Accumulation and fate of nano- and micro-plastics and associated contaminants in organisms

Francisca Ribeiro[1, 2], Jake O'Brien[1], Tamara Galloway[2] and Kevin V. Thomas[1]

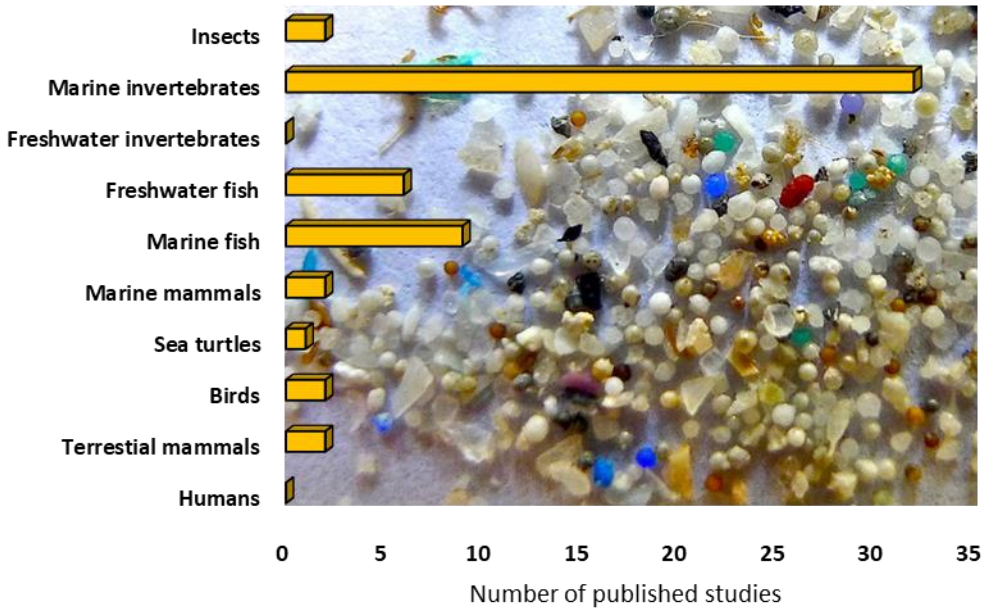
(1) Queensland Alliance for Environmental Health Science (QAEHS), The University of Queensland, 20 Cornwall Street, Woolloongabba, QLD, 4102, Australia

(2) College of Life and Environmental Sciences, University of Exeter, EX4 4QD, Exeter UK

HIGHLIGHTS

- Paucity of studies on the potential accumulation of microplastics in organisms
- Majority of studies to date have been performed on marine invertebrates
- Little information on marine vertebrates, mammals and humans
- In general, mechanisms of microplastics bioaccumulation and/or translocation are still poorly investigated and understood

GRAPHICAL ABSTRACT



Abstract

Following a decade of research into the potential environmental impacts of microplastics, there is still a significant gap in our knowledge about the processes by which microplastics pass across biological barriers, enter cells and are subject to biological processes. Here we summarize available research on the accumulation of microplastics, and their associated contaminants, in a range of different organisms, such as marine invertebrates, fish, sea turtles, marine and terrestrial mammals and humans. Analysis of the available research revealed that the majority of the data available on the accumulation of microplastics in both field and lab studies are for marine invertebrates, especially bivalves. An important aspect that could provide a measure of the risk of microplastics to exposed organisms is to understand their clearance and the effect it has on the inflammatory response and possible risk associated with exposure.. Evidence of microplastics accumulation in insects, birds, marine mammals and sea turtles is scarce, due to difficulty in sampling and extracting these particles form their stomachs and tissues. Information is sparse on the mode of accumulation of microplastics in both mammals and humans. There is some evidence to suggest possible uptake of plastic particles by the intestinal barrier and lungs, although this is far from conclusive. A step towards understanding microplastics mechanism of uptake would be the use of in vivo experimental testing using laboratory animals, however there are ethical implications associated with such studies. Further work is required in order to understand the mechanism of chemical partitioning as well as the role of contaminants when associated with a plastic. The methodologies that have been used to locate nano and microplastics in animal tissues have to date essentially been based on histology and imaging processes, although the intrinsic characteristics of the plastic pose technical limitations. Gaps in knowledge and recommendations for future research are provided, and attention is drawn to the urgent need to understand the mechanism of action of both nano- and micro-plastics and associated contaminants in a range of organisms.

Keywords: microplastics; accumulation; contaminants; analytical methods.

Contents

1. Introduction

Plastic production began in the 1950s with the commercial development of polyolefins, polypropylene and polyethylene (PlasticsEurope, 2017). Plastic use has increased globally, however rapid growth in production and distribution has resulted in serious environmental consequences (Lusher, 2015). The high durability and resistance of plastic polymers to degradation, coupled with high consumption and low recycling volumes, has contributed to the continuous increase of plastics in the environment (Keane, 2007). Global plastic production increases 9% every year, with 335 million tons produced in 2016 (PlasticsEurope, 2017).

Microplastics are distributed worldwide and have been found in all different environments and remote locations (Rochman, 2018). Microplastics have been reported in the marine environment (Andrady, 2011), freshwater systems such as lakes and rivers (Eerkes-Medrano *et al.*, 2015; Eriksen *et al.*, 2013), terrestrial systems (soil and sludge) (Lwanga *et al.*, 2017; Zubris & Richards, 2005), dust (Kole *et al.*, 2017) and air (Dris *et al.*, 2017).

