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Microbial hitchhikers of marine debris

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1 **Microbial hitchhikers of marine plastic debris: human exposure risks at**
2 **bathing waters and beach environments**

3

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10 **ABSTRACT**

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22 Keywords: marine plastic debris, microplastics, biofilms, microorganisms, pathogens,

23 bathing water quality, human health

24 **1. Introduction**

25

26 Marine plastic debris is an environmental pollutant of growing concern, with
27 detrimental effects on aquatic reptiles, birds and mammals already well documented
28 (Gregory, 2009; Derraik, 2002). The durable, light weight and inexpensive nature of
29 plastic has made it a ubiquitous choice for many industrial and consumer products
30 (Osborn and Stojkovic, 2014). Its widespread use has facilitated its entry and
31 accumulation within coastal waters and beach environments, with global changes in
32 rainfall, wind speed, and more frequent flood and storm events predicted to further
33 increase the amount of stranded and drifting plastics in the coastal zone (Young *et al*,
34 2011; Gulev and Grigorieva, 2004; Meier and Wahr, 2002; Goldenberg *et al*,
35 2001).

36 Globally, more than 200M tonnes of plastic is produced annually (Ivar do Sul
37 and Costa, 2014), with approximately 4.8 – 12.7M tonnes of plastic waste entering
38 the ocean in 2010 (Jambeck *et al*, 2015). Marine plastic debris includes large, macro
39 particles such as carrier bags, bottles, packaging, and fishing gear (Eriksen *et al*,
40 2014), and more commonly microplastics and nanoplastics (Andrady, 2011). Sources
41 of plastic debris entering the oceans are numerous, with the majority a result of
42 direct disposal of litter from land and at sea (including the illicit dumping of fishing
43 gear), the blowing of litter from landfill sites, public littering, and losses from ship
44 transport and accidental spills (Barnes *et al*, 2009). In a study assessing plastic debris
45 in Latin America and the wider Caribbean region, land-based plastic debris was
46 found to be the most abundant along continental shores (Ivar do Sul and Costa,
47 2007), whilst fishing-related plastics are often more prevalent on remote islands

48 (Barnes *et al*, 2009). Recently, there has been an increasing focus on “microplastics”,
49 which is defined by the National Oceanic and Atmospheric Administration (NOAA) as
50 plastic particles less than 5 mm in diameter (NOAA, 2009). Sources of microplastics
51 in marine environments include “primary” microplastics present in cosmetic care
52 products such as facial exfoliating scrubbers, clothes fibres washed into the sea
53 through sewage effluent from washing machines, and industrial discharge of virgin
54 plastic production pellets and those used as air-blasting media (Browne *et al*, 2011;
55 Cole *et al*, 2011; Fendall and Sewall, 2009; Derraik, 2002). In addition, “secondary”
56 microplastics frequently enter waterways through the breakdown of macro particles
57 by a combination of physical, biological and chemical processes (Ryan *et al*, 2009;
58 Thompson *et al*, 2004). Rivers, tides, wind, heavy rainfall, and storm and sewage
59 discharge facilitate dispersal of microplastics within the marine environment (Reisser
60 *et al*, 2013), with an estimated 5.25 trillion plastic particles weighing approximately
61 269,000 tonnes now floating in the sea (Eriksen *et al*, 2014).

62 The impacts of marine plastic debris go beyond simply posing a threat to
63 marine wildlife (Figure 1). Marine plastics can lead to significant economic losses by
64 interfering with the shipping and fishing industries – e.g. drifting fishing gear
65 becoming entangled on propellers and rudders; large floating debris causing damage
66 by collision; and ghostnets trapping commercial fish (Pichel *et al*, 2007; Sheavly and
67 Register, 2007). Furthermore, floating plastic bags are often the cause of blocked
68 and damaged water pumps, causing substantial vessel damage, costly repairs and
69 danger to operating staff (Sheavly and Register, 2007; Aliani *et al*, 2003). Plastic
70 debris also poses a significant threat to the tourism industry. For example, beaches
71 polluted with medical and sanitary waste poses a public health risk, devalues the

72 experience of beachgoers, and can often require costly beach-cleaning efforts
73 (Moore, 2008). With quantities of beach-cast plastic expected to rise due to
74 increased frequency of storm and severe weather events, coastal areas dependent
75 on tourism are likely to face a number of socio-economic challenges (McIlgorm *et al*,
76 2011).

