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Plastics and microplastics in the oceans: From emerging pollutants to emerged threat

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A B S T R A C T

Plastic production has increased dramatically worldwide over the last 60 years and it is nowadays recognized as a serious threat to the marine environment. Plastic pollution is ubiquitous, but quantitative estimates on the global abundance and weight of floating plastics are still limited, particularly for the Southern Hemisphere and the more remote regions. Some large-scale convergence zones of plastic debris have been identified, but there is the urgency to standardize common methodologies to measure and quantify plastics in seawater and sediments. Investigations on temporal trends, geographical distribution and global cycle of plastics have management implications when defining the origin, possible drifting tracks and ecological consequences of such pollution. An elevated number of marine species is known to be affected by plastic contamination, and a more integrated ecological risk assessment of these materials has become a research priority. Beside entanglement and ingestion of macro debris by large vertebrates, microplastics are accumulated by planktonic and invertebrate organisms, being transferred along food chains. Negative consequences include loss of nutritional value of diet, physical damages, exposure to pathogens and transport of alien species. In addition, plastics contain chemical additives and efficiently adsorb several environmental contaminants, thus representing a potential source of exposure to such compounds after ingestion. Complex ecotoxicological effects are increasingly reported, but the fate and impact of microplastics in the marine environment are still far to be fully clarified.

1. Introduction

The benefits of plastics, including versatility, resistance and durability to degradation, are well known and led to the actual definition of "age of Plastics", where almost everything contains this material. Plastic production increased dramatically worldwide over the last 60 years, passing from 0.5 million tons/yr^{-1} in 1960 to almost 300 million tons in 2013. Europe ranks second at global level with 20% of the total production, corresponding to 57 million tons of plastics produced in 2012; European plastic industry gives direct employment to over 1.45 million people, generating about 26.3 billion euro for public finance and welfare (Plastic Europe, 2014/2015).

Plastic materials also pose a serious threat to the marine environment when not properly disposed or recycled. Approximately 60–80% of the word's litter is in form of plastic (Derraik, 2002), and almost 10% of the annual production ends up into the oceans, where degradation of plastic objects can take several hundred years. The main inputs of plastics into the sea derive from beaches and land-based sources like rivers, storm water runoff, wastewater discharges, or transport of land litter by wind (Ryan et al., 2009). Maritime activities contribute with materials lost by professional and recreational fishing, and debris dumped by commercial, cruise or private ships (Cooper and Corcoran, 2010). Plastic accumulation in the marine environment produces several negative repercussions: from the aesthetic impact of litter and economic costs for beach cleaning, to adverse biological and ecological effects which, according to last conservative estimates from UNEP, would cause an overall economic damage to marine ecosystems of $13 billion each year (Year Book and Valuing Plastic, Nairobi, 2014).

Considering the new evidence on the multiple risks that plastics pose to the environment, marine protection projects such as the Marine Debris Program of the US National Oceanographic and Atmospheric Administration (NOAA), included plastics litter as an emerging form of contamination. The growth of scientific interest

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has been accompanied by important normative and political decisions. The European Marine Strategy Framework Directive (MSFD, 2008/56/EC) included marine litter among Descriptors used by member States to achieve good ecological status, and international Experts Committees (like ICES or GESAMP) worked to define standardized protocols for monitoring plastic pollution in environmental matrices. In 2013, Contracting Parties of the Barcelona Convention agreed on Marine Litter Regional Action Plan to prevent, reduce and remove marine litter from the Mediterranean through developing technical capacity, reducing knowledge gaps and providing financial resources. JPI Oceans launched in 2015 a €7.5 million call for proposals to increase the knowledge on microplastics in the marine environment. In the same year, the G7 Science Ministers meeting acknowledged the global risks posed by plastics to marine and coastal life, ecosystems and potentially human health, committing a priority Action Plan to Combat Marine Litter through innovation, education, research and outreach programs.

1.1. Plastics materials and microplastics formation

There are many typologies of plastic polymers and additives, which can be combined in objects with specific properties and characteristics. The most common polymers are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyamide (PA), polyethylene terephthalate (PET), polyvinyl alcohol (PVA). Once released in the ocean, their environmental fate primarily depends on the polymer density (Table 1), which influences buoyancy, position in the water column and the consequent possibility to interact with biota (Wright et al., 2013b). Polymers denser than seawater (like PVC) will sink, while those with lower density (e.g. PE and PP) will tend to float in water column. Processes like biofouling and the colonization of organisms on the plastic surface increase the weight of particles, thus accelerating their sinking on bottom sediments (Ye and Andrady, 1991; Lobelle and Cunliffe, 2011); also degradation, fragmentation and the leaching of additives can change the density of objects and their distribution along the water column.

