



Heriot-Watt University
Research Gateway

Agglomeration of nano- and microplastic particles in seawater by autochthonous and de novo-produced sources of exopolymeric substances

Citation for published version:

Summers, S, Henry, T & Gutierrez, T 2018, 'Agglomeration of nano- and microplastic particles in seawater by autochthonous and de novo-produced sources of exopolymeric substances', *Marine Pollution Bulletin*, vol. 130, pp. 258-267. <https://doi.org/10.1016/j.marpolbul.2018.03.039>

Digital Object Identifier (DOI):

[10.1016/j.marpolbul.2018.03.039](https://doi.org/10.1016/j.marpolbul.2018.03.039)

Link:

[Link to publication record in Heriot-Watt Research Portal](#)

Document Version:

Peer reviewed version

Published In:

Marine Pollution Bulletin

Publisher Rights Statement:

© 2018 Elsevier B.V.

General rights

Copyright for the publications made accessible via Heriot-Watt Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

Heriot-Watt University has made every reasonable effort to ensure that the content in Heriot-Watt Research Portal complies with UK legislation. If you believe that the public display of this file breaches copyright please contact open.access@hw.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

1 **Agglomeration of nano- and microplastic particles in seawater by**
2 **autochthonous and *de novo*-produced sources of exopolymeric**
3 **substances**

4
5

6 Stephen Summers^{1,2} Theodore Henry^{3,4} and Tony Gutierrez^{1*}

7

8 ¹ School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh,
9 EH14 4AS, United Kingdom;

10 ² The Singapore Centre for Environmental Life Sciences Engineering and the School of
11 Biological Sciences, Nanyang Technological University, Singapore 637551;

12 ³ School of Energy, Geoscience, Infrastructure and Society, Heriot-Watt University,
13 Edinburgh, EH14 4AS, United Kingdom.

14 ⁴ Department of Forestry Wildlife and Fisheries, Center for Environmental
15 Biotechnology, The University of Tennessee, Knoxville, TN, 36849, USA.

16

17

18

19

20 *Correspondence:

21 Dr. Tony Gutierrez

22 Tel.: +44 (0)131 451 3315

23 E-mail: tony.gutierrez@hw.ac.uk

24

25

26

27 **Abstract**

28 Microplastics (<5 mm) have often been studied under *in-vitro* conditions where plastics
29 have been investigated in isolation. However, in the natural environment microplastics readily
30 form agglomerates conferring the particles with properties different to their pristine
31 counterparts. Here, we examined the interaction of exopolymers with polystyrene nanoplastics
32 and microplastics. Formation of plastic agglomerates was examined using simulated sea surface
33 conditions. Flow cytometry coupled with microscopy revealed that nano- and microplastic
34 particle spheres form agglomerates in seawater with a mucilagenous material and an associated
35 microbial community. To characterise this material, differential staining methods revealed it to
36 be glycoprotein in composition. Exposing increasing concentrations of a marine bacterial
37 glycoprotein EPS to nano- or microplastics revealed that these types of polymers contribute to
38 the formation of plastic agglomerates. This work highlights the importance of EPS on the fate
39 of plastic and future research should take this into account when evaluating the impact of
40 plastics.

41

42 Running title: Agglomeration of nano /microplastics by bacterial EPS

43

44 Keywords: Microplastic; Nanoplastic; Marine Snow; Exopolysaccharide; EPS;
45 Glycoprotein; Marine Environment; Marine Pollution.

46

47 **Introduction**

48 Contamination of the natural environment by plastic debris is of increasing concern. With
49 the global production of plastics increasing from 230 million tonnes in 2005 to 322 million
50 tonnes in 2015 (PlasticsEurope, 2016), and considering the multitude of point sources for the
51 entry of plastics into the global ocean and seas (Eriksen et al., 2014; Lechner et al., 2014), there
52 has been a rise in studies attempting to better comprehend the risks this type of pollutant poses
53 to marine ecosystems (see Andrady, 2011 for a review).

54 In the case of laboratory-based studies, the plastics used were often sourced from
55 commercial suppliers, the reason for which often stems from the fact that they can be
56 manufactured with a prescribed uniform size and of known chemical composition that allows
57 for standardisation across studies. The composition of microplastics collected from the natural
58 environment, however, can exhibit a different surface chemistry and form a different entity
59 compared to their initial ‘pristine’ form (Fotopoulou and Karapanagioti, 2012; Li et al., 2018).
60 This is largely attributed to the natural weathering process and interaction of the plastics with
61 inert and living biogenic material upon their entry into marine waters. Hence, the use of pristine
62 plastic particles in laboratory studies to ascertain their toxicological effects to marine organisms
63 often discounts the fact that they are unlikely to exist in a pristine form after their entry into
64 marine waters.

65 Following the entry of nano- and microplastics into marine waters, or their formation from
66 disintegrating larger debris, the surface chemistry and/or physical state of the plastics will
67 experience changes within hours and consequently these changes will have an influence upon
68 their uptake/ingestion by marine organisms and subsequent toxicological effects. It is during
69 these initial hours that a biofilm will start to develop on the plastics surface (Fletcher, 1977;
70 Lobelle and Cunliffe, 2011), or if the particles are too small they would become enveloped as
71 part of a new or existing biofilm (Ikuma et al., 2015; Martel et al., 2014). Zettler et al. (2013)
72 reported that microorganisms are one of the first colonizers on the surface of plastic debris and
73 form an environment that has been referred to as the ‘plastisphere’. However, the diversity of
74 the microbial communities associated with plastics is strongly influenced by the surrounding
75 waters in which they are located, with plastic type and structure having a more minor influence
76 (Amaral-Zettler et al., 2015; Oberbeckmann et al., 2018; Zettler et al., 2013).

77 Recent work by Canesi et al. (2015) and Hentschel (2015) observed microplastics in an
78 agglomerated form in seawater. Whilst these studies did not explore the cause or mechanisms
79 underlying this plastic agglomeration, the process may be analogous to the formation of marine
80 snow in the ocean, which is a key component of the ‘biological pump’ that participates in the

81 redistribution of carbon in marine systems (Long and Azam, 2001; Shanks and Trent, 1980). In
82 addition, the process of microplastics, specifically latex beads, forming into agglomerates has
83 been referred to previously and used to monitor and record the ‘stickiness’ of various
84 exopolymers in the ocean, though not in the context of marine plastic pollution (Mari and
85 Robert, 2008). The encapsulation of plastic debris in marine snow was recently described for
86 waters collected at Avery point, Connecticut, USA (Zhao et al., 2017) – particles of
87 predominately polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET)
88 were found associated within the marine snow particles. If the formation of nano- and
89 microplastic agglomerates proceeds in a similar way to marine snow, this would likely
90 influence the buoyant density of the plastics within the water column, altering their sinking rate
91 (Kooi et al., 2017; Lobelle and Cunliffe, 2011). For example, Long et al. (2015) reported that
92 agglomeration of microplastics with diatoms significantly altered the sedimentation velocity of
93 plastics from tens to hundreds of meters per day. The density of the plastic material, though,
94 must be taken into consideration. For example, the common plastics identified within marine
95 snow by Zhao et al. (2017) were PP, PS and PET, which have densities of 0.9, 1.04 and 1.38
96 g/cm³, respectively. Since the density of seawater is 1.02 - 1.03 g/cm³, each of these three plastic
97 types would have a differential influence on marine snow buoyancy: PP would be expected to
98 increase buoyant density of marine snow, whereas PS would have a marginal negative effect,
99 and PET the greatest influence in lowering the buoyant density.