77 Plastic debris can also provide a novel mechanism for the spread of invasive
78 and alien species, in addition to that facilitated by natural substances like rafts of
79 vegetation, wood, or pumice (Bryan *et al*, 2012; Minchinton, 2006; Jokiel, 1990). A
80 diverse range of organisms have already been found colonising macro-plastics, and
81 in some cases have led to the introduction of non-native species into new habitats
82 (Gregory, 2009; Barnes 2002*a;b*). Until very recently, however, little attention has
83 been paid to the concept of plastic providing a novel means of spatial and temporal
84 transport for microorganisms across marine and coastal environments (Caruso,
85 2015). The physical properties of plastic can provide a unique habitat capable of
86 supporting diverse microbial communities (Zettler *et al*, 2013). The buoyant and
87 persistent nature of plastic could contribute significantly to the survival and
88 transport, across large distances, of microorganisms that associate with its surface.
89 The biofilms that make up this so-called “plastisphere” could also be a significant
90 reservoir for pathogenic microbes, faecal indicator organisms (FIOs) and harmful
91 algal bloom (HAB) species. Plastic debris could be acting as a potential vector for the
92 wide-scale dissemination of these organisms (Zettler *et al*, 2013; Masó *et al*, 2003).

93 A few recent studies have shown evidence for the formation of biofilms by
94 bacteria and FIOs (such as *E. coli*) on plastic water distribution pipes (Yu *et al*, 2010;
95 Lehtola *et al*, 2004), and the persistence of pathogens (such as *Vibrio* spp.) on plastic

96 debris (McCormick *et al*, 2014; Quilliam *et al.*, 2014; Zettler *et al*, 2013). However,
97 the ability of microorganisms to persist on beach-stranded plastic debris, and
98 increase dissemination of potentially pathogenic microbes in coastal zones needs
99 addressing to allow regulators and beach managers to make more informed
100 decisions about public safety at beach environments. In Europe, the quality of
101 bathing water and safety of beaches is governed by the EU Bathing Water Directive
102 (BWD; 2006/7/EC). The BWD sets standards for microbial water quality and guides
103 monitoring programmes to regulate bathing waters via the use of FIOs for the
104 assessment of faecal pollution. The BWD also requires the production of a Bathing
105 Water Profile (BWP) for all designated EU bathing waters (Mansilha *et al*, 2009),
106 which contains details on the nature of possible pollution sources that could have
107 negative impacts on a bather's health (Schernewski *et al*, 2012). Designations such as
108 the Blue Flag award are also largely driven by the BWD.

109 Epidemiological studies have reported the relationship between bathing
110 water quality and the occurrence of adverse human health effects such as
111 gastrointestinal (GI) symptoms, respiratory diseases, and eye, nose and throat
112 infections (Wade *et al*, 2006, Zmirou *et al*, 2003; Prüss, 1998). Whilst most of these
113 studies have focused on waters impacted by municipal-wastewater effluent, the
114 impacts of other diffuse sources of pollution remain relatively unexplored (Soller *et*
115 *al*, 2010). With the potential for plastic providing a possible site for pathogen and
116 FIO attachment and the dissemination of these organisms in the marine
117 environment, we require a better understanding of these processes in order to not
118 compromise beach safety and management of risks to bathing water quality.
119 Assessing beach and bathing environments for beach-cast plastic debris and

120 analysing it for associated pathogens could provide a better insight into the quality
121 of European bathing waters through the production of a more detailed BWP, as well
122 as to qualify plastic debris as a potential indicator and carrier of pathogens of risk to
123 human health. This could further help prevent economic losses associated with
124 beach closures, and enable beaches to maintain their Blue Flag status (Schernewski
125 *et al*, 2012; Wyer *et al*, 2010).

126 Beaches and coastal environments form some of the most ecologically and
127 socio-economically important habitats worldwide (Harley *et al*, 2006), and
128 ecosystem services in these areas are already facing significant pressure from
129 anthropogenic activities (Quilliam *et al*, 2015; Schlacher *et al*, 2007a; 2006). Against
130 a backdrop of climate change, and projected changes in ocean currents, upwellings,
131 water temperature and wind speed, the persistent multi-pollutant effects of plastic
132 debris in coastal environments increases the urgency to understand the risks of
133 human exposure to plastic pollution and inform more sustainable beach
134 management options. The aim of this review is to explore the potential for marine
135 plastics to serve as a mechanism for the persistence and transmission of FIOs and
136 pathogenic or harmful microorganisms, and the pathways of human exposure risk in
137 coastal environments.

138

139 **2. Biofilm formation and the “Plastisphere”: a man-made ecological habitat**

140

141 Biofilms are formed by the microbial secretion of extracellular polymeric
142 substances (EPS) that act as a type of architectural scaffolding, or support ‘glue’, for
143 microbial cells to ‘stick’ together, and further provide a protective environment that

144 enables microbial cells to grow in hostile habitats and facilitate dispersal (Hall-
145 Stoodley and Stoodley, 2005). Microorganisms can form biofilms on any artificial or
146 natural surface, such as medical implants, rocks, copper and plastic pipes of water
147 distribution systems (Costerton *et al*, 2005; Lehtola *et al*, 2004; Lee *et al*, 2003).
148 Some studies have demonstrated that the surfaces of different types of plastics, for
149 e.g. polyethylene (PE) and polyethylene terephthalate (PET), when submerged in
150 seawater can become rapidly colonised by heterotrophic bacteria, and that these
151 organisms are able to persist for longer periods than those in the surrounding
152 seawater (Webb *et al* 2009; Lobelle and Cunliffe, 2011). Interestingly, these studies
153 had found lower levels of EPS secretion and bacterial colonisation on these plastic
154 surfaces compared to that on other substrates.