Degradation is a sequence of chemical changes that drastically reduce the average molecular weight and mechanical integrity of the polymer, mostly modulated by reactions like photo- and thermal-oxidation, hydrolysis and biodegradation mediated by microbial activity (Singh and Sharma, 2008). Degradation rate can vary according to polymer typology, presence of chemical additives, oxygen availability to the system, environmental temperature. Compared to beaches, where temperature may raise up to 40 °C in summer, plastic weathering is markedly slower in colder seawater and marine sediments (Andrady, 2011). Coupled with physical abrasion, such as wave action and sand grinding, degradation leads to embrittlement and fragmentation. Extensively degraded plastics become brittle enough to fall apart into powdery fragments and microsized plastics, typically not visible to the naked eye, called microplastics (Barnes et al., 2009).

Table 1
Density range of most common polymers of environmental relevance.

Matrix	Density (g/cm^3)
Distilled water	1
Sea water	1.025
Polyethylene (PE)	0.93–0.98
Polypropylene (PP)	0.89–0.91
Polystyrene (PS)	1.04–1.11
Polyvinylchloride (PVC)	1.20–1.45
Polyamide (PA)	1.13–1.5
Polyethylene terephthalate (PET)	1.38–1.39
Polyvinyl Alcohol (PVA)	1.19–1.35

Microplastics are generally referred to particles with a grain size lower than 5 mm, although a recent definition suggests to consider fragments smaller than 1 mm (Browne et al., 2015). Their presence has been reported worldwide, from polar regions to the equator, from intertidal zone to abyssal sediments (Zarfl and Matthies, 2010; Lusher et al., 2015; Van Cauwenberghe et al., 2015a). Microparticles can be defined as primary or secondary, depending on their origin source (Barnes et al., 2009). Majority of microplastics in the oceans are secondary products derived from degradation and fragmentation of mesoplastics or larger fragments (Gregory and Andrady, 2003); primary microplastics, introduced directly into the oceans via runoff, are manufactured as micron-sized particles typically used as exfoliants for cosmetic formulations, in industrial abrasives and ‘sandblasting’ media, in textile applications and synthetic clothes (Gregory, 1996; Maynard, 2006; Fendall and Sewell, 2009).

Firstly reported in 2004 in beach sediments of the United Kingdom (Thompson et al., 2004), since then the scientific interest toward this issue has exponentially increased. Searching for “microplastic” in Scopus indexed journals, 9 documents were published until 2010 in Environmental Sciences, while the number increased to 129 in 2011–2015. Microplastics have thus passed from being considered emerging pollutants to be recognized as an emerged threat, with the urgent need to better assess their distribution in the oceans, as well as the ecotoxicological and ecological risks that these particles pose to the marine ecosystem.

2. Distribution, behaviour and occurrence of microplastics in marine environment

Quantitative estimates of the global abundance and weight of oceanic plastics are still limited and often controversial, particularly for the Southern Hemisphere and more remote regions (Lusher, 2015). Several global surveys were carried out in the last 5 years to assess the load of floating macro and microplastics (Cózar et al., 2014; Eriksen et al., 2014; Reisser et al., 2015), and a more limited number of studies focused on their presence in sediments and biota (Lusher et al., 2013; Avio et al., 2015b; Romeo et al., 2015; Van Cauwenberghe et al., 2015a). Data from different studies are often difficult to compare due to the lack of standardized sampling methodologies, normalization units and expression of data, as well as definition, size and characterization of described microplastics (Ryan et al., 2009).

Despite these technical aspects, distribution of plastics has been documented in several seas, with highly variable concentrations, normalized to either surface or volume units (Table 2). High concentrations of plastics debris were firstly observed in the North Pacific central gyre (Moore et al., 2001) and the term “ocean garbage patches” has then been introduced (Kaiser, 2010; Zhang et al., 2010). A minimum of 21.290 tons of floating plastic debris was estimated in the accumulation zone of the North Pacific subtropical gyre (Law et al., 2010). At now, a total of 5 ocean gyres have been identified (North Atlantic, South Atlantic, South Indian, North Pacific and South Pacific), and another garbage patch was predicted to occur in the Barents Sea (van Sebille et al., 2012).

Almost 270.000 tons of plastic would be currently floating in the oceans according to results of 24 expeditions (2007–2013) across all five sub-tropical gyres, costal Australia, Bay of Bengal and the Mediterranean Sea during which surface net tows and visual survey transects of large plastic debris were conducted (Eriksen et al., 2014). Based on the total number of the plastic particles and their weight, researchers calibrated these data on an oceanographic model of floating debris dispersal corrected for wind-driven vertical mixing, estimating a minimum of 5.25 trillion particles weighting 268.940 tons.

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Table 2
Average concentrations and weight of floating plastic items reported for different geographical areas.

