Trends in
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Opinion

Oceanic Hitchhikers – Assessing Pathogen Risks from Marine Microplastic

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As plastic debris in the environment continues to increase, an emerging concern is the potential for microplastic to act as vectors for pathogen transport. With aquaculture the fastest growing food sector, and microplastic contamination of shellfish increasingly demonstrated, understanding any risk of pathogen transport associated with microplastic is important for this industry. However, there remains a lack of detailed, systematic studies assessing the interactions and potential impacts that the attachment of human and animal pathogens on microplastic may have. Here we synthesise current knowledge regarding these distinct microplastic-associated bacterial communities and microplastic uptake pathways into bivalves, and discuss whether they represent a human and animal health threat, highlighting the outstanding questions critical to our understanding of this potential risk to food safety.

Highlights

Microplastics are a major source of anthropogenic contamination in the oceans. This contamination is now widespread, recalcitrant, and likely to continue unabated into the future.

Plastics represent an important environmental substrate for the colonisation of bacteria from the surrounding water column, with distinct communities, abundances, and population structures on the plastic surfaces.

There is the potential for microplastics to act as a long-distance transport mechanism for human and animal pathogens, potentially spreading pathogenic bacteria into new areas.

A variety of human pathogens have been found on microplastics in the open ocean, but we do not know their pathogenicity and virulence potential or what, if any, human pathogen transmission occurs via this route.

There is increasing scientific consensus that microplastics may act as vectors for the spread of antimicrobial-resistance genes.

Increasing Prevalence of Microplastic in Our Oceans

Plastic pollution is now ubiquitous within marine environments globally [1], with an estimated 15–51 trillion plastic particles floating on the surface of the world's oceans [2]. This likely represents only ~1% of the 4.8–12.7 million tons of the plastics thought to enter global oceans annually [3], with a significant input known to come via rivers, and the majority of microplastic eventually sinking via fouling, flocculation, and egestion processes [4]. Microplastic is now considered to be a global concern due to its widespread presence within aquatic and terrestrial food webs, including many commercially important species used for human consumption, encompassing zooplankton, bivalves, crustaceans, fish and other marine vertebrates [5]. Whilst a range of impacts of **macroplastic** (see Glossary) and **microplastic** upon organism health, and some effects on the ecosystem, have been reported (e.g., [6]), an emerging threat which, until recently, has received less attention is the potential for plastic debris to act as novel substrates for pathogens, in particular marine bacteria such as vibrios (e.g., [7,8]), and as carriers of antimicrobial-resistant bacteria. This is of particular concern for food safety given the growing body of evidence of microplastic uptake by commercial seafood and aquaculture shellfish species. Here, we synthesise the current understanding and discuss the critical knowledge gaps regarding the potential threat of transport of pathogens via microplastic and its risk to aquaculture species and food security.

Plastics Provide a Novel Substrate; the 'Plastisphere'

The surface properties of plastic are thought to play an important part in determining its ecological impacts [9]. The smooth, hydrophobic surfaces of virgin (unfouled) plastics have no net charge, but this rapidly changes once in seawater as organic matter, biomolecules, nutrients, and bacteria, as well as hazardous hydrophobic contaminants, quickly sorb to the polymer surface. This sorption of biological materials produces a unique **ecocorona** [9] which, as demonstrated by ecotoxicology studies, can influence both biological uptake of nanoparticles and their fate within

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tissues and cells (e.g., [10]). The selective binding of secretory molecules, including **infochemicals** or protein signalling molecules, to microplastic may also influence their ecological interactions within marine ecosystems. For example, dimethyl sulfide (DMS) – an infochemical, released during zooplankton grazing on phytoplankton, which stimulates feeding activity in a range of planktivorous species (e.g., [11]) – can be produced by the **fouling communities** present on microplastic [12]; this then increases the frequency of plastic ingestion by copepods [13] and seabirds [12]. Hence microplastic can take on a chemical profile that might mask its polymer properties and even act to facilitate accidental ingestion and uptake into tissues.