155 Successional changes in bacterial colonisation of plastics with varying degrees
156 of hydrophobicity have been demonstrated in seawater, with early-stage
157 colonisation often marked by higher species richness (Jones *et al* 2006; Jackson *et al*,
158 2001). *Alphaproteobacteria* was found to be the most dominant group of colonising
159 bacteria, with *Gammaproteobacteria* mainly occurring during the early colonisation
160 stages (Lee *et al*, 2008; Jones *et al*, 2006). However, a range of environmental factors
161 such as grazing pressure from macrozooplankton could also significantly influence
162 bacterial colonisation of plastic surfaces (Webb *et al*, 2009). *Gammaproteobacteria*
163 are an ecologically diverse group of gram-negative bacteria that contain a number of
164 pathogens harmful to human health, and which include *Salmonella* spp. and *Vibrio*
165 *cholera*. Since pathogenic strains of *Vibrio* are recognised to readily colonize a
166 diverse range of marine substrates, their potential to colonise plastic debris requires

167 urgent investigating, particularly in light of prescient knowledge that plastic debris
168 can be easily dispersed in the marine environment (Zettler et al., 2013).

169 Reports on biofilms on plastic waste in the environment are limited
170 (summarised in Table 1). Biofilm formation on plastic debris was first reported in
171 1972 in the Sargasso Sea, where bacterial communities were found colonising
172 floating microplastic particles (Carpenter *et al*, 1972; Carpenter and Smith, 1972).
173 Zettler *et al* (2013) conducted the first study of its kind, which characterised the
174 composition of microbial communities colonising micro and macro pieces of PE and
175 polypropylene (PP) collected from geographically distinct open ocean areas of the
176 North Atlantic Subtropical Gyre. The plastisphere community consists of a
177 morphologically diverse range of microbes that comprise a dense mix of eukaryotic
178 and prokaryotic cells, such as diatoms, coccolitophores, dinoflagellates, fungi and
179 bacteria (Reisser *et al* 2014; Zettler et al, 2013). The size, type and surface roughness
180 of marine plastic debris can also influence the diversity and abundance of the
181 microbial taxa that will colonise it (Carson *et al*, 2013).

182 Microbial assemblages associated with marine plastic are distinctly different
183 from those of the surrounding seawater (Harrison *et al*, 2014; McCormick *et al*,
184 2014; Oberbeckmann *et al*, 2014). PET drinking water bottles attached to buoys in
185 the North Sea, UK, showed clear differences in the composition of the plastisphere
186 community compared to microbial communities of seawater and those attached to
187 plankton and debris (Oberbeckmann *et al*, 2014). The study also illustrated temporal
188 differences in microbial community composition colonising the plastic bottles,
189 revealing a higher abundance of photosynthetic brown algae and cyanobacteria

190 during the summer months compared to a dominance of heterotrophic bacteria and
191 photosynthetic diatoms during the winter (Oberbeckmann *et al*, 2014).

192 In a study by Harrison *et al.* (2014) employing a laboratory-based microcosm
193 setup containing sterile artificial seawater and inoculated with low-density
194 polyethylene (LDPE) microplastics collected from marine sediments of the Humber
195 Estuary, UK, over time the microplastics were observed to become colonised by
196 morphologically distinct prokaryotic cells, predominantly bacteria. Molecular
197 analysis revealed significant differences between the bacterial communities found
198 attached to the LDPE microplastics and those within the sediment (Harrison *et al*,
199 2014). This finding corroborates that of McCormick *et al* (2014) who demonstrated
200 significant differences in microbial communities found on microplastics in an urban
201 Chicago River compared to those of the surrounding water column and suspended
202 organic matter. Harrison *et al* (2014) also highlighted significant time-dependent
203 variation in the structural community of the LDPE bacterial community. Initial
204 observations showed the existence of sediment type-specific communities present
205 on microplastics, with shifts towards “LDPE-specific” bacterial communities occurring
206 at days 7 and 14 of the experiment, indicating a possible adaptation and change in
207 community structure of these bacteria to microplastic waste (Harrison *et al*, 2014).
208 The tendency of microplastics to attract a bacterial community that differs from that
209 of the surrounding environment is further supported by a study conducted in a
210 freshwater system, where bacterial communities on plastic litter from the Chicago
211 River and Chicago’s Lake Michigan beaches differed significantly from those
212 colonising organic substances such as leaves and cardboard (Hoellein *et al*, 2014).
213 The prevailing evidence appears to indicate that the plastisphere microbial

214 communities are distinctly different from those found colonising other substrates or
215 within the same environment but not associated with the plastic debris, indicating
216 the possibility of specific adaptation to this novel man-made habitat. Plastics could
217 therefore provide a new ecological niche or biotope, which, owing to its longevity in
218 the environment, could help facilitate the persistence and transport of
219 microorganisms across oceans and into new geographic areas (De Tender *et al*,
220 2015).