Location	Average concentration	Average weight	Reference
Northwest Atlantic (coastal)	3 items/m^3		Carpenter and Smith, 1972
Northwest Atlantic (offshore)	67 items/m^2		Colton, 1974
Northeast Atlantic (Celtic sea)	2.46 items/m^3		Lusher et al., 2014
Western Mediterranean (Corsica)	0.116 items/m^2	2020 g/km^2	Collignon et al., 2012
Western Mediterranean (Sardinia)	0.062 items/m^2		Collignon et al., 2014
Western Mediterranean	135 items/m^2	187 g/km^2	Faure et al., 2015
	0.15 items/m^3		de Lucia et al., 2014
Northeast Pacific (South California)	8 items/m^3		Moore et al., 2002
North Pacific (central gyre)	334.3 items/m^2		Moore et al., 2001
Northeast Pacific	0.004–0.19 items/m^3		Doyle et al., 2011
North Pacific subtropical gyre	0.021–0.448 items/m^2		Goldstein and Goodwin, 2013
	>1000 items/m^2		Law et al., 2014
Australian coast	0.00085 items/m^3		Reisser et al., 2013
Ligurian/Sardinian sea	0.31 items/m^2		Fossi et al., 2012
South Pacific subtropical gyre	26,898 items/km^2	70.96 g/km^2	Eriksen et al., 2013
Mediterranean	0.243 items/m^2	423 g/km^2	Cózar et al., 2015
East China Sea	0.167 + −0.138 items/m^3		Moore et al., 2002
Yangtze estuary	4137.3 + 2461.5 items/m^3		Zhao et al., 2014
South Korea coast	13 + −11 items/m^2		Song et al., 2014
Lake Hovgol (Mongolia)	20.26 items/m^2		Free et al., 2014

A recent cruise reported the first quantification of floating plastics in the Mediterranean Sea with an average of 250.000 items/Km^2, and a frequency of occurrence in 100% of sampled sites (Cózar et al., 2015). These results were comparable to the accumulation zones of subtropical ocean gyres, although plastic debris in the Mediterranean waters was dominated by millimeter-sized fragments and with a higher proportion of large plastic objects, probably reflecting the closer connection with pollution sources. The accumulation of floating plastic in the Mediterranean Sea (between 1.000 and 3.000 tons) is likely related to the high human pressure and the peculiar hydrodynamic characteristics of this semi-enclosed basin, where outflow mainly occurs through a deep water layer. Given the biological richness and concentration of economic activities in the Mediterranean Sea, the effects of plastic pollution on marine and human life are expected to be of particular concern in this accumulating region.

Until a few years ago, no data on microplastics were available for polar regions and calculations on the plastic flux into the Arctic Ocean suggested negligible transport of such materials in this area (Zarfl and Matthies, 2010). However, analyses of ice cores collected from remote locations in the Arctic Ocean, revealed levels of microplastics ranging between 38 and 234 particles/m^3 (Obbard et al., 2014), two orders of magnitude greater than those previously reported in the Pacific gyre (Goldstein et al., 2012). Observations of floating plastics in surface Antarctic and Arctic waters (Barnes et al., 2010; Lusher et al., 2015), in the deep Arctic seafloor (Bergmann and Klages, 2012) and in stomachs of birds from the Canadian Arctic (Mallory et al, 2006; Provencher et al., 2009, 2010), further support polar areas as an additional global sink of plastics: this hypothesis has been corroborated by a modeling study suggesting the presence of a sixth garbage patch in the Barents Sea (van Sebille et al., 2012).

At local geographical scale, spatial patterns of microplastics distribution have been described along an estuarine shoreline (English Channel, UK), where proportionately more particles were deposited downwind, in habitats with slow-moving waters (Browne et al., 2010, 2011). High spatial variability was found in the western Mediterranean (Collignon et al., 2012, 2014; de Lucia et al., 2014), while no distinct geographical pattern was identified in a coastal area off South Korea (Song et al., 2014).

Temporal trends of plastic litter have also been discussed. In the North Pacific Subtropical Gyre, plastic concentration in 1999 was calculated at 335.000 items/km^2 (5.1 kg/km^2), approximately one order of magnitude higher than that measured in 1980 (Ryan et al., 2009; Moore et al., 2001). Similarly, a long-term study on microplastics in old plankton samples confirmed a time dependent increase from the 1960s up to 2000 (Thompson et al., 2004). On the other hand, particles of 1 mm size were found in 60% of plankton samples trawled between 1986 and 2008 in North Atlantic Subtropical Gyre, but no temporal trend was observed over that time (Law et al., 2010).

Beside floating particles in pelagic habitats, microplastics accumulate also on the seafloor sediments, posing additional risk to such ecosystems. Deposition of microplastics in sediments is influenced by many biologically-driven or physico-chemical transport mechanisms. Colonization by organisms, adherence to phytoplankton, the aggregation with organic debris and small particles in the form of marine snow will eventually enhance settling. Oceanographic processes which facilitate the transfer of microplastics to depth, include dense shelf water cascading (Canals et al., 2006), severe coastal storms (Sanchez-Vidal et al., 2012), offshore convection (Stabholz et al., 2013) and saline subduction (Talley, 2002). All these processes, induce vertical and horizontal transfers, from shallow ocean and coastal regions to deeper layers, of large volumes of particle loaded waters containing a full spectrum of grain sizes, from sand to clay (Galgani et al., 1995, 1996). Submarine canyons, acting as preferential conduits, may further enhance downwelling flows and increase the retention of microplastics at particular locations (White et al., 2007; Canals et al., 2013). Microplastic fragments more than larger items, are also likely to be influenced by advection and circulation patterns which contribute to explain the accumulation of particles in the deep sea, and the role of marine sediments as an important sink of these materials (Woodall et al., 2014; Van Cauwenberghe et al., 2015b).