Pathogenic Bacteria Attach to Microplastic

The attachment of harmful microbes to plastic debris was first observed by Masó *et al.* [14]. However, it was the landmark paper by Zettler *et al*. [7], first describing the '**plastisphere**', that highlighted the potential for marine microplastics to house distinct communities of microbes on their surfaces. It has since been widely demonstrated that, in seawater, plastic surfaces will quickly develop a conditioning film, and subsequently, a biofilm taxonomically distinct to that of the surrounding seawater [7,15]. Of particular concern are the increasing reports of the presence of numerous pathogenic microbes on both macro- and microplastic surfaces from across oceanic regions. Vibrios, in particular, have been found in high abundances within plastisphere communities, particularly in the summer months [7,8,16–18] (summarised in Table 1; [16–29]). Vibrios – not all of which are pathogenic – are generally sparse in the open ocean, preferring more estuarine salinities, yet strikingly high numbers of vibrios have been reported on microplastic from the mid-North Atlantic Ocean [7,8]. This, combined with the long-distance dispersal potential of floating microplastic [30], raises the important question as to whether the increasing amount of plastic waste in global oceans provides greater opportunities for vibrios and other pathogens to be transported and transmitted to potential hosts, leading to increased outbreaks of disease, compared to the opportunities provided by other, natural particles. Microbes, including vibrios, are known to be associated with a variety of natural substrates such as wood, cellulose, glass, planktonic organisms (Figure 1, Key Figure), and even birds [31–33], which act to increase the survival of vibrios and provide a means of transport across oceanic environments [31]. The total abundance of pathogenic microbes on particles of microplastic, compared to other, natural particles, may actually be similar. One recent **meta-analysis** concluded that the median relative abundances of a variety of potentially pathogenic species found on microplastic across the North Sea, the Baltic Sea, and the Yangtze Estuary were comparable with those present on natural particles sampled within the same regions [33]. But to address whether microplastic acts to increase the risk of pathogen transfer and disease occurrence, other than by simply providing increased availability of floating particles, there are a number of additional factors to consider, summarised in Figure 1: (1) the attachment processes and microbial interactions (e.g., rates of horizontal gene transfer, HGT) on the particle surface; (2) the rate and distance of transport of pathogen-colonised particles across oceans, and whether the plastisphere changes as plastics transit through different oceanographic regions; (3) vertical transport processes to the benthos, where ingestion and **trophic transfer** occurs; (4) the uptake and retention of particles into **mariculture** organisms and the likelihood of disease transfer occurring as a result; and (5) all of which might influence the risk to human consumers.

Biofilms Are Beneficiary to Pathogenic Microbes

Biofilms are complex structured groups of single or multiple species of microorganisms attached to a solid surface and encased in an extracellular polysaccharide matrix [34]. The unique ecocoronas and biofilms that arise rapidly on microplastic surfaces likely play a key role in supporting the unique plastisphere communities that subsequently form on them (summarised in Figure 2; [35,36]). The main drivers of this biofilm formation are the environmental conditions

Glossary

Benthic: associated within the ecological region occurring at the bottom of a body of water.
Conjugation: the transfer of genetic material between two microbes via direct cell–cell contact via the use of a sex pilus.
Ecocorona: particle surface coating comprising different components of natural organic matter (NOM) such as humic substances, extracellular polymeric substances, proteins etc.
Fouling communities: micro- and macro-organisms that are known to adhere to, and live on, any surface in an aquatic environment.
Infochemical: a chemical compound that carries information and acts as a form of communication, utilised by virtually all living organisms to locate food, avoid predators, find mates, or mediate metabolic functions.
Macroplastic: larger items of plastic debris; the term generally applies to items >1 cm in size.
Mariculture: cultivation of marine organisms.
Meta-analysis: a statistical procedure combining data from previous studies that address the same question.
Microplastic: smaller items of plastic debris; the term is generally applied to items <5 mm, although, in some definitions, the range is between 1 μm and 1000 μm.
Pathogenicity islands: a sub group of genomic islands that include at least one gene that provides pathogenicity or virulence to a microbe.
Plastisphere: a diverse microbial community located on the surface of a plastic particle.
Trophic transfer: in this instance, the transfer of plastic particles that have been ingested by a prey species to its higher trophic level consumer by predation.
Vector: a mediator that transports and transmits a pathogen from one area to another.

