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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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9

10 **Abstract**

11

12 Marine plastic debris is well characterized in terms of its ability to negatively impact
13 terrestrial and marine environments, endanger coastal wildlife, and interfere with
14 navigation, tourism and commercial fisheries. However, the impacts of potentially
15 harmful microorganisms and pathogens colonising plastic litter are not well
16 understood. The hard surface of plastics provides an ideal environment for
17 opportunistic microbial colonisers to form biofilms and might offer a protective
18 niche capable of supporting a diversity of different microorganisms, known as the
19 “Plastisphere”. This biotope could act as an important vector for the persistence and
20 spread of pathogens, faecal indicator organisms (FIOs) and harmful algal bloom
21 species (HABs) across beach and bathing environments. This review will focus on the
22 existent knowledge and research gaps, and identify the possible consequences of

23 plastic-associated microbes on human health, the spread of infectious diseases and
24 bathing water quality.
25
26
27 *Keywords:* marine plastic debris; microplastics; biofilms; pathogens; bathing water
28 quality; public health

29 1. Introduction

30

31 Marine plastic debris is an environmental pollutant of growing concern, with
32 its detrimental effects on aquatic and coastal wildlife already well documented
33 (Hammer et al., 2012; Gregory, 2009; Derraik, 2002). The durable, light weight and
34 inexpensive nature of plastic has made it a ubiquitous choice for many industrial and
35 consumer products (Osborn and Stojkovic, 2014). More than 200 M tonnes of plastic
36 are produced annually worldwide (Ivar do Sul and Costa, 2014), facilitating its entry
37 and accumulation in coastal waters and beach environments. Approximately 4.8 –
38 12.7 M tonnes of plastic waste entered the ocean from 192 coastal countries in 2010
39 alone (Jambeck et al., 2015), with global changes in rainfall, wind speed, and more
40 frequent flood and storm events predicted to further increase the amount of
41 stranded and drifting plastics in the coastal zone (Young et al., 2011; Gulev and
42 Grigorieva, 2004; Meier and Wahr, 2002; Goldenberg et al., 2001).

43

44 1.1 *Size, origin, accumulation and impacts of marine plastic debris*

45

46 Marine plastic debris includes large, macro particles such as carrier bags,
47 bottles and fishing gear (Eriksen et al., 2014), and now more frequently microplastics
48 and nanoplastics (Driedger et al., 2015; Andrady, 2011). Microplastics, defined
49 generally as plastic particles less than 5 mm in diameter (NOAA, 2009), include
50 “primary” microplastics present in cosmetic care products, clothes fibres, and the
51 industrial discharge of virgin plastic production pellets (Eerkes-Medrano et al., 2015;
52 Wagner et al, 2014; Browne et al., 2011; Cole et al., 2011; Fendall and Sewall, 2009),

53 along with “secondary” microplastics that frequently enter waterways through the
54 breakdown of macro particles by a combination of physical, biological and chemical
55 processes (Ryan et al., 2009; Thompson et al., 2004). The majority of plastic debris
56 entering the oceans are a result of the direct and improper disposal of terrestrial
57 waste and the discard of plastics at sea (Hammer et al., 2012; Barnes et al., 2009). In
58 addition, rivers, tides, wind, heavy rainfall, and storm and sewage discharge facilitate
59 the dispersal of both macro and microplastics within marine and freshwater
60 environments (Wagner et al., 2014; Reisser et al., 2013), with an estimated 5.25
61 trillion plastic particles weighing approximately 269,000 tonnes currently floating in
62 the sea (Eriksen et al., 2014). However, this number is likely to be much higher, with
63 a recent study by Van Sebille et al. (2015) estimating microplastic abundance
64 (defined here as those plastic particles <200 mm in diameter) to range from 15 to 51
65 trillion particles, and weighing between 93 to 236 thousand metric tonnes.

66 The impacts of marine plastic debris go beyond simply posing a threat to
67 marine wildlife (Figure 1). Marine plastics can lead to economic losses by interfering
68 with the shipping and fishing industries, and posing a significant threat to
69 recreational tourism (Pichel et al., 2007; Sheavly and Register, 2007). Beaches
70 polluted with medical and sanitary waste constitute a public health risk, devalue the
71 experience of beachgoers, and can often require costly beach-cleaning efforts
72 (Moore, 2008). With quantities of beach-cast plastic expected to rise due to more
73 severe weather events, coastal areas dependent on tourism are likely to face a
74 number of socio-economic challenges (McIlgorm et al., 2011).

75

76 *1.2 Plastic as a rafting material, the formation of biofilms, and the potential for*
77 *transport of harmful microorganisms*

78

79 Plastic debris can provide a novel mechanism for the spread of invasive and
80 alien species, in addition to that facilitated by natural substances like rafts of
81 vegetation, wood, or pumice (Bryan et al., 2012; Minchinton, 2006; Jokiel, 1990). A
82 diverse range of organisms has already been found colonising macro-plastics, and in
83 some cases has led to the introduction of non-native species into new habitats
84 (Gregory, 2009; Barnes 2002*a*; Barnes 2002*b*). Until very recently, however, little
85 attention has been paid to the concept of plastic providing a novel means of spatial
86 and temporal transport for microorganisms across marine and coastal environments
87 (Amaral-Zettler et al., 2015; Caruso, 2015). The physical properties of plastic can
88 provide a unique habitat capable of supporting diverse microbial communities
89 (Zettler et al., 2013; Harrison et al., 2011), with the buoyant and persistent nature of
90 plastic possibly contributing to the survival and long-distance transport of those
91 microbial hitchhikers that associate with its surface. The biofilms that colonise this
92 so-called plastisphere could also be a reservoir for pathogenic microbes, faecal
93 indicator organisms (FIOs) and harmful algal bloom (HAB) species. Plastic debris
94 could therefore be acting as a potential vector for the wide-scale dissemination of
95 these organisms (Oberbeckmann et al., 2015; Zettler et al., 2013; Masó et al., 2003).

