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Colonisation of plastic pellets (nurdles) by *E. coli* at public bathing beaches

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ABSTRACT

The hard surface of waterborne plastic provides an ideal environment for the formation of biofilm by opportunistic microbial colonisers, and could facilitate a novel means of dispersal for microorganisms across coastal and marine environments. Biofilms that colonise the so-called 'plastisphere' could also be a reservoir for faecal indicator organisms (FIOs), such as *Escherichia coli*, or pathogenic bacteria such as species of *Vibrio*. Therefore, the aim of this study was to map the spatial distribution of beach-cast plastic resin pellets (nurdles) at five public bathing beaches, and quantify their colonisation by *E. coli* and *Vibrio* spp. Nurdles were heterogeneously distributed along the high tide mark at all five beaches, and each beach contained nurdles that were colonised by *E. coli* and *Vibrio* spp. Knowledge of *E. coli* colonisation and persistence on nurdles should now be used to inform coastal managers about the additional risks associated with plastic debris.

1. Introduction

The most abundant form of litter in the marine environment is plastic, which due to its buoyant and persistent characteristics can provide an ideal environment for the formation of biofilm by opportunistic microbial colonisers. The interface between the plastic surface and the environment has been termed the 'Plastisphere' (Zettler et al., 2013), and such biofilm formation on marine plastics could provide plastisphere communities with a niche for protection from harmful UV irradiance and predation.

One of the most common types of plastic in the marine environment are virgin plastic resin pellets (or nurdles), a disc shaped plastic particle, typically 3–5 mm diameter, used as the raw material in the production of many thermally moulded plastic products. Nurdles can be accidentally released into the environment, and commonly enter marine systems through ship spills, road runoff, inland waterways, or wind transfer (Boucher and Friot, 2017). Once in the marine environment, nurdles can be deposited in coastal areas via winds, surface currents, and tides (Karkanorachaki et al., 2018), with the potential to be dispersed over long distances (Karlsson et al., 2018). Therefore, nurdles could act as a significant vector for hitchhiking microbes, and facilitate subsequent transport to beaches, bathing waters and shellfish harvesting waters (Keswani et al., 2016).

In Europe, the Bathing Water Directive (BWD), 2006/7/EC, uses faecal indicator organisms (FIOs), such as *Escherichia coli*, as the key monitoring parameter for regulating compliance of microbial water quality. However, whilst plastic litter in bathing water can influence the survival of *E. coli* (Quilliam et al., 2014), its ability to bind to, and persist in, the plastisphere of marine plastic debris is currently unknown. Thus, an improved understanding of the potential for nurdles to facilitate the survival of FIOs and human microbial pathogens (e.g. species of *Vibrio*), and thus increase human exposure routes by providing a vehicle for dispersal around coastal waters, is clearly an area of marine environmental pollution research that needs urgent investigation. Therefore, the aim of this study was to test the hypothesis that nurdles deposited on bathing beaches can act as a vector for *E. coli* persistence at public bathing beaches.

2. Methods

Nurdles were collected from five EU designated bathing beaches (Fig. 1) situated on the Forth Estuary (East Lothian, Scotland, UK), which are approximately 45 km downstream of the industrial town of Grangemouth, and 30 km downstream from the large urban settlement of Edinburgh. North Berwick (Milsey Bay) is the only sampling site in which the bathing water catchment contains both rural and urban land uses; the other four sites are all predominantly rural catchments. Waste water treatment works (WwTW) within the study area are located in the towns of Gullane and North Berwick, final effluent from these WwTW is discharged approximately 1 km off the coast. Combined sewer overflows (CSOs) and emergency overflows (EOs) are also present within the study area.

All field work was performed in the first two months of the Scottish bathing water season (June and July), when tourist numbers are