Table 1. Pathogens on Plastic. Summary of the Current Published Studies Reporting the Presence of Potential Pathogens on Both Environmental and *in situ* Macro- and Microplastic. The focus is on pathogens that were said to comprise higher than 1% of the operational taxonomic units (OTUs) for the particle they were discovered on.[a]

Potential pathogen	Plastic type	Plastic morphology	Location	Refs
Vibrio parahaemolyticus	PE, PP	PE fibres, PE fragments, PE films, PP fragments	North/Baltic Sea	[23]
Aeromonas salmonicida	Undetermined	Fragments	Northern Adriatic Sea	[24]
Vibrio spp. (*V. splendidus*), *Pseudoalteromonas* spp.	PE, PP, PS	Fragments	The Bay of Brest (France)	[16]
Vibrio spp. and *Escherichia coli*	Undetermined	Nurdles	Forth Estuary (Scotland)	[25][b]
Vibrio spp.	Undetermined	Fragments (75%)	Haihe Estuary	[17]
Vibrio spp.	PP, PVC	Microbeads	China coastline	[26]
Vibrio spp.	PE, PS	Microbeads	Baltic Sea	[27]
Vibrio spp., *Pseudoalteromonas*, *Shewanella* spp.	Undetermined	Film	Haihe Estuary	[28]
Pseudomonas alcaligenes	Unknown	Unknown	Singapore coastline	[29]
Arcobacter spp.	LDPE+	Fragment	Humber Estuary, UK	[19]
Escherichia coli and *Vibrio cholerae*, *Vibrio vulnificus*, *Vibrio mimicus*	PE, PP, PET	Fragments	Guanabara Bay, RJ, Brazil	[20]
Vibrio spp., *Pseudoalteromonas* spp. and *Alteromonas* spp.	Undetermined	Fragments	Sungo Bay, China	[18]
Tenacibaculum spp., *Phormidium* spp. and *Leptolyngbya* spp.	Undetermined	Undetermined	Western Mediterranean Sea	[21]
Vibrio spp.	PET	Plastic bottle	North Sea	[22]

[a]Abbreviations: LDPE, low-density polyethylene; PE, polyethylene; PET, polyethylene terephthalate, PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride.
[b]The entire bacterial community was not examined in this paper – rather, the percentage of plastic nurdles that were colonised by *Vibrio* spp. or *Escherichia coli*.

of the surrounding seawater, with polymer type thought to play a less significant role [8,15,27,28]. Living within a biofilm is highly beneficial and can cause microbes to become more infectious than when free-living (Figure 2), as reported for *Vibrio cholerae*, with increased levels of metabolic response and functional diversity [37]. Culturable vibrios proliferate rapidly when associated with aggregates compared to declining numbers when cultured in aggregate-free water. However, whether a primary biofilm is required for pathogenic species to be present within the plastisphere, and how biofilm formation processes might differ from those on natural particles, remains undetermined. HGT is thought to occur more frequently within microplastic biofilms than among free living microbes [38] and can lead to the formation of **pathogenicity islands** (PAIs) (demonstrated for *Vibrio vulnificus, Vibrio parahaemolyticus* and *Vibrio diabolicus*) [39]. Additionally, antimicrobial resistance bacteria (ARB) have been reported at concentrations 100–5000 times higher on microplastic surfaces than in the surrounding seawater [17,40]. Natural aggregates (marine snow, organic detritus etc.) can also display higher metabolic function and proliferation in some pathogenic microbes than is found in seawater [37]. However, in comparison with seawater and natural particles, the plastisphere community has shown significant elevations in the metabolic pathways that contribute to infectious diseases [18]. Therefore, microplastic may not act only as a vehicle for microbial pathogens, it may also enrich pathogenic strains which have acquired PAIs and other antimicrobial properties through HGT.

Is Microplastic a Hotspots of AMR?

Antimicrobial resistance (AMR) is recognised as a critical global issue, and there is increasing acceptance that the natural environment plays a role in the persistence and evolution of clinically relevant resistances. In the last decade there has been increasing concern that microplastic

Key Figure

Summary of the Potential Interactions of Vibrios with Microplastic Particles in Marine Ecosystems and How These Might Differ from Interactions with Natural Particles in Terms of the Following Factors