96

97 *1.3 Plastic debris and its unknown impact on beach and bathing environments*

98

99 A few recent studies have shown evidence for the formation of biofilms by
100 bacteria and FIOs (such as *E. coli*) on plastic water distribution pipes (Yu et al., 2010;
101 Lehtola et al., 2004), and the persistence of potentially harmful pathogens (such as
102 certain strains of *Vibrio* spp.) on plastic debris (McCormick et al., 2014; Zettler et al.,
103 2013), although this is speculative at best. However, the ability of microorganisms to
104 persist on beach-stranded plastic debris and increase dissemination of potentially
105 pathogenic microbes in coastal zones needs urgent addressing to allow regulators
106 and beach managers to make more informed decisions about public safety at
107 bathing environments. Beaches and coastal environments form some of the most
108 ecologically and socio-economically important habitats worldwide (Harley et al.,
109 2006), and ecosystem services in these areas are already facing significant pressure
110 from anthropogenic activities (Quilliam et al., 2015; Schlacher et al., 2007a; 2006). In
111 Europe, the quality of bathing water and safety of beaches is governed by the EU
112 Bathing Water Directive (BWD; 2006/7/EC). The BWD sets standards for microbial
113 water quality via the use of FIOs for the assessment of faecal pollution. The BWD
114 also requires the production of a Bathing Water Profile (BWP) for all designated EU
115 bathing waters (Mansilha et al., 2009), which contains details on the nature of
116 possible pollution sources that could have negative impacts on a bather's health
117 (Schernewski et al., 2012). Designations such as the Blue Flag award are also largely
118 driven by the BWD.

119 Epidemiological studies have reported the relationship between bathing
120 water quality and the occurrence of adverse human health effects such as
121 gastrointestinal (GI) symptoms, respiratory diseases, and eye, nose and throat
122 infections (Wade et al., 2006, Zmirou et al., 2003; Prüss, 1998). Whilst most of these

123 studies have focused on waters impacted by municipal-wastewater effluent, the
124 impacts of other diffuse sources of pollution remain relatively unexplored (Soller et
125 al., 2010). With the potential of plastic providing a possible site for pathogen and FIO
126 attachment, and the subsequent dissemination of these organisms in the marine
127 environment, a better understanding of these processes is required in order to
128 ensure beach safety. Assessing beach and bathing environments for stranded plastic
129 debris and analysing it for associated FIOs and pathogens could provide a better
130 insight into the quality of European bathing waters through the production of a more
131 detailed BWP, as well as enabling plastic debris to qualify as a potential indicator and
132 carrier of FIOs and pathogens that could present a risk to human health. This could
133 further help prevent economic losses associated with beach closures, and enable
134 beaches to maintain their Blue Flag status (Schernewski et al., 2012; Wyer et al.,
135 2010).

136 Against a backdrop of changing climate, the persistent multi-pollutant effects
137 of plastic debris in coastal environments increases the urgency to understand the
138 risks of human exposure to plastic pollution and inform more sustainable beach
139 management options. The aim of this review is to explore the potential of marine
140 plastics to serve as a mechanism for the persistence and transmission of FIOs and
141 potentially pathogenic or harmful microorganisms, and the pathways of human
142 exposure risk in coastal environments.

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147 **2. The Plastisphere: an anthropogenic ecological habitat**

148

149 Biofilms are formed by the microbial secretion of extracellular polymeric
150 substances (EPS), which include proteins, glycoproteins, and glycolipids (Flemming et
151 al., 2007) that act as a type of architectural scaffolding, forming a matrix around
152 microbes and enabling their attachment to a variety of different biotic and abiotic
153 surfaces (O'Toole et al., 2000). This helps provide a protective environment that
154 enables microorganisms to grow in hostile habitats and facilitates easy dispersal
155 (Hall-Stoodley and Stoodley, 2005). Microorganisms can form biofilms on any
156 artificial or natural surface, including medical equipment (such as catheters,
157 implants, and pacemakers) and copper and plastic pipes of water distribution
158 systems (Costerton et al., 2005; Lehtola et al., 2004). Studies have demonstrated
159 that the surfaces of different types of plastics, such as polyethylene (PE) and
160 polyethylene terephthalate (PET), are rapidly colonised by heterotrophic bacteria
161 when submerged in seawater and that these organisms are able to survive for longer
162 periods than those in the surrounding seawater (Webb et al., 2009; Lobelle and
163 Cunliffe, 2011). Interestingly, these studies also found significant changes in the
164 physiochemical properties of the plastic samples, with Webb et al. (2009) hinting at
165 the existence of plastic-degrading bacteria. There is now an increasing amount of
166 anecdotal evidence that suggests that microbes degrade marine plastic debris
167 (Reisser et al., 2014; Zettler et al., 2013; Webb et al., 2009), although this is not
168 supported by any actual data measurements, e.g. changes in tensile strength or
169 contact angle measurements.

