Pathogens transported by plastic debris: does this vector pose a risk to aquatic organisms?

Jake Bowley[1], Craig Baker-Austin[2], Steve Michell[1], Ceri Lewis[1]

[1]College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope, Exeter, EX4 4QD, UK

[2]Centre for Environment, Fisheries and Aquaculture (CEFAS), Weymouth, Dorset, DT4 8UB, UK

Abstract

Microplastics are small (<5 mm) plastic particles of varying shapes and polymer types that are now widespread global contaminants of marine and freshwater ecosystems. Various estimates suggest that several trillions of microplastic particles are present in our global oceanic system, and that these are readily ingested by a wide range of marine and freshwater species across feeding modes and ecological niches. Here, we present some of the key and pressing issues associated with these globally important contaminants from a microbiological perspective. We discuss the potential mechanisms of pathogen attachment to plastic surfaces. We then describe the ability of pathogens (both human and animal) to form biofilms on microplastics, as well as dispersal of these bacteria, which might lead to their uptake into aquatic species ingesting microplastic particles. Finally, we discuss the role of a changing oceanic system on the potential of microplastic-associated pathogens to cause various disease outcomes using numerous case studies. We set out some key and imperative research questions regarding this globally important issue and present a methodological framework to study how and why plastic-associated pathogens should be addressed.

Introduction:

Despite increased research effort and public attention, global plastic production and the amount of plastic debris entering and contaminating the world's oceans continues to increase. In 2020, 367 million metric tonnes (Mt) of plastics were produced and it is predicted that emissions into aquatic ecosystems could reach up to 90 Mt by the year 2030 if we continue business as usual [1,2]. Even with ambitious commitments to reduce plastic pollution set by the world's governments, we could still be releasing up to 53 Mt per year into aquatic ecosystems by 2030 [1]. Approximately 24.4 trillion pieces of microplastic particles are thought to now be floating within the upper level of the world's oceans [3]. This vast increase in ocean particulates provide novel and increased availability of surfaces available for pathogen attachment in oceanic and coastal waters, attracting great interest into the microbial communities that attach to ocean plastic surfaces.

First coined the 'plastisphere' by Zettler *et al.* [4], the community associated with this novel substrate is originally comprised of an assortment of bacteria that develops a biofilm, leading to subsequent attachment of eukaryotic organisms such as diatoms and even larval benthic organisms over time [5]. Plastic provides microbes a resilient substrate that has the potential to be transported across oceanographic regions and differing environmental conditions [5]. The microbial community on a microplastic surface has now been widely documented (see review by [5,6]) and shown to differ to that of its surrounding environment, largely dominated by Bacteroidetes, Cyanobacteria and Proteobacteria [7–10]. Of particular concern are the many potential pathogens that have been found incorporated within the plastisphere, namely *Vibrio spp.,* whose genera host a number of human and animal pathogens [11,12]. A recent systematic review by Junaid *et al*., [13] listed the potential pathogens that have been

documented as occurring within the plastisphere to date for both the aquatic and terrestrial environments, highlighting the following genera as most frequently associated with potentially harmful bacteria found attached to plastic surfaces within aquatic environments: *Vibrio, Pseudomonas, Acinetobacter, Arcobacter, Bacillus, Aquabacterium, Mycobacterium, Aeromonas, Tenacibaculum, Escherichia, Klebsiella*, and *Legionella* [13]. This raises the critical question as to whether plastic debris can act as a vector for potential pathogens, disseminating them throughout various aquatic environments and organisms, especially in comparison to natural particles.

As a result of microplastics persistence and ubiquity within marine and freshwater environments, a multitude of aquatic organisms with varying feeding modes, from the sea surface to the deepest part of the ocean are now known to readily ingest microplastic particles [14]. In particular, filter feeding organisms such as mussels and oysters will be subject to chronic exposures and uptake of microplastics [15]. This is occurring against the background of global ocean warming and acidification, which can alter or impair organisms' physiological processes and responses to any additional stressor. For example, one of the many impacts of ocean acidification can be reduced immunological response to infection [16,17]. The Anthropocene is becoming a pressing issue for these key aquatic species and understanding the interactions between these multiple stressors is imperative [18].

To address the important question as to whether the increasing microplastic burden in the global oceans might act as vectors of pathogen transfer to marine/aquatic species requires more than just assessing their presence on the particle surface. Here we will discuss four key processes that would be involved in any transfer of pathogenic bacteria from microplastics to hosts and subsequent disease outcome (summarised in Figure 1 [19]); 1) attachment of the

pathogen to the MP, 2) dispersal of the pathogen from the microplastic within an organism following ingestion or adhesion, 3) transfer of the pathogen into tissues and 4) disease outcome for the organism. The majority of studies to date have focused on describing the plastisphere community composition (also highlighted by Beloe *et al.*, [20] and reviewed by [5,6]), leaving the processes by which these communities attach and develop and then disperse over time still unexplored. It is also imperative to begin to understand whether they can be transferred into organismal tissues in sufficient numbers to cause disease outcomes and whether any of these processes differ at all from those that occur for the biofilms of natural particles.

Mechanisms of pathogen attachment to plastic surfaces

Moments after exposure of any surface, including those of plastics or natural particles, to aquatic environments a conditioning film comprised of various organic and inorganic macromolecules and dissolved solutes begins to form [21–23]. This conditioning film is complete within hours and continues until adsorption kinetics are no longer favourable [24]. The attachment of microbial cells to this conditioning layer occurs through a variety of processes that have been well documented [24–26]. An example of *Vibrio parahaemolyticus*, a known pathogen found within the plastisphere [27] attached to a polyamide nylon 6 microfiber is shown in Figure 2. The initiation of these processes is multifaceted, yet likely coordinated, requiring both environmental and genetic stimuli [28]. The production of exopolysaccharides, such as *Vibrio* polysaccharide (VPS) from *V. cholera*, is one such process indicative of a shift from a planktonic lifestyle to one associated with a biofilm community such as the plastisphere.