The largest sink for microplastics is the open ocean. The amount of plastic debris that reaches the marine environment is substantial and estimated between 4 and 12 million metric tons per annum (Derraik, 2002; Jambeck *et al.*, 2015; Thompson *et al.*, 2004). The primary sources of plastic debris in the sea are from fishing fleets (Cawthorn, 1989), marine recreational activities (Pruter, 1987; Wilber, 1987) (UNESCO, 1994), rivers and municipal drainage systems (Williams & Simmons, 1997). Major inputs of plastic litter from land sources typically occur in densely populated or industrialized areas (Derraik, 2002).

Plastic debris can be transported thousands of kilometres and contaminate relatively distant locations (Browne *et al.*, 2010) and accumulate along strandlines (Thornton & Jackson, 1998), in the open ocean (Shaw & Day, 1994), and on the seafloor (Galgani *et al.*, 2000). Most plastics are resistant to biodegradation, but they will break down gradually through mechanical action (Thompson *et al.*, 2004). When exposed to UV-B radiation, to the oxidative properties of the atmosphere and to the hydrolytic properties of seawater, these plastics become brittle and break into smaller pieces (Andrady, 2011), until they become microplastics (0.1-5000 μm) (Arthur *et al.*, 2009) or even nanoplastics (≤ 0.1 μm) (Lambert & Wagner, 2016). A secondary source of microplastics can be from industry (Lusher, 2015), from cleaning products or cosmetics (Fendall & Sewell, 2009), tyre wear (Kole *et al.*, 2017) or microfibers from machine-washed clothing (Browne *et al.*, 2011), that is directly released to the environment in the municipal effluent.

Nanoplastic manufacturing is also on the increase. Cosmetics, paints, adhesives, drug delivery vehicles, and electronics are just some examples (Koelmans *et al.*, 2015). The reduction in particle size, both by design or due to environmental degradation, may induce unique particle characteristics, that can influence their potential toxicity (Wright & Kelly, 2017).

Plastic ingestion is the main interaction between organisms and microplastics (Lusher, 2015), probably due to confusion with food (Andrady, 2011; Moore, 2008). Ingestion has been reported in marine mammals (Laist, 1997), cetaceans (Clapham *et al.*, 1999), birds (Mallory, 2008), sea turtles (Mascarenhas *et al.*, 2004), zooplankton (Cole *et al.*, 2013) , larvae and adult fish (Browne *et al.*, 2013; Lusher, 2015; Rochman *et al.*, 2014b). However, there are no reported studies on microplastic ingestion by other animals (e.g. terrestrial mammals, reptiles) or humans.

The potential for microplastics to cause injury to marine organisms has been widely documented leading to the following adverse effects: reduction of feeding rate (Wright *et al.*, 2013a), reduction of predatory performance (de Sá *et al.*, 2015), physical damage due to

accumulation (Avio *et al.*, 2015), induction of oxidative stress (Jeong *et al.*, 2017), effects on reproduction (Sussarellu *et al.*, 2016), decreased neurofunctional activity (Oliveira *et al.*, 2013; Ribeiro *et al.*, 2017), oxidative damage (Fonte *et al.*, 2016), development of pathologies (Rochman *et al.*, 2013), mortality (Mazurais *et al.*, 2015), among others.

Evidence of microplastics impact on freshwater biota is limited and has only been addressed in few studies (Duis & Coors, 2016). The same follows for terrestrial mammals, where there is only one study of the effects of microplastics in mice (Lu *et al.*, 2018). Information on the impact of microplastics on human health is still inexistent.

In addition to the physical impact caused by the intake of microplastics by organisms, microplastics themselves may be covered by biomolecules that interact with biological systems (Galloway *et al.*, 2017) and/or be a pathway for transfer of persistent organic pollutants (POPs) into their tissues (Browne *et al.*, 2013). The high surface/volume ratio of microplastics, curvature, reactivity and small size enable different uptake rates and biodistribution (Mattsson *et al.*, 2015), which makes them highly dynamic in the environment, altering microplastics bioavailability. The high accumulation potential of plastic provides a transport medium for contaminants as well as being a potential source of contaminants themselves. Degradation of microplastics to smaller particle sizes adds more surface area to sorb contaminants (Ogata *et al.*, 2009). This includes POPs, bioccumulative and toxic substances (Browne *et al.*, 2013; Engler, 2012).

To date, reviews on microplastics and associated contaminants in organisms have mainly focused on marine organisms and in summarizing ecotoxicological impact (Andrady, 2011; Barboza & Gimenez, 2015; Cole *et al.*, 2011; de Sá *et al.*, 2018), its uptake (Besseling *et al.*, 2013; Setälä *et al.*, 2014), effects (e.g. Cole et al., 2011; Auta et al., 2017; Horton et al., 2017), egestion (Brillant & MacDonald, 2002; Kaposi *et al.*, 2014; Setälä *et al.*, 2014; Ward & Kach, 2009) and the presence of plastic in several organs (Avio *et al.*, 2015; Lei *et al.*, 2018; Ribeiro *et al.*, 2017; Wright *et al.*, 2013a). Nonetheless, there has been no critical evaluation of the accumulation patterns and/or translocation of microplastics and associated contaminants inside organisms, neither data on the accumulation in other animal classes.

