



## Review

# Biosecurity implications of drifting marine plastic debris: Current knowledge and future research

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## ABSTRACT

The introduction and spread of marine non-indigenous species (NIS) and pathogens into new habitats are a major threat to biodiversity, ecosystem services, human health, and can have substantial economic consequences. Shipping is considered the main vector for marine biological invasions; less well understood is the increased spread of marine NIS and pathogens rafting on marine plastic debris (MPD). Despite an increasing research interest and recent progress in characterizing the plastisphere, this manuscript highlights critical knowledge gaps and research priorities towards a better understanding of the biosecurity implications of MPD. We advocate for future research to (i) investigate plastisphere community succession and the factors influencing NIS propagules and pathogens recruitment through robust experimental investigations; (ii) combine microscopy and molecular approaches to effectively assess the presence of specific taxa; (iii) include additional genetic markers to thoroughly characterize the biodiversity associated with MPD and explore the presence of specific marine pests.

## 1. Introduction

Since the mass production of plastic started in the 1950s, synthetic polymers have transformed many aspects of human's life. Originally plastics were predominantly used as lightweight replacements for steel or glass, largely for transportation and energy saving purposes. However, the remarkable range of plastic polymer properties (e.g. strength, malleability, electrical insulation properties, etc.) has rapidly expanded their use; production has increased from 0.5 to 348 million tons between 1950 and 2017 (Galgani et al., 2019). In contrast to their early use, now most plastics are employed for packaging and are discarded within a year of manufacture (Lebreton and Andrady, 2019). Most plastic monomers are derived from fossil hydrocarbons (ethylene, propylene) and they are predominantly destroyed through thermal processes such as incineration or pyrolysis. Consequently, most plastics are not recycled, instead accumulating in landfills or being dispersed into the environment (Cózar et al., 2014; Jambeck et al., 2015). Thompson (2006) estimated that up to 10% of the plastic produced worldwide ends up in the oceans, where it persists, accumulates, and adversely impact exposed biota (Keswani et al., 2016; Thiel et al., 2018). Recent

oceanographic models estimate that there is at least 5.25 trillion particles weighing approximately 270,000 tons of marine plastic debris (MPD) at sea (Eriksen et al., 2014). Plastic is now considered a major environmental stressor, along with climate change, ocean acidification, overfishing and habitat destruction (Salafsky et al., 2008; Halpern et al., 2012). Here, we (i) provide an overview of the extent MPD pollution; (ii) summarize the biological impacts of MPD; (iii) synthesize the existing reports of non-indigenous invasive species (NIS) and pathogens associated with MPD; and (iv) highlight important knowledge gaps and provides future directions to improve our understanding of the role of MPD in facilitating the spread of marine NIS.

### 1.1. Fragmentation of marine plastic debris (MPD)

Once in the environment, MPD is subject to environmental and biological factors such as photooxidation, wind and wave abrasion, hydrolysis, mineralization processes and microbial biodegradation (Shah et al., 2008; Andrady, 2011). Over time the structural integrity of plastics is compromised, and large items break down into microplastic-sized particles (MPs; <5 mm in size; Amaral-Zettler et al., 2016).

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Microplastics are now considered a global environmental threat, being the most abundant human-made debris encountered in natural systems (Thompson et al., 2009). Microplastics originate from multiple sources and can be divided into primary and secondary MPs. Primary MPs refer to the direct release of microplastic-sized particles such as plastic nurdles or microbeads, which are used in cosmetic personal care products as scrubbing agent, and in medicines as drug vectors (Patel et al., 2009). Boucher and Friot (2020) estimated 15 to 31% ocean plastic originates from a primary source, principally through water run-off (66%) and via wastewater treatment systems (25%). Secondary MPs originate from the fragmentation of larger plastic items (e.g. fishing gear, plastic bags, plastic bottles, tires and textiles) and are the dominant fraction in pelagic and coastal ecosystems (Browne et al., 2007; Barnes et al., 2009; Mason et al., 2016; Murphy et al., 2016; Sommer et al., 2018).

### 1.2. Occurrence of plastics in marine habitats

The abundance of different polymer types varies among marine habitats (Table 1). Low-density synthetic polymers are buoyant in seawater (i.e.  $<1 \text{ g/cm}^3$ ) and are likely to be washed ashore in coastal systems, whereas high density polymers (i.e.  $>1 \text{ g/cm}^3$ ) are more likely to sink in the water column. Plastic debris are now ubiquitous in the marine environment, and have been increasingly reported in habitats like mangroves (Barasarathi et al., 2014; Nor and Obbard, 2014), beaches (Browne et al., 2011; Poeta et al., 2014; Poeta et al., 2016), or coastal and deep-sea sediments (Erni-Cassola et al., 2019).