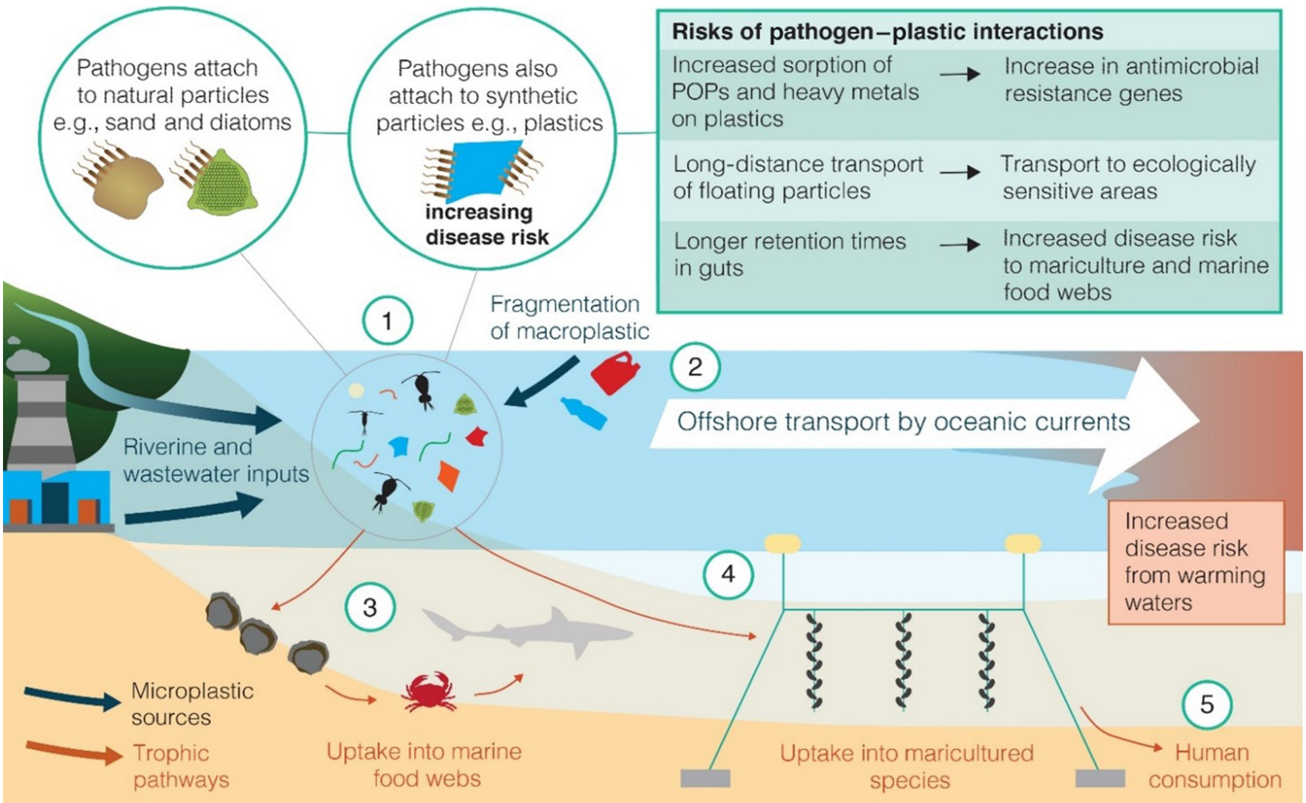

Figure 1. (1) The role of the polymer surface in influencing microbial attachment processes, survival over time, and microbial interactions such as rates of horizontal gene transfer on the particle surface. (2) The rate and distance of horizontal transport, and therefore the ultimate fate of pathogen-colonised floating particles across oceans, and whether this influences the plastisphere as the particle transits through different oceanographic regions. (3) Vertical transport of particles to the benthos, the ingestion by filter-feeding organisms and subsequent retention, egestion, or trophic transfer rates. (4) The uptake and retention of particles into mariculture organisms and the likelihood of disease transfer occurring as a result. (5) All the factors have potential to influence the likelihood of risk to human consumers. Abbreviation: POPs, persistent organic pollutants.

represents an important environmental niche for the development, retention, and spread of AMR. Yang *et al.* [40] studied the diversity, abundance, and co-occurrence of AMR genes (ARGs) and metal-resistance genes (MRGs) and their relationships within the microbial community, using metagenomic data from plastic particles sampled in the North Pacific Gyre. They found that the richness of both ARGs and MRGs in the microbiota on plastics was significantly greater than it was in seawater [40]. Laboratory-based studies have also shown that microplastic plays a role in influencing both the evolution of microbial communities and the exchange of genes, including ARGs. Increased frequency of plasmid transfer in bacteria associated with microplastic – compared with free-living bacteria or those in natural aggregates – has been observed and proposed to aid in the spread of AMR [38], though the mechanisms underpinning this phenomenon are