100 Our current understanding of the agglomeration of plastics, especially nanoplastics, in
101 seawater is in a nascent phase and warrants considerable attention. In this study, we examined
102 the formation of polystyrene nano- and microplastic agglomerates using methods for generating
103 artificial marine snow (Shanks and Edmondson, 1989) with natural seawater collected from the
104 northeast Atlantic. Since exopolymers (EPS), particularly transparent exopolymers (TEP),
105 produced by marine microorganisms constitutes a major fraction of the total pool of dissolved
106 organic matter (DOM) in the global ocean (Decho and Gutierrez, 2017; Hansell and Carlson,
107 1998; Jennings et al., 2017; Passow, 1994) and has been implicated in marine snow formation
108 (Engel, 2004; Mari et al., 2017), it was evaluated for its potential to influence the formation of
109 nano- and microplastic agglomerates.

110

111 **Materials and Methods**

112 **Isolation of EPS produced by strain TGOS-10**

113 The EPS produced by *Halomonas* sp. TGOS-10 was isolated by growing the strain in
114 ZM/10 supplemented with glucose to a final concentration of 0.1% (w/v). For this,

115 exponentially-growing cells of the strain were inoculated into a 3 L Erlenmeyer flask containing
116 1 L of the growth medium. The flask was then incubated with shaking (75 rpm) at 21°C in the
117 dark. Once growth had reached the stationary phase (2-3 days), the biomass was pelleted by
118 centrifugation (4000 x g; 20 min) and the supernatant filtered (0.2 µm) to remove residual cells.
119 Isolation of purified EPS was performed as previously described (Gutierrez et al., 2007).
120 Briefly, KCl (to 7% w/v) was dissolved in the cell-free supernatant volume prior to the addition
121 of two volumes of cold ethanol and the mixture left for 24 hours at 4°C. The precipitated
122 material was then pelleted by centrifugation (4000 x g; 20 min), the supernatant disposed of,
123 and then the pellet was extensively dialysed against milli-Q water (18 MΩ/cm quality) using a
124 1 kDa molecular-weight pore-size membrane (Spectra, Cole-Parmer, inc). The resultant
125 purified EPS was then lyophilised and stored at room temperature in a sealed container until
126 required.

127

128 **Roller-bottle incubations**

129 The potential for plastic particles to form agglomerates was investigated using a roller-
130 bottle design similar to that by Shanks and Edmondson (1989). This method maintains the
131 content of the bottles in constant gentle motion (~15-20 rpm) in order to simulate the natural
132 water column, whilst also reducing the potential of agglomerates from settling to the container
133 walls (Jackson, 1994, 2015).

134

135 *Natural seawater experiments (NSE)*

136 To determine if nano- and microplastic particles form into agglomerates in natural
137 seawater, four experimental treatments were set up. The seawater for these experiments was
138 collected from the Faroe-Shetland Channel (FSC) in the northeast Atlantic (60°38.12' N,
139 4°54.03' W) in September of 2015. Sea surface water samples were collected using Niskin
140 bottles from a depth of 5 m and maintained at 4°C until used. For these NSE experiments, the
141 first treatment (NSE-1) comprised filling 40 mL glass scintillation vials with 40 mL of the
142 collected natural seawater (negative controls). The remaining treatments comprised filling
143 scintillation vials with 40 mL of natural seawater and supplementing with polystyrene plastics
144 of different sizes (See table S1 for details on plastics) to a concentration of 5 µg mL⁻¹ in each
145 treatment vial: 50 nm (for treatment NSE-2), 1 µm (for treatment NSE-3) and 10 µm (for
146 treatments NSE-4).

147 In addition, the diameter and surface charge of the plastic used in these experiments was
148 measured using a ZetaSizer (Nano-ZS, Malvern, UK). All manipulations were carried out
149 aseptically and each treatment was performed using five replicates. The vials were sealed with
150 Teflon coated septa, leaving a 2 mL headspace, and placed onto a low-profile roller table at a
151 constant gentle rotation (~15-20 rpm) for 24 hours at 7°C, which is the *in-situ* temperature for
152 the FSC at the time the seawater was collected.

153 At the end of the incubation period (24 hours), the contents of each vial were filtered
154 through a 0.22 µm white Millipore filter, using a low-pressure vacuum to minimize the
155 disruption of marine snow/plastic agglomerates. Each filter was then examined using a bright-
156 field binocular dissection microscope. Three images (image area = 9 mm²) were recorded for
157 each of the five replicates, and the number of agglomerates formed and their size were measured
158 using Fiji image analysis software (Schindelin et al., 2012).

159

160 **Flow Cytometry**

161 A random subset of NSE samples were examined using flow cytometry to monitor the
162 agglomeration of the plastics after 24 hours. Only plastics smaller than 1000 nm were used to
163 prevent blockages within the injection port of the instrument. Therefore, an addition of a 500
164 nm plastics treatment was added to increase scope of the investigation. This was achieved using
165 a BD LSR Fortessa multi-colour cell analyser (Biosciences, UK). The nano- and micro plastics
166 were assessed on forward scatter (FC), side scatter (SC) and fluorescence intensity (Alexa Fluor
167 488, Cyan-green colour; excitation: 495 nm; emission: 519 nm). Plastic spheres were recorded
168 based on their FC and fluorescent intensity using 10000 events or a 2 minute duration if this
169 number of events could not be achieved. For the smaller 50 nm nanoplastics, FC was not an
170 appropriate method of detection due to the small size of the particles, therefore only
171 fluorescence intensity was used. Solutions of the nano- and microplastics were analysed to
172 examine the mean size and fluorescent intensity of the plastics in a singlet form. These singlet
173 stocks were used to set the gating to ensure that the majority of the singlet nano-plastics were
174 measured in gate Q4. This was repeated after 24 hours incubation within the natural seawater
175 to determine if agglomeration had occurred. Agglomeration was measured by changes in event
176 numbers occurring in gate Q2 and Q3, which measured increases in size and fluorescent
177 intensity, respectively (more plastics per aggregate). Gate Q1 measured for an increase in the
178 size of agglomerates with no increase in fluorescence, therefore a mostly marine snow
179 aggregate with little to no increase in plastic occurrence. All samples were filtered using a 100
180 µm gauze to prevent system blockages, though this limited the size of the agglomerates (to

181 <100 μm) that could be investigated by this method. All data was analysed and reported using
182 the FlowJo software (v10.3.0.) The relative proportion of events within each gate was reported
183 for both singlet and agglomerated samples.