Thus, this paper aims to: (*i*) compile, summarize and discuss current literature of field and laboratory research in terms of microplastics accumulation in all type of organisms; (*ii*) review the published studies about accumulation and fate of associated contaminants and (*iii*) based on the information provided, identify and critically discuss data gaps and promising areas for future research. Tables 1 and 3 summarize our findings on the evidence of

163 microplastics and associated contaminants accumulation in several species, respectively.
164 Table 2 only relates to observations on wild organisms.
165

166 ## 2. Field and laboratory research in terms of microplastics accumulation
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168 ### 2.1. Marine invertebrates and fish
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170 The small size of microplastics actively contributes to their bioavailability and
171 accumulation in organisms of lower trophic classes, from benthic and pelagic ecosystems
172 (Lusher, 2015) that are the basis of most food chains (Thompson *et al.*, 2004). Most
173 laboratory exposure experiments thus far have been performed on marine organisms.
174 Microplastics are known to be ingested by planktonic organisms (Fendall & Sewell, 2009;
175 Moore *et al.*, 2002), marine invertebrates (Murray & Cowie, 2011; Van Cauwenberghe &
176 Janssen, 2014; Welden & Cowie, 2016) and marine vertebrates (Abbasi *et al.*, 2018; Dantas
177 *et al.*, 2012). However, information concerning the extent of ingestion, accumulation,
178 translocation into organs and possible pathways of transition into cells is still scarce (Wright
179 *et al.*, 2013b).
180

181 #### 2.1.1. Microplastics interactions with the environment
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183 Plastic particles generally have smooth, hydrophobic surfaces with no net charge, but
184 when in seawater, they will interact with the surroundings, and become coated by a "eco-
185 corona" composed of substances, such as organic matter, nutrients, hydrophobic
186 contaminants and bacteria from the water column and sediments, which can accumulate on
187 the particle surface (Galloway *et al.*, 2017).
188 The transformation of many types of nanoparticles in the aquatic environment are
189 relatively well understood (e.g. the influence of natural organic matter in particle's
190 aggregation, rates of protein association, interaction with biological fluids, the formation of a
191 corona, etc) (Cai *et al.*, 2018; Cedervall *et al.*, 2007; Lead & Valsami-Jones, 2014; Mattsson
192 *et al.*, 2015; Monopoli *et al.*, 2012). Regarding microplastics there is only information on
193 weathering of polymers through photo-oxidation by ultraviolet light, which increases their
194 surface area and surface exposure, which may decrease the rate of release of sorbed
195 contaminants (Teuten *et al.*, 2007). There is however a lack of knowledge regarding the

196 types, rates and extent of transformations expected for both nano and microplastics in the
197 environment (Galloway *et al.*, 2017).

198 The high surface/volume ratio of microplastics, curvature, reactivity and small size
199 enable different uptake rates and biodistribution (Mattsson *et al.*, 2015), which makes them
200 highly dynamic in the environment, altering bioavailability. The environmental conditions
201 that may contribute to increase its bioavailability in the marine environment and/or settling of
202 nano and microplastics in the water column are dependent on the type of polymer, surface
203 chemistry and the extent of biofouling by microbial biofilms and rafting organisms (Turner,
204 2015). Particulate organic matter (POM), composed by faecal pellets from zooplankton and
205 fish, known as "marine snow" (Turner, 2015) can contribute to an aggregation of
206 microplastics as well.

207 Thus far, studies on the interaction of plastic particles with the surrounding
208 environment have focused on polystyrene (PS) microparticles. 30 nm PS nanoplastics rapidly
209 formed aggregates in seawater of millimetres in length (Wegner *et al.*, 2012) and 20 µm PS
210 microplastics showed a higher zeta potential value, which indicates a natural tendency to
211 aggregate in artificial seawater (Ribeiro *et al.*, 2017). Cai *et al.* (2018) studied the influence
212 of inorganic ions and natural organic matter (NOM) on the aggregation of PS nanoparticles
213 and observed an aggregation in iron (III) chloride ($FeCl_3$) solutions with an increase in ionic
214 strength. Strangely, it seems that NOM had an imperceptible effect on nanoplastic
215 aggregation.

216 As far as we are aware, only one study has reported interactions between layer
217 charged microplastics and biological systems. Della Torre *et al.* (2014) tested the
218 accumulation of both carboxylated (PS-COOH) and amine ($PS\text{-}NH_2$) polystyrene
219 nanoplastics inside the digestive tract of sea urchin embryos *Paracentrotus lividus*. PS-
220 COOH accumulated inside the embryo's digestive tract while $PS\text{-}NH_2$ were more dispersed.
221 This evidence suggests differences in surface charges of PS nanoplastics. It can thus be
222 hypothesised that the attachment of specific molecules to the particles may promote their
223 intake and accumulation, but this has not yet been investigated.

224

225 **2.1.2. Microplastics accumulation in marine invertebrates**

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227 Excretion products of bivalves, termed pseudofaeces, have two main functions: (i) to
228 act as a sorting process that separates edible organic particles from inorganic particles (e.g.
229 microplastics) (Beninger *et al.*, 1999) and/ or (ii) act as a cleaning mechanism that prevents

an overload of the gill with particulate material (Barker Jørgensen, 1981). Several studies with microplastics and marine invertebrates reported microplastics egestion in the form of pseudofaeces (Besseling *et al.*, 2013; Cole *et al.*, 2015; Cole *et al.*, 2013; Kaposi *et al.*, 2014; Setälä *et al.*, 2014; Ward & Kach, 2009; Wegner *et al.*, 2012). In some of these studies, egestion was only a few hours following the ingestion of microplastics (e.g. Chua et al., 2014; Ugolini et al., 2013). It is hypothesized that these organisms recognize the particles as a low nutritional food, which lead to their excretion. On the contrary, we can also face a situation of a prolonged gut residence time for microplastics. This was observed with *Nephrops norvegicus* captured from the field, where 70% of the control animals contained plastics which they had consumed prior to being captured, and had not digested during the two weeks starvation period prior to the experiment (Murray & Cowie, 2011). This indicates that microplastics are probably being retained and subjected to an extensive digestion at an energetic cost because of the low nutritional value (Wright *et al.*, 2013a). On the other hand, the elimination of mucus-embedded particles as pseudofaeces leads to the simultaneous ingestion of more particles (Barker Jørgensen, 1981).