Due to their accessibility and proximity to densely populated areas, beaches have been extensively surveyed and reported substantial plastic debris accumulation (Thompson et al., 2004; Barnes and Milner, 2005). Beaches are described as hotspots for MPD fragmentation, due to strong UV exposure combined with elevated temperature and oxygen availability that favor the oxidation and breakdown of plastic litter (Browne et al., 2007; Moore, 2008; Barnes et al., 2009; Andrady, 2011). In the open ocean, oceanic convergence zones such as gyres are well known plastic accumulation areas (Cózar et al., 2014) although recent findings suggest that plastic abundance in open ocean surface waters has been overestimated by an order of ten thousand tons (Cózar et al., 2014), raising new questions on the fate of marine plastics in the marine

environment. Consequently, Reisser et al. (2015) demonstrated that a vertical mixing of plastic particles was occurring in the North Atlantic gyre, affecting the number, mass and size distribution of buoyant plastics collected by surface nets. Additionally, Lobelle and Cunliffe (2011) and Kooi et al. (2017) discovered that biofilm formation on MPD decreases particle buoyancy, causing MPD to sink in the water column and possibly accumulate in deep-sea sediments. Woodall et al. (2014) reported that microplastic concentrations were up to four orders of magnitude higher in deep-sea sediments from the Atlantic Ocean, Mediterranean Sea, and Indian Ocean compared to contaminated surface waters. Similarly, Van Cauwenberghe et al. (2013) showed that microplastic accumulation in sediment is not only limited to the continental shelf, but that micro-size particles can also be collected in the abyss, ranging from 1100 to 5000 m depth. MPDs have now been reported across all seafloors, from Antarctica to the deepest oceanic trench on Earth (Bergmann et al., 2017; Peng et al., 2018).

### 1.3. Biological impacts of MPD

Plastic debris harm a wide range of organisms, including seabirds (Ryan, 1987; Codina-García et al., 2013; Battisti et al., 2019a, 2019b), marine mammals (Jacobsen et al., 2010; Williams et al., 2011; Poeta et al., 2017), turtles (Nelms et al., 2016; Wilcox et al., 2018), reptiles (Staffieri et al., 2019) and fish (Neves et al., 2015; Rummel et al., 2016), often resulting from animals mistaking debris for prey. Additionally, the small size of MPs mean they are bioavailable to a wide range of marine organisms, including primary producers (Elser et al., 2007) allowing plastics to be transferred by herbivory and predation across trophic levels (Gregory, 2009; Pietrelli et al., 2017; Iannilli et al., 2018). Environmental MPs are rapidly colonized by epibiont communities, which can mimic the taste and smell of planktonic organisms' prey. Microplastics ingestion has been described for 39 zooplankton species from 28 taxonomic orders, with negative effects for feeding behavior, growth, reproduction, and lifespan (Botterell et al., 2019). Ingestion rates comparing aged and pristine MP particles by three copepod taxa showed they selectively prey on aged MPs, inferring that biofilm formation on MPs differentially affect copepod chemo-detection activity (Vroom et al., 2017). Prata et al. (2019a, 2019b) highlighted growth inhibition,

**Table 1**  
Distribution of common plastic types in marine settings in relation to their density and intended application.

Plastic type	Application	Density ( $\text{g/cm}^3$ )	Occurrence in marine settings	References
Polyethylene – PE	Plastic bags, storage containers, cosmetic products	0.91–0.95	Surface water, deep-sea sediments, beaches, mangrove	Browne et al. (2011), Reisser et al. (2013), Nor and Obbard (2014), Auta et al. (2017), Munari et al. (2017), Erni-Cassola et al. (2019)
Polypropylene – PP	Rope, bottle caps, fishing gears, strapping, cosmetic products	0.90–0.92	Surface water, deep-sea sediments, beaches, mangrove	Browne et al. (2011), Reisser et al. (2013), Nor and Obbard (2014), Auta et al. (2017), Munari et al. (2017), Erni-Cassola et al. (2019)
Polystyrene (expanded) – EPS	Cool boxes, floats, cups	1.01–1.05	Surface water, beaches, and mangrove sediments	Barasarathi et al. (2014), Fok and Cheung (2015)
Polystyrene – PS	Utensils, containers, disposable cups, plates, cutlery	1.04–1.09	Surface water, beaches, estuarine and subtidal sediments, water column	Barasarathi et al., 2014, Fok and Cheung (2015)
Polyvinyl chloride – PVC	Film, pipe, containers, window frame, flooring	1.16–1.30	Sediments, mangrove	Auta et al. (2017), Lusher et al. (2017)
Polyamide or nylon	Fishing nets, rope	1.13–1.15	Sediments, mangrove, beaches, estuarine and subtidal sediments, wastewater	Browne et al. (2011), Auta et al. (2017) Battisti et al. (2019a, 2019b)
Polyethylene terephthalate – PET	Bottles, strapping, textiles	1.34–1.39	Sediments	Auta et al. (2017)
Polyester resin + glass fiber	Textiles, boats, scrubbers	$>1.35$	Wastewater, beaches, estuarine and subtidal sediments, surface water	Browne et al. (2011), Erni-Cassola et al. (2019)
Cellulose acetate	Cigarette filters	1.22–1.24	Wastewater, beaches, estuarine and subtidal sediments	Wright et al. (2015)
Acrylic	Textiles	1.17–1.20	Beaches, estuarine and subtidal sediments	Browne et al. (2011), Erni-Cassola et al. (2019)
Polylactic acid (PLA)	Food containers, bottles, disposable cutlery	1.210–1.430	Sediments	Green et al. (2016)