In nature, biofilms are comprised of a complex consortium including multiple species [29,30]. Within this community there are various cell to cell interactions that will affect the formation and composition of the biofilm. These are largely in the shape of inter- and intraspecies interactions including predation, quorum sensing and synergistic partnerships [29]. Along with enhanced communication between cells, biofilms offer many advantages including defence from external pressures such as decreased predation and reduction in sensitivity to antibiotics and host immune attacks, as well as enhanced metabolite exchange and access to nutrients [31]. Plastisphere communities are comprised of a complex and diverse grouping of bacteria including potential pathogens and it has been suggested that multi-species biofilms facilitate persistence of pathogenic cells within the biofilm community [32]. Evidence also suggests that mechanisms associated with biofilm formation can also induce the expression of genes required for virulence and in the transfer of AMR genes [33,34].

It has become clear that the prevailing environmental conditions of surrounding seawater can strongly influence the mature bacterial community composition, whereas substrate/polymer type mainly affect the early stages of biofilm formation [8,35,36]. A few studies, however, have suggested that surface plastic type can select for differing bacterial clusters and even that some bacteria may selectively attach to a specific polymer [37,38]. Natural particles, such as glass, wood and feathers have been used as comparative surfaces to determine whether plastic offers new ecological niche to a specific consortium of bacteria. The evidence to date from these studies suggests that the bacterial communities of plastic and natural substrates are similar, with limited plastic specific bacteria being found thus far [6,39,40]. Elucidating how similar or different pathogen attachment to microplastics is to that of natural particles is a crucial component to explaining the impacts of this new ecological niche.

For the plastisphere, it is unknown whether or not pathogens are likely to be early or secondary colonisers, with this likely being species specific. For instance, vibrios attachment to microplastics have been discovered to be highly dynamic during the early stages of biofilm formation, yet poorly connected with the biofilm community [41]. The survival and longevity of pathogens such as vibrios within the plastisphere community once established is not well understood. The paradigm that the plastisphere community is strongly shaped by the surrounding environmental conditions, and hence is ever changing across varying marine environments, has been supported by review in 2020 [42], which analysed 35 plastisphere studies, and a more recent study which demonstrated a large community shift when the plastisphere community was exposed to changing salinity [43] These findings are important when considering whether pathogens might be transported to new locations via floating microplastics and ocean currents. Whilst a number of reviews suggest that microplastics do not pose more of a risk than natural controls with regards to potential pathogen colonisation [6,20,39]. However, another more recent review has reported the contrary with Metcalf *et al*., [44] reporting that 62% of the studies examining potential pathogens on plastics and natural controls had higher abundances on the plastic particles than the natural controls. Vibrios have been reported to occur in high abundance on microplastic fragments collected from open ocean trawls [4,5], despite vibrio abundances generally found to be low in open ocean waters compared to coastal marine ecosystems [45], suggesting that microplastics may be acting as a vector for transport. However, these studies did not compare the plastisphere to any natural particles, and it is difficult to ascertain where these particles originated from, making conclusions as to plastics role as a vector challenging.

The presence of potential pathogens described at the genus level within the plastisphere does not necessarily determine that pathotypes of any given genera are present. There are around

half a dozen papers that have been able to assign pathogenic genes to specific bacteria present on microplastic surfaces sampled from the environment, such as *Vibrio cholerae* and *Aeromonas salmonicida*, but were unable to confirm if these genes were being actively expressed at the time of sampling due to the lack of metatransciptomics used in most studies to date [46–49]. Antimicrobial resistant genes (ARGs) and bacteria (ARBs) have also been discovered within the plastic biofilm [50], with a number of studies reporting higher abundance of these (up to 5000 times) in the plastisphere community when compared to the surrounding water [51–53]. This might be expected due to the role of biofilms in enhancing horizontal gene transfer (HGT), a process that has been described to occur at a higher rate in microplastics when compared to the surrounding environment [54]. The converse has also been reported, however, with some studies findings these ARGs and ARBs in higher numbers on natural particle controls or in seawater compared to the plastisphere [50]. Alas, the activity of these ARGs within the natural controls has yet to be studied in conjunction with actual pathogens within the plastisphere. An important point to make here is that biofilm formation has been suggested to increase the affinity of pollutants (such as heavy metals) to microplastics [55]. As well as this, Liu *et al.,* [56] described that biofilms can have a positive effect upon the adsorption and concentration of these heavy metals on microplastics [57]. With heavy metals having the potential to aid in the proliferation of antibiotic resistance in pathogenic bacteria, it remains important to gain a further understanding into the presence and activity of ARGs within the plastisphere. Wu *et al.* [58] is one of the first papers to describe ARGs that were being actively expressed within the plastisphere community. They detected 75 genes with transcriptional activity, offering evidence that the plastispheres antibiotic resistome was actively expressed at the time of sampling. Interestingly, opportunistic human

pathogens belonging to the Enterobacteriaceae family were discovered to host ARGs within the plastisphere [58].

Mechanisms required for attachment and subsequent phenotypic changes for pathogens within aquatic settings are well described. However, key stages that may occur within the plastisphere biofilm are largely understudied. For example, bacterial adhesion to specific surfaces can actually cause an increase in competence for the uptake of foreign DNA, such as vibrios attachment to chitin [59]. This combined with the evidence that HGT and ARGs are increased within the plastisphere community, raises questions on the role that these pathogens play within the biofilm community. But then, for any attached pathogen to be of concern for any organisms coming into contact with or ingesting them, dispersal of pathogen from the particle needs to occur followed by uptake into the organismal tissues or circulatory system. Detachment from a biofilm can generally be described as active (bacteria driven) or passive (driven by external factors). These processes within the plastisphere biofilm are currently unknown, representing a major knowledge gap.