The ability for marine invertebrates, such as bivalves to distinguish between organic and inorganic particles, but not microplastics, poses the question of what is the mechanism they use to do so. It has been suggested that the shape and charge of particles may play a role in the ingestion and consequently translocation in the organism (Browne *et al.*, 2008), but this hypothesis hasn't been tested thus far.

Several ecotoxicology studies have documented microplastic accumulation in a diverse group of organisms. Evidence of accumulation and the techniques to assess the presence of microplastics in different tissues and organs are described in Tables 1 and 2, for lab and field organisms, respectively. There are different routes of possible microplastic uptake. For bivalves, a possible pathway for microplastic uptake was proposed by Ribeiro *et al.* (2017) for the clam *Scrobicularia plana*, where the particles are first trapped in the gills; the first organ in contact with particles. They can also be ingested through the inhalant siphon, transported to the mouth and once in the haemolymph, transferred to the digestive tract for intracellular digestion (Hughes, 1969). Upon ingestion, microplastics can also cause physical injury to the intestinal tract (Laist, 1997). Since microplastics cannot undergo total digestion (Andrady, 2011), once in the digestive gland, most of them are eliminated (Ribeiro *et al.*, 2017). A different potential uptake of microplastics by the mussel *Mytilus edulis* was suggested by von Moos *et al.* (2012). The first uptake pathway is mediated by the gill surface (by microvilli), which transports the particles into the gills by endocytosis, that is probably a

considerable pathway for dust and smaller plastic particles. The second, occurs via ciliae movement which transfers the particles to the digestive system: stomach and intestine, and consequently the primary and secondary ducts in the digestive tubules. From there, microparticles can be taken up and accumulate in the lysosomal system. von Moos *et al.* (2012) also observed particles in the connective tissue, which were likely eliminated by the epithelial cells of the ducts and phagocytosed by the eosinophilic granulocytes. These granulocytes migrated into the tissue and formed the observed granulocytomas. Translocation through the digestive gland has also been reported for PS micro and nanoplastics in bivalves (Browne *et al.*, 2008; Ward & Kach, 2009). According to the literature, translocation of microplastics between the gastro-intestinal system and tissues has been suggested for mussels with particles of 2 and 4 µm (Browne *et al.*, 2008; von Moos *et al.*, 2012). There is some evidence that particles larger than 10-20 µm are not capable of being translocated from the intestinal tract to the tissues (Hussain *et al.*, 2001). The results from Devriese *et al.* (2015) suggest that microplastics bigger than 20 µm are not able to translocate into the tissues of the shrimp *C. crangon.* However, Ribeiro *et al.* (2017) identified polystyrene in the digestive gland of the clam *S. plana,* which indicates that possibly the tested 20 µm PS microparticles were present in this organ. Watts *et al.* (2014) showed that the shore crab *Carcinus maenas* can ingest microplastics through ingestion with food (evidence in the foregut) and also through inspiration across the gill cavity.

An interesting scenario has been presented by Murray and Cowie (2011), that found smaller concentrations of microplastics in the Norway lobster, *Nephrops norvegicus* that had recently moulted. This occurs during the yearly moult where the carapace and part of the stomach are replaced (Farmer, 1973). During this process, the upper portion of the the lobsters' chitinous teeth, known as a gastric mill, is lost at each moult which may be essential to maintain an effective digestion (Welden *et al.*, 2015). Welden and Cowie (2016) also analysed *N. norvegicus*, sampled from the Clyde Sea Area in Scotland, and determined that ecdysis (the process invertebrates use to cast off their outer cuticle) is the primary route of microplastic loss. Once again, they observed that animals that had recently moulted contained lower levels of microplastics than the ones that didn't.

2.1.3. Microplastics accumulation in marine vertebrates

In respect to vertebrates, Mattsson *et al.* (2017) reported the presence of amino modified polystyrene nanoparticles in the brain of the fish *Carassius carassius,* after being

fed with *Daphnia magna* previously exposed to nanoplastics. Behavioural changes in the fish were observed, which suggests that their brains were affected by the particles (Mattsson *et al.*, 2017). They also noticed changes in the brain structure and water content in the fish that had ingested microplastics. If this has been tested, it could be a possible way to demonstrate if nanoplastics can pass across the blood-brain barrier in fish or not. Collard *et al.* (2017) detected microplastics in the liver of the European anchovie, *Engraulis encrasicolus,* collected from the field. It was proposed that the larger particles found in the liver may result from the agglomeration of smaller particles and/or they simply pass through the intestinal barrier by endocytosis, phagocytosis or another mechanism. In the freshwater fish, *Danio rerio,* polystyrene microplastics (5 µm) were translocated into the liver within two days (Lu *et al.*, 2016)

The mechanism(s) by which microplastics enter non-digestive tissues is unclear but can be related to translocation or adherence (Abbasi *et al.*, 2018). Laboratory experiments have demonstrated the occurrence of microplastics in the circulatory system or non-digestive organs of marine animals, such as in the haemolymph (Browne *et al.*, 2008; Farrell & Nelson, 2013; Ribeiro *et al.*, 2017), in the lymphatic system (von Moos *et al.*, 2012), the gills (Avio *et al.*, 2015; Karami *et al.*, 2016), the liver (Lu *et al.*, 2016) and the brain (Mattsson *et al.*, 2017). The particles used in these studies were all less than tens of micrometres in diameter, which is probably the reason why they were able to pass through the gills or gut epithelium through cell internalization and possible subsequent translocation (Abbasi *et al.*, 2018).