Table modified from Lusher et al. (2017).

photosynthesis reduction, oxidative stress, and morphological changes in microalgae populations exposed to MPs. Most studies have focused on organism-level responses to MP exposure, making it challenging to infer the effects at a population or ecosystem scale. Setälä et al. (2014) showed for the first time that polystyrene microspheres could be transmitted from copepods to mysid shrimps in laboratory conditions. Additionally, a study conducted on scat samples from captive grey seals and whole digestive tracts of the Atlantic mackerel they were fed upon revealed an indirect, yet alarming potential pathway of MP transfer to top predators (Nelms et al., 2018). To date, most findings originate from laboratory investigations and might not reflect the complexity of interactions between MPD (including MPs) and marine organisms in natural systems. While numerous studies confirm ingestion of plastics by marine organisms across trophic levels, many questions remain regarding the potential of plastics to bioaccumulate within the food chain (Provencher et al., 2019).

Recently, some investigators have raised concern about plastics' ability to adsorb a range of anthropogenic pollutants (Teuten et al., 2009). Persistent organic pollutants (POPs) are stable, lipophilic, and can bond to and concentrate on the hydrophobic surfaces of MPD. Plastic debris accumulate and remove dissolved pollutants in seawater, but once ingested by marine organisms, they can become bio-available within the digestive tract of host organisms (Koelmans et al., 2013; Bakir et al., 2014). Plastic-associated POPs include polycyclic aromatic hydrocarbons (PAHs), agricultural waste such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (dichlorodiphenyltrichloroethane – DDT, dichlorodiphenyldichloroethylene – DDE), and heavy metals (copper, zinc, lead, cadmium, etc.), which have accumulated in marine sediments for decades, partly due to their use in anti-fouling coatings (Brennecke et al., 2016). Others have demonstrated the negative effects of plastic additives such as phthalates, bisphenol A, PBDE on a wide range of

marine organisms (Rochman, 2015; Hermabessiere et al., 2017). For a comprehensive analysis on the impacts of plastic additives, see Anbumani and Kakkar (2018), Wang et al. (2019).

## 2. Biosecurity implications of marine plastic debris

### 2.1. Transfer of non-indigenous species (NIS)

Biological invasions of NIS refer to when organisms arrive, establish and spread into new environments (Carlton, 1989). This can occur via two distinct processes: range expansion and introduction. Range expansion is a mechanism allowing marine populations to naturally spread to new oceanic regions, by their extension to adjacent suitable habitats and via natural vectors (currents, debris, hitchhiking on other mobile organisms). In contrast, introduction is predominantly an anthropogenic mechanism of species transfer, which often occur across natural barriers and vast distances (Carlton, 1987). Introductions of NIS have increased since the 1980s, with ballast water discharge and ship hull fouling identified as the key vectors (Levine and D'Antonio, 2003; David and Perković, 2004; Drake and Lodge, 2004). In addition, secondary processes can facilitate NIS establishment and proliferation in new habitats (Fig. 1). Artificial stationary structures in urban areas (e.g. ports and marinas) and offshore facilities are hubs of NIS settlement, and reservoirs for their secondary spread through natural processes (e.g., tidal movements, currents, transfer by wind), vessel operations (Kinloch et al., 2003; Olenin et al., 2017), or increasingly via attachment to marine debris (Ibabe et al., 2020). Numerous studies have shown that the transfer and proliferation of NIS has adverse impacts on biodiversity, economies and can alter ecosystem functioning (Bax et al., 2003; Pimentel et al., 2005; Molnar et al., 2008). Marine plastic debris are now considered as important emerging vector for the spread of NIS since it is long-lived, capable of slowly drift across vast ocean regions (Thiel and



**Fig. 1.** Interactions of conventional mechanisms for non-indigenous species (NIS) introduction (e.g. via ships' ballast water and hull fouling, aquaculture, recreational craft and fishing activities), as well as emerging (e.g. marine plastic debris) vectors of spread in estuarine and coastal areas.



Gutow, 2005; NOAA, 2017), and may act as an ideal substrate for NIS recruitment (NOAA, 2017).