What are the mechanisms causing bacterial dispersal from the plastisphere biofilm?

Currently, very few studies have focused on the dispersal or detachment stage of the plastisphere biofilm, yet this is key to elucidating the potential for the plastisphere community to transfer to aquatic microorganisms following ingestion or adhesion of microplastic particles. Dispersal of cells from biofilms is mediated by physical processes including shearing or via processes that are actively regulated. In the marine environment the role of fluid flow over biofilms suggests that shearing processes are likely to play a considerable role in dispersal. However, from knowledge of regulated processes of biofilm dispersal, several

scenarios can be envisaged. These are; dispersion, detachment and desorption [60]. Collectively, dispersal occurs through a variety of cues, signals and regulators that causes specialised dispersal cells to leave the mature biofilm and enter back into a planktonic lifestyle [61]. Active dispersion is generally the result of specific signal transduction cues leading to physiological adjustments such as downregulation of biofilm matrix production and pilus retraction [62]. Detachment relates to the attrition of the biofilm matrix via shear forces, where sloughing of the cells releases them into the adjacent aquatic environment [61]. Finally, desorption generally relates to the disassociation of sessile cells in the early stages of biofilm formation. This may be an active, regulated process if it is considered as a reversal or inhibition of the initial attachment process such as inhibition or downregulation of adhesins such as LapA [63]. Interrogation of the expression status of genes associated with biofilm dispersal in the context of the plastisphere will aid in determining the potential effects of pathogens embedded in this environment.

The ingestion of a variety of plastics by a wide range of aquatic organisms in their natural habitats is now documented for over 200 different species from all regions of the ocean [64]. Upon ingestion, these microplastics will enter the organism's digestive tracts and gut and either remain there indefinitely, or pass through the guts and be removed via depuration, which is the case for many invertebrates [15]. The surrounding environmental conditions for the plastisphere community will change upon ingestion of the microplastic particle; for example, internal/gut pH, nutrient levels and oxygen concentration (as highlighted in concept Figure 1). One of the key methods of dispersal is the use of enzymes to break down the extracellular matrix of the biofilm [65] (Figure 1). A multitude of biofilm species have been shown to secrete enzymes specific for extracellular matrix degradation as a result of changes to environmental conditions within and exterior to the biofilm [65]. Also, in *Vibrio cholerae*,

it has been shown that the bile salts within the host's intestinal cavity promotes biofilm dispersal [66]. Changes in the gut pH may also induce the expression of virulence associated genes giving more potential for these plastisphere transported microorganisms to cause disease. Interestingly, some pathogenic strains of bacteria (i.e. *Vibrio cholerae*) have actually enabled themselves to use acidic conditions within the stomach to regulate virulence for efficient infection [67,68].

Host colonisation and disease

Only one study to date has directly demonstrated the transfer of bacterial cells from a plastic particle to a host organism, demonstrating the transfer of *E. coli* cells to the temperate coral *Astrangia poculata,* after ingestion of biofilmed microbeads [69]. This study also demonstrated disease outcome following this transfer, whereby the corals colonised by the *E. coli* cells consequently died after 4 weeks of exposure, in contrast to corals that ingested virgin plastics which survived post depuration. Likewise, a recent study looked at the effects of biofilmed plastic on the Mussel *Mytilus galloprovincialis* and found plastic ingestion caused a significant immune response in comparison to mussels exposed to virgin plastics. As well as this, they discovered that exposure to biofilm-associated microplastics also increased bacterial diversity on the gills within the mussels when compared to Mussels exposed to sterile microplastics [70]. Not only showing pathogen transfer but also providing evidence of disease is a critically important area of research that requires further investigation.

Pathogen-plastic-biota interactions under global ocean change?

231 The ocean environment in which any pathogen-microplastic-organism interactions will occur
232 is changing at an unprecedented rate as a result of ocean warming, acidification and
233 deoxygenation [71]. These changes in the physico-chemical properties of seawater have the
234 potential to influence both the abundance and global distribution of marine pathogens, and
235 the physiological responses of any organisms they interact with. For example, ocean
236 acidification (OA), the decline in ocean pH and associated changes in carbonate chemistry due
237 to increasing uptake of atmospheric carbon dioxide, has been shown to supress
238 immunological responses in a variety of marine organisms [16,72,73]. Near-future OA
239 conditions can alter microbial communities within organisms, including host-pathogen
240 interactions [74], and susceptibility to a known bivalve bacterial pathogen was found to
241 increase when exposed under ocean acidification conditions for the Blue Mussel, *Mytilus*
242 *edulis* [75]. Ocean acidification may also directly cause tissue damage in organisms such as
243 fish, potentially contributing to a weakened immune system that creates opportunities for
244 bacterial invasion [76]. Understanding these potential interactions between the physiological
245 effects of ocean acidification and exposure to the plastisphere community, in a multi stressed
246 ocean are important.