170 Successional changes in bacterial colonisation of artificial surfaces including
171 glass, stainless steel and polycarbonate sheets have been demonstrated in seawater,
172 with early-stage colonisation often marked by higher species richness (Jones et al.,
173 2006; Jackson et al., 2001). *Alphaproteobacteria* was found to be the most dominant
174 group of colonising bacteria on acryl, glass, steel and polycarbonate substrata, with
175 *Gammaproteobacteria* mainly occurring during the early colonisation stages in the
176 first 9 hours, indicating that initial colonisation might be substrate-specific (Lee et al.,
177 2008; Jones et al., 2006). *Gammaproteobacteria* are an ecologically diverse group of
178 Gram-negative bacteria that contain a number of potentially pathogenic strains of
179 *Salmonella* spp. and *Vibrio* spp. that might be harmful to human health. Since certain
180 strains of *Vibrio* spp. are recognised to readily colonise plastics, the potential of
181 pathogenic species of *Vibrio*, including for example *Vibrio cholerae* that causes
182 cholera, to colonise plastic requires urgent investigation, particularly in light of
183 prescient knowledge that plastic debris can easily be dispersed in the marine
184 environment (Zettler et al., 2013).

185 Reports of biofilms on plastic waste in the environment are limited
186 (summarised in Table 1). Biofilm formation on plastic debris was first reported in
187 1972 in the Sargasso Sea, where bacterial communities were found colonising
188 floating microplastic particles (Carpenter et al., 1972; Carpenter and Smith, 1972).
189 Zettler et al. (2013) conducted the first high-throughput sequencing study of its kind,
190 which characterised the composition of microbial communities colonising six micro
191 and macro pieces of PE and polypropylene (PP) collected from geographically distinct
192 open ocean areas of the North Atlantic Subtropical Gyre. The plastisphere
193 community consisted of a morphologically diverse range of microbes that comprise a

194 dense mix of eukaryotic and prokaryotic cells, such as diatoms, coccolithophores,
195 dinoflagellates, fungi and bacteria (Zettler et al., 2013). However, how
196 representative these results are in relation to the wider plastisphere communities
197 remains unclear, since these were generated from just six plastic fragments collected
198 from only one environment

199 Amaral-Zettler et al. (2015) provide a more comprehensive study of the
200 bacterial communities found on plastic debris collected from two different
201 environments, the North Pacific and North Atlantic subtropical gyres, using DNA
202 sequencing techniques. Their findings, although lacking taxonomic details, highlight
203 significant differences between bacteria found in the water column and those
204 attached to plastic debris, along with differences in plastisphere-communities
205 collected from the two different ocean basins (Amaral-Zettler et al., 2015). Polymer
206 type appeared less important in determining bacterial colonisation, with significant
207 differences only occurring between polystyrene and PE, or polystyrene and
208 polypropylene (Amaral-Zettler et al., 2015). This finding lies in accordance with that
209 made by Carson et al. (2013), who highlight the possible influence of size, type and
210 surface roughness of marine plastic debris on the diversity and abundance of the
211 colonising microbial taxa, with polystyrene exhibiting higher bacterial abundance.
212 Another study conducted by Reisser et al. (2014) on plastic particles collected in
213 Australian waters yielded similar results as those from Zettler et al. (2013). However,
214 it should be noted that both the Carson et al. (2013) and Reisser et al. (2014) studies
215 are based solely on morphological data, with only Zettler et al. (2013) and Amaral-
216 Zettler et al. (2015) employing sequencing techniques.

217 Microbial assemblages associated with marine plastics are also distinctly
218 different from those of the surrounding seawater (Amaral-Zettler et al., 2015;
219 Harrison et al., 2014; McCormick et al., 2014; Oberbeckmann et al., 2014; Zettler et
220 al., 2013). PET drinking water bottles attached to buoys in the North Sea, UK,
221 showed clear differences in the composition of the plastisphere community
222 compared to microbial communities of seawater and those attached to plankton and
223 debris (Oberbeckmann et al., 2014). The study also illustrated temporal differences
224 in microbial community composition colonising the plastic bottles, revealing a higher
225 abundance of photosynthetic brown algae and cyanobacteria during the summer
226 months compared to a dominance of heterotrophic bacteria and photosynthetic
227 diatoms during the winter (Oberbeckmann et al., 2014).

228 In a study by Harrison et al. (2014) employing a laboratory-based microcosm
229 setup containing sterile artificial seawater and inoculated with low-density
230 polyethylene (LDPE) microplastics, colonisation of plastics by morphologically
231 distinct prokaryotic cells, predominantly bacteria, occurred over time. Further
232 molecular analysis revealed significant differences between the bacterial
233 communities found attached to the LDPE microplastics and those within the
234 sediment (Harrison et al., 2014). This finding corroborates that of McCormick et al.
235 (2014) who demonstrate significant differences in microbial communities found on
236 microplastics in an urban Chicago River compared to those of the surrounding water
237 column and suspended organic matter. Harrison et al. (2014) also highlight
238 significant time-dependent variation in the structural community of the LDPE
239 bacterial community. Initial observations showed the existence of sediment type-
240 specific communities present on microplastics, with shifts towards “LDPE-

241 associated” bacterial communities occurring at days 7 and 14 of the experiment,
242 indicating a possible adaptation and change in community structure of these
243 bacteria to microplastic waste (Harrison et al., 2014). The tendency of microplastics
244 to attract a bacterial community that differs from that of the surrounding
245 environment is further supported by a study conducted in a freshwater system,
246 where bacterial communities on plastic litter from the Chicago River and Chicago’s
247 Lake Michigan beaches differed significantly from those colonising organic
248 substances such as leaves and cardboard (Hoellein et al., 2014). The prevailing
249 evidence appears to indicate that plastisphere communities are distinctly different
250 from those found colonising other substrates or within the same environment but
251 not associated with the plastic debris, indicating the possibility of specific adaptation
252 to this man-made habitat. Plastic could therefore provide a new ecological niche or
253 biotope, which, owing to its longevity in the environment, could help facilitate the
254 persistence and transport of microorganisms across oceans and into new geographic
255 areas (De Tender et al., 2015). Further research is needed in order to establish
256 whether this novel transport mechanism could lead to the spread and prolonged
257 persistence of disease-causing organisms in marine environments.

