu g\ L^{-1}$	sd	DIN µM	sd	DON µM	sd	DIP µM	sd	DOP µM	sd	DOC µM	sd	POC/PON M: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

Table 2. Statistical parameters of the stickiness regression calculations.

				Stn. Lag				
Day	Intercept	se	p-value	Slope	se	p-value	r^2	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

				Stn. Ind				
Day	Intercept	se	p-value	Slope	se	p-value	r^2	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

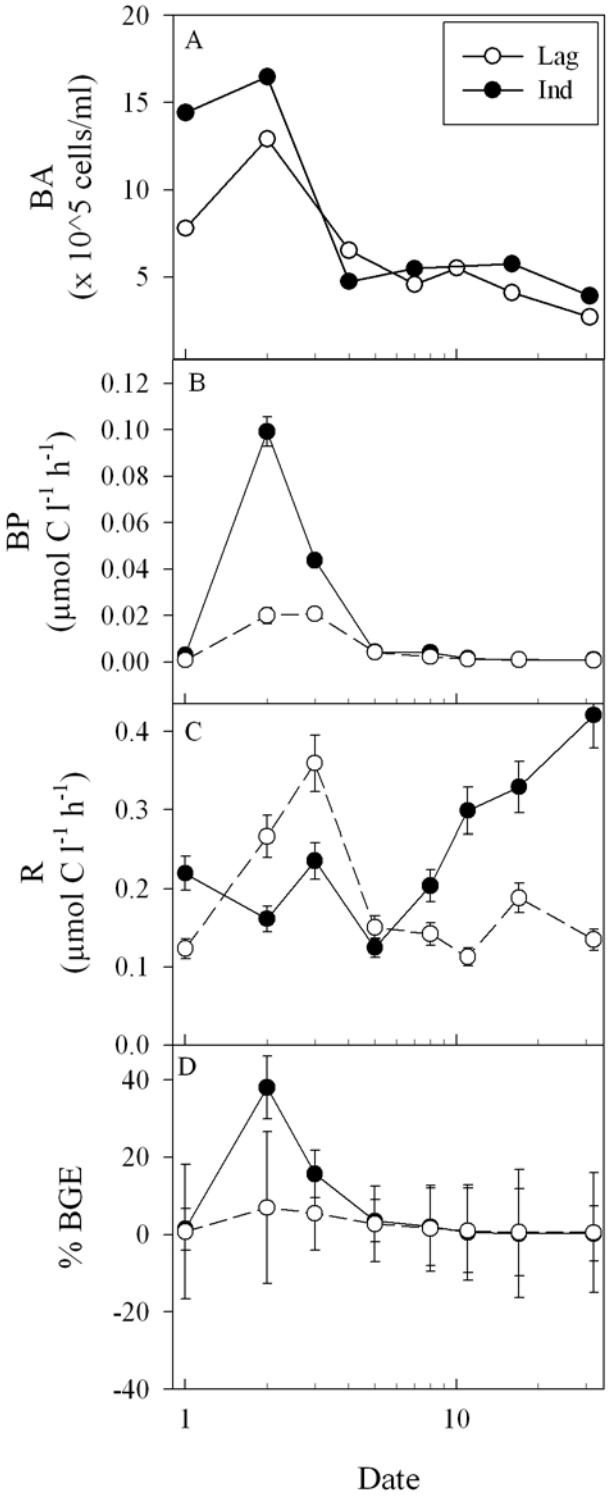
A
Lag
Ind
BA
(x 10^5 cells/ml)
20
15
10
5
B
BP
(µmol C l-1 h-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
C
R
(µmol C l-1 h-1)
0.4
0.3
0.2
0.1
0.0
D
% BGE
40
20
0
-20
-40
1
10
Date