## 2.2. Plastic debris: an emerging vector for the transport of non-indigenous macro-organisms

The first reports of marine organisms (bacteria, diatoms, hydroids) encrusted on small floating plastic debris were presented in the early 1970s (Carpenter et al., 1972). Since then, a plethora of studies have reported the role of MPD in carrying a wide range of marine organisms (Table 2 and Table 3). Barnes (2002) investigated colonization rates of marine biota on plastic debris from thirty isolated islands across all oceans. Barnes findings suggested that litter of human origin has roughly doubled the chances for marine biota to propagate across sub-tropical environments, and more than tripled it at high latitudes ( $>50^\circ$ ). In March 2011, the Great Japan Tsunami (GJT) flushed 5 million tons of debris into the Pacific Ocean, of which 47% were plastics. As a result, considerable amount of debris were observed on the Pacific coast of North America and Hawaii from 2012 to 2015 (Therriault, 2017). Molecular analysis coupled with taxonomic observations revealed nearly 300 debris associated marine species, including some of the world's most notorious NIS: the blue mussel *Mytilus galloprovincialis*, the ascidian *Didemnum vexillum*, the sea star *Asterias amurensis*, the brown algae *Undaria pinnatifida*, and the green algae *Codium fragile* (Lowe et al., 2000; Lowe et al., 2000). Additionally, Carlton et al. (2017) made several key observations after the GJT: (i) an unexpected number of exotic organisms from Japan continued to arrive on American shorelines  $>5$ -years after the event; (ii) several invasive species, including the mussel *M. galloprovincialis*, and the barnacle *Megabalanus rosa* along with other opportunistic colonizers were able to survive the journey; and (iii)

numerous taxa, from at least 13 different phyla and orders were described as reproductively active upon arrival.

There is other evidence for probable NIS transport associated with plastic debris over wider marine geographical areas. For example, the bryozoan *Thalamoporella evelinae*, which originates from Brazil, was observed encrusted on MPD on Florida's beaches (Winston et al., 1997). The invasive sea anemone *Diadumene lineata*, detected in large numbers attached to discarded fishing/trawling nets in the Northwestern Hawaiian Islands, was suspected to have drifted from as far as Japan (Zabin et al., 2004). Barnes and Fraser (2003) observed a plastic band on the shorelines of Adelaide Island (Antarctica) on which they recorded the presence of 10 exotic species, including bryozoans, mollusks, cnidarians, polychaetes, and sponges. These findings highlight the capability of small MPD to carry highly diverse assemblage of encrusted organisms in remote places like Antarctica. Previous studies hypothesized extensive time in open-oceanic (or oligotrophic) water would reduce the probability of hitchhiker's survival and ability to spread in new habitats (Lewis et al., 2003; Lewis et al., 2005). However, recent observations from the debris generated by the GJT and other oceanic studies illustrate the capability of MPD to carry viable, and reproductively active exotic hitchhikers over long distances and for considerable amount of time. Hence, MPD needs to be considered not only as an emerging secondary vector for regional expansion of marine species, but also as a vector for primary introduction of NIS into remote regions.

Marine plastic debris can contribute to the secondary spread of NIS between invasion hotspots (e.g. ports and marinas). A recent study from the Cantabrian coast (Miralles et al., 2018) identified three invasive invertebrates (*Austrominius modestus*; *Magallana gigas*; and *Amphibalanus amphitrite*) by genetic barcode analysis attached to two types of floating MPD (bottles and fishing gear). Similarly, Ibabe et al. (2020) used next

**Table 2**

Inventory of studies reporting exotic and non-indigenous species (NIS) associated with marine plastic debris. HTS: High-Throughput Sequencing; PET: polyethylene terephthalate; PC: polycarbonate; PS: polystyrene; PVC: polyvinyl chloride. Note: This table excludes review papers.