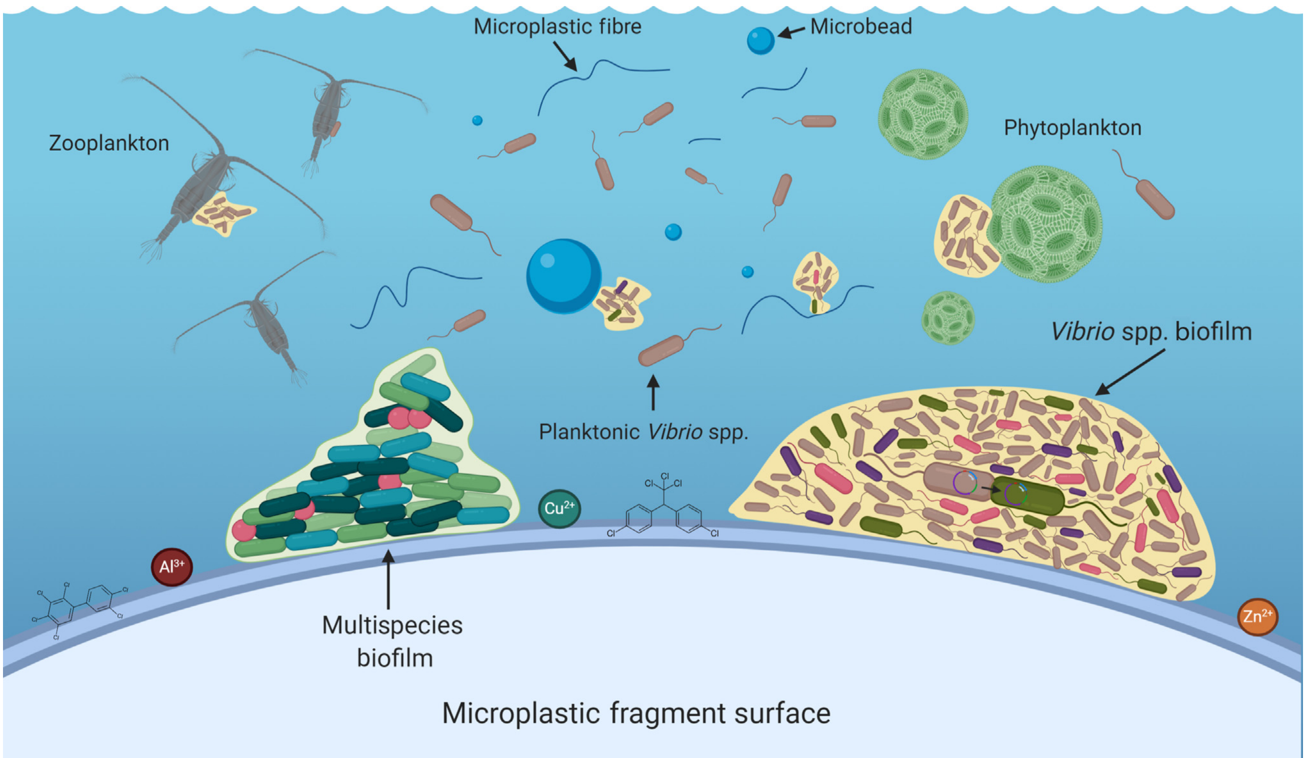

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Figure 2. Summary of the Potential Microbe–Microplastic Interactions That Occur at the Polymer Surface, and Biofilm-formation Processes for Microplastic Present in Seawater in Comparison to Those That Occur on Natural Particles such as Zooplankton (e.g., Copepods) and Phytoplankton. Various strains are displayed in the *Vibrio* spp. (pink, purple, and green cells) biofilm, with plasmid transfer depicted occurring between cells via conjugation. The heavy metals aluminium, copper, and zinc, along with PCB156 and DDT, are shown to be sorbed onto the plastic surface which may influence selection processes and horizontal gene transfer within attached microbial communities. For example, concentrations of Zn (14 815 μg/g) on microplastic in sediment from the Beijing River, China, concentrations of PAHs (>5000 ng/g) on microplastic in East Asia, and concentrations of PCBs (>2000 ng/g) on microplastic in coastal waters of São Paulo State, Brazil, have been reported (reviewed in [36]). Abbreviations: PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls. Created with biorender.com.

currently unclear. Certainly, because microplastics can serve as **vectors** for harmful microorganisms – often in close association with other microbes and sorbed contaminants (Figure 2), including metals which often coselect for AMR – microplastics could act as a microcosm for more effective gene exchange between bacteria. In particular, bacterial communities present in biofilms are extremely effective at spreading and sharing ARGs [41] through HGT mechanisms such as **conjugation**. Elucidating both the role of biofilms and the prevalence and types of gene exchange taking place on microplastic particles are exciting and ongoing areas of research.