Plastic particles sized in the micrometer range were observed in deep-sea sediments collected at 1.100–5.000 m from four locations in Atlantic and Mediterranean (Van Cauwenberghe et al., 2013). Microplastics in form of fibers were up to four orders of magnitude more abundant (per unit volume) in deep-sea sediments from the Atlantic Ocean, Mediterranean Sea and Indian Ocean than in contaminated sea-surface waters, providing additional evidence for a large and hitherto unknown repository of microplastics (Mordecai et al., 2011; Van Cauwenberghe et al., 2013; Pham et al., 2014; Tubau et al., 2015). With the expansion of sea floor explorations, benthic microplastics are progressively being revealed to be more widespread than previously assumed with temporal accumulation trends matching the increasing plastic production worldwide (Claessens et al., 2011; Mordecai et al., 2011; Anastasopoulou et al., 2013; Ramirez-Llodra et al., 2013).

It is now crucial to establish consistent methodologies to allow robust temporal and spatial comparisons, to address how abundance and composition of microplastics vary with depth, location, topography and habitat. Microplastics behaviour in marine environment should be considered in a dynamic and changing perspective, since initially floating particles can sink to sediments, potentially being remobilized to water column by bioturbation, resuspension or hydrodynamic conditions.

3. Occurrence of microplastics in wild marine organisms

A recent analysis revealed that 663 marine species experience adverse effects from interaction with plastic (CBD, 2012), a 40% increase compared to a previous census (Laist, 1997). Entanglement in and ingestion by large organisms can have fatal but also sub lethal consequences, compromising their ability to capture and digest food, sense of hunger, escape from predators, decrease of body condition and impairment of locomotion, including migration. Marine mammals, seabirds, turtles, fishes are the most impacted organisms by macro debris (Laist, 1997; Derraik, 2002; Allsopp et al., 2006) with an impressive percentage of affected individuals in some species: at least 96% of North Sea fulmars have been reported to contain at least one piece of plastic in the stomach. Since plastic waste production is continuously increasing, it is expected that also the number of influenced species will grow in the future.

The knowledge on the presence of plastics in small fish and invertebrates has been hampered by the greater technical difficulty in isolation and identification of microscopic particles from tissues (Cole et al., 2014; Avio et al., 2015b). Ingestion is the most likely interaction with microplastics for many organisms particularly when feeding mechanisms do not allow to discriminate between particles (Moore et al., 2001). Absorption of microplastics by organisms from the primary trophic level, e.g. phytoplankton and zooplankton, could be a pathway for transfer into the food chain. Some organisms such as shore crabs (*Carcinus maenas*) and filter feeding bivalves not only ingest microplastics along with food, but also contain these particles in the gills due to ventilation mechanisms (Browne et al., 2008; Moore, 2008; Watts et al., 2014).

Microplastics ingestion has been described in several marine species, including invertebrates and fish (Thompson et al., 2004; Von Moos et al., 2012; Avio et al., 2015a,b), but studies on wild organisms are less common than those obtained in laboratory conditions. As a few examples, gooseneck barnacle *(Lepas* spp.) from the North Pacific subtropical gyre contained microplastics in 33.5% of examined individuals: from 1 to 3 items were observed in gastrointestinal tracts, indicating that ingestion of microplastics is a quite common phenomenon in these filter feeding organisms, with unknown trophic impacts on such rafting communities (Goldstein and Goodwin, 2013). Plastic pellets were also found in the stomachs of mass stranded Humboldt squids (*Dosidicus gigas*) (Braid et al., 2012), a large predatory cephalopod which usually feeds at depth between 200 and 700 m: the route of ingestion was unclear since the squid may have fed directly on sunken microplastics or, indirectly, through preying organisms with pellets in their digestive system. Fibers of monofilament plastics, probably derived from fibers of trawls and fragments of plastic bags, were identified in the intestines of the Norway lobster, *Nephrops norvegicus* (Murray and Cowie, 2011): these organisms have various feeding modes, including scavenging and predation, but are not adapted to cut flexible filamentous materials which are not eliminated by normal digestive processes (Murray and Cowie, 2011). Microplastics were identified in other organisms with commercial value and consumed whole, like field-caught brown shrimps (*Crangon crangon*) and farmed bivalves, highlighting also potential implications for human health (De Witte et al., 2014; Pott, 2014; Van Cauwenberghe and Janssen, 2014; Galloway, 2015; Van Cauwenberghe et al., 2015a).

The above studies revealed that invertebrates may be efficiently used as indicator species for environmental microplastics, although additional studies are required to understand the flux of such particles within water column and sediments, the interactions between different species of benthic infauna feeding in/or manipulating the sediment; these organisms can both ingest and excrete microplastics, and the whole individuals or their fecal pellets may in turn be eaten by secondary consumers, thus affecting higher trophic levels.