Type of study	Polymer type considered	Methods applied	NIS considered	Geographic region	References
Field sampling	Not specified	Microscopy and HTS	Barnacle - <i>Amphibalanus amphitrite</i> , <i>Microeuraphia permitini</i> , <i>Striatobalanus amaryllis</i> ; bryozoan - <i>Parasmittina egyptica</i> , <i>Microsporella browni</i>	Iranian coast of the Persian Gulf	Shabani et al. (2019)
Field sampling	Not specified	Microscopy	Ascidians - <i>Ciona intestinalis</i> ; bryozoan - <i>Bugula neritina</i> , <i>Bugula flabellata</i> ; algae - <i>Codium fragile</i>	Bay of Coquimbo - Chile	Astudillo et al. (2009)
Field sampling	Not specified	HTS	Mussel - <i>M. galloprovincialis</i> ; bryozoan - <i>Scruparia ambigua</i> , <i>Bugula</i> sp.; barnacle - <i>Mageobalanus rosa</i> ; amphipod - <i>Jassa marmorata</i> ; isopod <i>Ianiropsis serricaudis</i>	Pacific coast of America and Hawaii	Carlton et al. (2017)
Field sampling	Not specified	Microscopy and HTS	Barnacle - <i>Megalobalanus rosa</i> , <i>Amphibalanus amphitrite</i> ; mussel - <i>M. galloprovincialis</i> ; Bryozoan - <i>Victorella</i> spp.; coral pathogen - <i>Halofolliculina</i> spp.	Eastern and Western Pacific sampling	Goldstein et al. (2014)
Field sampling	Not specified	Microscopy and HTS	Oyster - <i>Crassostrea gigas</i> , <i>Austrominius modestus</i>	Bay of Biscay (Spain) - Atlantic Ocean	Rech et al. (2018a, 2018b)
Field sampling	Not specified	HTS	Barnacle - <i>Austrominius modestus</i> and <i>Amphibalanus amphitrite</i> ; oyster - <i>Magallana gigas</i>	Cantabrian coast (Atlantic Ocean)	Miralles et al. (2018)
Field sampling	Not specified	HTS	Barnacle - <i>Amphibalanus amphitrite</i> , <i>Austrominius modestus</i> , <i>Balanus trigonus</i> ; <i>Hesperibalanus fallax</i> ; hydroids - <i>Hydroids elegans</i> , <i>Hydroids sanctaecrucis</i> ; oyster - <i>Magallana angulata</i>	Venetian lagoon; Portuguese Algarve region	Rech et al. (2018a, 2018b)
Field sampling	Not specified	Taxonomic assignment	Mussel - <i>Perna viridis</i>	Colombian Caribbean Sea	Gracia and Rangel-Buitrago (2020)
Field sampling	Not specified	Taxonomic assignment	Mussel - <i>Gregariella ehrenbergi</i> ; gastropod - <i>Cellana rota</i> ; crabs - <i>Eriphia verrucosa</i> , <i>Sphaerostium nitidus</i> ; barnacle - <i>Balanus trigonus</i> , <i>Amphibalanus reticulatus</i> , <i>Perforatus perforatus</i>	Israeli coast - Mediterranean Sea	Ivkić et al. (2019)
Field sampling	Not specified	Taxonomic assignment	Tubeworm - <i>Sabella spallanzanii</i>	Coromandel peninsula - New-Zealand	Campbell et al. (2017)
Field sampling	Not specified	Taxonomic assignment	Oyster - <i>Pinctada imbricata</i>	Uruguayan coast - South Atlantic Ocean	Marques and Breves (2015)
Experimental study	PET, PVC, PS, PC	Microscopy	Bryozoan larvae - <i>Bugula flabellata</i> and <i>Bugula neritina</i>	Aquaria	Pinochet et al. (2020)
Field sampling	Not specified	HTS	Brown algae - <i>Sargassum muticum</i> ; freshwater crayfish - <i>Pacifastacus leiusculus</i> ; cephalopod - <i>Illex argentinus</i>	Northern coast of Spain (Atlantic Ocean)	Ibabe et al. (2020)
Field sampling	Styrofoam	Taxonomic assignment	Invasive coral - <i>Tubastraea coccinea</i>	Brazil - South Atlantic Ocean	Faria and Kitahara (2020)
Field sampling	Not specified	Taxonomic assignment	Invasive corals - <i>Tubastraea coccinea</i> ; <i>T. tagusensis</i>	Brazil - South Atlantic Ocean	Mantelatto et al. (2020)

**Table 3**

Inventory of studies on pathogens and harmful microalgae associated with marine plastic debris. PE: polyethylene; PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride; PET: polyethylene terephthalate; Oxo-PE: oxo-polyethylene; SEM: scanning electron microscopy; HTS: High-Throughput Sequencing; MALDI-TOF MS: Matrix Assisted Laser Desorption/Ionisation – Time of Flight Mass Spectroscopy; qPCR: quantitative polymerase chain reaction; ATM: atomic force microscopy.

Type of study	Polymer type considered	Method applied	Pathogens/microalgae considered	Geographic region	Reference
Field collection	Not specified	Microscopy and HTS	Potentially harmful epibenthic dinoflagellates <i>Coolia</i> , <i>Amphidinium</i> , and <i>Prorocentrum</i>	Southern Pacific	Larsson et al. (2018)
Field collection	Not specified	SEM	Potential harmful dinoflagellates <i>Ostreopsis</i> sp. and <i>Coolia</i> sp., <i>Alexandrium taylorii</i>	Catalan Coast - Mediterranean Sea	Masó et al. (2003)
Field collection	PE, PP	SEM and HTS	<i>Vibrio</i> sp.	North Atlantic Ocean	Zettler et al. (2013)
Field collection	PE, PP, PS, PVC, Polyamide	MALDI-TOF MS	<i>Vibrio</i> spp. ( <i>Vibrio parahaemolyticus</i> )	North and Baltic Sea	Kirstein et al. (2016)
Field collection	PE, PP, PS	HTS	<i>Vibrio</i> sp. ( <i>V. aestuarius</i> , <i>V. splendidus</i> )	Bay of Brest, France	Frère et al. (2018)
Field collection	PE, PP, PS, PET, Polyamide	HTS	Fish pathogen <i>Aeromonas salmonicida</i>	North Adriatic	Viršek et al. (2017)
Field collection	PE, PP	qPCR	<i>Pseudo-nitzschia</i> spp., <i>Ostreopsis</i> sp., <i>Ostreopsis ovata</i>	Mediterranean Sea	Casabianca et al. (2019)
Field collection	Not specified	SEM	Harmful diatom - <i>Ceratoneis closterium</i>	Mediterranean Sea	Masó et al. (2016)
Field collection	PE, PP, PS	SEM and HTS	Fecal indicators, potential human pathogens ( <i>Mycobacterium</i> and <i>Staphylococcus</i> spp.)	Naples, Italy - Mediterranean Sea, proximity with sewage discharge	Basili et al. (2020)
Field collection	PE, PP, PET	PCR	<i>Escherichia coli</i> , <i>Vibrio</i> sp. ( <i>V. cholerae</i> , <i>V. vulnificus</i> , <i>V. mimicus</i> )	Guanabara Bay (Brazil)	Silva et al. (2019)
Experimental study	PE, OXO-PE, aged-PE, Polyester	ATM, Epifluorescence Microscopy and HTS	Putative pathogenic bacteria: <i>Vibrio</i> sp., <i>Tenacibaculum</i> sp., <i>Staphylococcus aureus</i>	Aquarium with natural circulating water	Dussud et al. (2018a, 2018b)
Field collection	Not specified	HTS	<i>Vibrio</i> spp.	Yellow Sea (China)	Zhang et al. (2020)
Experimental study	Not specified	SEM, HTS	Potential pathogens bacteria <i>Vibrio</i> spp., <i>Pseudoalteromonas</i> spp., and <i>Alteromonas</i> spp.	Sungu Bay (China)	Sun et al. (2020)
Field collection	PE, PP, PS	HTS, Epifluorescence Microscopy	<i>Vibrio</i> sp.; fish pathogen <i>Tenacibaculum</i> sp., <i>Leptolyngbya</i> sp.	Western Mediterranean basin	Dussud et al. (2018a, 2018b)
Experimental study	PET	SEM, HTS	<i>Vibrio</i> spp.	North Sea	Oberbeckmann et al. (2016)
Field collection	Not specified	Bacterial isolation	<i>E. coli</i> and <i>Vibrio</i> spp.	Forth Estuary (Scotland)	Rodrigues et al. (2019)
Field collection	PE, PP	HTS	Salmon pathogens <i>Aeromonas salmonicida</i>	Norway (North Sea)	Radisic et al. (2020)