221 There is growing commercial interest in plastic biodegradation, with current
222 research focussing on identifying the types of microorganisms capable of degrading
223 plastics (Loredo-Treviño *et al*, 2012). Numerous studies have shown many different
224 species of marine bacteria with the capacity to degrade hydrocarbons. Species of
225 hydrocarbon- degrading bacteria, belonging to over 20 genera and distributed across
226 some of the major bacterial Classes (*Alpha-*, *Beta-* and *Gammaproteobacteria*;
227 *Actinomycetes*; *Flexibacter-Cytophaga- Bacteroides*), have been isolated and
228 described (Floodgate, 1995; Head and Swannell, 1999; Head *et al.*, 2006; Yakimov *et*
229 *al.*, 2007). These organisms are strongly enriched for during an oil spill at sea and
230 they play an important role in the biodegradation of the oil (Gutierrez *et al*, 2014;
231 Gertler *et al*, 2012). To our knowledge, the marine environment is the only place
232 where we find bacteria with the ability to utilize hydrocarbons almost exclusively as
233 a sole source of carbon and energy. Considering that plastic is composed of
234 hydrocarbons, these types of bacteria could have important implications with
235 respect to their role in degrading plastic debris. There are reports of changes in the
236 surface topography of PET samples colonised by microorganisms, and microbial cells
237 have been identified within pits and grooves, suggesting microbial degradation of

238 the plastic surface (Reisser *et al* 2014; Webb *et al* 2009). However, the mechanics of
239 biodegradation of marine plastic debris, and the underlying processes that influence
240 this behaviour, are areas that need much further investigation to fully exploit the
241 implications this can have on the environment.

242

243 **3. Plastic dispersal: dissemination of pathogenic and harmful microbes**

244

245 There are many examples on the introduction of invasive species into new
246 habitats through colonisation of natural substances, such as wood, dead plants and
247 pumice (Bryan *et al*, 2012; Minchinton, 2006; Van Duzer, 2004), and the ability of
248 intertidal species to travel great distances offshore on floating rafts of seaweed
249 (Ingólfsson, 2000). An increase in anthropogenic waste, in particular plastic litter,
250 also provides another mechanism for facilitating the dispersal of non-native species
251 in marine environments (Gregory, 2009; Jokiel, 1990). The buoyancy and durability
252 of plastic makes it an ideal alternate substratum for a variety of colonisers, with
253 plastics often shown to have higher diversity of species compared to other floating
254 substrates, though this is likely to be dependent on the location and experimental
255 sampling time (Bravo *et al*, 2011). The non-biodegradable nature of plastic increases
256 its longevity in the marine environment, and which in turn significantly increases its
257 potential for wide-scale dispersal of alien and invasive species (Barnes 2002a,b;
258 Winston *et al*, 1997; Jokiel, 1990; Gregory, 1978). Increased survival and long-
259 distance transport of native benthic invertebrates has been observed following their
260 attachment to marine plastic debris (Barnes and Milner, 2005), with one study
261 reporting the introduction of pathogens into a coral reef ecosystem through drifting

262 plastic litter (Goldstein *et al*, 2014). Colonisation of a single piece of plastic by at
263 least ten different species of marine animals has also been reported at remote
264 locations such as the Southern Ocean, an area that has a relatively low input of
265 anthropogenic litter (Barnes and Fraser, 2003). The size of the encrusting colonies
266 indicated that this piece of plastic had been afloat for at least a year, indicating the
267 potential for plastic-colonising organisms to survive and adapt at sea for many
268 months, and potentially years (Barnes and Fraser, 2003). This provides important
269 evidence that microbial “hitchhikers” on marine plastic debris could be widely
270 disseminated, with the increasing amounts of global marine plastic providing
271 opportunities for the transport of species into new habitats (De Tender *et al*, 2015).