247 Increasing sea surface temperatures are perhaps the most obvious and pervasive impact of
248 climate change in coastal ecosystems worldwide, particularly in light of recent observations
249 demonstrating significant warming in over 70% of the world's coastlines [77]. Climate change
250 plays a significant role in determining the dynamics of many bacterial pathogens and for some
251 disease agents is becoming increasingly well understood [78,79]. Many diseases are expected
252 to increase in range and severity with projected climate changes [79–81]. There is a growing
253 body of evidence to indicate that climatic warming may allow certain Vibrio strains to emerge
254 in new areas. For instance, a highly pathogenic variant of *Vibrio parahaemolyticus* belonging

255 to the clonal complex ST36 and termed the Pacific Northwest strain emerged on the
256 Northeast coast of the United States of America during the unusually warm spring of 2012
257 [82,83]. It is likely that localised climate warming played some role in the epidemic ignition of
258 this strain [79]. Climate warming can potentially have impacts on the evolution of bacterial
259 pathogens in the environment, but there is no evidence to date as to how this might affect
260 the plastisphere community. Marine bacteria such as vibrios have some of the fastest
261 replication times of any studied bacteria with studies of *V. parahaemolyticus* and other *Vibrio*
262 species have replication times as little as 8-9 minutes [84,85]. Increased environmental
263 temperatures may also amplify and accentuate microbial evolution. Potential underlying
264 mechanisms include elevated temperatures facilitating horizontal gene transfer of mobile
265 genetic elements of resistance, and increased pathogen growth rates promoting
266 environmental persistence, carriage and transmission [86,87]. There are therefore, a number
267 of potential mechanisms by which ocean warming and the plastisphere associated with the
268 increasing microplastic burden in the global ocean might interact. Assessing these
269 interactions represent a key research avenue.

270

271 **Future directions**

272 Plastics have become an important component of everyday life since the start of their mass
273 production in the middle of the 20th Century. Critically, microplastic contamination is
274 geographically widespread, longstanding and likely to increase in the future. Several factors
275 make the study of pathogens (both human and animal) and their interactions with plastics in
276 the environment absolutely critical. Firstly, and perhaps most importantly, the sheer volume
277 of floating plastics entering the global oceanic system alone is staggering. In 2021 it was

predicted that there are 24.4 trillion plastic particles in the world's upper oceans, not accounting for sinking plastic. Since then, the numbers have increased and are expected to continue on an upward trajectory if the causes and sources are not mitigated [88]. As a global environmental contaminant this alone represents an unprecedented surface area and milieu for the colonisation, attachment, dispersal and potential spread of various bacterial pathogens. Unfortunately, it is not known to what extent this additional input of human plastic waste into the natural environment has altered the potential spread of human and animal diseases, and how these risks have changed since the advent of plastic contamination of the environment in the last few decades. This alone is probably one of the key questions that needs to be addressed by the scientific community. Although there is now a glut of studies that demonstrate the presence of pathogens on plastic particles (of differing levels of weathering), many studies are anecdotal, lack robust experimental comparators and generally fail to provide conclusive evidence to infer risk, such as biologically plausible pathways of disease transmission by plastic-associated pathogens. Complicating matters further, several confounding factors also exist; climate change (including coastal warming, extreme weather events and ocean acidification), a global increase in intensive aquaculture, as well as demand for aquaculture products, which make the study of pathogens associated with microplastics all the more necessary.

Whilst the rate, type and diversity of microbial colonisation of microplastics is well documented in the literature, there are still some key knowledge gaps that need to be addressed: 1) robust (laboratory-based) evidence for the selection of pathogens on both plastic and natural particle surfaces as well as antibiotic resistant bacteria, 2) and their associated rate and type of gene exchange on both plastic and natural particles; 3.) Studies examining the four key stages of a vector; 1.) Attachment of the pathogens 2) dispersal of the

pathogen within an organism, 3) transfer of the pathogen into tissues and 4) disease outcome as highlighted in Figure 1. These all represent key research challenges. Of note, although many anecdotal studies exist (e.g. showing presence of human pathogens and/or AMR genes on microplastics fragments), very few carefully controlled laboratory studies such as those studying the relative colonisation and spread of different bacterial pathogens have been published to date. Across various scientific disciplines the minimum technical requirements necessary for the publication of work using approaches such as genome sequencing and PCR have been established now for some time. This framework is essential for ensuring the validity of the approaches used, harmonises specific scientific definitions, increases experimental transparency and helps promote consistency between different laboratories. We suggest that a similar set of commonly defined minimum requirements are needed in the study and publication of research in environmental plastic research, and should focus on the types of samples studied (e.g. polymer type, size, weathering rate), choice of controls (natural, virgin plastic, glass etc.), microbial detection and characterisation methods (e.g. PCR, genome sequencing) and whether the work uses environmental and/or laboratory-based studies. Given these huge uncertainties and complexities it is likely that studying plastic-associated pathogens and deriving more fully an understanding of how and potentially why plastic contamination poses a risk in the environment will require merging disparate scientific disciplines - such as biological oceanography, ecotoxicology, microbiology, molecular biology, and genomics, among others.

Summary

- Plastic contamination of the environment is a significant and growing global problem.
- There is now a strong body of evidence suggesting that the plastisphere community can include a range of potential pathogens.
- With widespread ingestion of these plastic particles by aquatic species, the potential for dispersal of the plastisphere community within the gut of an organism following ingestion remains a key knowledge gap.
- A changing oceanic system can have an impact on the immune physiology of some marine species as well as increase pathogen prevalence in marine ecosystems thus raising the potential for interaction between these stressors.
- Methodological frameworks for developing research in this area should include the four key vector stages; 1.) Attachment of the pathogens 2) dispersal of the pathogen within an organism, 3) transfer of the pathogen into tissues and 4) disease outcome.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

All authors wrote and edited the manuscript.

Acknowledgements

J.B. is funded by insurance firm AXA XL's Ocean Risk Scholarships Programme who we thank for their support of this work. C.L. is supported by Natural Environment Research Council U.K. grant NE/S003975/1. Thanks to Christian Hacker and the University of Exeter Bioimaging Centre for their help with the SEM imaging.