184

185 ***Synthetic seawater experiments (SSE)***

186 To determine the influence of bacterial EPS on the formation of plastic agglomerates, the
187 partially purified EPS from *Halomonas* sp. TGOS-10 was dissolved in sterile ONR7a synthetic
188 seawater (Dyksterhouse et al., 1995) to $1500 \mu\text{g mL}^{-1}$. This EPS working volume was aliquoted
189 into a series of 22 mL glass Hungate tubes to achieve the following final concentrations of the
190 EPS in ONR7a to 20 mL total volume per tube: 0, 0.1, 1, 10, 100 and $1000 \mu\text{g mL}^{-1}$. To each
191 tube, 10 μL of the 1.0 μm microplastics stock solution ($5 \mu\text{g mL}^{-1}$ final concentration) was
192 added. Each EPS treatment was prepared in triplicate, and all the tubes were incubated on the
193 roller table for 24 hours at 7°C . After 24 hours, each tube was filtered through 5.0 μm
194 Nucleopore filters under a low-pressure vacuum to recover microplastic spheres incorporated
195 into any agglomerates ($>5 \mu\text{m}$) that formed, whilst ‘pristine’ microplastics – i.e. those not
196 associated with agglomerates, would pass through the filters with the filtrate.

197

198

199 ***Sedimentation Velocity Experiments (SVE)***

200 The influence of plastics on the buoyant density of marine snow particles was measured
201 and compared to marine snow particles containing no plastic. Seawater for these experiments
202 was collected at a depth of 1 m from the Leven docks on the Firth of Forth estuary ($56^\circ 11.11'$
203 N, $03^\circ 00.09'$ W) on October 17, 2016, using sterile Duran bottles. For these sedimentation
204 velocity experiments (SVE), a series of amber 40 mL scintillation glass vials were filled with
205 40 mL of the seawater and supplemented with fluorescent plastic particles of diameter 50 nm
206 (SVE-1 treatment), 1 μm (SVE-2 treatment), or 10 μm (SVE-3 treatment). The final
207 concentration of plastic spheres in each treatment vial was $5 \mu\text{g mL}^{-1}$. The control treatment
208 comprised the same seawater without the addition of plastics (SVE-4). All experimental
209 manipulations were carried out aseptically and each treatment was performed in triplicate. The
210 tubes were sealed using Teflon septa, with a 2 mL headspace provided and placed onto a roller
211 table at a constant gentle rotation ($\sim 15\text{-}20 \text{ rpm}$) for 7 days at 10°C .

212 After 7 days, the experiment was terminated and the whole volume from each of the
213 treatments was filtered through 5.0 μm Nucleopore filters and then immediately examined

214 using a binocular dissection microscope. Each visible agglomerate that was identifiable under
215 the microscope was carefully manipulated using a blunt needle to separate it from nearby
216 agglomerates and to allow size and area measurements to be taken. Each agglomerate was
217 individually transferred into a 2 L sedimentation tube containing sand-filtered natural seawater
218 for sedimentation velocity measurements. Measurements for sinking rate were of individual
219 agglomerates after allowing them to equilibrate for up to 30 seconds and then letting them sink
220 vertically within the tube. The time taken for each agglomerate to sink 40 mm was visually
221 observed and recorded. Agglomerates that came within 1 cm of the sedimentation tube walls,
222 or that were observed to disintegrate, were discounted. The sedimentation velocities of at least
223 ten individual agglomerates were examined for each SVE treatment.

224

225 **Microscopy**

226 To directly visualise the agglomerates under the microscope, a separate approach to the
227 NSE experiments was conducted. For this, a series of clear glass Hungate tubes containing 22
228 mL of FSC water were prepared and further amended with 10 μL of either 50 nm, 1 μm , or 20
229 μm plastic spheres to give a final plastic concentration of 5 $\mu\text{g mL}^{-1}$ for each plastic size per
230 tube. Each Hungate tube was wrapped in aluminium foil to eliminate potential light-induced
231 influences, sealed with Teflon-coated septa and placed onto a low-profile roller table at a
232 constant gentle rotation ($\sim 15\text{-}20$ rpm) for 7 days at 7°C .

233 Tubes were visually inspected daily using a blue light transilluminator to monitor for signs
234 of agglomerate formation. Agglomerates that were visible to the eye after 7 days were collected
235 using a glass Pasteur pipette, mounted on a glass slide and then carefully washed with
236 phosphate-buffered saline (10 mM Phosphate, 137 mM NaCl, and 2.7 mM KCl) for subsequent
237 staining of agglomerates. This was performed with the amino-acid specific dye Coomassie
238 Brilliant Blue G (CBBG) at pH 7.4 (Long and Azam, 1996) or with the cationic copper
239 phthalocyanine dye Alcian Blue (AB) at pH 2.5 (Alldredge et al., 1993). AB is commonly used
240 for staining acidic sugars of EPS or TEP in seawater, whereas CBBG is used for staining the
241 proteinaceous component of these polymeric substances. Following staining, each agglomerate
242 was counterstained with the nucleic acid stain acridine orange at 4 $\mu\text{g mL}^{-1}$ to examine for the
243 presence and abundance of prokaryotic cells.

244

245 **Statistics**

246 To determine if plastic size influenced the abundance of formed agglomerates, the numbers
247 of agglomerates formed in the NSE experiments were examined using a Kruskal-Wallis test, as

248 these data did not meet the assumption required for parametric analysis. An additional
249 Wilcoxon post-hoc test was carried out to determine the difference between each of the means.
250 The equivalent spherical diameter (ESD) of the agglomerates were compared by an analysis of
251 variance test (ANOVA) for each plastic sphere size used; the data was log transformed to meet
252 the assumptions for parametric analysis. A Tukey post-hoc test was carried out to identify which
253 groups were significantly different.

254 For the SSE experiments, the data was analysed using a polynomial regression analysis to
255 infer any relationship between EPS concentration and total number of agglomerates that
256 formed. A pairwise t-test was used to test for differences between mean ESD and EPS
257 concentration. A polynomial regression analysis was used to infer any relationship between
258 surface tension and EPS concentration. The difference in sedimentation velocity for
259 agglomerates, containing different sizes of plastic spheres, was analysed using a pairwise t-test
260 of the ratios between size and sedimentation velocity. All statistical analyses were performed
261 using R (R Core Team, 2014).

262

263 **Results and Discussion**

264 *Plastic characterisation*

265 The individual characteristics of the plastics used were recorded using dynamic light
266 scattering (DLS) and zeta potential (Table 1). The smaller plastics, purchased as 50 nm and 500
267 nm were found to have a diameter that was more than double that expected on the pack. This
268 may be due to the size and density of these plastics, as at this size the PS plastics were starting
269 to exhibit a sedimentation effect. As the movement of the larger plastics was more than could
270 be attributed to just Brownian motion, the DLS method was not suitable for accurately
271 measuring this dimension in this case. However, for the smaller plastics (50 and 500 nm), these
272 were colloidally stable, permitting a more accurate measure of their size. This colloidal stability
273 matched the zeta potential measurement obtained, with the smallest plastic size measured
274 corresponding to having the largest zeta potential measured. As the size of the plastics
275 increased, the charge reduced until the value of ± 30 mV was achieved, at which point the
276 colloidal stability was affected enough to permit agglomeration.

