The need to investigate continuums of plastic particle diversity, brackish environments and trophic transfer to assess the risk of micro and nanoplastics on aquatic organisms

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Abstract

Plastic particles are ubiquitous in marine and freshwater environments. While many studies have focused on the toxicity of microplastics (MPs) and nanoplastics (NPs) in aquatic environments there is no clear conclusion on their environmental risk, which can be attributed to a lack of standardization of protocols for *in situ* sampling, laboratory experiments and analyzes. There are also far more studies concerning marine environments than fresh or brackish waters despite their role in the transfer of plastics from continents to oceans

We systematically reviewed the literature for studies: (1) using plastics representative of those found in the environment in laboratory experiments, (2) on the contamination of plastic particles in the continuum between fresh and marine waters, focusing in particular on estuaries and (3) on the continuum of contamination of plastic particles between species through trophic transfer in aquatic environments. We found that the exposure of aquatic organisms in the laboratory to plastic particles collected in the environment are very scarce. Moreover, plastic exposures of estuarine species in the laboratory are generally carried out for a single salinity and a single temperature that do not reflect the fluctuating environmental conditions of estuaries. Finally, the trophic transfer of plastic particles is mainly studied in the laboratory through simple food chains which are not representative of the complexity of the trophic networks observed in the aquatic environment. We pointed out that future studies in the laboratory should include both MPs and NPs sampled in the environment and focus on the precise characterization of the composition and surface of these plastics as well as on their absorbed pollutants, additives or biofilms. Moreover, investigations must be continued concerning the toxicity of plastic particles in brackish water environments such as estuaries and the trophic transfer of plastic particles in complex food chains.

Keywords: microplastics, nanoplastics, environmental representativeness, estuaries, trophic transfer

Capsule:

This paper presents the current state of knowledge on the environmental representativeness of plastic particles used in laboratory experiments, contamination in estuaries and trophic transfer.

Introduction

Over the past decades, significant research efforts have been made to investigate the sources, composition, effects and fate of plastic particles in aquatic environments (Auta et al., 2017; Rezania et al., 2018). Plastic particles whose size are less than 5 mm have been commonly described as microplastics (MPs) (Barnes et al., 2009; Browne et al., 2007). Recently, smaller plastic particles ranging in size from 1nm to 1000 nm have been defined as nanoplastics (NPs) (Gigault et al., 2018). Both MPs and NPs can come from primary sources, *i.e.* intentionally designed, typically from cosmetics and cleaning agents, scrubbers in air-blasting or plastic waste production (Auta et al., 2017; Browne et al., 2011; Gall and Thompson, 2015; Gregory, 1996; Wang et al., 2016). Secondary sources of MPs and NPs correspond to the fragmentation of larger plastics through physical, biological and chemical processes (Andrady, 2015; Rezania et al., 2018). This plastic debris in aquatic environments is various in composition with the most common polymers being polypropylene (PP), polyethylene (PE) and polyvinyl chloride (PVC) (Sadri and Thompson, 2014; Schwarz et al., 2019). Paradoxically, while freshwater environments represent important transport routes for plastic particles, data on sources and composition of plastic particles is scarce when compared to the marine environment (J. Li et al., 2018; Wagner et al., 2014). In the freshwater environment, the main sources come from treatment plants and runoff from urban, agricultural, tourist and industrial areas (Wagner and Lambert, 2018). Both marine and freshwater organisms can ingest plastic particles by filtration, by adsorption or by consuming contaminated prey (Setälä et al., 2018). However, mechanisms of transfer of MPs and NPs within aquatic food chains are far from understood (Nelms et al., 2018).

A very large number of studies has focused on the toxicity of plastic particles on aquatic organisms, with many more studies focused on marine organisms than those dedicated to fresh or brackish waters. However, there is no clear conclusion on their effects (Bouwmeester et al., 2015; Rios Mendoza et al., 2018). Major difficulties include the lack of standardization of protocols related to *in situ* sampling, laboratory experiments as well as subsequent analyzes (Bouwmeester et al., 2015; Rios Mendoza et al., 2018). Regarding laboratory experiments, plastic particles tested are often not representative of those found in the aquatic environments particularly in terms of size, diversity, composition and possible presence of additives and adsorbed pollutants. Studies on the impacts of plastic particles on aquatic organisms mainly concern MPs with research on NPs being uncommon (da Costa et al., 2016). Moreover,