References

1. Borrelle SB, Ringma J, Law KL, Monnahan CC, Lebreton L, McGivern A, et al. 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science*. 369:1515–8. 10.1126/science.aba3656

2. Plastic Europe. 2021. Plastics - the Facts 2021. 2021.

3. Isobe A, Azuma T, Cordova MR, Cózar A, Galgani F, Hagita R, et al. 2021. A multilevel dataset of microplastic abundance in the world's upper ocean and the Laurentian Great Lakes. *Microplastics and Nanoplastics*. 1:1–14. 10.1186/s43591-021-00013-z

4. Zettler ER, Mincer TJ, Amaral-Zettler LA. 2013. Life in the "plastisphere": Microbial communities on plastic marine debris. *Environ Sci Technol*. 47:7137–46. 10.1021/es401288x

5. Amaral-Zettler LA, Zettler ER, Mincer TJ. 2020. Ecology of the plastisphere. *Nat Rev Microbiol*. 18:139–51. 10.1038/s41579-019-0308-0

6. Oberbeckmann S, Labrenz M. 2020. Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. *Ann Rev Mar Sci*. 14. 10.1146/annurev-marine-010419

7. Dussud C, Meistertzheim AL, Conan P, Pujo-Pay M, George M, Fabre P, et al. 2018. Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. *Environ Pollut*. 236:807–16. 10.1016/j.envpol.2017.12.027

8. Oberbeckmann S, Kreikemeyer B, Labrenz M. 2018. Environmental factors support the formation of specific bacterial assemblages on microplastics. *Front Microbiol*. 8:1–12. 10.3389/fmicb.2017.02709

9. Xu X, Wang S, Gao F, Li J, Zheng L, Sun C, et al. 2019. Marine microplastic-associated bacterial community succession in response to geography, exposure time, and plastic type in China's coastal seawaters. *Mar Pollut Bull*. 145:278–86. 10.1016/j.marpolbul.2019.05.036

10. Lear G, Kingsbury JM, Franchini S, Gambarini V, Maday SDM, Wallbank JA, et al. 2021. Plastics and the microbiome: impacts and solutions. *Environ Microbiomes*. 16:1–19. 10.1186/s40793-020-00371-w

11. Austin B. 2010. Vibrios as causal agents of zoonoses. *Vet Microbiol*. 140:310–7. 10.1016/j.vetmic.2009.03.015

12. Bonnin-Jusserand M, Copin S, Le Bris C, Brauge T, Gay M, Brisabois A, et al. 2019. Vibrio species involved in seafood-borne outbreaks (Vibrio cholerae, V. parahaemolyticus and V. vulnificus: Review of microbiological versus recent molecular detection methods in seafood products. *Crit Rev Food Sci Nutr*. 59:597–610. 10.1080/10408398.2017.1384715

13. Junaid M, Ali J, Sadaf M, Liu S, Wang J. 2022. Enrichment and dissemination of bacterial pathogens by microplastics in the aquatic environment. *Sci Total Environ*. 830:1–15. 10.1016/j.scitotenv.2022.154720

14. Santos RG, Machovsky-Capuska GE, Andrades R. 2021. Plastic ingestion as an evolutionary trap: Toward a holistic understanding. *Science*. 373:56–60. 10.1126/science.abh0945

15. Ward JE, Rosa M, Shumway SE. 2019. Capture, ingestion, and egestion of microplastics by suspension-feeding bivalves: a 40-year history. *Anthr Coasts*. 2:39–49. 10.1139/anc-2018-0027

16. Wang Q, Cao R, Ning X, You L, Mu C, Wang C, et al. 2016. Effects of ocean acidification

407 on immune responses of the Pacific oyster *Crassostrea gigas*. *Fish Shellfish Immunol*. 49:24–
408 33. 10.1016/j.fsi.2015.12.025

409 17. Wu F, Xie Z, Lan Y, Dupont S, Sun M, Cui S, et al. 2018. Short-Term Exposure of *Mytilus*
410 *coruscus* to Decreased pH and Salinity Change Impacts Immune Parameters of Their
411 Haemocytes. *Front Physiol*. 9:1–11. 10.3389/fphys.2018.00166

412 18. Waters CN, Zalasiewicz J, Summerhayes C, Barnosky AD, Poirier C, Gałuszka A, et al.
413 2016. The Anthropocene is functionally and stratigraphically distinct from the Holocene.
414 *Science*. 351. 10.1126/science.aad2622

415 19. Eggermont M, Cornillie P, Dierick M, Adriaens D, Nevejan N, Bossier P, et al. 2020. The
416 blue mussel inside: 3D visualization and description of the vascular-related anatomy of
417 Mytilus edulis to unravel hemolymph extraction. *Sci Rep*. 10:1–16. 10.1038/s41598-020-
418 62933-9

419 20. Beloe CJ, Browne MA, Johnston EL. 2022. Plastic Debris As a Vector for Bacterial Disease:
420 An Interdisciplinary Systematic Review. *Environ Sci Technol*. 56:2950–8.
421 10.1021/acs.est.1c05405

422 21. Bhagwat G, O'Connor W, Grainge I, Palanisami T. 2021. Understanding the Fundamental
423 Basis for Biofilm Formation on Plastic Surfaces: Role of Conditioning Films. *Front Microbiol*.
424 12:1–10. 10.3389/fmicb.2021.687118

425 22. Compère C, Bellon-Fontaine M, Bertrand P, Costa D, Marcus P, Poleunis C, et al. 2001.
426 Kinetics of conditioning layer formation on stainless steel immersed in seawater. *Biofouling*.
427 17:129–45. 10.1080/08927010109378472

428 23. Loeb GI, Neihof R a. 1975. Marine Conditioning Films. *Adv Chem*. 1975. p. 319–35.