Microplastic as a Novel Vector of Pathogen Transport

Many plastics are positively buoyant before being fouled, allowing for greater transport via surface waters [42]. Rivers are a major source of microplastics and pathogenic microbes in coastal waters [43,44]. A large quantity of riverine microplastic particles originate from sewage effluent, and it has been reported that the plastisphere communities that are attached to these particles differ from the organisms in the surrounding environment downstream of the effluent; the plastisphere has also been reported to contain pathogenic species [45] – thus raising the question of the role of the world's rivers in transporting pathogenic microbes. Floating plastic debris has already been linked to the transport of invasive species across oceanic barriers (e.g., [46]) yet it is unclear to what extent the plastisphere is maintained as particles transit across vast distances; do plastisphere communities adapt and change to the prevailing environment as they are transported through different oceanic regions? Current evidence suggests that the plastisphere's microbial community is predominantly shaped by the geographical location and environmental conditions of the seawater where the microplastic resides, leading to the suggestion that plastisphere communities will adapt and change to prevailing conditions as they are transported through oceanic regions; this has been shown in a riverine system [33,45]. This follows similar arguments made for the chemical contaminants sorbed to a microplastic surface remaining in equilibrium with the surrounding seawater [47]. Yet attachment/succession processes are not well defined for plastisphere communities, and there are increasing reports of potential pathogens colonising microplastic in areas with few, if any, previously described instances of the potential pathogen (Table 1). However, studies to date that have discovered pathogenic species have looked at the pathotypes only and not the whole microbial community; this does not allow for complete comparisons when looking at the context of the plastisphere as a whole [20,23]. Nevertheless, this continually expanding area of research shows that the attachment of pathogenic microbes to microplastic requires more systematic evaluation.

Microplastic Is Readily Ingested by Marine Species

Benthic filter feeders, such as bivalves (e.g., mussels and oysters), provide a number of key ecological services – specifically, filtration of the surrounding water [48] and supporting bentho-pelagic coupling of nutrients. This filter-feeding strategy is very susceptible to microplastic uptake, with a large number of both laboratory and field studies demonstrating that mussels and oysters readily take up microplastic particles from their surrounding seawater (Figure 3; [49,50]), with average microplastic contamination ranging from 1.5 [51] to 7.64 [52] particles per individual, but with as high as 178 particles per individual having been recorded [53]. Size [54], shape [54], and polymer type of microplastic particle appear to influence particle uptake – microfibres being the most common shape recovered; however, a variety of fragments, films, and pellets are also commonly present [49]. The true exposure rate of bivalves to microplastic is more difficult to assess since most microplastic will pass through guts and be excreted over time, likely according to the shape and size of particle, making the data on gut contents a snapshot of the microplastic content of the individual at the point of sampling.

Microplastic as a Vector of Pathogen Transport into Seafood Species

In the face of exploitation of wild stocks and a growing global human population, aquaculture is deemed to be the only viable solution to meet future sustainable seafood production quotas [55]. The UN Food and Agriculture Organisation (FAO) has recognised the need for a doubling of production by 2050 to meet global demand, with a 28 million tonne shortfall projected within the next decade [56]. Aquaculture is now the fastest growing food sector, overtaking wild captures in terms of seafood production for human consumption [57], with many currently unexploited opportunities for countries like those in the Pacific and Caribbean [58]. The promotion of non-fed, filter-feeding bivalves arguably offers the primary route to sustainable intensification of production globally. Disease is one of the biggest issues faced by the aquaculture industry [59]. *Vibrio* spp. are a large contributor to disease in cultured bivalves, often causing mass mortality within larvae and even some cases of mortality within adult populations [60,61].

These outbreaks cause severe economic losses throughout the aquaculture sector globally, hence any factors that may increase disease are a serious concern. Reports documenting the oyster pathogen *Vibrio splendidus* on microplastic adds to the growing concern of any potential role of microplastic in pathogen transfer [16]. Microplastic has been commonly detected in commercial edible bivalves, including mussels, oysters, and clams. Overlaying modelled global sea-surface microplastic against aquaculture production (Figure 3) highlights a number of areas of