Figure 1

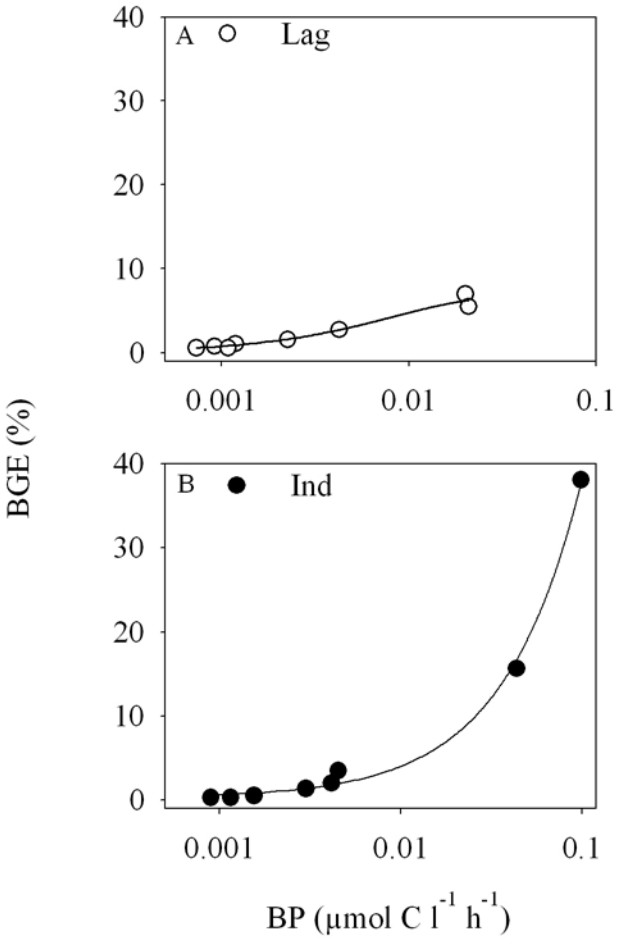

Figure 2.

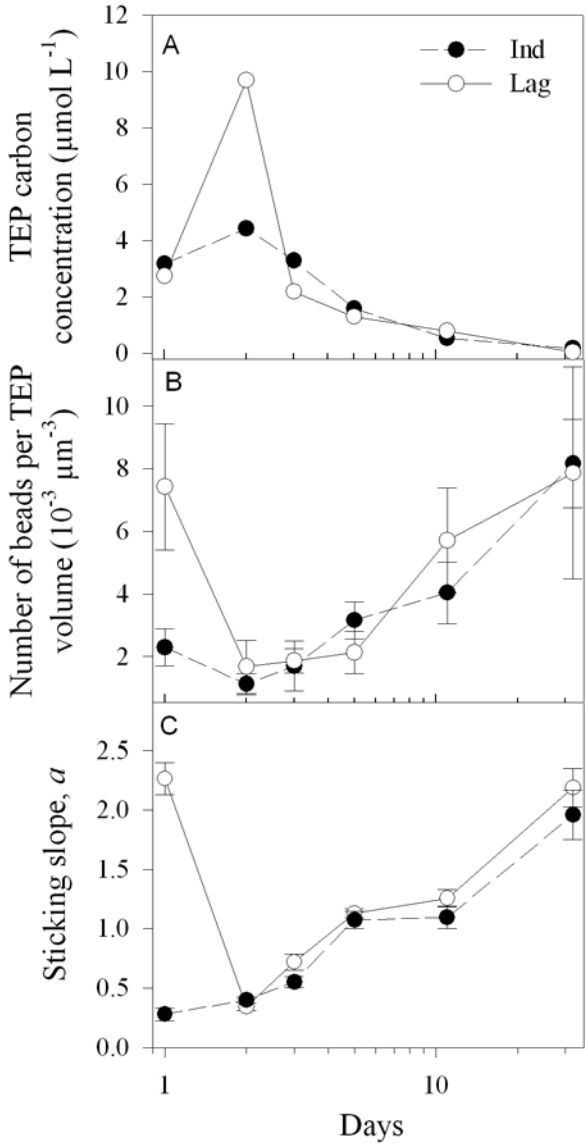
A
B
C
Ind
Lag
TEP carbon concentration (µmol L-1)
Number of beads per TEP volume (10-3 µm-3)
Sticking slope, a
Days
1
10
0
2
4
6
8
10
12
0.0
0.5
1.0
1.5
2.0
2.5

Figure 3.

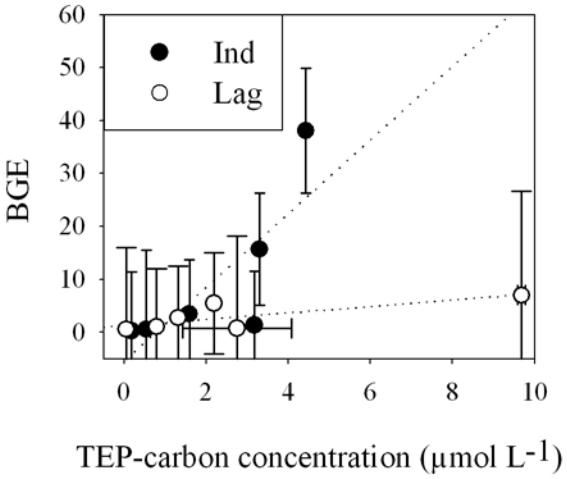
60
50
40
30
20
10
0
BGE
Ind
Lag
0
2
4
6
8
10
TEP-carbon concentration (µmol L-1)

Figure 4