10.1021/ba-1975-0145.ch016

24. Lawrence JR, Neu TR, Paule A, Korber DR, Wolfaardt GM. 2015. Aquatic Biofilms: Development, Cultivation, Analyses, and Applications. *Man Environ Microbiol*. ASM Press; 2015. p. 4.2.3-1-4.2.3-33. 10.1128/9781555818821.ch4.2.3

25. Palmer J, Flint S, Brooks J. 2007. Bacterial cell attachment, the beginning of a biofilm. *J Ind Microbiol Biotechnol*. 34:577–88. 10.1007/s10295-007-0234-4

26. Okshevsky M, Meyer RL. 2015. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. *Crit Rev Microbiol*. 41:341–52. 10.3109/1040841X.2013.841639

27. Kirstein I V., Kirmizi S, Wichels A, Garin-Fernandez A, Erler R, Löder M, et al. 2016. Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio spp.* on microplastic particles. *Mar Environ Res*. Elsevier Ltd; 120:1–8.

28. Hall CW, Mah T-F. 2017. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol Rev*. 41:276–301. 10.1093/femsre/fux010

29. Giaouris E, Heir E, Desvaux M, Hébraud M, Møretrø T, Langsrud S, et al. 2015. Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. *Front Microbiol*. 6:1–26. 10.3389/fmicb.2015.00841

30. Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. 2020. Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. *Front Microbiol*. 11:1–20. 10.3389/fmicb.2020.00928

31. Santos ALS dos, Galdino ACM, Mello TP De, Ramos LDS, Branquinha MH, Bolognese AM,

et al. 2018. What are the advantages of living in a community? A microbial biofilm perspective! *Mem Inst Oswaldo Cruz*. 113:1–7. 10.1590/0074-02760180212

32. Sanchez-Vizuete P, Orgaz B, Aymerich S, Le Coq D, Briandet R. 2015. Pathogens protection against the action of disinfectants in multispecies biofilms. *Front Microbiol*. 6:1–12. 10.3389/fmicb.2015.00705

33. Çam S, Brinkmeyer R. 2020. Differential expression of vvhA and CPS operon allele 1 genes in *Vibrio vulnificus* under biofilm and planktonic conditions. *Antonie Van Leeuwenhoek*. 113:1437–46. 10.1007/s10482-020-01452-z

34. Ma L, Konkel ME, Lu X. 2021. Antimicrobial Resistance Gene Transfer from *Campylobacter jejuni* in Mono- and Dual-Species Biofilm. Elkins CA, editor. *Appl Environ Microbiol*. 87:1–17. 10.1128/AEM.00659-21

35. Coons AK, Busch K, Lenz M, Hentschel U, Borchert E. 2021. Biogeography rather than substrate type determines bacterial colonization dynamics of marine plastics. *PeerJ*. 9:1–27. 10.7717/peerj.12135

36. Delacuvellerie A, Ballerini T, Frère L, Matallana-Surget S, Dumontet B, Wattiez R. 2022. From rivers to marine environments: A constantly evolving microbial community within the plastisphere. *Mar Pollut Bull*. 179:1–11. 10.1016/j.marpolbul.2022.113660

37. Pinto M, Langer TM, Hüffer T, Hofmann T, Herndl GJ. 2019. The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. *PLoS One*. 14:1–20. 10.1371/journal.pone.0217165

38. Hansen J, Melchiorsen J, Ciacotich N, Gram L, Sonnenschein EC. 2021. Effect of polymer type on the colonization of plastic pellets by marine bacteria. *FEMS Microbiol Lett*. 368:1–9.

10.1093/femsle/fnab026

39. Wright RJ, Erni-Cassola G, Zadjelovic V, Latva M, Christie-Oleza JA. 2020. Marine Plastic Debris: A New Surface for Microbial Colonization. *Environ Sci Technol*. 54:11657–72. 10.1021/acs.est.0c02305

40. Scales BS, Cable RN, Duhaime MB, Gerdts G, Fischer F, Fischer D, et al. 2021. Cross-Hemisphere Study Reveals Geographically Ubiquitous, Plastic-Specific Bacteria Emerging from the Rare and Unexplored Biosphere. *mSphere*. 6:1–17. 10.1128/mSphere.00851-20

41. Kesy K, Labrenz M, Scales BS, Kreikemeyer B, Oberbeckmann S. 2020. Vibrio Colonization Is Highly Dynamic in Early Microplastic-Associated Biofilms as Well as on Field-Collected Microplastics. *Microorganisms*. 9:76. 10.3390/microorganisms9010076

42. Wright RJ, Langille MGI, Walker TR. 2020. Food or just a free ride? A meta-analysis reveals the global diversity of the Plastisphere. *ISME J*. 15. 10.1038/s41396-020-00814-9

43. Song J, Beule L, Jongmans-Hochschulz E, Wichels A, Gerdts G. 2022. The travelling particles: community dynamics of biofilms on microplastics transferred along a salinity gradient. *ISME Commun*. 2:35. 10.1038/s43705-022-00117-4

44. Metcalf R, Oliver DM, Moresco V, Quilliam RS. 2022. Quantifying the importance of plastic pollution for the dissemination of human pathogens: The challenges of choosing an appropriate 'control' material. *Sci Total Environ*. 810:1–11. 10.1016/j.scitotenv.2021.152292

45. Yooseph S, Nealson KH, Rusch DB, McCrow JP, Dupont CL, Kim M, et al. 2010. Genomic and functional adaptation in surface ocean planktonic prokaryotes. *Nature*. 468:60–6. 10.1038/nature09530

46. Silva MM, Maldonado GC, Castro RO, de Sá Felizardo J, Cardoso RP, Anjos RM dos, et al.

2019. Dispersal of potentially pathogenic bacteria by plastic debris in Guanabara Bay, RJ, Brazil. *Mar Pollut Bull*. 141:561–8. 10.1016/j.marpolbul.2019.02.064

47. Radisic V, Nimje PS, Bienfait AM, Marathe NP. 2020. Marine Plastics from Norwegian West Coast Carry Potentially Virulent Fish Pathogens and Opportunistic Human Pathogens Harboring New Variants of Antibiotic Resistance Genes. *Microorganisms*. 8:1–13. 10.3390/microorganisms8081200