272 Relatively little is known about the growth and dispersal dynamics of
273 pathogenic and harmful microorganisms colonising the plastisphere, and the
274 increased risk of human exposure from this poorly understood vector. Plastic-
275 associated microbes from the Chicago River were dominated by taxa of potential
276 pathogens and plastic decomposers, although these were less diverse than those of
277 the surrounding water column and associated suspended organic matter
278 (McCormick *et al*, 2014). LDPE-associated bacterial colonies were reported to be
279 dominated by *Arcobacter* (Harrison *et al* (2014), and pathogenic species of *Vibrio*
280 were found to dominate PP samples where they covered nearly 24% of the plastic
281 surface (Zettler *et al.*, 2013). Several *Vibrio* species, such as *V. cholerae* that causes
282 cholera and *V. fluvialis* that can cause bloody diarrhoea and gastroenteritis, are
283 known human pathogens, so their potential to colonise onto plastic marine litter
284 represents an underexplored pathway for their dispersal. Essentially, plastic debris
285 could represent a poorly-understood vehicle for the transport of these disease-

286 causing organisms, particularly due to the ability of plastics to persist for significantly
287 longer periods of time compared to other natural substances such as wood and
288 feathers (Caruso, 2015; Zettler *et al*, 2013).

289 Drifting plastic debris can also be colonised by HAB species, such as
290 *Ostreopsis* sp. and *Coolia* sp., in addition to resting cysts of unknown dinoflagellates,
291 and temporary cysts and vegetative cells of *Alexandrium taylori* (Masó *et al*, 2003).
292 Experiments using *A. taylori* cultured in plastic flasks showed the tendency of
293 temporary cysts to attach to plastic surfaces (Masó *et al*, 2003), providing important
294 insight toward our understanding on the global increase in HABs due to their
295 dispersion via anthropogenic means. There is presently very little information on the
296 role of plastic litter in the dispersion of HAB species, particularly in comparison to
297 other natural debris (Carson *et al*, 2013), and therefore further studies will be
298 needed to better understand this.

299

300 **4. Implications for bathing water quality: human health and beach management**

301

302 Faecal indicator organisms (FIOs), such as *E. coli* and intestinal enterococci,
303 are widely used to monitor the quality of bathing waters and beach environments.
304 These microorganisms mainly inhabit the mammalian gut, but can be delivered to
305 the wider aquatic environment from numerous diffuse and point sources including
306 sewage discharge, agricultural storm run-off, and sewer overflows (Oliver *et al*, 2015;
307 Kay *et al*, 2008; Oliver *et al*, 2005). The rate of FIO delivery to receiving waters will
308 therefore vary according to land-use and seasonal climatic conditions, e.g. patterns
309 of localised storm events. The survival of FIOs in sand and water at beach

310 environments is well documented (Halliday *et al*, 2015; Heaney *et al*, 2014), with
311 Bonilla *et al* (2007) demonstrating significantly higher levels of bacteria in dry (2- to
312 23-fold) and wet (30- to 460-fold) sand compared to seawater. The harbouring of
313 FIOs and human pathogens by certain species of freshwater macroalgae and beach-
314 cast wrack (seaweed) have also been reported (Quilliam *et al*, 2014; Imamura *et al*,
315 2011; Ishii *et al*, 2006).

316 Beaches and bathing waters attract millions of tourists, swimmers,
317 volunteers, and beach-goers each year and are a significant point of contact
318 between humans and potential sources of pollution. Swimming is one of the most
319 popular recreational activities at beaches (Wade *et al*, 2006), and epidemiological
320 evidence shows a relationship between poor water quality and the occurrence of GI
321 illnesses (Wade *et al*, 2010; Colford *et al*, 2007; Wade *et al*, 2006; Kay *et al*, 2004).
322 Recreational water sports that are associated with varying degrees of potential
323 water ingestion/contact, such as fishing, boating, wading and kayaking, are another
324 emerging risk factor contributing towards possible GI illness (Dorevitch *et al*, 2011).
325 However, beachgoers usually spend more time on the beach and strandline than in
326 the water, with young children engaged in playing on the sand at the water's edge,
327 whereas adults and elderly people are often found sunbathing (Heaney *et al*, 2012).
328 Beach sands are known to harbour both FIOs and human pathogens in localised
329 'hotspots', often in concentrations much higher than those found in bathing waters
330 (Sabino *et al*, 2014; Bonilla *et al*, 2007). Studies have demonstrated the occurrence
331 of GI symptoms and diarrhoea in people exposed to sand via digging, building
332 sandcastles and burying their bodies in sand at beaches with potential FIO
333 contamination from nearby sewage treatment plants, with children found to be at

334 higher susceptibility for contracting such illnesses (Heaney *et al*, 2012; Heaney *et al*,
335 2009).