Alternatively, it has recently been suggested that adherence is an additional process by which fibrous microplastics may associate with organs, independently of the digestive system, as found in seaweeds (Gutow *et al.*, 2016). This was observed in mussels exposed to microfibers, where about 50 % of the microplastic uptake was through adherence in foot and mantle, and thus, it was the adherence instead of ingestion, that led to the accumulation of microplastics in organs that are not part of the digestive tract (Kolandhasamy *et al.*, 2018). There is currently discussion among the scientific community on the accumulation of microplastics in fish, since most of the research reported that microplastics seems to remain in the digestive tract or other organs such as the brain or the liver (mentioned above) and do not move into muscle tissue, which is basically what we eat. Adherence itself, however, poses a totally new scenario that needs to be considered, where microplastics might be transferred from other organs and get attached to the muscle, which may pose a risk to human health when ingested.

2.1.4 Depuration

Depuration is usually defined as an elimination process for intestinal contents (clearing) through defecation, when in the absence of food. It constitutes an essential part for the understanding of the accumulation of nano and microplastics, since it can help in the recovery of the exposed organisms and decrease the risk of these contaminants.

Few studies have evaluated the effects of a depuration period after an exposure to microplastics. Besseling *et al.* (2013) observed that no plastic remained in the worms that survived the 28 days assay, after the depuration overnight. Plastic particles were only found in organisms that were removed during the exposure period because of mortality or escape. This result indicates that *Arenicola marina* ingested PS microparticles although they didn't accumulate because they were egested. Other studies also reported egestion of microplastics, although it wasn't a complete egestion (Cole *et al.*, 2013; Setälä *et al.*, 2014; Ward & Kach, 2009). On the other hand, experiments with *Scrobicularia plana* and PS microbeads (Ribeiro *et al.*, 2017) suggested that 7 days of depuration weren't enough for the animal to egest the particles, since after this time, polystyrene was still detected in both the gills and digestive gland. Thus, in respect to depuration of nano and microplastics, there is not a consensus among the available literature.

2.2. Birds

Numerous studies have dealt with the ingestion of marine debris by sea birds (Kühn *et al.*, 2015), where microplastics, essentially pellets and user-fragments, have been isolated from birds targeted for dietary studies, cadavers, regurgitated samples and faeces (Bond *et al.*, 2014; Codina-García *et al.*, 2013; Herzke *et al.*, 2016; Tanaka *et al.*, 2013). After ingestion, seabirds appear to be able to remove microplastics from their digestive tracks by regurgitation (Lindborg *et al.*, 2012). On the other hand, it suggests that parents may expose their offspring to plastics during feeding. This is supported by Kühn and van Franeker (2012) that found more plastic in the intestine's juveniles than in adults. This can indicate that possibly microplastics contamination in birds occurs mostly between generations and that the regurgitation process may lead to a breakdown of microplastics into even smaller particles. The majority of birds examined did not die as a direct result of microplastic uptake, thus it can be concluded that microplastic ingestion does not affect seabirds as severely as macroplastic ingestion (Lusher, 2015). Most studies of microplastics in seabirds only

366 analysed microplastics in the digestive tract (Herzke *et al.*, 2016) and faeces (Reynolds &
367 Ryan, 2018) and thus, at this stage, there is no evidence that microplastics can cross the
368 intestine barrier and/or enter the blood stream and accumulate in different organs. To date,
369 there have been no studies demonstrating nanometre-sized microplastics in sea bird guts or
370 faeces.
371
372

2.3. Marine mammals and sea turtles

The uptake of microplastics by marine mammals is likely to occur through filter feeding, inhalation or via trophic transfer from prey (Lusher, 2015). However, information on microplastic uptake by marine mammals is still scarce because it is difficult to extract and assess microplastics from their stomachs due to the large size and decomposition rates. Plus, strandings are unpredictable and sporadic (Lusher, 2015). Diversely, 56% of 48 cetacean species analysed yet had large plastic items in their stomachs (Baulch & Perry, 2014; Kühn *et al.*, 2015). To the best of our knowledge, only two studies reported microplastics in cetaceans: Lusher *et al.* (2015) was the first study to report the presence of microplastics in an adult true's beaked female whale (*Mesoplodon mirus*); Rebolledo *et al.* (2013) confirmed microplastics presence in stomachs and intestines of harbour seals (*Phoca vitulina*) and Lusher *et al.* (2018) analysed 528 stranded and bycaught individuals and 21 contained microplastics, mostly fibres and fragments. Cetaceans were also suggested as sentinels for microplastic pollution by Fossi *et al*., (2014, 2012) though the assessment of phthalate concentrations in the blubber of stranded fin whales (*Balaenoptera physalus*). However, it is not possible to determine whether the origin of phthalates is derived from plastic or not, since exposure routes can be via microplastics, large plastic particles or simply from direct uptake of chemicals from the surrounding seawater (Lusher, 2015). Further work is essential to assess the risks of microplastics to marine mammals and what happens to the particles after its ingestion.