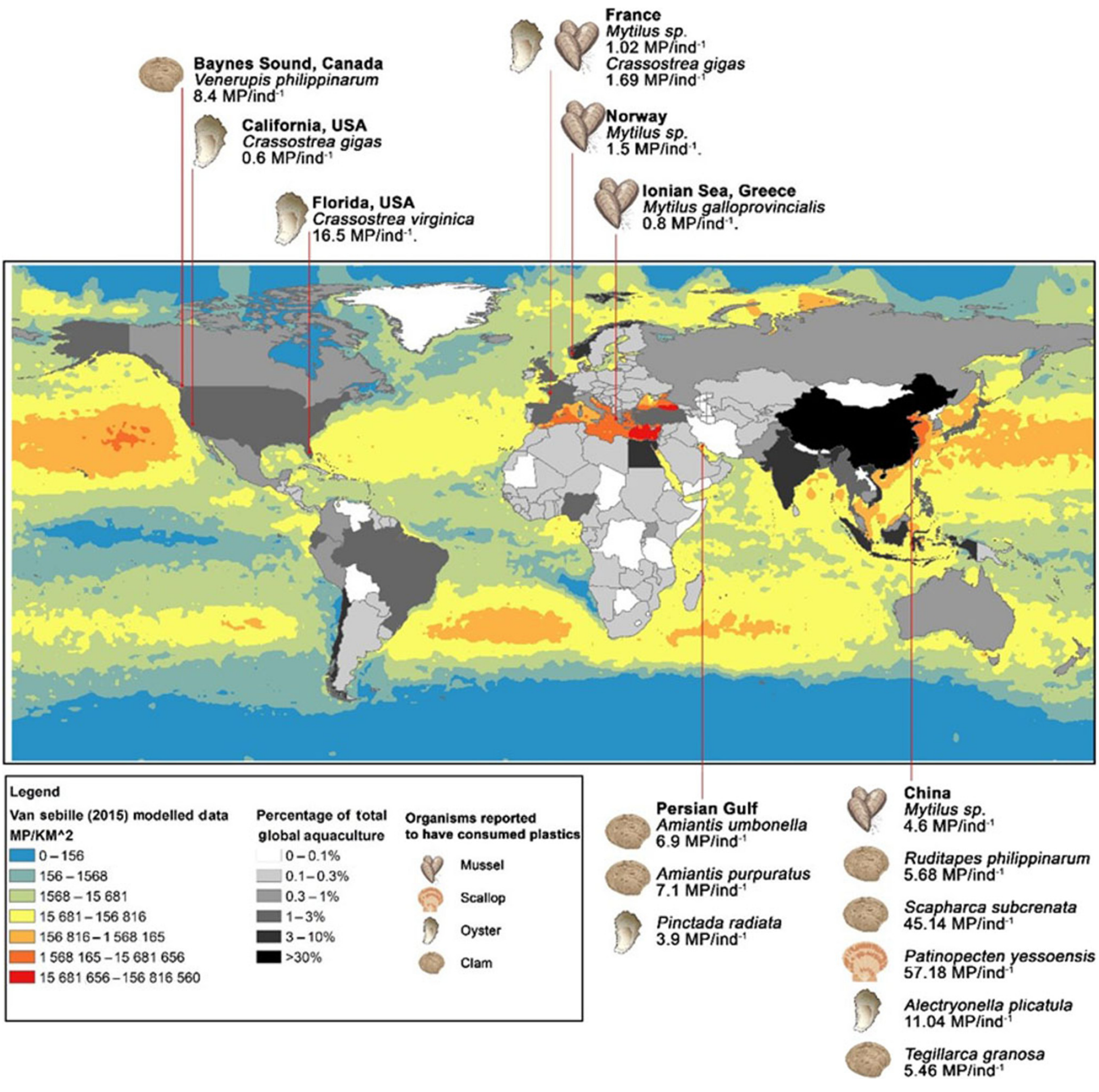

Figure 3. Modelled Sea-surface Microplastic Abundances (Particles km-2) Using Data from the Van Sebille Model (2015) with the Abundances Plotted on a Log Scale (Blue = Low, Red = High). Countries are colour-coded by the percentage of global aquaculture output that they produce (map reproduced from [50]). Finally, organisms that have been reported to have ingested microplastic in the available literature are marked with their location, species, and average number of particles found per individual (data and references in Table S1 in the supplemental information online). The organisms have been grouped into common classifications using symbols for ease of interpretation. All data were modelled and plotted using ArcGIS version 10.6.1, and countries' shapefiles were obtained from ArcGIS Online service. Abbreviations: MP/ind-1, microplastics per individual; MP/KM_2, microplastics per kilometer squared.