277

278 *Formation of nano- and microplastic agglomerates in seawater*

279 The formation of agglomerates occurred within 24 hours in all the NSE treatments, as
280 confirmed by flow cytometry (Figure 1). It was clear that the stock 50 nm particles were tightly
281 grouped together in Q4 (Figure 1A; 99.6%). However, after 24 hours of exposure to natural

282 seawater, agglomeration could potentially be measured (Figure 1B) with the proportion of
283 events measured in Q4 dropping to 94.4%. While this was only a marginal difference in
284 number, the plot indicates that the distribution of the size and fluorescent intensity was
285 beginning to shift from that observed from the 50 nm stock solution. This sized plastic is below
286 the detectable limits of this cell analyser (FC), therefore these data are only an indication that
287 agglomeration may be occurring.

288 The 500 nm plastics appeared to have formed density spots of both singlets and
289 agglomerates (Figure 1C and 1D) in both Q4 and Q3 respectively, during both pre- and post-
290 incubation. This indicates that some agglomeration had taken place prior to the incubation of
291 the plastics in seawater. In this case, the proportions of the events measured in each gate are
292 more indicative of agglomerate formation as 60.0% of events are in Q4, showing most the 500
293 nm plastics are unbound in singlet form; with the remaining 39.9% of events showing some
294 agglomeration already. However, following 24 hours incubation in the natural seawater the
295 proportion of plastics in Q3, indicating agglomeration, had risen from 39.9% to 93.9%. For the
296 largest plastic measured (1000 nm; Figure 1E and 1F) the difference between pristine plastic
297 and incubated plastics was not clear. The proportions in Q4 and Q2 were similar, before and
298 after incubation, with 87.1% and 82.1% of events in Q4 (singlet) and 12.8% and 15.4% present
299 in the gate believed to display the agglomerates. In addition, there appears to be an increase in
300 FC of the events in Q4 as the contour plot started to become evident in Q1. This indicates that
301 larger agglomerates were forming, yet their fluorescent signature was either not detected by the
302 cell analyser or aggregates were being formed with non-fluorescent particles.

303 Agglomerates formed from the NSE experiments were examined using a bright field
304 microscope. The size and quantity of the agglomerates were recorded. There was a significant
305 difference in the size of the agglomerates when different sized plastics were used (Figure 2A;
306 ANOVA, $F_{(1-753)} = 12.746$, $p = <0.001$). However, as shown in Figure 2B, there was overall no
307 effect conferred by the size of the plastics to the number of agglomerates measured.
308 Furthermore, the larger agglomerates that formed in vials containing 50 nm plastic spheres were
309 the only treatment that reported differing agglomerate ESDs from the control treatment
310 (pairwise t-test, $p = < 0.001$; mean ESD (SD): NSE-1 = 121.84 μm (83.70); NSE-2 = 197.64
311 μm (132.16); NSE-3 = 122.9 μm (92.07); NSE-4 = 122.25 μm (106.67)). These results
312 demonstrate that agglomerates containing larger plastic spheres were not significantly different
313 from those formed in control incubations (NSE-1).

314 Whilst we are unable at present to definitively explain why the 50 nm plastic spheres
315 promoted the formation of larger agglomerates, we offer possible hypotheses. The plastic

316 spheres that were added to the treatments had been standardised for mass per volume, which
317 would result in a greater number of plastic spheres present in the 50 nm treatment (10^{10} mL⁻¹)
318 compared to the 1 μ m (10^6 mL⁻¹) and 10 μ m (10^3 mL⁻¹) treatments. This higher plastic particle
319 number in the former treatment will have altered the collision probability between the spheres
320 and particulate organic matter (e.g. EPS, TEP) in forming agglomerates (Alldredge and
321 McGillivray, 1991; Alldredge et al., 1993). The initial stages of agglomerate formation would
322 have favoured the higher particle density within the NSE-2 treatment. Over time, it may be
323 expected that the rate of agglomerate formation in these incubations would sharply drop and
324 allow the agglomerates that formed in the other treatments to possibly reach an equal size to
325 that observed in the NSE-2 treatment. Furthermore, the shear stress experienced by
326 agglomerates would be expected to decline over time, and in turn increase the probability of
327 larger agglomerates to form (Jackson, 2015). Additionally, the dilution effect in the ocean water
328 column will likely reduce the collision rate of plastic particles to forming agglomerates
329 compared to that occurring in a confined volume as in a laboratory setting (Alldredge and
330 McGillivray, 1991). Therefore, examination of particulate density as well as mass density is an
331 important variable to be investigated in future studies.

332 The total number of agglomerates that formed in these NSE incubations did not
333 significantly differ as a function of plastic particle size (Figure 2B; Kruskal-Wallis, $H = 4.9$, d.f.
334 = 3, $p > 0.05$). In addition, all four treatments also presented a high level of variability in these
335 data (mean \pm SD): NSE-1 = 27.62 ± 26.44 ; NSE-2 = 33.50 ± 14.14 ; NSE-3 = 46.15 ± 26.73 ;
336 NSE-4 = 32.78 ± 17.94 .

337 Overall, the total numbers of agglomerates detected across all treatments ranged between
338 33-47 per mL. The controls had a mean of 27 agglomerates per mL; though smaller than the
339 treatments this was not significant. Given the relatively small size of these agglomerates (100-
340 200 μ m in diameter), the concentrations of agglomerates that formed were not unexpected;
341 numerous small particle clusters will form initially and then over time will collide and stick
342 together to form fewer, yet larger, agglomerates (Logan and Wilkinson, 1990). As the
343 agglomerates grow larger and fewer in number, the fact that they reached a maximal abundance
344 and size may be explained by the finite concentration of EPS in these experiments. In addition,
345 the larger agglomerates may experience a greater shear stress, resulting in an equilibrium state
346 of more abundant yet smaller agglomerates present within these microcosms (Barton et al.,
347 2014). As there was no significant difference in the number of agglomerates formed between
348 the plastic size and control treatments, this is evidence that the plastic is not influencing the
349 formation of agglomerates differently to that of marine snow in the controls. While nanoplastics

350 have yet to be recorded in the natural marine environment, concentrations of microplastics from
351 highly polluted areas have been recorded at $\sim 10^5$ particles m^{-3} (Lozano and Mouat, 2009). While
352 this concentration is lower than the particle densities used in the microcosms in this study
353 containing the 10 μm plastic spheres (10^9 particles m^{-3}), the microplastics used in the
354 microcosms are 1/10th of the diameter of those reported by Barton et al. (2014). Therefore the
355 final volume of the smaller plastics was one order of magnitude greater than that currently
356 observed for larger plastics.