Some of the earliest studies on ingestion of microplastics by wild-caught fish include coastal species from the USA (Carpenter et al., 1972) and the U.K. (Kartar et al., 1973, 1976). More recent evidences reported microplastic (fibers, fragments and films) in mesopelagic fish from the North Pacific Central Gyre (Boerger et al., 2010; Davison and Asch, 2011; Choy and Drazen, 2013), and in estuarine fish which are subjected to high riverine input (Possatto et al., 2011; Dantas et al., 2012; Morritt et al., 2014). Ingestion of microplastics was observed in bottom-feeding fish from a tropical estuary in Northeast Brazil, containing nylon fragments in 13.4% of the examined stomachs (Ramos et al., 2012); in addition to the hypothesis that fish mistakenly identify these fragments as prey items, the authors suggested that ingestion might have occurred through consumption of fish already containing these microplastics, or during suction of sediments.

Lusher et al. (2013) described microplastic polymers in 10 fish species from the English Channel. Among the 504 examined specimens, 37% had ingested a variety of polymers, the most common being polyamide and the semi-synthetic material rayon. Similarly, microplastics were recorded in 35% of planktivorous fish examined from the North Pacific central gyre (Boerger et al., 2010), while fish from the northern North Sea ingested these items at significantly lower level (1.2%) compared to specimens from the southern North Sea (5.4%) (Foekema et al., 2013).

Recent analyses on the stomach content of three different species of large pelagic Mediterranean species (*Xiphias gladius, Thunnus thynnus, Thunnus alalunga*) demonstrated the occurrence of microplastics in the 18,5% of collected organisms, providing the first evidence of microplastics ingestion for Mediterranean top predators (Romeo et al., 2015). Microplastics were also extracted from fish with high commercial interest along the Central-North Adriatic Sea with 28% of analyzed organisms containing these particles in their gastrointestinal tract (Avio et al., 2015b). Microplastics were found in all analyzed species (*Sardina pilchardus, Squalus acanthias, Merluccius merluccius, Mullus barbatus* and *Chelidonichthys lucerna*), the frequency of fish positive to microplastics ingestion was higher in benthic compared to pelagic species but the number of items was typically greater in pelagic organisms. These results suggested the effects of a different distribution of microplastics in the marine environment, where floating microplastics are commonly aggregated in patches (elevated density but irregular spatial distribution), while sinking particles are more homogeneously distributed in sediments (Avio et al., 2015b).

Beside studies describing the presence of microplastics in tissues of marine organisms, a few investigations considered the potential for microplastics to be transferred between trophic levels following ingestion. Many zooplankton species undergo diurnal migrations, possibly acting as vectors of microplastics to greater

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depths and relative inhabitants, either through predation or production of fecal pellets sinking to the seafloor (Wright et al., 2013a). Field observations highlighted the presence of microplastics in the scats of fur seals (*Arctocephalus* spp.) and the authors suggested that microplastics had initially been ingested by the plankton-feeding fish, *Mycophii,* which is the main prey consumed by fur seals (Eriksson and Burton, 2003). In feeding experiments under controlled conditions, microplastics previously been ingested by blue mussels (*Mytilus edulis*), were identified in the gut and haemolymph of the shore crab (*Carcinus maenas*) (Farrell and Nelson, 2013): caution was suggested in interpreting these results due to large variability in the amount of microspheres in tissues samples, the low number of analyzed individuals, and the exposure levels exceeding those from natural field conditions. Fish fed with *Nephrops norvegicus* containing polypropylene filaments, were found to ingest but not to excrete the microplastic strands (Murray and Cowie, 2011), further corroborating the potential for trophic transfer.

4. Biological hazards from plastic and microplastic pollution

The impact of large plastic debris on the marine environment has long been the subject of environmental debates (Cole et al., 2011). Well recognized effects include loss of aesthetic perception and environmental value, economic repercussions for the tourism and for numerous marine-related industries (e.g. shipping, fishing, energy production, aquaculture), and significant biological concerns for the injury and death of marine birds, mammals, fish and reptiles (Moore, 2008; Gregory, 2009; Lozano and Mouat, 2009; Lusher et al., 2015). In recent years, new environmental hazards have been highlighted following the scientific evidence of the widespread and ubiquitous presence of plastic and microplastic pollution in the marine environment (Derraik, 2002; Barnes et al., 2009; Sivan, 2011). Beside the transport of non-native or pathogen species to new habitats on floating plastic debris (Barnes, 2002; Derraik, 2002; Zettler et al., 2013), microplastics might release plasticizers and adsorbed pollutants after the ingestion by a wide variety of marine organisms. In this respect, microplastics may introduce species, biological toxins and chemicals in the food chain, with still unpredictable ecological effects for bioaccumulation and biomagnification (Teuten et al., 2009).

4.1. *Plastics as vector of alien species*

The huge amount of plastic debris into the ocean has massively increased the opportunity for the dispersal of marine organisms through rafting material, representing a potential mechanism for invasions by alien species in new habitats (Barnes, 2002; Barnes and Milner, 2005; Andrady, 2011).