generation sequencing (NGS) to characterize biofouling samples collected on different plastic items (plastic bags, textiles, plastic bottles, fishing gear, EPS) near the port of Gijón (Spain). Results from this investigation revealed that the macrofauna profiles identified on the litter were associated with distance from the local port, thus confirming that MPD can be vectors for species dispersal at a regional scale.

Despite an increasing research interest to understand the capability of MPD to carry unwanted organisms, research on plastic associated NIS transfer is still limited (Table 2). Most reports on NIS attachment on MPD are restricted to opportunistic collections of marine debris, and often provide a limited description of the litter (e.g. plastic bottle, plastic bag, fishing gear). Polymer chemical properties are considered key factors affecting the preferential recruitment of organisms on different types of debris. The thorough characterization of the polymer types associated with NIS is an essential step to improve our understanding of which types of MPD are most likely to enhance NIS spread in marine ecosystems.

### 2.3. Methodological advances for plastisphere analyses

Recent analytical advances have created new opportunities to characterize epiplastic community composition (Table 3). Zettler et al. (2013) published the first comprehensive characterization of epiplastic assemblages, in which they coined the term “Plastisphere”: a diverse microbial assemblage of heterotrophs, autotrophs, predators, and symbionts attached to MPs. Techniques such as scanning electron microscopy (SEM) have been consistently applied to characterize the composition and structure of plastic assemblages and communities, also allowing observation of microstructures and specific interactions between plastisphere members (Table 3). Besides, advances in molecular methods have also markedly improved our understanding of the

biodiversity of plastic associated communities (Bott et al., 2010; Chariton et al., 2010; Pochon et al., 2013; Zaiko et al., 2016; Taberlet et al., 2018). Targeted methods such as quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR), routinely applied to detect human pathogens (Espy et al., 2006), are increasingly implemented for marine pest surveillance (Bott et al., 2010; Wood et al., 2013; Zaiko et al., 2018; Wood et al., 2019). In addition to targeted approaches, the recent advances in High-Throughput Sequencing (HTS) and metabarcoding techniques prompted their application for characterizing biodiversity in complex environmental matrices, including plastisphere macrofaunal communities (Zaiko et al., 2016; Briand et al., 2018). Recently, Lacerda et al. (2020) used DNA metabarcoding to characterize plastic-specific fungal assemblages from different types of plastics collected in the South Atlantic and Antarctic Peninsula, and discovered fungal groups not yet described on MPD. Other researchers tried to elucidate the factors shaping plastisphere communities by combining field collection and in or ex situ experimental assays. Some studies have reported differences in microbial communities associated with various polymer types (Zettler et al., 2013; Oberbeckmann et al., 2018). In contrast, other investigators reported no significant differences based on substrate type (Bryant et al., 2016; Kirstein et al., 2018), or indicated that the composition of microbial assemblages was mostly driven by seasonal and spatial factors (Oberbeckmann et al., 2014; Oberbeckmann and Labrenz, 2020). Overall, it is difficult to estimate how these complex factors shape plastisphere communities, and how they ultimately affect the dispersal of specific bacterial taxa attached on MPD (Oberbeckmann et al., 2018). The rapid development of analytical methods provide opportunities to: (i) Effectively capture the entire biodiversity of plastisphere assemblages, flagging the presence of putative pests; and (ii) Accurately assess and confirm the presence and abundance of specific taxa on different type of substrates (Zaiko et al., 2018).