336 With plastics now widely present in sediments and beach sands, and
337 representing a potential unknown reservoir of pathogens and FIOs, a series of
338 emerging research questions relating to plastics as a vector for wider public health
339 risks need critical investigation. Furthermore, increasing amounts of floating plastic
340 debris in bathing waters could also contribute to negative health impacts on bathers
341 and recreational water users, owing to the yet unquantified potential of plastic litter
342 to harbour and transmit diseases. The abundance of stranded and drifting plastic
343 debris (both macro and micro particles) along beaches and coastal areas is expected
344 to increase with projected increases in sea level, wind speed, wave height, and
345 altered rainfall conditions (Browne *et al*, 2015; Young *et al*, 2011; Gulev and
346 Grigorieva, 2004; Meier and Wahr, 2002). This is also likely lead to even greater
347 human exposure to washed-up plastic debris. The majority of studies of marine
348 plastic debris have mainly focused on its occurrence in coastal waters and open
349 ocean areas such as gyres. Limited research, however, has been performed to
350 investigate stranded beach plastics at designated bathing waters or other public
351 beaches (Table 2). Of these limited studies, the majority had investigated abundance
352 and distribution of plastic debris, with a variety of citizen science-based studies
353 further complementing these assessments (Hoellein *et al*, 2015; Eastman *et al*, 2014;
354 Smith *et al*, 2014; Hidalgo-Ruz and Thiel, 2013). Links between the colonisation of
355 stranded plastic litter with human pathogens and FIOs, and the impact this could
356 have on beachgoers and their health, have not yet been established, even despite
357 the likelihood of public exposure to beach-cast plastic waste being much higher

358 compared to litter in the open ocean. Strandlines are also marked by large quantities
359 of beach-cast wrack and plastics, both of which could contain potential human
360 pathogens (Quilliam *et al.*, 2014). Faecal loading from animals, such as gulls,
361 waterfowl and dogs, significantly contributes towards elevated FIO abundance on
362 beaches and in recreational waters (Wither *et al.*, 2013; Edge and Hill, 2007; Wither
363 *et al.*, 2005; Lévesque *et al.*, 2000). This could further facilitate the colonisation of
364 beach-cast plastic litter with pathogens, and which could be prone to further
365 dispersed elsewhere by wind, an incoming tide, or other means.

366 The ingestion of colonised plastic debris (particularly microplastics) by fish
367 and marine birds that mistake it as food represents a potential pathway for disease-
368 carrying plastic particles to enter the food chain and be dispersed to other
369 environments. Recent evidence has demonstrated that deposit-feeders, such as
370 mussels and shrimps, can ingest microplastics (Li *et al.*, 2015; Cauwenberghe *et al.*,
371 2015; Setälä *et al.*, 2014; Browne *et al.*, 2008), highlighting the potential for the
372 transfer of microplastics from one trophic level to another. Therefore, as
373 microplastics and stranded plastic debris are so prevalent on beaches, surface
374 waters, marine sediments and in the water column, it is important that we develop a
375 better understanding on the fate of plastics colonised by FIOs and pathogens, and
376 their potential to become incorporated into the food chain and to persist in the gut
377 of animals. Clearly, this could have far-reaching consequences for human health,
378 commercial fisheries and the environment (Lattin *et al.*, 2004; Thompson *et al.*, 2004;
379 Moore *et al.*, 2001).

380 Microplastics from cosmetic care products and fibres in clothing are not
381 effectively removed by wastewater treatment plants (WWTPs) and accumulate in

382 the environment (McCormick *et al*, 2014), with 250% more microplastics found in
383 coastal WWTP disposal sites compared to reference sites in the United Kingdom
384 (Browne *et al*, 2011). Microplastics entering aquatic systems from WWTPs have been
385 in close contact with human faeces, hence facilitating their potential to be colonised
386 by FIOs and a range of human faecal pathogens (Oberbeckmann *et al*, 2015). The
387 potential for sewage-exposed microplastics to harbour pathogens has only recently
388 been explored, and there are reports of microplastics with high levels of colonisation
389 by members of the *Campylobacteraceae* downstream of a WWTP (McCormick *et al*,
390 2014). This reinforces the need for further work to understand the mechanisms by
391 which microorganisms, especially pathogens, in sewage hitchhike onto microplastic
392 particles and find their way onto beaches and surrounding bathing environments.
393 Targeted research in these areas could have significant societal impact, perhaps
394 most notably by advancing beach management protocols and providing improved
395 evidence to informing EU BWPs for increased public protection.

396

397 **5. Conclusion**

398

399 The negative impacts of marine plastic debris are widespread, but not yet
400 fully understood. Marine and freshwater plastic debris is constantly being modified
401 by the chemical and physical environment; therefore biofilm communities colonising
402 plastics need to be dynamic with an ability to adapt to their changing environment.
403 The potential for complex interactions between plastic waste and microorganisms of
404 human health significance are currently poorly understood, yet the implications from
405 this in disease transmission are significant. Promoting increased knowledge of both

406 the role and importance of plastic surfaces in facilitating the survival and transfer of
407 pathogens, particularly with respect to plastisphere-pathogen associations, currently
408 represents an emerging research agenda in the wider field of health-related water
409 microbiology. Quantifying the spatial and temporal shifts in human exposure
410 pathways to human pathogens that might occur from macro to micro plastic debris,
411 and the changing magnitude of risks to human health, will be challenging.
412 Recognising human health impacts is clearly important, but the nature of risk
413 associated with this novel transport mechanism capable of transferring associated
414 microorganisms across large geographic ranges also introduces new regulatory
415 challenges associated with the environmental and socio-economic protection of
416 bathing waters and waters of significant recreational interest.