258 There is also a growing commercial interest in plastic biodegradation, with
259 current research focussing on identifying the types of microorganisms capable of
260 degrading plastics (Loredo-Treviño et al., 2012). Numerous studies have shown
261 several different species of marine bacteria with the capacity to degrade
262 hydrocarbons. Species of hydrocarbon-degrading bacteria belonging to over 20
263 genera and distributed across some of the major bacterial Classes (*Alpha-*, *Beta-* and
264 *Gammaproteobacteria*; *Actinomycetes*; *Flexibacter-Cytophaga- Bacteroides*), have

265 been isolated and described (Yakimov et al., 2007; Head et al., 2006; Head and
266 Swannell, 1999; Floodgate, 1995). These organisms are strongly enriched for during
267 an oil spill at sea and play an important role in the biodegradation of oil (Gutierrez et
268 al., 2014; Gertler et al., 2012). To our knowledge, the marine environment is the only
269 place where we find bacteria with the ability to utilize hydrocarbons almost
270 exclusively as a sole source of carbon and energy. Considering that plastic is
271 composed of hydrocarbons, these types of bacteria could have important
272 implications with respect to their role in degrading plastic debris. There are reports
273 of changes in the surface topography of plastic samples colonised by
274 microorganisms, and microbial cells have been identified within pits and grooves,
275 suggesting possible microbial degradation of plastic surfaces (Reisser et al., 2014;
276 Zettler et al., 2013; Webb et al., 2009), again however lacking any real evidence.
277 Only a handful of studies have investigated biodegradation through actual
278 measurement. A recent study by Nauendorf et al. (2016) examining mass loss,
279 changes in surface wettability and surface chemical composition of biodegradable
280 plastic bags and PE recovered from sediments from the Western Baltic Sea, found no
281 signs of biodegradation after 98 days. However, Yoshida et al. (2016), have recently
282 discovered the existence of a new bacterium, *Ideonella sakaiensis* 201-F6, which is
283 able to completely degrade PET within six weeks. The mechanics of biodegradation
284 of marine plastic debris, and the underlying processes that influence this behaviour,
285 are areas that clearly need much further investigation to fully exploit the
286 implications this can have on the environment.

287 Current research relating to plastisphere communities often fails to consider
288 the likely impacts of associated chemical co-pollutants present on plastics that may

289 also play a role in determining the community structure of the attached biofilm.
290 Plastic debris, including microplastics, contain numerous organic contaminants such
291 as, for example, polychlorinated biphenyls (PCBs), petroleum hydrocarbons and
292 bisphenol A, which are either added during the plastic manufacturing process or
293 absorbed from the surrounding environment (Koelmans et al., 2016; Teuten et al.,
294 2009). Plastic debris is therefore a known vector of such chemical pollutants (Cole et
295 al., 2011). Studies have already demonstrated the negative impacts associated with
296 such additives on wildlife, humans and the environment (Van der Meulen et al.,
297 2014; Teuten et al., 2009), with a large amount of these chemicals known to desorb
298 when the plastic is ingested by marine species and eventually bioaccumulate in the
299 food chain (Engler, 2012). Future research should consider the combined biotic and
300 chemical load present on plastic debris and the consequent role microbial
301 hitchhikers play in either mitigating this problem by biodegradation or aggravating it
302 through increased biofilm binding. This could also help in trying to establish a more
303 accurate risk assessment of plastic debris by taking into consideration both the
304 effects of potentially harmful plastic-associated microbes as well as chemical co-
305 pollutants.

306

307 **3. Plastic dispersal: Dissemination of pathogenic and harmful microbes**

308

309 The introduction of invasive species into new habitats through colonisation of
310 natural substances, such as wood, dead plants and pumice (Bryan et al., 2012;
311 Minchinton, 2006; Van Duzer, 2004), and the ability of intertidal species to travel
312 great distances offshore on floating rafts of seaweed (Ingólfsson, 2000) are well