Several studies have reported the ingestion of macroplastics by marine turtles (Derraik, 2002; Kühn *et al.*, 2015), however microplastics have only been found in the stomach of the herbivorous green turtle (*Chelonia mydas*) (Caron *et al.*, 2018; Tourinho *et al.*, 2010) and in sea turtles (*Caretta caretta) (Pham et al., 2017).* Savoca *et al.* (2018) studied the concentration of phthalates in sea turtles and found significant concentrations in their liver and gonads. Although it is an interesting method to assess plastic debris exposure, once again we cannot extrapolate these results as indicative of microplastics in these tissues. Thus, further studies are necessary to evaluate the presence of microplastics in sea turtle tissues. If microplastics are not egested by sea turtles, both the effects and the harm caused by a possible accumulation of the particles is still unknown.

Additional work is required to understand the extent of the harm caused by microplastics in marine mammals and sea turtles.

2.4. Terrestrial mammals

Most published studies to date have focused on the effects of microplastics on aquatic organisms, but data regarding the potential accumulation and the potential health risks in terrestrial mammals and humans are absent (Deng *et al.*, 2017). Fewer studies have yet been able to extrapolate the results obtained with lower trophic animals, such as adverse effects related to the uptake of particles, to higher levels of biological organisation (Galloway *et al.*, 2017). Thus far, there is a huge knowledge gap regarding the translocation of microparticles across different tissues (Revel *et al.*, 2018). Deng *et al.* (2017) tested the effects and possible accumulation and distribution of PS microbeads in mice. Results indicated an accumulation in the liver, kidney and gut, depending on particle size, with the smaller particles (5 µm) showing the highest accumulation concentration (Table 1). A different study investigated the uptake of 2 µm latex particles by young adult rats, which revealed an uptake by the small intestine (Carr *et al.*, 2012). Plastic particles appeared in the hepatic portal vein (Volkheimer, 1974) of a dog, which can then end up in the liver, since this vein transports blood from the gastrointestinal tract, gallbladder, pancreas and spleen to the liver. To the best of our knowledge these are the only published studies about microplastic accumulation in terrestrial mammals. More data would be of valuable knowledge, since the physiology of this animals is very similar to humans, and thus, results could be extrapolated.

2.5. Humans

In respect to studies involving humans, there are several papers related to medicine and drug development that report the translocation of polylactide-co-glycolide microparticles across the digestive tract into the lymphatic system (Hussain *et al.*, 2001) and in the mucosal colon tissue (Schmidt *et al.*, 2013), however none of these studies refers specifically to plastic particles. Besides the proved particle translocation across the gut, a possible route for microplastics exposure may be through the air, where they can be inhaled and induce lesions in the respiratory system (Prata, 2018). An increasing incidence of cancer was observed in synthetic textile workers (e.g. Hours et al., 2007, Mastrangelo *et al*., 2002, Gallagher *et al*., 2015) and respiratory problems in PVC workers (e.g. Arnaud *et al*., 1978, Cordasco *et al*., 1980, Lee *et al*., 1989). Although these workers could be also exposed to high amounts of organic solvents, a potential exposure to chronic concentrations of airborne microplastics

could be the responsible for causing lung injuries dependent on individual susceptibility and particle properties (Prata, 2018), but further research is necessary to access this.

Phthalates are used as plasticizers to soften plastic products. Several papers have reported their presence in human breast milk (e.g. Fromme *et al*., 2011; Main *et al*., 2006), blood (e.g. Högberg *et al*., 2008) and urine (e.g. Jornet-Martínez et al., 2015). Although this cannot be considered an indicator of the presence of plastic particles in these biological fluids, it does suggest a lead to the next logical step, which is to analyse human samples, such as breast milk, urine, stool and blood, to look for the presence of microplastics. House dust, for example, has been shown to contain high levels of phthalate plasticisers (Abb *et al.*, 2009; Butte & Heinzow, 2002) and the possible association between allergic symptoms in both children and adults and the concentration of phthalates in dust collected from their houses (Bamai *et al.*, 2014; Bornehag *et al.*, 2004). It would be interesting to investigate the presence of microplastics in indoor dust and explore whether or not the presence of phthalates in an indoor environment is associated with the existence of microplastics in house dust.

Toxicity and/ or possible inflammation, uptake and accumulation in different organs, fluids or tissues and risk of exposure should be estimated in order to understand the mechanism and potential effects of nano and microplastics in humans (Wright & Kelly, 2017). While the physical properties of microplastics pose a risk to human and environmental health, the effect of the associated contaminants within/sorbed to the plastics must also be taken into account to not underestimate the risk they pose to human and environmental health (Rainieri *et al.*, 2018).

3. Associated contaminants and leaching of plastic additives

Besides the injuries caused by microplastic ingestion, microplastics also have the potential to cause harm by leaching chemical additives either incorporated during manufacture or adsorbed from the environment (von Moos *et al.*, 2012). These additives may be incorporated to extend the life of the plastic by providing resistance to heat, oxidation or microbial degradation (Browne *et al.*, 2007; Cole *et al.*, 2011; Thompson *et al.*, 2009). Hence, the plastic degradation times can last longer and the additives may leach out, becoming a potential hazardous to biota (Barnes *et al.*, 2009; Chua *et al.*, 2014; Lithner *et al.*, 2009).