417 Understanding the ecology of the plastisphere community will further inform
418 regulators and environment managers of the risks from particular types and sizes of
419 plastics, and the effects of environmental stressors such as temperature and
420 exposure to higher UV radiation on the survival of plastic colonising pathogens and
421 harmful microorganisms. Advances in plastisphere ecology will also contribute
422 towards our knowledge of biodegradation of plastics and its adsorbed pollutants,
423 and could provide useful information for future remediation strategies.

424

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427 Stirling etc

Table 1: Studies investigating environmental plastic debris and biofilm formation

Area Sampled	Size of Plastic	Microbial Issue Investigated	Reference
Open ocean	Micro (0.25-0.5cm)	Colonisation of plastic particles by diatoms & hydroids	Carpenter and Smith, 1972
Coastal waters	Micro (0.1-2mm)	Bacterial colonisation of polystyrene particles	Carpenter <i>et al</i> , 1972
Open ocean	Macro & Micro	Characterization of microbial plastisphere community	Zettler <i>et al</i> , 2013
Coastal waters	Macro (30x30cm)	Bacterial colonisation of polyvinylchloride by <i>Rhodobacterales</i>	Dang <i>et al</i> , 2008
Open ocean	Macro & Micro (<5mm)	Abundance, diversity & variation of microbial community	Carson <i>et al</i> , 2013
Open ocean	Macro & possibly micro (<2cm)	Characterization of microorganisms colonising plastic debris; relationship between size of plastic & number of observed taxa	Goldstein <i>et al</i> , 2014
Open ocean	Macro (15x10cm)	Variation of biofilm community on HDPE, LDPE, & PP coupons with season and polymer type	Artham <i>et al</i> , 2009
Coastal waters	Macro	Potential of floating plastics to disperse toxic algal species	Masó <i>et al</i> , 2003
Coastal waters	Macro	Biofilm formation on polystyrene particles by bacteria & diatoms	Briand <i>et al</i> , 2012
Laboratory experiment using seawater	Macro (PET bottle pieces)	Biofilm formation & attachment of marine bacteria to PET surfaces	Webb <i>et al</i> , 2009
Coastal and ocean waters	Macro & micro	Characterization of microorganisms colonising plastic debris	Reisser <i>et al</i> , 2014
Open ocean	Macro (PET bottles)	Seasonal and spatial differences in biofilm diversity	Oberbeckmann <i>et al</i> , 2014
Seafloor	Macro (> 25mm) & micro (< 5mm)	Comparison of the plastisphere community to bacterial community of beach microplastics, sediment & surrounding seawater	De Tender <i>et al</i> , 2015

Coastal waters	Macro (PE plastic food bags)	Early stages of microbial biofilm formation on marine plastics	Lobelle and Cunliffe, 2011
Beach sediments	Micro (<5mm)	Bacterial colonisation of low-density polyethylene (LDPE) microplastics from three different sediment types	Harrison <i>et al</i> , 2014
Urban river	Micro	Assessment of microplastic abundance in urban river & composition of bacterial biofilms on plastics	McCormick <i>et al</i> , 2014

Table 2: Studies conducted on plastic debris from public bathing water beaches (excluding citizen science volunteer data studies).

Area sampled	Size of plastic	Issue investigated	Reference
Beach sediments	Pellets (0.1-0.5cm)	Potential of PP plastic pellets to transport toxic chemicals	Mato <i>et al</i> , 2001
Beach sediments	Macro & micro (1-15 mm)	Abundance of small plastic debris on Hawaiian beaches	McDermid and McMullen, 2004
Beach, estuarine and subtidal sediments	Micro	Abundance and extent of microplastic pollution	Thompson <i>et al</i> , 2004
Coastal beach sediments and seawater	Micro (>1.6µm)	Presence and abundance of microplastics	Ng and Obbard, 2006
Beach shorelines	Macro (> 1mm) & micro (< 1mm)	Influence of wind on spatial patterns of plastic debris	Browne <i>et al</i> , 2010
Beach	Virgin pellets, small (< 20mm) & micro (<20mm)	Size & distribution of plastic fragments on Brazilian beach	Costa <i>et al</i> , 2010
Beach shoreline sediments	Micro (<1 mm)	Spatial distribution of microplastics along six different continents	Browne <i>et al</i> , 2011
Beach sediments	Micro (<5 mm)	Bacterial colonization of low-density polyethylene (LDPE) microplastics from 3 different sediment types	Harrison <i>et al</i> , 2014
Beach shoreline and coastal waters (70-100m)	Macro	Distribution of anthropogenic litter in freshwater system & microbial interactions	Hoellein <i>et al</i> , 2014
Beach	Macro	Predicting short-term quantities of plastic debris washing ashore on beaches using a particle tracking model (PTM) & webcam monitoring	Kako <i>et al</i> , 2014
Beach	Macro	Colonisation of plastic litter by <i>E. coli</i> and <i>Vibrio</i> spp.	Quilliam <i>et al</i> , 2014