313 described. An increase in anthropogenic waste, in particular plastic litter, provides
314 another mechanism for facilitating the dispersal of non-native species in marine
315 environments (Gregory, 2009; Jokiel, 1990). The buoyancy and durability of plastic
316 makes it an ideal alternate substratum for a variety of colonisers, with plastic often
317 shown to have a higher diversity of species compared to other floating substrates,
318 though this is likely to be dependent on the location and experimental sampling time
319 (Bravo et al., 2011). The non-biodegradable nature of plastic increases its longevity
320 in the marine environment, which in turn significantly increases its potential for
321 wide-scale dispersal of alien and invasive species (Barnes 2002a,b; Winston et al.,
322 1997; Jokiel, 1990; Gregory, 1978). Increased survival and long-distance transport of
323 native benthic invertebrates has been observed following their attachment to
324 marine plastic debris (Barnes and Milner, 2005), with one study reporting the
325 introduction of pathogens into a coral reef ecosystem through drifting plastic litter
326 (Goldstein et al., 2014). Colonisation of a single piece of plastic by at least ten
327 different species of marine animals (including Bryozoans, Porifera, Annelida,
328 Cnidaria, and Mollusca) has also been reported at remote locations such as the
329 Southern Ocean, an area that has a relatively low input of anthropogenic litter
330 (Barnes and Fraser, 2003). The size of the encrusting invertebrate colonies indicated
331 that this particular piece of plastic had been afloat for at least a year, illustrating the
332 potential for plastic-colonising organisms to survive and adapt at sea for many
333 months, and potentially years (Barnes and Fraser, 2003). This provides important
334 evidence that microbial hitchhikers on marine plastic debris could be widely
335 disseminated, with the increasing amounts of global marine plastic providing ample
336 opportunities for the transport of species into new habitats (De Tender et al., 2015).

337 Relatively little is known about the growth and dispersal dynamics of
338 potentially pathogenic and harmful microorganisms colonising the plastisphere, and
339 the increased risk of human exposure from this poorly understood vector. Plastic-
340 associated microbes from the Chicago River, a freshwater environment, were found
341 to contain taxa of potential pathogens and plastic decomposers, although these
342 were less diverse than those of the surrounding water column and suspended
343 organic matter (McCormick et al., 2014). The authors found a high abundance (7.4%)
344 of the family Campylobacteraceae colonising microplastics released from a nearby
345 sewage treatment plant, certain taxa of which are known to cause human GI
346 infections (McCormick et al., 2014). This suggests the potential of microplastics to be
347 colonised by waste-water associated microbes that could have a negative impact on
348 human health and might contribute towards the transport of disease-causing
349 organisms in the environment. However, entrance of these plastic particles into
350 marine systems would likely increase die-off of the associated freshwater microbes
351 attached to plastics, and hence the potential for wider dispersal of these possibly
352 pathogenic microorganisms remains unclear. *Aeromonas*, *Acrobacter* and
353 *Pseudomonas* were also found in higher abundance on microplastics, all of which
354 could contain possible pathogenic strains (McCormick et al., 2014). Other studies
355 also indicate the ability of plastic debris to be colonised by potential pathogens, with
356 LDPE-associated bacterial colonies found in coastal sediments dominated by
357 *Arcobacter* and *Colwellia* spp., amounting to 84-93% of sequences (Harrison et al.,
358 2014), and possibly pathogenic species of *Vibrio* found to dominate one of the PP
359 samples in the Zettler et al. (2013) study, where they covered nearly 24% of the
360 plastic surface. Whilst this illustrates the potential of plastic debris to be colonised by

361 potentially harmful microbes, how representative this pathogenic *Vibrio* is with
362 respect to the wider plastisphere communities remains unknown since this was
363 found on just one of the six collected plastic fragments. Several *Vibrio* species, such
364 as *V. cholerae* the causal agent of cholera and *V. fluvialis* that can cause bloody
365 diarrhoea and gastroenteritis, are known human pathogens, so their potential to
366 colonise marine plastic litter presents an yet unexplored pathway for dispersal.
367 Therefore, plastic debris could represent a vehicle for the transport of these disease-
368 causing organisms, particularly due to the ability of plastics to persist for significantly
369 longer periods of time compared to other natural substances such as wood and
370 feathers, and their widespread global distribution across marine and terrestrial
371 environments (Caruso, 2015; Zettler et al., 2013).

372 Drifting plastic debris can also be colonised by HAB species, such as
373 *Ostreopsis* sp. and *Coolia* sp., in addition to resting cysts of unknown dinoflagellates,
374 and temporary cysts and vegetative cells of *Alexandrium taylori* (Masó et al., 2003).
375 Experiments using *A. taylori* cultured in plastic flasks showed the tendency of
376 temporary cysts to attach to plastic surfaces (Masó et al., 2003), providing an
377 important insight towards understanding the global increase in HABs due to their
378 dispersion via anthropogenic means. There is presently very little information on the
379 role of plastic litter in the dispersion of HAB species, particularly in comparison to
380 other natural debris (Carson et al., 2013), and further studies are needed to better
381 understand this. Furthermore, more emphasis should be placed on characterising
382 plastic-associated eukaryotic microbes using sequencing techniques, which
383 represents another substantial knowledge gap needed to fully understand the
384 diverse and complex nature of the plastisphere communities.

385

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

387

388 FIOs such as *E. coli* and intestinal enterococci are widely used to monitor the
389 quality of bathing waters and beach environments. These microorganisms mainly
390 inhabit the mammalian gut, but can be delivered to the wider aquatic environment
391 from numerous diffuse and point sources including sewage discharge, agricultural
392 storm run-off, and sewer overflows (Oliver et al., 2015; Kay et al., 2008; Oliver et al.,
393 2005). The rate of FIO delivery to receiving waters will vary according to land-use
394 and seasonal climatic conditions, e.g. patterns of localised storm events. The survival
395 of FIOs in sand and water at beach environments is well documented (Halliday et al.,
396 2015; Heaney et al., 2014), with Bonilla et al. (2007) demonstrating significantly
397 higher levels of bacteria in dry (2- to 23-fold) and wet (30- to 460-fold) sand
398 compared to seawater. The harbouring of FIOs and potential human pathogens by
399 certain species of freshwater macroalgae and beach-cast wrack (seaweed) have also
400 been reported (Quilliam et al., 2014; Imamura et al., 2011; Ishii et al., 2006). Van der
401 Meulen et al. (2014) found 150 different bacterial species colonising microplastics
402 found in the Interreg region, including those associated with causing diseases in
403 humans such as *E. coli* and *Pseudomonas anguilliseptica*.