48. Rasool FN, Saavedra MA, Pamba S, Perold V, Mmochi AJ, Maalim M, et al. 2021. Isolation and characterization of human pathogenic multidrug resistant bacteria associated with plastic litter collected in Zanzibar. *J Hazard Mater*. 405:124591. 10.1016/j.jhazmat.2020.124591

49. Laverty AL, Primpke S, Lorenz C, Gerdts G, Dobbs FC. 2020. Bacterial biofilms colonizing plastics in estuarine waters, with an emphasis on Vibrio spp. and their antibacterial resistance. Kelly JJ, editor. *PLoS One*. 15:e0237704. 10.1371/journal.pone.0237704

50. Yu X, Zhang Y, Tan L, Han C, Li H, Zhai L, et al. 2022. Microplastisphere may induce the enrichment of antibiotic resistance genes on microplastics in aquatic environments: A review. *Environ Pollut*. 310:119891. 10.1016/j.envpol.2022.119891

51. Zhang Y, Lu J, Wu J, Wang J, Luo Y. 2020. Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system. *Ecotoxicol Environ Saf*. 187:109852. 10.1016/j.ecoenv.2019.109852

52. Hou D, Hong M, Wang Y, Dong P, Cheng H, Yan H, et al. 2021. Assessing the Risks of Potential Bacterial Pathogens Attaching to Different Microplastics during the Summer–Autumn Period in a Mariculture Cage. *Microorganisms*. 9:1–15.

517 10.3390/microorganisms9091909

518 53. Zhang S-J, Zeng Y-H, Zhu J-M, Cai Z-H, Zhou J. 2022. The structure and assembly
519 mechanisms of plastisphere microbial community in natural marine environment. *J Hazard*
520 *Mater*. 421:1–13. 10.1016/j.jhazmat.2021.126780

521 54. Arias-Andres M, Klümper U, Rojas-Jimenez K, Grossart H-P. 2018. Microplastic pollution
522 increases gene exchange in aquatic ecosystems. *Environ Pollut*. 237:253–61.
523 10.1016/j.envpol.2018.02.058

524 55. Stenger KS, Wikmark OG, Bezuidenhout CC, Molale-Tom LG. 2021. Microplastics
525 pollution in the ocean: Potential carrier of resistant bacteria and resistance genes. *Environ*
526 *Pollut*. 291:1–12. 10.1016/j.envpol.2021.118130

527 56. Liu S, Shi J, Wang J, Dai Y, Li H, Li J, et al. 2021. Interactions Between Microplastics and
528 Heavy Metals in Aquatic Environments: A Review. *Front Microbiol*. 12:1–14.

529 57. Nguyen CC, Hugie CN, Kile ML, Navab-Daneshmand T. 2019. Association between heavy
530 metals and antibiotic-resistant human pathogens in environmental reservoirs: A review.
531 *Front Environ Sci Eng*. 13:46. 10.1007/s11783-019-1129-0

532 58. Wu X, Liu Z, Li M, Bartlam M, Wang Y. 2022. Integrated metagenomic and
533 metatranscriptomic analysis reveals actively expressed antibiotic resistomes in the
534 plastisphere. *J Hazard Mater*. 430:1–12. 10.1016/j.jhazmat.2022.128418

535 59. Pruzzo C, Vezzulli L, Colwell RR. 2008. Global impact of *Vibrio cholerae* interactions with
536 chitin. *Environ Microbiol*. 10:1400–10. 10.1111/j.1462-2920.2007.01559.x

537 60. Petrova OE, Sauer K. 2016. Escaping the biofilm in more than one way: Desorption,
538 detachment or dispersion. *Curr Opin Microbiol*. 30:67–78. 10.1016/j.mib.2016.01.004

539 61. McDougald D, Rice SA, Barraud N, Steinberg PD, Kjelleberg S. 2012. Should we stay or
540 should we go: mechanisms and ecological consequences for biofilm dispersal. *Nat Rev*
541 *Microbiol*. 10:39–50. 10.1038/nrmicro2695

542 62. Teschler JK, Nadell CD, Drescher K, Yildiz FH. 2022. Mechanisms Underlying *Vibrio*
543 *cholerae* Biofilm Formation and Dispersion. *Annu Rev Microbiol*. 76:503–32.
544 10.1146/annurev-micro-111021-053553

545 63. Gjermansen M, Nilsson M, Yang L, Tolker-Nielsen T. 2010. Characterization of starvation-
546 induced dispersion in *Pseudomonas putida* biofilms: genetic elements and molecular
547 mechanisms. *Mol Microbiol*. 75:815–26. 10.1111/j.1365-2958.2009.06793.x

548 64. Gall SC, Thompson RC. 2015. The impact of debris on marine life. *Mar Pollut Bull*.
549 92:170–9. 10.1016/j.marpolbul.2014.12.041

550 65. Karatan E, Watnick P. 2009. Signals, Regulatory Networks, and Materials That Build and
551 Break Bacterial Biofilms. *Microbiol Mol Biol Rev*. 73:310–47. 10.1128/MMBR.00041-08

552 66. Hay AJ, Zhu J. 2015. Host Intestinal Signal-Promoted Biofilm Dispersal Induces Vibrio
553 cholerae Colonization. *Infect Immun*. 83:317–23. 10.1128/IAI.02617-14

554 67. Sistrunk JR, Nickerson KP, Chanin RB, Rasko DA, Faherty CS. 2016. Survival of the Fittest:
555 How Bacterial Pathogens Utilize Bile To Enhance Infection. *Clin Microbiol Rev*. 29:819–36.
556 10.1128/CMR.00031-16

557 68. Chiang I-L, Wang Y, Fujii S, Muegge BD, Lu Q, Tarr PI, et al. 2021. Biofilm Formation and
558 Virulence of Shigella flexneri Are Modulated by pH of Gastrointestinal Tract. *Infect Immun*.
559 89:1–11. 10.1128/IAI.00387-21