357

358 ***Microscopic examination of microplastic agglomerates***

359 Microscopic examination of the 20 μm plastic agglomerates that formed in our experiments
360 with natural seawater from the FSC were found to be free from natural particulate matter, such
361 as plankton casts and faecal matter, that is normally observed associated with marine snow
362 (Smetacek, 1985). The agglomerates were barely visible to the naked eye during the initial 24
363 hours of the incubations, but clearly visible when illuminated under blue light transillumination,
364 which was indicative that the agglomerates were largely composed of microplastic particles. At
365 day 7 (the termination of the experiment), the agglomerates visually appeared larger (~ 0.5 – 1
366 mm) – a size that is consistent with the size definition for marine snow (>0.5 mm) in the ocean.
367 When observed under the light microscope, these 7-day agglomerates were almost entirely
368 composed of the plastic particles, held together by what appeared to be a biopolymeric
369 substance. When stained with the polysaccharide-specific dye AB (Figure 3A), or with the
370 amino acid-specific dye CBBG (Figure 3B), this confirmed the biopolymer enveloping the
371 particles was of glycoprotein composition. Since EPS produced by marine bacteria is
372 commonly of glycoprotein composition (Gutierrez et al., 2007; Hassler et al., 2011; Mancuso
373 Nichols et al., 2004), we suspect that the biopolymer forming these microplastic agglomerates
374 may predominantly be of bacterial origin. Whether it was present as part of the DOM pool in
375 the water from the FSC at the time of collection, or it was produced via *de novo* synthesis by
376 endogenous EPS-producing bacteria during these incubations, is unknown to us as we did not
377 measure changes in EPS or TEP concentrations. Nonetheless, this glycoprotein polymer
378 appeared to act as a ‘glue’ by way of interconnecting, encapsulating or trapping (like a net) the
379 microplastic particles into an amorphous matrix. The presence and persistence of EPS has been
380 shown to enhance the agglomeration of particulate matter and micro-algal cells (Grossart et al.,
381 2006), and we show here that autochthonous EPS in seawater, in particular that of glycoprotein
382 composition and possibly of bacterial origin, is a major component of nano- and microplastic
383 agglomerates.

384 Staining the agglomerates with acridine orange revealed they contained a community of
385 associated microorganisms which, based on their size (average 0.5-2.0 μm), were prokaryotes
386 (Figure 4). Due to the background fluorescence contributed by the plastic particles, it was
387 logistically impossible to provide an accurate microbial cell count associated with any one
388 agglomerate. This was also confounded by the spatial localisation of cells likely hidden within
389 and behind the agglomerates and their encapsulated plastic spheres. The EPS, and potentially
390 also the associated microbial community assuming they are not plastic-degraders, may offer
391 the microplastic particles a degree of protection from the weathering forces at sea. Coombes et
392 al. (2011), for example, observed that the colonization of engineered materials by marine
393 microorganisms (i.e. biofilms) can offer bioprotection from the weathering of such materials
394 when left exposed at intertidal zones. Other studies have also reported bioprotective qualities
395 of biofilms for other materials, such as silicates (Di Bonaventura et al., 1999; Gowell et al.,
396 2015). Although we did not carry out a sequencing survey to analyse the phylogenetic identity
397 and abundance of the microbial community associated with these microplastic agglomerates,
398 this is a key area for future investigation as it could reveal insight into a functional bioprotective
399 role, as well as whether the agglomerates might act as carriers for the transportation of
400 pathogens (Lyons et al., 2005).

401

402 ***Role of bacterial EPS in microplastic agglomerate formation***

403 From our observations of plastic agglomerate formation in the NSE incubations and
404 analysis of their composition, which revealed they are composed largely of glycoprotein
405 biopolymer, we hypothesised that marine bacterial EPS may be a key agent in the
406 agglomeration of plastic particles within the marine environment. To evaluate this, we used a
407 glycoprotein polymer produced by a marine EPS-producer, *Halomonas* sp. TGOS-10. Using
408 increasing concentrations (0.01 to 1000 $\mu\text{g mL}^{-1}$) of this *Halomonas* EPS when dissolved in
409 ONR7a synthetic seawater, we found a significant relationship between the numbers of
410 microplastic agglomerates that formed and the concentration of EPS (Regression analysis, R^2_{adj}
411 = 0.054, $p = 0.022$). Higher numbers of agglomerates formed as a function of increasing EPS
412 concentration, from the lowest EPS concentrations used (0.01 $\mu\text{g mL}^{-1}$) to 1000 $\mu\text{g mL}^{-1}$ (Figure
413 5A). EPS concentrations higher than 1 $\mu\text{g mL}^{-1}$ did not yield significantly higher numbers of
414 agglomerates relative to the untreated controls. This may be due to the finite number of unbound
415 plastic particles becoming a limiting factor at these higher EPS concentrations – i.e. there was
416 likely no available unbound microplastic particles to enhance the size of existing agglomerates
417 in these incubations that contained EPS at $>1 \mu\text{g mL}^{-1}$. Hence, whilst the size of plastic

418 agglomerates had been visually observed to increase in size over time in our microscopic
419 examination of incubations with just natural seawater, our results with a purified form of
420 bacterial EPS suggests that its concentration in seawater may contribute a limiting factor in
421 microplastic agglomerate formation.

422 Noteworthy was the number of agglomerates recorded for the negative control, which was
423 the highest across all the treatments. While the EPS clearly appears to act as a binding agent,
424 specifically in holding the plastic particles together into agglomerates, it is feasible that EPS
425 may impart a bio-dispersant function, potentially limiting the hydrophobic properties of the
426 plastic surfaces, which at low EPS concentrations may outweigh the ‘sticky’ properties of the
427 polymer. Therefore, in the absence of the EPS in the control treatment, the hydrophobic
428 chemistry of the plastic spheres may be enough to mediate efficient agglomerate formation.
429 This resulted in 136.07 (± 34.33) agglomerates that formed per mL in the EPS negative control.
430 We emphasize, however, that these negative controls are not a realistic representation of the
431 natural seawater environment because they were devoid of biopolymers that are a key feature
432 to any natural aquatic environment. With surface seawater from the North Sea having a reported
433 DOC concentration of $\sim 65\text{--}75\ \mu\text{M}$ (Thomas et al., 2005) and EPS contributing to $\sim 65\%$ of this
434 carbon budget (Underwood et al., 2010), the calculated EPS concentrations of $350\text{--}750\ \mu\text{g mL}^{-1}$
435 ¹ are comparable to those used in this study. Therefore, whilst pristine plastics can agglomerate
436 in EPS-free synthetic seawater, our results highlight the importance of marine EPS in the
437 formation of plastic agglomerates in the ocean, and we have shown here, for the first time, that
438 this occurs with nanoplastics. Hence, nano- and microplastics encapsulated within a matrix of
439 natural seawater biopolymer should be considered in any study deemed to understand the fate
440 of these pollutants in marine environments.

441 We had expected that at lower concentrations of EPS, smaller sized agglomerates would
442 form, and the converse occur at higher EPS concentrations. Our results, however, did not show
443 this. The mean ESD of microplastic agglomerates showed no difference across the range of
444 EPS concentrations evaluated (ANOVA, $F_{(6-10906)} = 1.227$, $p = 0.289$; Figure 5B). As the number
445 of agglomerates increased with increasing concentrations of EPS, it was assumed that the EPS
446 was acting as a cohesive agent. This, however, did not appear to significantly influence the size
447 of the agglomerates, which may be due to sheer forces that the agglomerates would have
448 experienced within the bottles during these incubations (Jackson, 2015; Long et al., 2015).
449 Furthermore, as this experiment employed a single size and concentration of plastic spheres,
450 the experimental set up was standardised for both mass concentration as well as particle
451 numbers. Therefore, this lack of any significant difference in agglomerate ESD supports the

452 hypothesis that the larger agglomerates reported from the NSE experiments with the 50 nm
453 spheres could be due to particle numbers being higher, and the resulting increase in surface area
454 as a binding site, compared to the other treatments; as particle densities in synthetic seawater
455 were uniform, it would explain the apparent uniformity in agglomerate ESD.