Frère et al. (2018) sampled microplastics in the Bay of Brest (Atlantic Coast, France) and applied HTS to depict microbial composition of plastic-associated biofilm, and the factors influencing their composition and structure. Using HTS techniques, potentially pathogenic *Vibrio* strains, classified as oyster pathogens were detected on the surface of most MPs (77% of the samples). In parallel, other molecular studies have reported the presence of *Vibrio* species attached to MPD, in the Mediterranean Sea (Dussud et al., 2018a, 2018b), in the Atlantic Ocean (Zettler et al., 2013; Frère et al., 2018), in the Chinese Sea (Zhang et al., 2020), and in the North Sea (Oberbeckmann et al., 2016). Looking at fecal bacterial contamination in Guanabara Bay (Brazil), Silva et al. (2019) reported the presence of *Escherichia coli* and *Vibrio* species within biofilm samples collected on plastic debris. Furthermore, the authors reported virulence gene expression for the two species, suggesting that dispersal of potentially pathogenic bacteria is occurring via MPD. Similarly, Rodrigues et al. (2019) collected plastic nurdles on five bathing beaches in Scotland with the same observations: each sampling location contained nurdles colonized by *E. coli* and *Vibrio* spp.

To date, molecular methods have largely been applied for determining plastisphere microbiomes and pathogenic organisms and are underutilized for understanding diversity and spread of macroscopic taxa associated with MPD (Miralles et al., 2018; Rech et al., 2018a, 2018b; Ibabe et al., 2020). Consequently, applying those techniques in different combinations would help to better understand the spatial and temporal patterns of biofouling formation on MPD, the role of different types of plastic litter in species spread, and the interaction between micro-, meio- and macro-plastisphere and implications for potential survival of pests while rafting on MPD. Ultimately, this might help address the current knowledge gaps around MPDs-mediated species transfer (see below) and will have practical application in refining the risk assessment and species distribution models accounting for this novel vector of spread.

### 3. Knowledge gaps and future directions

#### 3.1. Towards a better understanding of microplastics role in the transfer of unwanted organisms

While there is increasing reports of opportunistic pathogens and potential harmful microalgae associated with MPs (Wright et al., 2020), our understanding of their specific role in transferring NIS propagules is still very limited. The potential of MPs as a vector of NIS transfer largely depends on the ability of early life stages macroinvertebrates (larvae, eggs, and other cryptic stages) to recruit on MPs biofilms. Morpho-taxonomic approaches are often used to identify NIS associated with MPD (see Table 2), but application of such techniques to accurately detect cryptic stages of NIS in microplastic biofilms are very limited. Recent molecular tools have the potential to offset the limitation of traditional methods to identify specific taxa associated with MPs. However, the existing methods to capture surface and sub-surface MPs (manta trawl, plankton and neuston nets) inevitably damage the natural state of MPs biofilm during the sampling process, which often result in nets clogging and induce high risks of cross-contamination between particles (Lenz and Labrenz, 2018). While scientific reports call for a standardization of methodologies for the capture, identification and quantification of field-collected MPs (Löder and Gerdt, 2015; Prata et al., 2019a, 2019b), our ability to delineate patterns of settlement or risks associated with a particular type of plastic polymer collected in the field are particularly limited by this technical impediment. Recent biotechnological advances, and particularly membrane-free separation techniques such as microfluidics, have the potential to improve our ability to continuously and quantitatively sort and separate MPs particles (Fu et al., 2020). To date, manipulative experiment with MPs in the field remain challenging, and to our knowledge, only one in-situ study compared the recruitment of microbial communities and putative pathogens (Sun et al., 2020). Therefore, there is a need for more

experimental studies investigating community succession patterns, and the underlying factors influencing the recruitment of NIS propagules and pathogens on MPs.

Numerous studies have reported the presence of opportunistic pathogens and harmful microalgae associated with plastic debris but failed to identify organisms at the species level (Table 3). Only recently, targeted approaches such as qPCR have been used to quantify harmful microalgae species producing toxins attached to MPs (Casabianca et al., 2019). Nevertheless, to our knowledge, no study has yet used ddPCR technique, which produce more precise, reproducible and statistically significant results to investigate specific recruitment of selected species (Taylor et al., 2017). Targeted approaches (e.g. qPCR, ddPCR) can achieve high detection sensitivity and specificity at species level and should be more abundantly implemented in the near future to fully characterize the MPs plastisphere, and in particular detect and quantify the species of concern associated with MPs. A systematic combination of microscopic (e.g. confocal, SEM, etc.) and molecular (e.g. qPCR, ddPCR, metabarcoding) analytical tools could help better understand the range of taxa (from viruses to metazoans), their structure and specific interactions within the plastisphere. While most investigations are using common genetic markers (bacterial 16S rRNA and eukaryotic 18S rRNA genes) to study the plastisphere's biodiversity, future studies should also include additional markers, including ribosomal Internal Transcribed Spacers (ITS) for fungi, chloroplastic Rubisco (rbcL) for diatoms, and mitochondrial Cytochrome c Oxidase I (COI) for metazoan species. There is a clear need to better evaluate the overall utility of a multi-marker metabarcoding approach towards 'whole-microecosystem' surveying of MPD and MPs in the marine environment. Furthermore, the aforementioned techniques are also applicable beyond the marine realm, and could be also applied to understand the ecological impacts of plastic debris in freshwater habitats (Cera et al., 2020).