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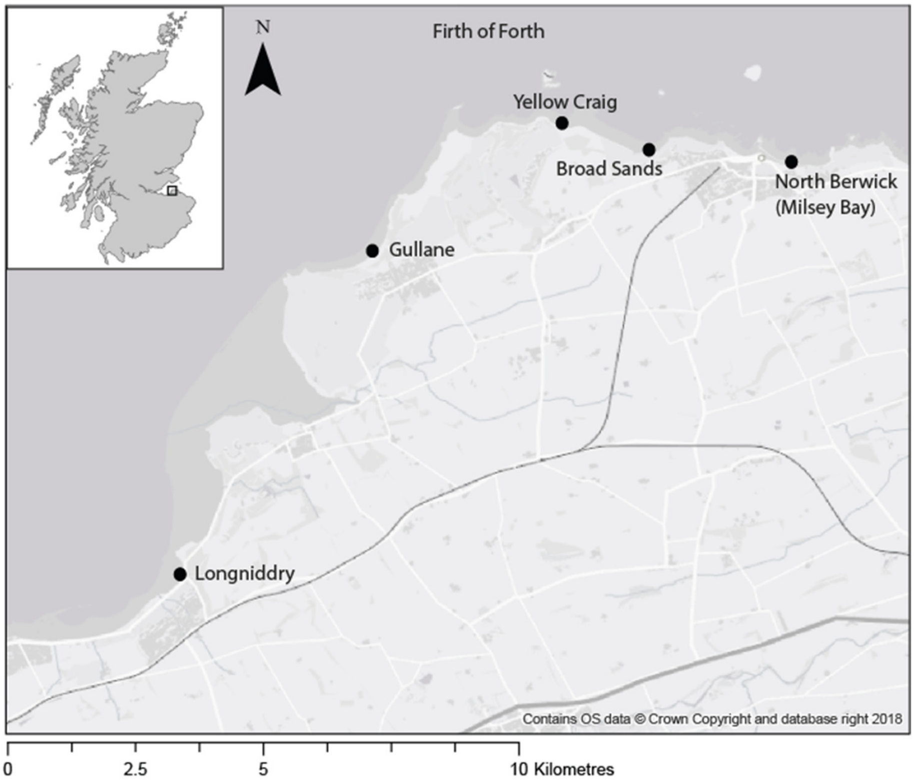

Fig. 1. Spatial locations of the public bathing beaches sampled in this study.

generally at their highest. At each beach, 1 m^2 quadrats were used to sample nurdles in a transect along the high tide line. In areas of higher nurdle density, e.g. areas with seaweed or other organic debris, quadrats were laid more frequently to gain a more focused snapshot of changes in spatial distribution. The top 5 cm of sand within each quadrat was passed through a 2 mm mesh sieve to separate nurdles from sand and other debris. The total number and colour composition of nurdles in each quadrat was recorded, together with the grid reference and distance along the transect. In cases where nurdle counts were in excess of 500 m^{-2}, the number of nurdles was recorded as > 500 m^{-2}. Nurdles from each beach were collected with sterile forceps and a composite sample from each beach transferred to a 50 ml plastic tube wrapped in aluminium foil to avoid exposure to sunlight. All samples were stored at 5 °C and processed within 18 h.

In the laboratory, nurdles were thoroughly rinsed with sterile distilled H_2O, and pre-enriched in LB broth (Fisher Bio-reagents, UK) at 37 °C in a rotating incubator (60 rev min^{-1}) for 18 h, in five individual pseudoreplicate bottles (150–250 nurdles per bottle) per beach. Following the pre-enrichment step, the nurdles were thoroughly rinsed with sterile distilled H_2O to remove any microorganisms not bound within the biofilm and, using sterile forceps, twelve nurdles from each bottle were aseptically placed onto the surface of two different types of selective agar: membrane lactose glucuronide agar (MLGA) for *E. coli*, and thiosulphate citrate bile salts agar (TCBS) (Oxoid, UK) for *Vibrio* spp. Each nurdle was gently pressed into the surface of the agar in order to maximise the possible growth from the biofilm on the surface of the nurdle. All plates were inverted and incubated at 37 °C for 18–24 h. The halos of either *E. coli* or *Vibrio* spp. growing around individual nurdles were enumerated; all plates contained individual nurdles with and without this diagnostic bacterial growth, demonstrating that the pre-enriched media was not acting as an inoculant for uncolonised nurdles, and only those nurdles that were colonised by biofilm at the beach produced bacterial growth on the media.

3. Results and discussion

Plastic nurdles were found at all five public bathing beaches, and were distributed heterogeneously on the sand along the high tide mark (Fig. 2). At each beach, the highest densities of nurdles were found in discrete areas either behind rocks or amongst organic debris and detached seaweeds. The presence of detached seaweed and other debris in these areas may facilitate the settlement of buoyant plastics as the tide goes out (Turner and Holmes, 2011). The direction of prevailing winds and major coastal currents are also key factors influencing the distribution and accumulation of nurdles in coastal zones (Ryan et al., 2018), and beach geomorphology and physical features such as ridges can trap nurdles as the tide retreats (Browne et al., 2015). The dominant winds in Scotland blow from the west to the east, and it is likely that the prevailing winds and dominant eastern currents of the Forth estuary will have played a role in the spatial distribution and accumulation of nurdles at the eastern end of most of these beaches.