404 Beaches and bathing waters attract millions of tourists, swimmers,
405 volunteers, and beach-goers each year and are a significant point of contact
406 between humans and potential sources of pollution. Swimming is one of the most
407 popular recreational activities (Wade et al., 2006), and epidemiological evidence
408 shows a relationship between poor water quality and the occurrence of GI illnesses

409 (Wade et al., 2010). Recreational water sports that are associated with varying
410 degrees of potential water ingestion/contact, such as fishing, boating, wading and
411 kayaking, are another emerging risk factor contributing towards possible GI illness
412 (Dorevitch et al., 2011). However, beachgoers usually spend more time on the beach
413 and strandline than in the water, with young children engaged in playing in the sand
414 at the water's edge, and adults and the elderly often found sunbathing (Heaney et
415 al., 2012). Beach sands are known to harbour both FIOs and human pathogens in
416 localised 'hotspots', often in concentrations much higher than those found in
417 bathing waters (Sabino et al., 2014; Bonilla et al., 2007). A few studies have
418 demonstrated the occurrence of GI symptoms and diarrhoea in people exposed to
419 sand via digging, building sandcastles and burying their bodies in sand at beaches
420 with potential FIO contamination from nearby sewage treatment plants, with
421 children found to have a higher susceptibility for contracting such illnesses (Heaney
422 et al., 2012; Heaney et al., 2009).

423 With plastics now widely present in sediments and beach sands (Van
424 Cauwenberghe et al., 2015; Imhof et al., 2013), and representing a potential
425 unknown reservoir of FIOs and pathogens, a series of emerging research questions
426 relating to plastics as a vector for wider public health risks need critical investigation.
427 Furthermore, increasing amounts of floating plastic debris in bathing waters could
428 also contribute to negative health impacts on bathers and recreational water users,
429 owing to the yet underexplored potential of plastic litter to harbour and transmit
430 diseases. The abundance of stranded and drifting plastic debris (both macro and
431 micro particles) along beaches and coastal areas is expected to increase with
432 projected increases in sea level, wind speed, wave height, and altered rainfall

433 conditions (Browne et al., 2015; Young et al., 2011; Gulev and Grigorieva, 2004;
434 Meier and Wahr, 2002). This is likely to lead to even greater human exposure to
435 washed-up plastic debris. The majority of studies on marine plastic debris have
436 focused on its occurrence in coastal waters and open ocean areas such as gyres.
437 Limited research, however, has been performed to investigate stranded beach
438 plastics at designated bathing waters or other public beaches (Table 2). Of these
439 limited studies, the majority have investigated abundance and distribution of plastic
440 debris, with a variety of citizen science-based studies further complementing these
441 assessments (Hoellein et al., 2015; Eastman et al., 2014). Links between the
442 colonisation of stranded plastic litter with human pathogens and FIOs, and the
443 impact this could have on beachgoers and their health, have not yet been
444 established, despite the likelihood of public exposure to beach-cast plastic waste
445 being much higher compared to litter in the open ocean. Strandlines are also marked
446 by large quantities of beach-cast wrack and plastics, both of which could contain
447 potential human pathogens (Quilliam et al., 2014). Faecal loading from animals, such
448 as gulls, waterfowl and dogs, significantly contributes towards elevated FIO
449 abundance on beaches and in recreational waters (Edge and Hill, 2007; Wither et al.,
450 2005; Lévesque et al., 2000). This could further facilitate the colonisation of beach-
451 cast plastic litter with FIOs and potential pathogens, which could then be prone to
452 dispersal by wind, an incoming tide, or other means.

453 The ingestion of colonised plastic debris (particularly microplastics) by fish
454 and marine birds that mistake it as food represents another potential pathway for
455 disease-carrying plastic particles to enter the food chain and be dispersed to other
456 environments (Oberbeckmann et al., 2015). Recent evidence has demonstrated that

457 deposit-feeders, such as mussels and shrimps, can ingest microplastics (Li et al.,
458 2015; Van Cauwenberghe et al., 2015; Setälä et al., 2014; Browne et al., 2008),
459 highlighting the potential for the transfer of microplastics from one trophic level to
460 another. Therefore, as microplastics and stranded plastic debris are so prevalent on
461 beaches, surface waters, marine sediments and in the water column, it is important
462 that we develop a better understanding of the fate of plastics colonised by FIOs and
463 pathogens, and their potential to become incorporated into the food chain and to
464 persist in the gut of animals. Clearly, this could have far-reaching consequences for
465 human health, commercial fisheries and the environment (Lattin et al., 2004;
466 Thompson et al., 2004; Moore et al., 2001).