Table 3 combines existing reports of pathogens and microalgae associated with marine plastic debris. Most reports have focused on characterizing bacterial communities using the common genetic marker 16S to assess the presence of potential pathogens inhabiting the plastisphere. In the biosecurity context, molecular tools have been applied to detect putative pests, including invasive species and pathogens other than bacteria. Thus, there is a need for a thorough analysis of invasive/pathogenic organisms other than bacteria associated with the debris, by the mean of molecular tools – particularly targeted approaches and metabarcoding.

#### 3.2. Lessons learnt from investigations of biofouling communities associated with marine plastic debris

To date, only a very limited number of studies have investigated the settlement behavior of invasive organisms on different types of man-made substrates under controlled conditions (e.g. Pinochet et al., 2020, Table 2). Investigating the succession of plastisphere communities on MPD is an important first step to understand their ecological impact, fate in marine settings, and their capability to recruit and carry invasive species within or across broad geographic regions. Because MPD are exposed to complex biological and physico-chemical factors in natural systems, it remains challenging to estimate their residence-time in the water column (Fazey and Ryan, 2016). Yet, most studies assessing the biodiversity associated with MPD use samples collected opportunistically in the field. While some authors reported differences in community composition in response to spatial and seasonal factors (Oberbeckmann et al., 2014; Oberbeckmann and Labrenz, 2020), there is still a lack of geographically representative studies between oceanic regions (Table 2). For example, only one study reported NIS attachment to MPD in the South Pacific Ocean (Campbell et al., 2017), and most reports originate from the northern hemisphere (Table 2). Future work should investigate the specific patterns in community succession (e.g. species turnover, structural and compositional changes in biofouling communities over time) through robust experimental designs, measuring the



specific factors shaping biofouling communities on artificial substrates (e.g. physical and chemical properties, time, and location of exposure). This approach should include the use of appropriate controls and establish regular sampling of associated biota over extended timeframes with an array of molecular and conventional analytical methods, such as before-after-control-impact/treatment assessments (Eberhardt, 1976), mechanistic or correlative models (Schoener, 1986).

Application of a wider range of 'omics' techniques and particularly transcriptomics would be beneficial for enhancing knowledge on the functionality of MPD-associated biological systems. Transcriptomics refers to the measure of an organism's genes expression (Lowe et al., 2017). In the biosecurity context, transcriptomics could be useful for measuring the alteration of gene expression resulting from adaptation (i. e. increasing invasiveness), toxin production or pathogenic activity of certain taxa (Toffano-Nioche et al., 2012), and whether these can be induced by exposure to specific chemical compounds released from plastics (Rochman, 2015). By combining and integrating different omics techniques (e.g. genomics, transcriptomics and metabolomics), future studies will provide critical new insights into the functioning, mechanisms of physiological adaptations, and thus, evolutionary selectivity of marine organisms associated with MPD (Kirstein et al., 2018; Jacquin et al., 2019).

Plastics have become an integral part of our everyday life over the last five decades. Even with recent efforts in reducing excessive plastic production, and particularly single-use items, plastic debris will persist and adversely impact our ecological health for decades. Therefore, understanding the biosecurity implications associated with this newly introduced and ubiquitous material is a crucial step forward to understand, monitor, and eventually mitigate its impacts at the global scale. We call for more experimental research, using established and emerging analytical tools to improve our capacity to answer the many knowledge gaps around the biosecurity implications and ecological fate of MPD in marine habitats.

#### List of acronyms

DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
ddPCR	droplet digital polymerase chain reaction
EPS	expanded polystyrene
GJT	Great Japan Tsunami
HTS	High-Throughput Sequencing
MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionisation – Time of Flight Mass Spectroscopy
MPD	marine plastic debris
MPs	microplastics
NIS	non-indigenous species
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PBDEs	polybrominated diphenyl ethers
PE	polyethylene
PET	polyethylene terephthalate
PLA	polylactic acid
POPs	persistent organic pollutants
PP	polypropylene
PS	polystyrene
PVC	polyvinyl chloride
qPCR	quantitative polymerase chain reaction
SEM	scanning electron microscopy

#### CRedit authorship contribution statement

**François Audrézet:** Conceptualization, Investigation, Data curation, Writing – original draft preparation; **Xavier Pochon:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration; **Anastasija Zaiko:** Conceptualization, Writing – review

& editing, Supervision; **Gavin Lear:** Writing – review & editing; **Susanna A. Wood:** Writing – review & editing; **Louis Tremblay:** Writing – review and editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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