Translucent nurdles were the most common colour, although the total colour composition was not significantly different between the beaches (Table 1). The consistency of colour composition suggests that translucent nurdles are produced in higher numbers than other colours, perhaps due to their versatility in the manufacturing process (Turner and Holmes, 2011; Karkanorachaki et al., 2018). Importantly, translucent nurdles are inconspicuous in beach sand and could easily be overlooked, particularly if other organic debris is present. This can pose a challenge, both for accurate quantification and their removal during beach cleaning (Hidalgo-Ruz et al., 2012). The density of nurdles in this study was relatively low compared with previous reports (Ryan et al., 2018); although as these beaches are popular with tourists, it is likely that intensive beach cleaning or mechanical grooming had been undertaken prior to the bathing season. However, as the tides submerge these beaches twice a day, there is the opportunity for the constant addition and removal of beach cast nurdles with the ebb of the tide.

Nurdles colonised by *E. coli* and *Vibrio* spp. were found at all five beaches (Table 2), with high levels of *Vibrio* colonisation (> 75%

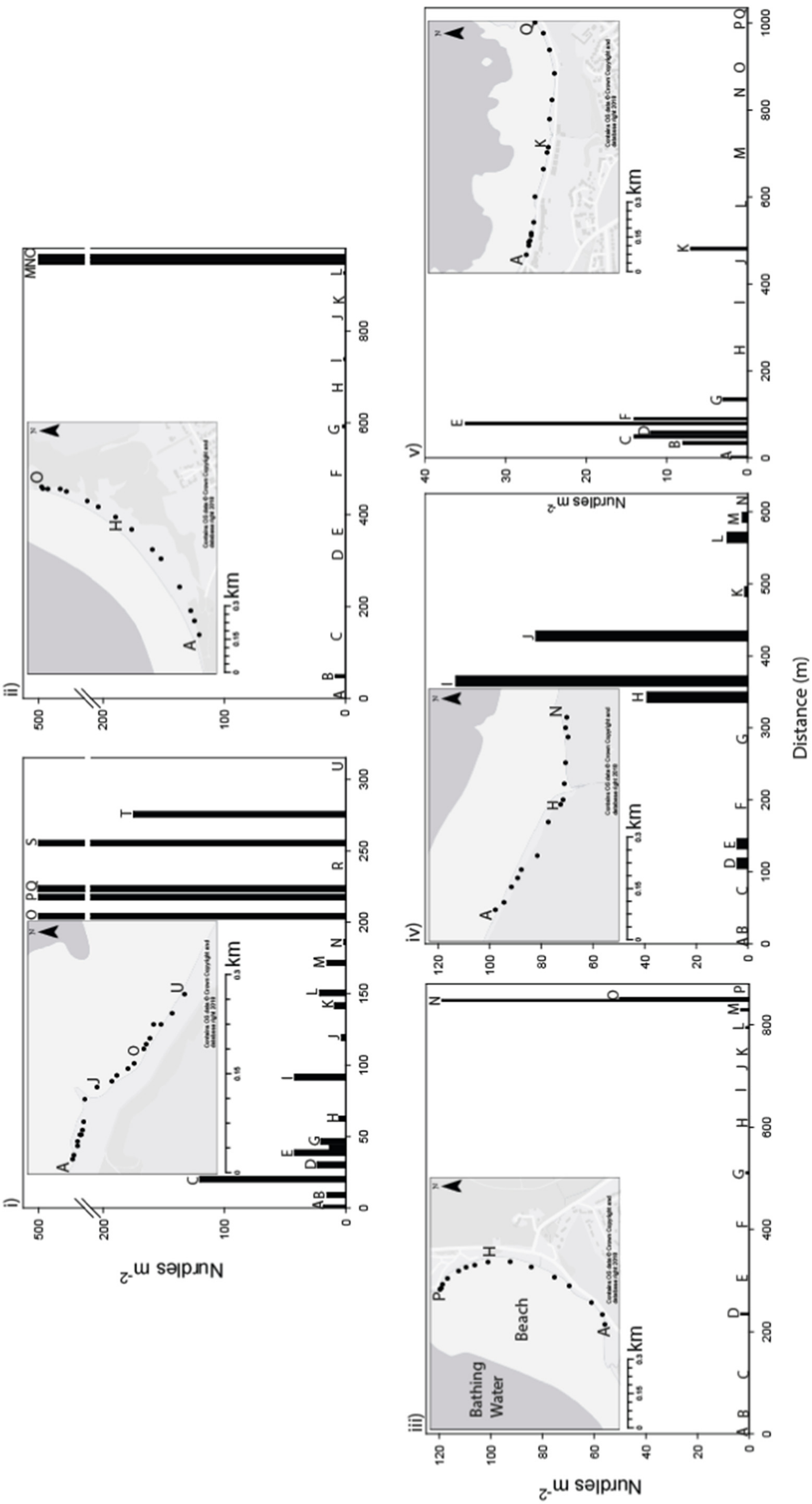

Fig. 2. Spatial distribution of beach-cast nurdles from five UK public bathing beaches: Yellow Craig (i); Gullane (ii); Longniddry (iii); Broad Sands (iv); North Berwick (v). Each map insert shows the quadrat sampling points, which are coupled with the accompanying letters above the bars.