Besides plastic can be a potential source of contaminants itself, because the plastic particles float on the sea surface, they can easily sorb contaminants. The combination of increased surface area due to weathering, long exposure times in the marine environment, and the hydrophobicity of organic xenobiotics may facilitate adsorption of these contaminants to microplastics at concentrations significantly higher than those detected in seawater and potential accumulation in organisms (Ogata *et al.*, 2009). This includes persistent organic pollutants (POPs) and bioccumulative and toxic substances (Browne *et al.*, 2013; Engler, 2012), including polychlorinated biphenyls (PBTs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs) and other petroleum hydrocarbons (Chua *et al.*, 2014; Mato *et al.*, 2001; Rios *et al.*, 2007; Teuten *et al.*, 2009). Other pollutants known to sorb into these plastics include heavy metals such as lead, cadmium, zinc and nickel (Holmes *et al.*, 2012; Rochman *et al.*, 2014a) and organic contaminants such as drugs (Fonte *et al.*, 2016; Guilhermino *et al.*, 2018; Qu *et al.*, 2018).

So far, it has been demonstrated that polyethylene (PE) pellets have higher affinity for PCBs than polypropylene (PP), both in the field and laboratory experiments (Endo *et al.*, 2005; Teuten *et al.*, 2007), but the kinetics of different microplastics types and distinct contaminants has not been fully addressed.

Animals exposed to a higher concentration of microplastics with adsorbed chemicals may be at greater risk, because the kinetics may favour the desorption of contaminants from the ingested microplastics to the tissues (Avio *et al.*, 2015; Browne *et al.*, 2013; Chua *et al.*, 2014; Teuten *et al.*, 2007), confirming the hypotheses that microplastics can act as a vector and source of hydrophobic organic contaminants (HOCs) to marine organisms and induce inflammation and/ or toxicity. To date, most laboratory studies used clean organisms exposed to contaminated microplastics (Table 3), which can favour a chemical transfer to the tested organisms (Koelmans, 2015). Several studies so far, showed that the tested chemicals desorbed from the plastic and transferred into animal's tissues. Frequently, the contaminant is transferred into tissues (Browne *et al.*, 2013; Chua *et al.*, 2014; O'Donovan *et al.*, 2018), accumulated (Ma *et al.*, 2016; Wardrop *et al.*, 2016), transferred to the next generation (Batel *et al.*, 2018) or induces damage (Karami *et al.*, 2016; Rainieri *et al.*, 2018; Rochman *et al.*, 2013). But the way these contaminants reach organs or tissues and if it is directly related with microplastics spread and accumulation is not yet very clear.

Most of the available information of transfer of contaminants from microplastics to organisms refers to marine invertebrates, but when it comes to the safety of seafood

ingestion, more work should be done regarding microplastics and associated chemicals in fish since it can pose a risk to human health. Current studies of microplastics and associated contaminants in fish detected concentrations of these compounds in the intestine (Chen *et al.*, 2017; Khan *et al.*, 2015), gills (Batel *et al.*, 2018), liver (Karami *et al.*, 2016; Rainieri *et al.*, 2018; Rochman *et al.*, 2013) and brain (Chen *et al.*, 2017), but none of them addressed concentration of these pollutants in the edible part such as the muscle or the skin.

On the other hand, theoretical studies predict that ingested microplastics contaminated by pollutants would not favour chemical transfer to the tissues because concentrations of these pollutants would be in equilibrium with their environment (Browne *et al.*, 2013). Nonetheless, equilibrium scenarios can be problematic because they assume pollutants and organisms are evenly distributed (Engler, 2012). It has been discussed (Koelmans, 2015) that microplastics ingestion may increase bioaccumulation for some chemicals, such as additives or plasticizers, yet decrease the body burden of these chemicals if they have opposing concentration gradients between plastic and biota lipids (Gouin *et al.*, 2011; Koelmans *et al.*, 2013; O'Connor, 2014). Whether plastic acts as a source or a sink of pollutants depends on the gradient between the chemical concentration in the plastic and the surrounding water. Furthermore, recent modelling studies (Koelmans *et al.*, 2014; Koelmans *et al.*, 2013; Zarfl & Matthies, 2010) have concluded that, given the low abundance of plastic when compared to natural pathways (water, sediment), the contribution of plastic to chemical transport of HOCs in the oceans, and subsequent exposure and bioaccumulation by marine organisms is probably small.

4. Analytical methods

Lab studies that have attempted to trace the pathways of microplastics and associated contaminants uptake have used a wide range of aquatic (including invertebrates and vertebrates) and terrestrial organisms (mice), types of plastic (PS, PE, PVC, PP, PA) and duration of exposure (Tables 1 and 3). Imaging approaches have been mainly used to trace microplastics inside organs and tissues of organisms, such as histological techniques (e.g. Avio *et al*., 2015; Pedà *et al*., 2016; Wright *et al*., 2013a), scanning electron microscopy (SEM) (e.g. Abbasi *et al*., 2018; Murray & Cowie, 2011), Raman (e.g. Van Cauwenberghe *et al*., 2015; Watts *et al*., 2014), optical (e.g. Welden & Cowie, 2016; Devriese *et al*., 2015) and

fluorescent microscopy (e.g. Della Torre, 2014; Lu *et al.* 2016). However, technical limitations have interfered in the comprehension of accumulation, translocation and fate of microplastics, mainly due to the physical characteristics of the particles. To be able to track microplastics inside of a living organism, they must be stained or fluorescently marked in order to be easily identified by advanced microscopy techniques. On the other hand, in order to follow the path and fate of nano and microplastics it becomes necessary to conduct an exposure experiment with a sufficient number of individuals and days, to be able to sample and dissect animals at different stages, which can be quite time consuming.