456 Although we did not perform viscosity measurements, we noted by visual inspection that
457 the viscosity of the ONR7a medium increased, especially at the higher EPS concentrations. An
458 increase in viscosity could impede particle mobility and collision in the liquid medium.
459 Additionally, an increase in the viscosity of the solution could exert a shear stress on the
460 agglomerates, sufficient to affect the maximal size the agglomerates could otherwise potentially
461 reach – smaller agglomerates would remain stable under the prevailing conditions (Alldredge
462 et al., 1990; Tiselius and Kuylenstierna, 1996).

463 To ascertain if increasing concentrations of the *Halomonas* EPS affects the surface tension
464 of the ONR7a liquid medium, and potentially also influence microplastic agglomerate
465 formation, we measured the surface tension of each of the EPS solutions. Figure 6 shows the
466 surface tension for the various EPS concentrations in ONR7a. At the lower EPS concentrations
467 used (0.0 – 1 $\mu\text{g mL}^{-1}$), the surface tension remained relatively constant, averaging around 72.6
468 to 73.6 mN m^{-1} . However, at EPS concentrations of 10 $\mu\text{g mL}^{-1}$, the surface tension increased
469 to 78.5 mN m^{-1} , and further increased to 84.4 mN m^{-1} when an EPS concentration of 100 μg
470 mL^{-1} was used. At the highest EPS concentration measured (1000 $\mu\text{g mL}^{-1}$), however, the
471 surface tension of the liquid was 77.9 mN m^{-1} , which was 6.5 mN m^{-1} lower compared to that
472 for the EPS concentration at 100 $\mu\text{g mL}^{-1}$. Overall, there was a significant relationship between
473 surface tension and EPS concentrations employed in the SSE treatments ($R^2_{\text{adj}} = 0.153$, p
474 < 0.001). The effects of surface tension on particle agglomeration have been studied within the
475 fields of particle analysis and fluid dynamics, which show that increasing surface tension is
476 related to the increased agglomeration of oil associated minerals in solution (Duzyol and Ozkan,
477 2010; Ozkan et al., 2005).

478

479 ***Influence of plastic encapsulation on marine snow sinking velocities***

480 Seawater from the Firth of Forth, and the particulates and plastics therein, bound with the
481 autochthonous EPS material to form agglomerates. Agglomerates comprising mainly of natural
482 particulate matter exhibited reduced cohesive stability. As observed for several of the control
483 agglomerates and those with 50 nm plastics, they broke apart easily into smaller sizes.
484 Therefore, only three agglomerates from each of these treatment types were assessed in these

485 SVE experiments. The larger plastic particles (1 and 10 μm spheres) were incorporated into
486 agglomerates and found to maintain a structural integrity throughout the experiment. During
487 the experiment, two of the agglomerates, both containing 10 μm plastic spheres, adhered to or
488 came within 1 cm of the walls of the sedimentation tubes. Due to the increase in drag that this
489 can exert on a particle (Winet, 1973), they were excluded from further analyses.

490 The nano-scale (50 nm) plastic spheres formed agglomerates that were smaller than those
491 observed in incubations containing the micron-scale (1 and 10 μm) plastic spheres and in the
492 control incubations with no plastic added. The small size of these agglomerates appeared to
493 have influenced their sinking velocities, which were 39.4 m day^{-1} , 113.4 m day^{-1} , 77.4 m day^{-1}
494 and 56.3 m day^{-1} , respectively for agglomerates formed in the 50 nm, 1000 nm, 10000 nm
495 and control treatments (Figure 7). For comparison, we show our data alongside two well cited
496 articles that report sedimentation velocities for artificial marine snow – 67.1 m day^{-1} (Shanks
497 and Trent, 1980) and 74.9 m day^{-1} (Alldredge et al., 1990).

498 Unlike the NSE and SSE experiments, in which agglomerates formed within 24 hours, we
499 performed these SVE experiments for 7 days to allow for larger agglomerates to form within a
500 size range reminiscent of marine snow ($>500 \mu\text{m}$). This, combined with the increased presence
501 of particulate matter from the water used here from the Firth of Forth estuary, resulted in
502 agglomerates that were markedly different in appearance and size.

503 As the sedimentation velocity of a sinking particle is directly proportional to its size, we
504 could not investigate sedimentation velocity in isolation. Therefore, the ratio between the ESD
505 and the velocity was assessed using a pairwise t-test to determine if any agglomerate was
506 significantly different to those from treatments containing different sized plastics. Our results
507 showed no significant difference as the sedimentation velocities were due to their size and not
508 the plastic contained therein. Moreover, the size difference of the agglomerates was not
509 significantly different between the different treatments and negative controls. The lack of any
510 significant difference in sedimentation velocity means that, at least with these plastic spheres,
511 the rate of DOM transport to the benthos is not affected. However, the density of different
512 plastic types may contribute to influencing the sedimentation velocities of DOM in the ocean,
513 as changes in ballast or buoyancy properties resulting from the abundance and densities of the
514 incorporated plastics will influence sedimentation (Kooi et al., 2017; Lagarde et al., 2016).
515 Moreover, the buoyant density of these agglomerates may be influenced by the composition or
516 chemistry of the EPS or TEP – the glue holding them together – since this gel-like substance
517 can significantly alter the sedimentation velocity of agglomerates (Azetsu-Scott and Passow,

518 2004; Kooi et al., 2017). However, the behaviour of these particles may not be consistent, as
519 the time taken for the agglomerates to reach significant depths can facilitate changes in the
520 biofilms, such as metabolisation of labile carbon or alterations to the microbial community
521 capable of sustaining metabolic processes at bathymetric pressures. This can result in
522 unpredictable migration velocity of the particles, which in turn would influence their
523 sedimentation. Kooi et al. (2017) reports that plastic agglomerates can alter from positive to
524 negative buoyancy, with the opposite being true also, in a cyclic manner resulting in an
525 unpredictable trajectory. Therefore, any alteration to the buoyancy of the agglomerate may be
526 mitigated or enhanced by the EPS, thus opening a new area of investigation that explores
527 biofilm formation with various plastic types.

528

529 **Acknowledgments**

530 This research was supported by a Natural Environment Research Council grant (project
531 RealRiskNano; NE/N006526/1). We thank chief scientist Alejandro Gallego of Marine
532 Scotland Science, and the ship and scientific crew of R/V *Scotia* for their support and in
533 accommodating our research needs during a research cruise to the FSC. We would like to also
534 acknowledge the help of Karina Salek in the preparation and purification of the *Halomonas*
535 EPS. Additionally, we would like to thank Raif Yucel and Linda Duncan for their help in
536 generating the flow cytometry data. Finally, we would like to thank the anonymous reviewers
537 of this manuscript, as their thoughts and comments have improved the content and overall
538 presentation of the study.