Table 1
Total colour composition of nurdles collected from five bathing beaches.

	Colour (%)						
	Translucent	White	Black	Yellow	Blue	Green	Other
Broad Sands	93	2	0	3	1	1	0
Gullane	93	1	1	2	1	0	2
Longniddry	87	0	1	6	4	0	2
North Berwick	88	1	4	0	1	0	6
Yellow Craig	92	2	0	3	3	0	0

Table 2
Colonisation of nurdles by *E. coli* and *Vibrio* spp.

	Colonisation (%)	
	E. coli	*Vibrio* spp.
Broad Sands	12	78
Gullane	43	80
Longniddry	15	95
North Berwick	9	82
Yellow Craig	24	50

colonised) at four of the beaches. Colonisation by either *E. coli* or *Vibrio* spp. did not significantly differ between the different coloured nurdles and colonisation by *E. coli* was consistently lower than colonisation by *Vibrio* spp. (Table 2). Plastic nurdles deposited at the high tide mark on beaches could have become colonised by *E. coli* during their time in the sea, or by direct contact at the beach, e.g. by either bird or dog faeces. However, plastic debris can quickly become colonised by biofilm in the environment (Amaral-Zettler et al., 2015), and it is most likely that *E. coli* colonisation occurred in faecally-contaminated seawater, arising from either agricultural run-off or sewage discharge. Microbial colonisation of environmental plastics is influenced by the time the plastic has been in the environment, with those that have been in the environment for longer more likely to come into contact with a diverse range of microorganisms (Kirstein et al., 2018). Furthermore, older, and more degraded plastics often have pits, grooves and scratches on the surface, which increases the surface area for microbial colonisation (Fotopoulou and Karapanagioti, 2012). Most beaches are subject to semidiurnal high tides, and consequently beach cast nurdles can be submerged twice a day. Submergence by potentially contaminated water could promote *E. coli* recruitment into the biofilm and nurdles could then either remain on the beach or be washed back out to sea with the ebb of the tide.

Vibrio spp. are ubiquitous in the marine environment, and the high colonisation of nurdles in this study suggests that *Vibrio* spp. may make up part of the autochthonous biofilm community on the majority of plastics found on beaches. Whilst the culture-dependent methods used here do not differentiate between *Vibrio* spp., potentially pathogenic species (e.g. *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus*) have previously been isolated from plastic debris in coastal and estuarine regions of the North Sea (Kirstein et al., 2016). *Vibrio* spp. are more likely to colonise plastics that already contain a biofilm compared with bare microplastics (Yokota et al., 2017), although whether *E. coli* similarly prefers to bind to an existing biofilm, or whether they can efficiently bind to uncolonised plastic debris remains unknown. The type of plastic, together with seasonal and spatial factors are important for promoting microbial colonisation of plastics in the open ocean (Kirstein et al., 2018), with evidence that bacteria more readily colonise plastics in low nutrient environments (Amaral-Zettler et al., 2015). Subsequently, the longer a nurdle has been in the environment, the higher the chance of it becoming colonised by microbial biofilm and the greater the risk of being colonised by potential pathogens or FIOs prior to it being deposited on beaches.

This under-investigated point of contact between humans and potentially plastic polluted beach sands, could lead to higher exposure rates of the public to reservoirs of potential pathogens and FIOs associated with plastic debris (Keswani et al., 2016). At EU designated bathing waters, improvements could be made to Bathing Water Profiles (BWPs) by detailing beach stranded plastics as a possible source of FIOs and potential pathogens, which are harmful to human health. This could improve beach management strategies for the protection of public health, through increased removal effort, particularly of inconspicuous microplastics such as nurdles, from beach sands at designated bathing waters.

4. Conclusion

There is currently a significant lack of data on the negative implications of marine plastics capable of supporting diverse microbial communities and potentially disseminating FIOs and/or pathogenic microorganisms within the marine and coastal environment. The potential for the wider global dissemination of FIOs and human pathogens by marine plastic debris vectors is further exacerbated by future climate change scenarios, in particular the projected increases in surface water temperature, which could increase the geographical range of new and emerging diseases into coastal and marine environments. Changes in climate are also driving an increased frequency and intensity of coastal inundation, and storm surges could amplify the risk of plastic debris being delivered to the beach environment, particularly during the absence of seasonal beach cleaning by local authorities. Knowledge of *E. coli* colonisation and persistence on nurdles should now be used to inform bathing water managers about the additional risks associated with plastic debris and allow appropriate regulations to be implemented to help reduce the risks of illness from contact with nurdles at public beaches.

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