560 69. Rotjan RD, Sharp KH, Gauthier AE, Yelton R, Lopez EMB, Carilli J, et al. 2019. Patterns,

561 dynamics and consequences of microplastic ingestion by the temperate coral, *Astrangia*
562 *poculata*. *Proc R Soc B Biol Sci*. 286:1–9. 10.1098/rspb.2019.0726

563 70. Bandini F, Hchaichi I, Zitouni N, Missawi O, Cocconcelli S, Puglisi E, et al. 2021. Bacterial
564 community profiling of floating plastics from South Mediterranean sites: First evidence of
565 effects on mussels as possible vehicles of transmission. *J Hazard Mater*. 411:1–12.
566 10.1016/j.jhazmat.2021.125079

567 71. IPCC et al. 2021. Climate Change 2021: The Physical Science Basis. Contribution of
568 Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate
569 Change [Internet]. Manag. Risks Extrem. Events Disasters to Adv. Clim. Chang. Adapt. Spec.
570 Rep. Intergov. Panel Clim. Chang. 2021. 10.1017/CBO9781139177245.003

571 72. Hernroth B, Baden S, Tassidis H, Hörnaeus K, Guillemant J, Bergström Lind S, et al. 2016.
572 Impact of ocean acidification on antimicrobial activity in gills of the blue mussel (*Mytilus*
573 *edulis*). *Fish Shellfish Immunol*. 55:452–9. 10.1016/j.fsi.2016.04.007

574 73. Liu S, Shi W, Guo C, Zhao X, Han Y, Peng C, et al. 2016. Ocean acidification weakens the
575 immune response of blood clam through hampering the NF-kappa β and toll-like receptor
576 pathways. *Fish Shellfish Immunol*. 54:322–7. 10.1016/j.fsi.2016.04.030

577 74. Zha S, Liu S, Su W, Shi W, Xiao G, Yan M, et al. 2017. Laboratory simulation reveals
578 significant impacts of ocean acidification on microbial community composition and host-
579 pathogen interactions between the blood clam and *Vibrio harveyi*. *Fish Shellfish Immunol*.
580 71:393–8. 10.1016/j.fsi.2017.10.034

581 75. Asplund ME, Baden SP, Russ S, Ellis RP, Gong N, Hernroth BE. 2014. Ocean acidification
582 and host–pathogen interactions: blue mussels, *Mytilus edulis*, encountering *Vibrio tubiashii*.

583 *Environ Microbiol*. 16:1029–39. 10.1111/1462-2920.12307

584 76. Frommel AY, Maneja R, Lowe D, Malzahn AM, Geffen AJ, Folkvord A, et al. 2012. Severe
585 tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat Clim Chang*.
586 2:42–6. 10.1038/nclimate1324

587 77. Lima FP, Wethey DS. 2012. Three decades of high-resolution coastal sea surface
588 temperatures reveal more than warming. *Nat Commun*. 3:1–13. 10.1038/ncomms1713

589 78. Vezzulli L, Colwell RR, Pruzzo C. 2013. Ocean Warming and Spread of Pathogenic Vibrios
590 in the Aquatic Environment. *Microb Ecol*. 65:817–25. 10.1007/s00248-012-0163-2

591 79. Baker-Austin C, Trinanes J, Gonzalez-Escalona N, Martinez-Urtaza J. 2017. Non-Cholera
592 Vibrios: The Microbial Barometer of Climate Change. *Trends Microbiol*. 25:76–84.
593 10.1016/j.tim.2016.09.008

594 80. Koelle K, Pascual M, Yunus M. 2005. Pathogen adaptation to seasonal forcing and
595 climate change. *Proc R Soc B Biol Sci*. 272:971–7. 10.1098/rspb.2004.3043

596 81. Baker-Austin C, Trinanes JA, Taylor NGH, Hartnell R, Siitonen A, Martinez-Urtaza J. 2013.
597 Emerging Vibrio risk at high latitudes in response to ocean warming. *Nat Clim Chang*. 3:73–
598 7. 10.1038/nclimate1628

599 82. Baker-Austin C, Stockley L, Rangdale R, Martinez-Urtaza J. 2010. Environmental
600 occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European
601 perspective. *Environ Microbiol Rep*. 2:7–18. 10.1111/j.1758-2229.2009.00096.x

602 83. Martinez-Urtaza J, Blanco-Abad V, Rodriguez-Castro A, Ansede-Bermejo J, Miranda A,
603 Rodriguez-Alvarez MX. 2012. Ecological determinants of the occurrence and dynamics of
604 Vibrio parahaemolyticus in offshore areas. *ISME J*. 6:994–1006. 10.1038/ismej.2011.156

84. Aiyar SE, Gaal T, Gourse RL. 2002. rRNA Promoter Activity in the Fast-Growing Bacterium *Vibrio natriegens*. *J Bacteriol*. 184:1349–58. 10.1128/JB.184.5.1349-1358.2002

85. Joseph SW, Colwell RR, Kaper JB. 1982. *Vibrio Parahaemolyticus* and Related Halophilic Vibrios. *CRC Crit Rev Microbiol*. 10:77–124. 10.3109/10408418209113506

86. MacFadden DR, McGough SF, Fisman D, Santillana M, Brownstein JS. 2018. Antibiotic resistance increases with local temperature. *Nat Clim Chang*. 8:510–4. 10.1038/s41558-018-0161-6

87. Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, et al. 2019. Scientists' warning to humanity: microorganisms and climate change. *Nat Rev Microbiol*. 17:569–86. 10.1038/s41579-019-0222-5

88. Jambeck J, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. 2015. Plastic waste inputs from land into ocean. *Mar Pollut*. 347:3–6. 10.1126/science.1260352