Organisms ranging from algae to reptiles (i.e. iguanas) have been observed to raft on floating objects, but the most common species include barnacles, polychaete worms, bryozoans, hydroids and mollusks (Barnes, 2002). At now, 32 reports documented the transport of 270 species belonging to 85 taxa, and 3 studies specifically distinguished the presence of 5 invasive species (CBD, 2012). It is however likely that this phenomenon is still underestimated due to the limited number of studies and observations to species level.

The occurrence of diatoms, hydroids, and bacteria was initially documented in early 70s on small plastic fragments (0.1–5 mm long) collected by plankton nets (Carpenter and Smith, 1972; Carpenter et al., 1972). Additional studies on microplastic fouling emerged in the 2000's (Carson et al., 2011; Goldstein et al., 2013; Zettler et al., 2013), and the first comprehensive characterization of epi-plastic microbial communities introduced the concept of "Plastisphere" (Zettler et al., 2013). Scanning electron microscopy (SEM) and next-generation sequencing on polyethylene and polypropylene particles (approx. 2–20 mm long) from the North Atlantic revealed a unique, diverse, and complex microbial community with approximately 8600 differential OTUs from bacteria, diatoms, cyanobacteria and predatory ciliate. This pioneer study confirmed that plastic debris may act as a vector for dispersal of these organisms which, on their side, can influence biodegradation rate and leaching of contaminants from polymers (Zettler et al., 2013).

Scanning electron microscopy (SEM) allowed to characterize organisms on the surface of 68 small plastic particles (length range = 1.7–24.3 mm, median = 3.2 mm) from inshore and offshore waters around the Australian continent (Reisser et al., 2014). Authors described new taxa associated with millimeter-sized microplastics: diatoms were the most represented group of plastic colonizers, with 14 genera, followed by 'epiplastic' coccolithophores (7 genera), bryozoans (4 genera), hydroids (3 genera), polychaetes (2 genera), in addition to individual genera for barnacles (*Lepas* spp.), dinoflagellate (*Ceratium*), isopod (*Asellota*), marine insect eggs (*Halobates* sp.), and numerous rounded, elongated, and spiral cells putatively identified as bacteria, cyanobacteria, and fungi. Furthermore, a variety of plastic surface microtextures, including pits and grooves conformed to the shape of microorganisms, supporting an important role that such biota may play in plastic degradation (Reisser et al., 2014).

All these studies highlighted that anthropogenic polymers have created a new pelagic habitat for microorganisms and invertebrates. The ecological ramifications of this phenomenon for species dispersal, ocean productivity, palatability and trophic transfer of microplastics in food webs, degradation and leaching of plastic-associated pollutants, remain an open field of research.

4.2. *Microplastics and chemicals*

Although plastics were typically considered as biochemically inert (Roy et al., 2011; Teuten et al., 2009), plastic additives are incorporated into polymers during manufacturing processes to improve their properties or extend resistance to heat (e.g. by using polybrominated diphenyl ethers), oxidative damage (with nonylphenol) and microbial degradation (with triclosan) (Browne et al., 2007; Thompson et al., 2009). Phthalates, used as emollients to soften plastics can constitute up to 50% of the polymer weight in some PVC objects (Oehlmann et al., 2009; Talsness et al., 2009).

These additives are of environmental concern since they can increase the degradation times of plastic, but are also desorbed from the polymer at rates depending on the pore size of the synthetic matrix, the amount and typology of the additive, and various environmental factors which influence the size and weathering of plastic (Ng and Obbard, 2006; Moore, 2008; Barnes et al., 2009; Teuten et al., 2009 Lithner et al., 2011).

Commonly used additives, including phthalates, bisphenol A (BPA), alkylphenols, polybrominated diphenyl ethers are hazardous to biota acting as endocrine-disrupting chemicals that can mimic, compete with, or disrupt the synthesis of endogenous hormones (Talsness et al., 2009). These compounds have been measured at high concentrations in plastic fragments sampled both at remote and urban beaches, as well as in those floating in the open ocean (Hirai et al., 2011): alkylphenols were identified at values up to 3940 ng/g, bisphenol A up to 35 ng/g with outliers up to 700 ng/g, whereas levels of PBDEs were found to range between 0.1 and 400 ng/g with outliers up to 9900 ng/g (Hirai et al., 2011; Rochman et al., 2014). Adverse effects of plasticizers were observed in the ng/l–μg/l range and associated to endocrine disruption and transcriptional down-regulation of choriogenin (Chg H) and

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vitellogenin I (Vtg I) in fish exposed to polyethylene (Rochman et al., 2014). The increasing concern on the endocrine-disrupting effects of some plastic additives prompted industry to seek alternative chemicals. In this respect, bisphenol A is now banned in several "BPA-free" labeled products, although some of the chemical substitutes are also bisphenols, e.g. bisphenol S (BPS) and bisphenol F (BPF). A recent comparison of their hormonal activity revealed the endocrine potency to be in the same order of magnitude of BPA, with similar mode of action (estrogenic, antiestrogenic, androgenic, and antiandrogenic) and physiological effects in organisms (Rochester and Bolden, 2015). Data on chronic effects of traditional and new additives are actually lacking for aquatic species in long-term exposures (Oehlmann et al., 2009; Rochester and Bolden, 2015).