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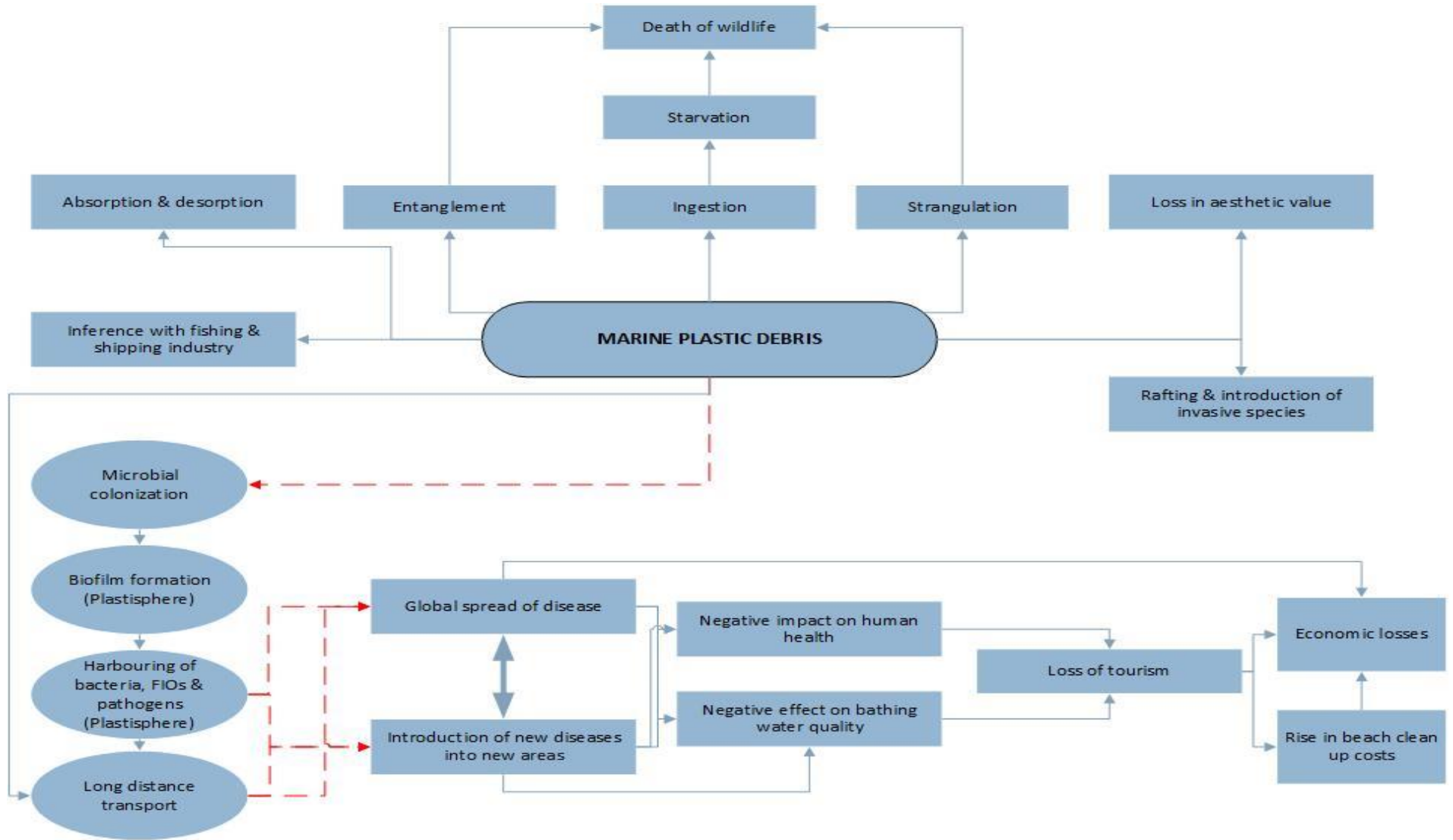


Figure 1: Impacts and interactions of marine plastic debris. Blue arrows indicate known effects; red dotted arrows indicate the yet unexplored effects/interactions as mediated by marine plastic debris.