467 Furthermore, microplastics from cosmetic care products and fibres in
468 clothing are not effectively removed by Waste Water Treatment Plants (WWTPs) and
469 accumulate in the environment (McCormick et al., 2014), with 250% more
470 microplastics found in coastal WWTP disposal sites compared to reference sites in
471 the United Kingdom (Browne et al., 2011). Microplastics entering aquatic systems
472 from WWTPs have been in close contact with human faeces, hence facilitating their
473 potential to be colonised by FIOs and a range of human faecal pathogens
474 (Oberbeckmann et al., 2015). The potential for sewage-exposed microplastics to
475 harbour possible pathogens has only recently been explored, with McCormick et al.
476 (2014) reporting high levels of members of Campylobacteraceae colonising
477 microplastics downstream of a WWTP. This reinforces the need for further work to
478 understand the mechanisms by which microorganisms, especially pathogens, in
479 sewage “hitchhike” on microplastic particles and find their way onto beaches and
480 surrounding bathing environments. At present there is very limited information

481 available to assess whether the presence of microbial pathogens and FIOs on plastic
482 debris represents a real risk to human health, and it is therefore currently not yet
483 possible to establish a complete risk assessment on the multi-scale effects of plastic
484 debris (Van der Meulen et al., 2014). Targeted research in these areas could have
485 significant societal impact, perhaps most notably by advancing beach management
486 protocols and providing improved evidence to informing EU BWPs for increased
487 public protection.

488

489 **5. Conclusion**

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491 The negative impacts of marine plastic debris are widespread, but not yet
492 fully understood. Marine and freshwater plastic debris is constantly being modified
493 by the chemical and physical environment; therefore, biofilm communities
494 colonising plastics need to be dynamic with an ability to adapt to their changing
495 environment. The potential for complex interactions between plastic waste and
496 microorganisms of human health significance are currently poorly understood, yet a
497 number of emerging studies indicate the ability of potential pathogens to attach to
498 plastic debris and possibly be transported to new environments. However, further
499 work is essential in order to determine the implications this has in terms of disease
500 transmission and whether this linkage significantly impacts human health. Promoting
501 increased knowledge of both the role and importance of plastic surfaces in
502 facilitating the survival and transfer of pathogens, particularly with respect to
503 plastisphere-pathogen associations, currently represents an emerging research
504 agenda in the wider field of health-related water microbiology. Quantifying the

505 spatial and temporal shifts in human exposure pathways to pathogens that might
506 occur from macro to micro plastic debris, and the changing magnitude of risks this
507 presents to human health, will be challenging. However, the nature of threat
508 associated with this novel transport mechanism capable of transferring
509 microorganisms across large geographic ranges also introduces new regulatory
510 challenges associated with the environmental and socio-economic protection of
511 bathing waters and waters of significant recreational interest.

512 Understanding the ecology of the plastisphere community will further inform
513 regulators and environment managers of the risks from particular types and sizes of
514 plastics, and the effects of environmental stressors such as temperature and
515 exposure to higher UV radiation on the survival of plastic-colonising pathogens and
516 harmful microorganisms. Future research should entail studying microbial
517 interactions with plastic debris at all sites of its accumulation including soils,
518 sediments, beaches, rivers, open oceans and the deep sea in order to allow a more
519 comprehensive assessment of plastic-associated communities and its potential
520 negative impacts on the environment and public health. Advances in plastisphere
521 ecology will also contribute towards our knowledge of biodegradation of plastic and
522 its adsorbed pollutants, and could provide useful information for future remediation
523 strategies.

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Table 1: Studies investigating aquatic plastic debris and biofilm formation

Area sampled	Size of plastic	Microbial issue investigated	Reference
Open ocean	Micro (0.25-05.cm)	Colonisation of plastic particles by diatoms & hydroids	Carpenter and Smith, 1972
Open ocean	Macro (15x10cm)	Variation of biofilm community on High density polyethylene(HDPE), Low density polyethylene (LDPE) & PP coupons with season & polymer type	Artham et al., 2009
Open ocean	Macro & Micro	Characterization of microbial plastisphere community	Zettler et al., 2013
Open ocean	Macro & Micro (<5mm)	Abundance, diversity & variation of microbial community	Carson et al., 2013
Open ocean	Macro & possibly Micro (<2cm)	Characterization of microorganisms colonising plastic debris; relationship between size of plastic & number of observed taxa	Goldstein et al., 2014
Open ocean	Macro (PET bottles)	Seasonal & spatial differences in biofilm diversity	Oberbeckmann et al., 2014
Open ocean & coastal waters	Macro & Micro (<5mm)	Differences in composition of plastisphere community with respect to biogeographic origin & polymer type	Amaral-Zettler et al., 2015
Coastal waters	Micro (0.1-2mm)	Bacterial colonisation of polystyrene particles	Carpenter et al., 1972
Coastal waters	Macro	Potential of floating plastics to disperse toxic algal species	Masó et al., 2003
Coastal waters	Macro (30x30cm)	Bacterial colonisation of polyvinylchloride by <i>Rhodobacterales</i>	Dang et al., 2008
Coastal waters	Macro (PE plastic food bags)	Early stages of microbial biofilm formation on marine plastics	Lobelle and Cunliffe., 2011