In addition to the leaching of additives, chemical risk of microplastics derives also from the adsorption of a wide array of organic and inorganic contaminants on these particles (Wang et al., 2016). Due to the great specific surface area and the affinity of organic pollutants for the hydrophobic characteristics of polymers, concentrations of environmental chemicals on microplastics are often orders of magnitude higher than those detected in seawater (Ogata et al., 2009). The presence of organisms fouled on the plastic debris, including diatoms, hydroids, filamentous algae and tarry residues, could act as additional sorbent for contaminants (Endo et al., 2005; Zettler et al., 2013). In general, the combination of physical, chemical and biological factors allow concentrations of chemical pollutants to increase over time via sorption by particles and accumulation by biofilms (Wang et al., 2016).

The possibility for plastic particles to adsorb chemical pollutants from the surrounding environment has been widely characterized in laboratory conditions. Different polymers, like polyvinyl chloride, polyethylene, polypropylene, polystyrene, were shown to have a high sorption capacity for DDTs, polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes and chlorinated benzenes (Bakir et al., 2012; Lee et al., 2014; Avio et al., 2015a). Consistent with these studies, persistent organic pollutants (POPs), like polychlorinated biphenyls (PCBs), organo-halogenated pesticides, nonylphenol, PAHs and dioxins have been detected in plastic pellets stranded on different beaches around the world (Endo et al., 2005; Ogata et al., 2009; Hirai et al., 2011; Heskett et al., 2012). Polycyclic aromatic hydrocarbons on beached plastic fragments showed mainly petrogenic signature, with total PAH concentrations up to 45.000 ng/g (Hirai et al., 2011; Antunes et al., 2013; Mizukawa et al., 2013); polychlorinated biphenyls and organochloride pesticides were detected at values up to 450 ng/g and 200 ng/g, respectively (Hirai et al., 2011; Karapanagioti et al., 2011; Antunes et al., 2013; Mizukawa et al., 2013).

Metals also showed a strong adsorption capacity to plastic with measured levels up to 300 μg/g for Al, Fe, Cu, Pb and Zn and up to 80 ng/g for Cd, Cr, Co, Ni in beached pellets (Holmes et al., 2012). Little is known about mechanisms of metal adsorption which is higher in weathered compared to virgin plastics, due to an increased polarity of particles (Mato et al., 2001; Turner and Holmes, 2015): environmental conditions like pH, have also been suggested as crucial factors for the adsorption of metals onto plastics polymer (Turner and Holmes, 2015).

Despite the importance of microplastics in adsorption and transport of xenobiotic pollutants, it has long been debated whether they also represent a source of chemical exposure within marine food webs. In this respect, the ingestion of microplastics by biota, could highlight an additional concern for their potential toxicological effects to the organisms (Bowmer and Kershaw, 2010). Various indirects evidences, including the use of a thermodynamic approach and of models simulating physiological conditions in the gut, suggested that both adsorbed pollutants and chemical additives of plastics might be released to organisms after ingestion (Gouin et al., 2011; Tanaka et al., 2013; Bakir et al., 2014). The first direct demonstration of a similar possibility was provided in mussels, *Mytilus galloprovincialis*, exposed to PAH-contaminated microplastics (polyethylene and polystyrene), which revealed a marked bioaccumulation of adsorbed chemicals in both digestive gland and gills (Avio et al., 2015a).

Further studies are actually needed to better elucidate the magnitude of chemical load on environmental microplastics and the real potential to transfer such compounds to marine biota.

4.3. Microplastics and biological effects

Until a few years ago scientific interest was mostly focused toward the ecological impacts of macro-debris, while recently the ecotoxicological effects of microplastics are drawing researchers attention (Browne et al., 2015). In field conditions, it is difficult to distinguish possible adverse effects modulated by exposure to microplastics from those caused by other, including chemical, stressors.

In this respect, several laboratory studies were carried out to better characterize and predict the potential ecotoxicological risk of microplastics in the marine environment. Adsorption and effects of microplastics at the base of the marine food web were demonstrated by the presence of charged nano-polystyrene beads into the cellulose of the Chlorophycea alga, *Scenedesmus* spp., with consequent inhibition of photosynthesis and onset of oxidative stress (Bhattacharya et al., 2010). Ingestion of microplastics was shown to affect the physiology and health of marine zooplankton, e.g. decreasing feeding rate and fecundity success (Cole et al., 2013, 2015). Furthermore, adult females and nauplius larvae of the copepod, *Tigriopus japonicas*, survived acute exposure, but higher mortality rates were observed following a two-generation chronic toxicity tests (Lee et al., 2013).