high aquaculture production in microplastic hotspots where pathogen transfer could theoretically occur, such as China, where 57.18 microplastic particles per individual in the commercially important Yesso clam have been reported [62]. Interestingly, the Mediterranean also has high microplastic abundances, yet, in this region, the number of microplastic particles per mussel is relatively low (Figure 3), highlighting the complex spatiotemporal dynamics of microplastic pollution [2]. The global distribution of microplastic may need to be considered in the future development of aquaculture sites if pathogen transfer is demonstrated to be a risk. Critical to elucidating this threat is the knowledge gap as to whether ingestion of pathogen-contaminated microplastic can actually lead to disease transfer and, if so, the exposure required. Many studies have now postulated that pathogen transfer from plastic to ingesting organisms may occur [7,16,23] but have not demonstrated this experimentally. Pathogen-colonised plastic debris is strongly associated with increased infection rates for corals in the Asian-Pacific region where the likelihood of disease within corals increases from 4% to 89% when associated with overlying plastic debris [63]. In the one study to date directly demonstrating pathogen transfer via microplastic ingestion, transfer of *Escherichia coli* from the surface of microplastic to the gut tissues of the northern star coral was demonstrated visually using green fluorescent protein (GFP)-tagged *E. coli* following microplastic ingestion [64]. This study provides compelling proof-of-concept that pathogen transfer from particle to digestive tissue can occur, providing mechanistic evidence supporting the proposed role of microplastic as a pathogen vector. Whether this occurs under natural settings in other marine organisms, such as cultivated bivalves, and its relevance to infection rates and human health outcomes, is unknown and is a vital area for future research.

Critical Knowledge Gaps and Novel Approaches for Addressing Them

Many fundamental questions regarding the role of bacteria and microplastic in the open ocean remain unanswered (see Outstanding Questions). Recent advances in analytical techniques may now allow us to more fully understand these bacteria and their interactions with microplastic particles across different environmental frameworks, providing answers to these questions. Perhaps the most obvious advance in microbiology in the last decade has been the revolution in genomics and whole-genome sequencing. A standard (~4 Mb) bacterial genome can now be sequenced inexpensively within hours, and then assembled and analyzed in far less time. Previous next-generation sequencing (NGS) approaches have produced much of our current knowledge describing the bacterial composition of plastisphere communities (summarised in Table 1). Yet advances in sequencing technologies are moving at breakneck speed and so too are analysis tools to scrutinise these datasets and look at more detailed microbial interactions on these surfaces. One of the most exciting developments is nanopore sequencing technologies – which can produce long-read length sequences quickly and cheaply, facilitating more rapid genome assembly [65]. Because these instruments are also portable, there is now potential to use these methods in field-based applications, enabling far greater geographical coverage of data to be achieved within reasonable project budgets and timeframes. Improvements in microscopy, such as confocal and epifluorescent microscopy, will also allow researchers to scrutinise microplastic particles to determine the types of bacteria inhabiting them in both time and space. Coupled together, such approaches will allow us to analyse and identify key bacteria present on microplastic fragments, and potentially quantify their relative abundances, as well as different ARGs, MRGs, and virulence genes.

Concluding Remarks and Future Perspectives

From a mechanistic perspective, there is growing evidence to suggest that microplastic fragments represent a potential reservoir of pathogens and ARB on these distinct human-made matrices that differ markedly from natural particles (Figure 2). Whilst attachment of *Vibrio* spp. and other pathogens to microplastic is well evidenced, the overarching effects that this may cause for any potential transfer to bivalve aquaculture are yet to be described. Additionally, the factors promoting bacterial attachment to microplastic are also unknown and require immediate attention. The potential economic losses that this may cause to the aquaculture sector, as well as the implications for human health, are great and so further work is urgently required in order to

Outstanding Questions

How is the fate and transport of pathogens across oceans and into key marine species by microplastics different, if at all, to that by natural particles?

Do the bacterial communities on microplastics represent only the area they are currently in, for example, tropical, subtropical, cold environments etc., or are microbial communities translocated by microplastic dispersal?

How much gene exchange is facilitated by microplastic fragments? Is it a concern for human health?

Do marine viruses also attach to microplastics? If so, to what extent are these animal and human viruses/enveloped and non-enveloped viruses etc., and are these viruses viable/infectious?

Does pathogen transfer from microplastic to ingesting organism occur and, if so, does this increase the likelihood of disease occurrence.

How can we adequately assess human health risks from microplastics in the marine environment and within aquaculture species?

gain a conclusive insight into this increasing threat. This is an area of ongoing research that will require collaborative efforts between ecotoxicologists, microbiologists, oceanographers, marine biologists, and ecologists, among others.

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Supplemental Information

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