Concerning histology techniques, since the traditional histology uses solvents and paraffin, which can affect the plastic, the use of cryohistology is suggested by Paul-Pont *et al.* (2018) to avoid this problem. Another thing that needs to be considered is the collection of samples and contamination control (Paul-Pont *et al.*, 2018). Samples should be collected carefully in order to avoid external contamination as rinsed before dissection, to limit the transfer of microplastics located outside of the tissues (Browne *et al.*, 2008). There is also a lack of information on the analysis of tissues of control organisms by microscopy, which would be a valuable comparison between unexposed and exposed individuals in terms of microplastics accumulation (Paul-Pont *et al.*, 2018),

In respect to the associated contaminants to the plastic, most animal tissues are analysed through gas chromatography mass spectroscopy techniques (GC-MS) or High-Performance Liquid Chromatography (HPLC) (Table 3). Regarding the concentration found in animal's tissues, the current methods seem to work very well and give reliable results in terms of chemical concentration. Most of the current literature refers to marine invertebrates and analyzed specific tissues of the organism (e.g. Avio *et al*., 2015; Paul-Pont *et al*., 2016; O'Donovan *et al*., 2018), which is the most valuable thing to do since it is important to understand where these contaminants and additives tend to accumulate, especially when the plastic microparticles acts as a vehicle.

5. Conclusions, knowledge gaps and recommendations for future studies

A large number of organisms are exposed to microplastics with the occurrence, effects and accumulation of microplastics, especially in the aquatic environment, well established (de Sá *et al.*, 2018). Based on experimental data and field observations, there is a clear knowledge gap with respect to the information regarding the surface interactions of

microplastics in the natural environment and their fate and implications to organisms. The influence particle surface can have on the ingestion of microplastics, through the formation of a biological layer of molecules attached to the plastic, or the effect that particle's agglomeration can have on the translocation has not been studied yet. Although considerable progress has been made over the past years, the information referring to the lab exposure experiments conducted so far is still scarce and it seems they are very diverse in terms of experimental design and model organism chosen. The route by which microplastics enter living systems has not yet been identified and the observation of translocation in organisms can be very challenging. There is the need to implement a multidisciplinary approach to assess whether or not microplastics of different types, sizes and shapes can be transferred into tissues of organisms, other than the digestive tract, and then through the food web to humans.

More information on the depuration of microplastics is imperative to understand their consequences to living organisms. Lab exposure experiments with several depuration times should be performed in order to understand if, in fact animals are able to completely eliminate them through egestion or if they stay in the system and, consequently accumulate in different organs or tissues. This is extremely important to assess whether or not, if a long depuration period concerning shellfish, contributes to a crease of the risk of its consumption by other animals of the trophic food web or humans.

It is also necessary to infer if the ingestion of contaminated microplastics enhances the elimination rate by organisms and if depuration is the major modulating factor on the depuration of persistent hydrophobic chemicals in the real environment. Regarding the fate of associated contaminants to microplastics, in the future, it would be interesting to perform bioaccumulation studies with a different perspective to infer the relative importance of microplastics versus sediments/water as vectors for pollutants to animal's tissues and investigate whether microplastics act as a sink of hydrophobic organic compounds (HOCs) in organisms with a high internal concentration of pollutants.

The biggest problem associated with the studies of microplastics accumulation and translocation is the lack of analytical methods to identify these nano and microplastics inside the living systems, especially in situ. More research and development of new and improved methods are needed in the coming years. They will be fundamental to understand the mechanism or mechanisms by which microplastics and associated contaminants operate in organisms.

Most of the studies that show an evidence of nano or microplastics accumulation are based in marine invertebrates, especially bivalves. Surprisingly there are not enough studies

with high commercial value species of seafood. They are part of the human diet, and thus, the incidence of microplastics in the non-digestive tissues of shellfish can have implications to human health through seafood consumption and, consequently, biomagnification. More studies on the translocation and accumulation of nano and microplastics in edible animal parts are needed.

Finally, there is still a major knowledge gap concerning the impact of microplastics on mammals and humans. If microplastics pose a risk to human health or not is still unknown. In fact, it is hypothesized that these particles enter the human body through food, water and dust, but what happens next in terms of particle uptake, inflammation and toxicity is still unknown. As a start, more in vivo animal studies would provide important information to understand the mode of action of microplastics in a living system similar to humans. A different approach such as the growth of human cell lines and their interaction with nano and microplastics would provide insights about translocation and cell uptake.

Based on this review, we have identified some key knowledge gaps that need to be considered, in order to better understand the accumulation, mechanisms and fate of microplastics in organisms:

a) Perform further laboratory studies to understand if the translocation of microplastics is possible and what particle sizes are able to move across the gut into tissues;
b) Understand if microplastics can pass other biological barriers besides the intestinal tract;
c) Collect more data on nanoplastics. Infer if nanoplastics are taken up by cells and if so, what is the cellular mechanism of uptake;
d) Understand the risk associated to nanoplastics accumulation in tissues, in terms of toxic response and inflammation;
e) Understand what is the role of size, shape and eco-corona of nano and microplastics in organism's uptake and accumulation;
f) Perform realistic exposure experiments in respect to the transfer of contaminants associated with microplastics;
g) Development new methods to identify plastic particles in different tissues;
h) Understand what the implication of depuration of microplastics is. Does elimination occur? And if so, how long does it take;

i) Gather more information on microplastics accumulation in species of high level of biological organization such as birds, sea turtles, marine and terrestrial mammals;

j) Perform lab exposure experiments using animal testing;

k) Assess if microplastics are able to accumulate in the human body, namely in tissues and/or specific organs, such as the lungs. Try to understand is there is an inflammatory response induced by microplastics.

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