539

540 **Supplementary data**

541 Details of some of the methods used in this study have been included in the supplementary
542 documents.

543

544 **References**

- 545 Alldredge, A.L., Granata, T.C., Gotschalk, C.C., Dickey, T.D., 1990. The physical strength of
546 marine snow and its implications for particle disaggregation in the ocean. *Limnol Oceanogr* 35,
547 1415-1428.
- 548 Alldredge, A.L., McGillivray, P., 1991. The attachment probabilities of marine snow and their
549 implications for particle coagulation in the ocean. *Deep-Sea Res* 38, 431-443.
- 550 Alldredge, A.L., Passow, U., Logan, B.E., 1993. The abundance and significance of a class of
551 large, transparent organic particles in the ocean. *Deep-Sea Res PT I* 40, 1131-1140.

552 Amaral-Zettler, L.A., Zettler, E.R., Slikas, B., Boyd, G.D., Melvin, D.W., Morrall, C.E.,
553 Proskurowski, G., Mincer, T.J., 2015. The biogeography of the Plastisphere: implications for
554 policy. *Front Ecol Environ* 13, 541-546.

555 Andrady, A.L., 2011. Microplastics in the marine environment. *Mar Poll Bull* 62, 1596-1605.

556 Azetsu-Scott, K., Passow, U., 2004. Ascending marine particles: Significance of transparent
557 exopolymer particles (TEP) in the upper ocean. *Limnol Oceanogr* 49, 741-748.

558 Barton, A.D., Ward, B.A., Williams, R.G., Follows, M.J., 2014. The impact of fine-scale
559 turbulence on phytoplankton community structure. *Limnology and Oceanography: Fluids and*
560 *Environments* 4, 34-49.

561 Canesi, L., Ciacci, C., Bergami, E., Monopoli, M.P., Dawson, K.A., Papa, S., Canonico, B.,
562 Corsi, I., 2015. Evidence for immunomodulation and apoptotic processes induced by cationic
563 polystyrene nanoparticles in the hemocytes of the marine bivalve *Mytilus*. *Mar Environ Res*
564 111, 34-40.

565 Coombes, M.A., Naylor, L.A., Thompson, R.C., Roast, S.D., Gómez-Pujol, L., Fairhurst, R.J.,
566 2011. Colonization and weathering of engineering materials by marine microorganisms: An
567 SEM study. *Earth Surf Process* 36, 582-593.

568 Decho, A.W., Gutierrez, T., 2017. Microbial extracellular polymeric substances (EPSs) in
569 ocean systems. *Frontiers in microbiology* 8, 922.

570 Di Bonaventura, M.P., Del Gallo, M., Cacchio, P., Ercole, C., Lepidi, A., 1999. Microbial
571 formation of oxalate films on monument surfaces: bioprotection or biodeterioration?
572 *Geomicrobiol J* 16, 55-64.

573 Duzyol, S., Ozkan, A., 2010. Role of hydrophobicity and surface tension on shear flocculation
574 and oil agglomeration of magnesite. *Sep Purif Technol* 72, 7-12.

575 Dyksterhouse, S.E., Gray, J.P., Herwig, R.P., Lara, J.C., Staley, J.T., 1995. *Cycloclasticus*
576 *pugetii* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from marine
577 sediments. *Int J Syst Evol Micr* 45, 116-123.

578 Engel, A., 2004. Distribution of transparent exopolymer particles (TEP) in the northeast
579 Atlantic Ocean and their potential significance for aggregation processes. *Deep Sea Research*
580 *Part I: Oceanographic Research Papers* 51, 83-92.

581 Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F.,
582 Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic
583 pieces weighing over 250,000 tons afloat at sea. *PLoS ONE* 9, e111913.

584 Fletcher, M., 1977. The effects of culture concentration and age, time, and temperature on
585 bacterial attachment to polystyrene. *Canadian journal of microbiology* 23, 1-6.

586 Fotopoulou, K.N., Karapanagioti, H.K., 2012. Surface properties of beached plastic pellets.
587 *Mar Environ Res* 81, 70-77.

588 Gowell, M.R., Coombes, M.A., Viles, H.A., 2015. Rock-protecting seaweed? Experimental
589 evidence of bioprotection in the intertidal zone. *Earth Surf Process* 40, 1364-1370.

590 Grossart, H.P., Czub, G., Simon, M., 2006. Algae-bacteria interactions and their effects on
591 aggregation and organic matter flux in the sea. *Environmental Microbiology* 8, 1074-1084.

592 Gutierrez, T., Mulloy, B., Black, K., Green, D.H., 2007. Glycoprotein emulsifiers from two
593 marine *Halomonas* species: Chemical and physical characterization. *J Appl Microbiol* 103,
594 1716-1727.

595 Hansell, D.A., Carlson, C.A., 1998. Deep-ocean gradients in the concentration of dissolved
596 organic carbon. *Nature* 395, 263-266.

597 Hassler, C.S., Alasonati, E., Nichols, C.M., Slaveykova, V., 2011. Exopolysaccharides
598 produced by bacteria isolated from the pelagic Southern Ocean—role in Fe binding, chemical
599 reactivity, and bioavailability. *Mar Chem* 123, 88-98.

600 Hentschel, L.-h., 2015. Understanding species-micro plastics interactions: A laboratory study
601 on the effects of microplastics on the Azorean barnacle, *Megabalanus azoricus*, Faculty of
602 Business and Science. University of Akueyri, pp. 1-101.

603 Ikuma, K., Decho, A.W., Lau, B.L., 2015. When nanoparticles meet biofilms—interactions
604 guiding the environmental fate and accumulation of nanoparticles. *Frontiers in microbiology* 6,
605 591.

606 Jackson, G.A., 1994. Particle trajectories in a rotating cylinder: implications for aggregation
607 incubations. *Deep Sea Research Part I: Oceanographic Research Papers* 41, 429-437.

608 Jackson, G.A., 2015. Coagulation in a rotating cylinder. *Limnology and Oceanography:*
609 *Methods* 13, 194-201.

610 Jennings, M.K., Passow, U., Wozniak, A.S., Hansell, D.A., 2017. Distribution of transparent
611 exopolymer particles (TEP) across an organic carbon gradient in the western North Atlantic
612 Ocean. *Mar Chem* 190, 1-12.

613 Kooi, M., van Nes, E.H., Scheffer, M., Koelmans, A.A., 2017. Ups and downs in the ocean:
614 Effects of biofouling on the vertical transport of microplastics. *ENVIRON SCI TECHNOL* 51,
615 7963-7971.

616 Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic
617 interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density
618 appear strongly dependent on polymer type. *Environ Pollut* 215, 331-339.

619 Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M.,
620 Schludermann, E., 2014. The Danube so colourful: a potpourri of plastic litter outnumbers fish
621 larvae in Europe's second largest river. *Environ Pollut* 188, 177-181.

622 Li, S., Liu, H., Gao, R., Abdurahman, A., Dai, J., Zeng, F., 2018. Aggregation kinetics of
623 microplastics in aquatic environment: Complex roles of electrolytes, pH, and natural organic
624 matter. *Environ Pollut* 237, 126-132.

625 Lobelle, D., Cunliffe, M., 2011. Early microbial biofilm formation on marine plastic debris.
626 *Mar Poll Bull* 62, 197-200.

627 Logan, B.E., Wilkinson, D.B., 1990. Fractal geometry of marine snow and other biological
628 aggregates. *Limnol Oceanogr* 35, 130-136.