The uptake and consequences of microplastic ingestion have also been investigated in a number of benthic invertebrates exposed under laboratory conditions (Cole et al., 2011; Wright et al., 2013a). These studies demonstrated that lugworms (*Arenicola marina*), amphipods (*Orchestia gammarellus*), sea cucumbers (*Holothuria* sp.), sea urchins (*Tripneustes gratilla*) and mussels (*Mytilus* sp) feed directly on microplastics which may be partly excreted through the intestinal tract (Thompson et al., 2004; Graham and Thompson, 2009; Wegner et al., 2012; Kaposi et al., 2014; Avio et al., 2015a). Adverse effects were reported after the ingestion of such particles in lugworms, with a positive correlation between weight loss and concentration of microplastics spiked in sediments (Besseling et al., 2013). In the same organisms exposure to 5% unplasticised polyvinyl chloride (U-PVC) caused inflammation processes, a significant decrease of feeding activity and reduction of energy reserves, while onset of oxidative stress was caused by ingestion of μm-sized PVC microplastics (Browne et al., 2013; Wright et al., 2013a).

Recent studies provided experimental evidences that ingested microplastics can be retained within the organisms and even translocated between different tissues. In mussels, *Mytilus edulis*, plastic particles (3 and 9.6 μm) were accumulated in digestive tissues and translocated to haemolymph after three days (Browne et al., 2008). In the same organisms, the uptake of microplastics caused notable histological changes in digestive cells with strong inflammatory responses, formation of granulocytomas and lysosomal destabilization that increased with exposure time (Von Moos et al., 2012). A wide range of molecular, biochemical and cellular alterations were assessed in *M. galloprovincialis* exposed to polyethylene and polystyrene microplastics, both virgin or previously contaminated with pyrene (Avio et al., 2015a). Transcriptomic profile showed the modulation of several pathways involved in the responses to microplastics which partly differed according to the presence or absence of adsorbed chemical. Transcriptional responses were mostly related to lysosomal metabolism, immunological function and apoptotic events; additional genes differentially expressed after microplastics ingestion were those involved in antioxidant defense, detoxification and repair of DNA damage. The effects of virgin and contaminated microplastics were also confirmed at the cellular level by changes of several biomarkers. "Clean" particles caused alterations of immune system parameters, a strong destabilization of lysosome membrane and loss of DNA integrity, while contaminated particles also increased the frequency of micronuclei and inhibited the activity of acetylcholinesterase. The overall results were elaborated in a quantitative hazard model, based on weighted criteria which evaluate biological relevance and magnitude of observed effects: the summarized hazard for biomarker responses ranged from slight to moderate for virgin polymers, and from major to severe for contaminated microplastics, further corroborating that these particles could be potentially hazardous for marine organisms (Avio et al., 2015a).

A more limited number of studies evaluated the toxic effects of microplastics in fish. Common goby, exposed to polyethylene with or without pyrene, showed a significant decrease in the activity of AchE, confirming the susceptibility of this enzyme involved in neurotransmission signaling (Oliveira et al., 2013). Early signals of endocrine disruption appeared in the Japanese medaka exposed to μm-sized polyethylene particles (Rochman et al., 2014), while larvae of *Dicentrarchus labrax* did not exhibit effects on growth and inflammatory responses but a significant correlation of cytochrome P4501A1expression with the number of polyethylene microbeads scored *per* larva (Mazurais et al., 2015).

Effects of microplastics in sediments were investigated in mesocosm experiments by comparing three different typologies of polymers, the biodegradable polylactic acid and the conventional polyethylene and polyvinylchloride. Metabolic rates of lugworms, *Arenicola marina* increased at higher concentrations of microplastics in wet sediment (0.02, 0.2 and 2%) while microalgal biomass decreased (Green et al., 2015). The overall results showed as both conventional and biodegradable microplastics in sandy sediments can affect the health and behaviour of lugworms and reduce primary productivity of these habitats. Responses were strongest to polyvinylchloride, highlighting that various materials may have differential effects which should not be generalized when characterizing the ecological and toxicological risks of microplastic pollution in the marine environment.

The above studies clearly demonstrated that microplastics should not be considered as biologically inert materials, often highlighting the cellular pathways adversely affected after their ingestion by marine organisms. Growing scientific evidence corroborates the ecotoxicological hazard of microplastics, whether due to a simple mechanical or physical damage induced by these particles, or for a more complex activation of molecular, biochemical and cellular pathways.

5. Conclusion and recommendations

Plastic pollution in the marine environment is now recognized as a real threat with a global-scale distribution and adverse effects spanning from molecular level, physiological performance and organisms health, up to the loss of ecosystemic services. Due to the long-life of plastics on marine ecosystems, harm to marine life would continue for many decades even if the production and disposal of plastics suddenly stopped.

In this respect, it is imperative that severe measures are taken to address the problem at both international and national levels. Further studies are needed to better elucidate factors influencing the occurrence of microplastics in marine organisms, and modulation of biological effects. New scientific data should sustain input for conservation management, provide marine scientists with better evidence for political authorities responsible for normative guidelines, and strengthen the basis for educational campaigns.

At the same time, the rise of public awareness on environmental microplastics should also stimulate technological innovation to reduce the use and consumption of plastics, minimize their input into the environment, stimulate a new approach toward collection and re-use of stranded materials.

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