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

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ABSTRACT

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

1. Introduction

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

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

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

Recent work by Canesi et al. (2015) and Hentschel (2015) observed microplastics in an agglomerated form in seawater. Whilst these studies

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did not explore the cause or mechanisms underlying this plastic agglomeration, the process may be analogous to the formation of marine snow in the ocean, which is a key component of the 'biological pump' that participates in the redistribution of carbon in marine systems (Long and Azam, 2001; Shanks and Trent, 1980). In addition, the process of microplastics, specifically latex beads, forming into agglomerates has been referred to previously and used to monitor and record the 'stickiness' of various exopolymers in the ocean, though not in the context of marine plastic pollution (Mari and Robert, 2008). The encapsulation of plastic debris in marine snow was recently described for waters collected at Avery point, Conneticut, USA (Zhao et al., 2017) – particles of predominately polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET) were found associated within the marine snow particles. If the formation of nano- and microplastic agglomerates proceeds in a similar way to marine snow, this would likely influence the buoyant density of the plastics within the water column, altering their sinking rate (Kooi et al., 2017; Lobelle and Cunliffe, 2011). For example, Long et al. (2015) reported that agglomeration of microplastics with diatoms significantly altered the sedimentation velocity of plastics from tens to hundreds of meters per day. The density of the plastic material, though, must be taken into consideration. For example, the common plastics identified within marine snow by Zhao et al. (2017) were PP, PS and PET, which have densities of 0.9, 1.04 and 1.38 g/cm^3, respectively. Since the density of seawater is 1.02–1.03 g/cm^3, each of these three plastic types would have a differential influence on marine snow buoyancy: PP would be expected to increase buoyant density of marine snow, whereas PS would have a marginal negative effect, and PET the greatest influence in lowering the buoyant density.

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

2. Materials and methods

2.1. Isolation of EPS produced by strain TGOS-10

The EPS produced by *Halomonas* sp. TGOS-10 was isolated by growing the strain in ZM/10 supplemented with glucose to a final concentration of 0.1% (w/v). For this, exponentially-growing cells of the strain were inoculated into a 3 L Erlenmeyer flask containing 1 L of the growth medium. The flask was then incubated with shaking (75 rpm) at 21 °C in the dark. Once growth had reached the stationary phase (2–3 days), the biomass was pelleted by centrifugation (4000 ×g; 20 min) and the supernatant filtered (0.2 μm) to remove residual cells. Isolation of purified EPS was performed as previously described (Gutierrez et al., 2007). Briefly, KCl (to 7% w/v) was dissolved in the cell-free supernatant volume prior to the addition of two volumes of cold ethanol and the mixture left for 24 h at 4 °C. The precipitated material was then pelleted by centrifugation (4000 ×g; 20 min), the supernatant disposed of, and then the pellet was extensively dialysed against milli-Q water (18 MΩ/cm quality) using a 1 kDa molecular-weight pore-size membrane (Spectra, Cole-Parmer, inc). The resultant purified EPS was then lyophilised and stored at room temperature in a sealed container until required.

2.2. Roller-bottle incubations

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

2.3. Natural seawater experiments (NSE)

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

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

At the end of the incubation period (24 h), the contents of each vial were filtered through a 0.22 μm white Millipore filter, using a low-pressure vacuum to minimize the disruption of marine snow/plastic agglomerates. Each filter was then examined using a bright-field binocular dissection microscope. Three images (image area = 9 mm^2) were recorded for each of the five replicates, and the number of agglomerates formed and their size were measured using Fiji image analysis software (Schindelin et al., 2012).

2.4. Flow cytometry

A random subset of NSE samples were examined using flow cytometry to monitor the agglomeration of the plastics after 24 h. Only plastics smaller than 1000 nm were used to prevent blockages within the injection port of the instrument. Therefore, an addition of a 500 nm plastics treatment was added to increase scope of the investigation. This was achieved using a BD LSR Fortessa multi-colour cell analyser (Biosciences, UK). The nano- and micro plastics were assessed on forward scatter (FC), side scatter (SC) and fluorescence intensity (Alexa Fluor 488, Cyan-green colour; excitation: 495 nm; emission: 519 nm). Plastic spheres were recorded based on their FC and fluorescent intensity using 10,000 events or a 2 min duration if this number of events could not be achieved. For the smaller 50 nm nanoplastics, FC was not an appropriate method of detection due to the small size of the particles, therefore only fluorescence intensity was used. Solutions of the nano- and microplastics were analysed to examine the mean size and fluorescent intensity of the plastics in a singlet form. These singlet stocks were used to set the gating to ensure that the majority of the singlet nano-plastics were measured in gate Q4. This was repeated after 24 h incubation within the natural seawater to determine if agglomeration had occurred. Agglomeration was measured by changes in event numbers occurring in gate Q2 and Q3, which measured increases in size and fluorescent intensity, respectively (more plastics per aggregate).

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