Coastal waters	Macro	Biofilm formation on polystyrene particles by bacteria & diatoms	Briand et al., 2012
Coastal & ocean waters	Macro & Micro	Characterization of microorganisms colonising plastic debris	Reisser et al., 2014
Beach sediments	Micro (<5mm)	Bacterial colonisation of LDPE microplastics from three different sediment types	Harrison et al., 2014
Marine sediments	Macro (PE bags & biodegradable bags)	Colonisation & degradation of PE & biodegradable plastic bags by microbes in oxic & anoxic marine sediments	Nauendorf et al., 2016
Seafloor	Macro (>25mm) & Micro (<5mm)	Comparison of plastsphere community to bacterial community of beach microplastics, sediment & surrounding seawater	De Tender et al., 2015
Laboratory experiment using seawater	Macro (PET bottle pieces)	Biofilm formation & attachment of marine bacteria to PET surfaces	Webb et al., 2009
Urban river	Micro	Assessment of microplastic abundance in urban river & composition of bacterial biofilms on plastics	McCormick et al., 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 et al., 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 et al., 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 et al., 2010
Beach	Virgin pellets, small (< 20mm) & micro (<20mm)	Size & distribution of plastic fragments on Brazilian beach	Costa et al., 2010
Beach shoreline sediments	Micro (<1 mm)	Spatial distribution of microplastics along six different continents	Browne et al., 2011
Beach sediments	Micro (<5 mm)	Bacterial colonization of low-density polyethylene (LDPE) microplastics from 3 different sediment types	Harrison et al., 2014
Beach shoreline and coastal waters (70-100m)	Macro	Distribution of anthropogenic litter in freshwater system & microbial interactions	Hoellein et al., 2014
Beach	Macro	Predicting short-term quantities of plastic debris washing ashore on beaches using a particle tracking model (PTM) & webcam monitoring	Kako et al., 2014
Beach	Macro	Colonisation of plastic litter by <i>E. coli</i> and <i>Vibrio</i> spp.	Quilliam et al., 2014

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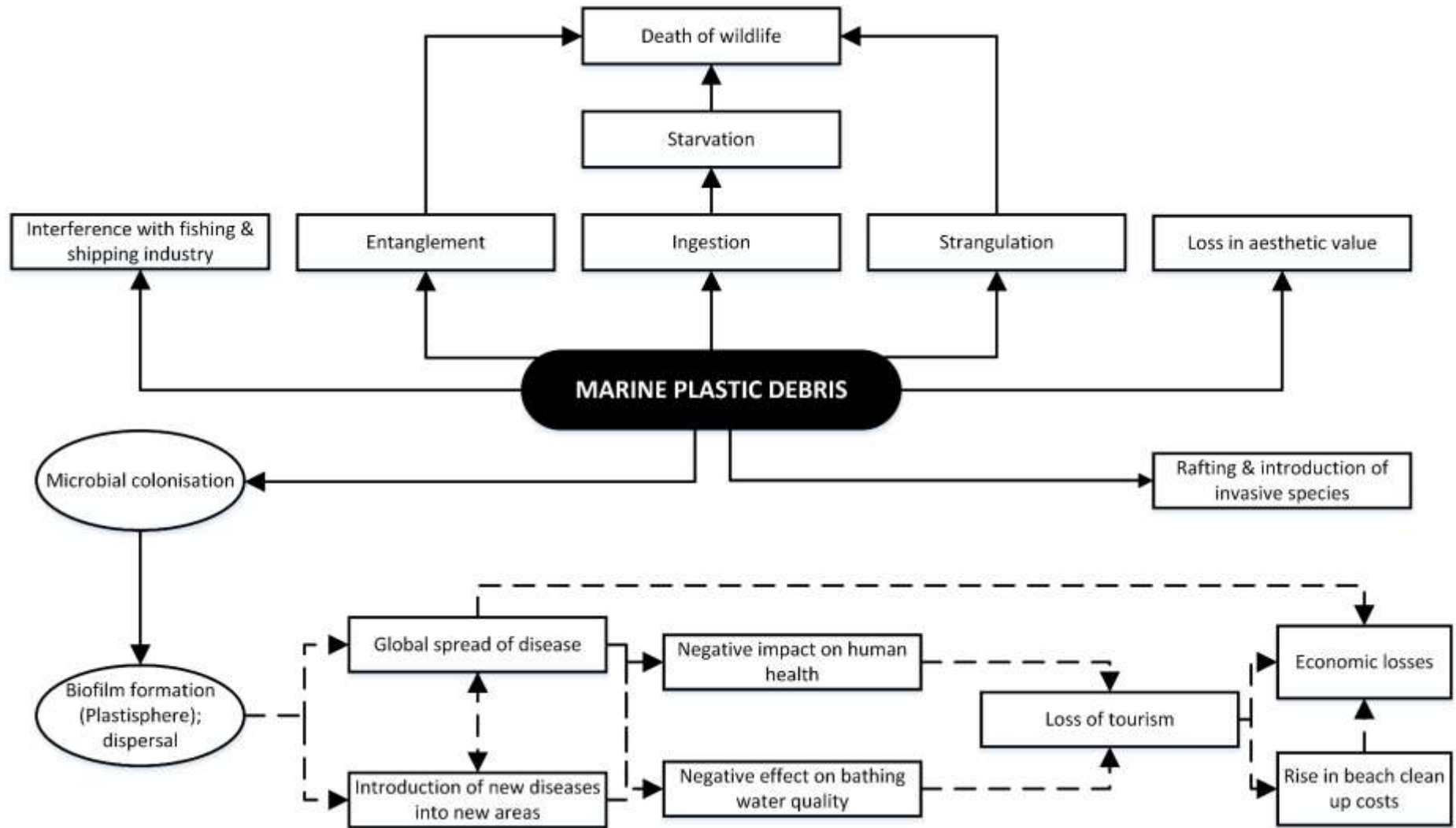


Figure 1: Impacts and interactions of marine plastic debris. Solid black arrows indicate known effects; dotted black arrows indicate the yet unexplored effects/interactions as mediated by marine plastic debris.