629 Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015.
630 Interactions between microplastics and phytoplankton aggregates: Impact on their respective
631 fates. *Mar Chem* 175, 39-46.

632 Long, R., Azam, F., 1996. Abundant protein-containing particles in the sea. *Aquat Microb Ecol*
633 10, 213-221.

634 Long, R.A., Azam, F., 2001. Microscale patchiness of bacterioplankton assemblage richness in
635 seawater. *Aquat Microb Ecol* 26, 103.

636 Lozano, R., Mouat, J., 2009. Marine litter in the North-East Atlantic Region: Assessment and
637 priorities for response. KIMO International.

638 Lyons, M.M., Ward, J.E., Smolowitz, R., Uhlinger, K.R., Gast, R.J., 2005. Lethal marine snow:
639 Pathogen of bivalve mollusc concealed in marine aggregates. *Limnol Oceanogr* 50, 1983-1988.

640 Mancuso Nichols, C., Garon, S., Bowman, J., Raguénès, G., Guezennec, J., 2004. Production
641 of exopolysaccharides by Antarctic marine bacterial isolates. *J Appl Microbiol* 96, 1057-1066.

642 Mari, X., Passow, U., Migon, C., Burd, A.B., Legendre, L., 2017. Transparent exopolymer
643 particles: Effects on carbon cycling in the ocean. *Progress in Oceanography* 151, 13-37.

644 Mari, X., Robert, M., 2008. Metal induced variations of TEP sticking properties in the
645 southwestern lagoon of New Caledonia. *Mar Chem* 110, 98-108.

646 Martel, J., Peng, H.-H., Young, D., Wu, C.-Y., Young, J.D., 2014. Of nanobacteria,
647 nanoparticles, biofilms and their role in health and disease: facts, fancy and future.
648 *Nanomedicine* 9, 483-499.

649 Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2018. Environmental Factors Support the
650 Formation of Specific Bacterial Assemblages on Microplastics. *Frontiers in Microbiology* 8.
651 Ozkan, A., Aydogan, S., Yekeler, M., 2005. Critical solution surface tension for oil
652 agglomeration. *Int J Miner Process* 76, 83-91.
653 Passow, U., 1994. Distribution, size, and bacterial colonization of transparent exopolymer
654 particles (TEP) in the ocean. *Marine Ecology Progress Series* 113, 185-198.
655 PlasticsEurope, 2016. *Plastics - the facts 2016: An analysis of European plastics production,*
656 *demand and waste data*, PlasticsEurope, pp. 1-38.
657 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch,
658 S., Rueden, C., Saalfeld, S., Schmid, B., 2012. Fiji: an open-source platform for biological-
659 image analysis. *Nature methods* 9, 676-682.
660 Shanks, A.L., Edmondson, E.W., 1989. Laboratory-made artificial marine snow: a biological
661 model of the real thing. *Mar Biol* 101, 463-470.
662 Shanks, A.L., Trent, J.D., 1980. Marine snow: sinking rates and potential role in vertical flux.
663 *Deep-Sea Res* 27, 137-143.
664 Smetacek, V., 1985. Role of sinking in diatom life-history cycles: ecological, evolutionary and
665 geological significance. *Mar Biol* 84, 239-251.
666 Team, R.C., 2014. *R: A language and environment for statistical computing*. R Foundation for
667 Statistical Computing, Vienna, Austria. 2013.
668 Thomas, H., Bozec, Y., de Baar, H.J., Elkalay, K., Frankignoulle, M., Schiettecatte, L., Kattner,
669 G., Borges, A., 2005. The carbon budget of the North Sea. *Biogeosciences*.
670 Tiselius, P., Kuylenstierna, B., 1996. Growth and decline of a diatom spring bloom
671 phytoplankton species composition, formation of marine snow and the role of heterotrophic
672 dinoflagellates. *J Plankton Res* 18, 133-155.
673 Underwood, G.J., Fietz, S., Papadimitriou, S., Thomas, D.N., Dieckmann, G.S., 2010.
674 Distribution and composition of dissolved extracellular polymeric substances (EPS) in
675 Antarctic sea ice. *Marine Ecology Progress Series* 404, 1-19.
676 Winet, H., 1973. Wall drag on free-moving ciliated micro-organisms. *Journal of Experimental*
677 *Biology* 59, 753-766.
678 Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the "plastisphere": microbial
679 communities on plastic marine debris. *ENVIRON SCI TECHNOL* 47, 7137-7146.
680 Zhao, S., Danley, M., Ward, J.E., Li, D., Mincer, T.J., 2017. An approach for extraction,
681 characterization and quantitation of microplastic in natural marine snow using Raman
682 microscopy. *Analytical Methods*.
683
684
685
686

687 **Figures and Tables**

688

689 **Figure 1** Countour quadrant plots showing singlet and agglomerated plastics monitored
690 using flow cytometry. A, C and E are the initial stock solutions. B, D and F are after 24 hour
691 exposure to sea water in a roller bottle. The plastic sizes used were 50 nm (A, B), 500 nm (C,
692 D), and 1000 nm (E, F).

693

694 **Figure 2.** Microplastic agglomerates formed in natural seawater after 24 hours incubation.
695 The microplastic agglomerates were measured for size (A) and abundance (B) for four
696 treatments comprising of a control and three plastic sphere sizes (50 nm, 1 μ m, and 10 μ m).
697 Values shown are the replicate means ($n = 5$), and error bars represent standard error.
698 Significant differences between treatment and control are indicated by p value.

699

700 **Figure 3.** Microplastic agglomerates formed in natural seawater after a 7-day incubation
701 were visualised widefield light microscopy. The microplastic particles stained with Alcian Blue
702 (A) and Coomassie Brilliant Blue G (B), indicating the biopolymer is of glycoprotein
703 composition. Scale bars, 100 μ m.

704

705 **Figure 4.** Nano- and microplastic agglomerates (green spheres) stained with the nucleic
706 acid-specific stain acridine orange (orange cells) and observed under a fluorescence
707 microscope. Plastic particles are: A = 20 μ m, B = 1 μ m and C = 50 nm plastic spheres; Scale
708 bars, 5 μ m. Images are composites of images from filters FITC and Rhodamin (ex 440nm, em
709 510nm and ex 546 nm, em 580 nm, respectively).

710

711 **Figure 5.** Total number of microplastic agglomerates (A), and the mean equivalent
712 spherical diameter (ESD) of the microplastic agglomerates (B) that formed in treatments with
713 increasing concentrations of bacterial EPS. Values are the mean of triplicate measurements,
714 and error bars signify standard error. Significant differences between treatment and control are
715 indicated by p value.

716

717 **Figure 6.** Surface tension of ONR7a synthetic seawater containing increasing
718 concentrations of the *Halomonas* EPS and with/without added microplastics. Values are the
719 mean of triplicate measurements, and error bars signify 95% confidence interval.

720

721 **Figure 7.** Mean equivalent spherical diameter of agglomerates formed over a 7-day
722 incubations (A), and sedimentation velocity of these agglomerates (B). Two well-cited data sets
723 were added for comparison.

724

725 **Table 1.** The characteristic data of the nan- and micro-plastic particles used throughout the
726 different experiments of this study.

727

728