experiments often focus on short periods and are usually not environmentally representative. Thus, many biochemical biomarkers involved in antioxidant pathways, oxidative damage and neurotoxicity that are commonly used in ecotoxicology respond little or not to plastic particles exposure in aquatic organisms in laboratory tests. Effects on these biomarkers are often evident at high concentrations, generally several fold greater than those found in the environment (Prokić et al., 2019). The concentrations of MPs used in these studies range from a few mg/L to 20 g/L while the range of concentrations measured in surface water is 0.0002-0.51 mg/L (Paul-Pont et al., 2018; Prokić et al., 2019). Nevertheless, it should be noted that plastic particles quantification in aquatic environments is likely to be underestimated because of a lack of adequate methods to detect and quantify the small size fractions (< 1mm) (Erni-cassola et al., 2017; Paul-Pont et al., 2018). For example, using a combined approach with different sampling devices, Dris et al. (2015) quantified nearly 230 times more MPs in a plankton net (80-µm mesh) than in a mantal trawl (330-µm mesh) in the river Seine. Recently, studies on the toxicity of NPs have emerged and, at the moment, there are some controversies concerning ecotoxicological experiments with nanomaterials (Handy et al., 2008). Indeed, the behavior of manufactured NPs in environmental matrices is complex and not fully understood as it may be influenced by different parameters such as particle characteristics (shape, size, surface area and charge) and abiotic factors (pH, salinity, natural organic matter) under laboratory conditions (Handy et al., 2008). Moreover, when the toxicity of MPs and NPs from the environment is tested in the laboratory on aquatic organisms, plastic particles are not often chemically characterized even though they can be associated with additives, pollutants or heavy metals (Yu et al., 2019), leading to possible synergistic effects. Thus, the toxicity of plastic particles must be assessed in their entirety and not through studies which are compartmentalized and/or not representative of the plastic particles found in the environment.

This review focuses on three aspects that should be considered for future studies in risk assessment of plastic particles in the aquatic environment: (1) testing a continuum of sizes of plastic particles ranging from NPs to MPs, favoring particles from the natural environment, (2) studying the impact of plastic particles on the continuum from freshwater to marine waters, in particular by focusing on the interface environments of estuaries and (3) investigating a continuum of contamination by plastic particles between species by trophic transfer along aquatic food chains. Articles which deal with these aspects were selected based on a bibliographic research on Web of Science and PubMed platforms (03/2020)

(Supplementary material S1). The literature search is presented in Figure 1. The total number of reviewed articles is 108.

1. Evaluation of the toxicity of plastic particles mimicking the ones found in the environment: importance of investigating a nano - microplastic size continuum

Research efforts have been made to better understand the effects of plastic particles on aquatic organisms. Most laboratory studies on aquatic species initially focused on the effects of MPs on ingestion, bioaccumulation and key physiological functions. Negative effects were notably demonstrated such as a reduced food consumption (Cole et al., 2015; Murray and Cowie, 2011; Watts et al., 2015), intestinal damages, lesions and microbiota dysbiosis in the gut (Jin et al., 2018; Lei et al., 2018; Pedà et al., 2016), a depletion of energy reserves (Bour et al., 2018; Gardon et al., 2018; Wright et al., 2013) and a decrease of reproductive output (Cole et al., 2015; Sussarellu et al., 2016). Studies regarding the effects of NPs emerged in recent years but are limited when compared to MPs (Ferreira et al., 2019). Studies revealed that NPs ingestion has a high toxic potential and can induce a delay in embryo development (Pinsino et al., 2017), larval malformations (Della Torre et al., 2014), induction of neurotoxic effects and oxidative stress (Gambardella et al., 2017; González-Fernández et al., 2018) and a decrease in fecundity (Lee et al., 2013). In addition, NPs present a high surface area-to-volume ratio and can significantly adsorb higher levels of contaminants like organic toxic chemicals and heavy metals than MPs (Bergmann et al., 2015; da Costa et al., 2016; Velzeboer et al., 2014). While MPs and NPs differ regarding their toxic impacts and behaviour (da Costa et al., 2016), studies characterizing the effects of both MPs and NPs on the same batch of individuals and under the same laboratory conditions are very uncommon. Moreover, most of the plastics used for the exposure of aquatic organisms in the laboratory are virgin plastics, usually in the form of perfectly calibrated spheres and used at concentrations much higher than those measured in the environment (Burns and Boxall, 2018; Paul-Pont et al., 2018; Phuong et al., 2016). Indeed, the concentrations used in laboratory studies which often range from a few hundred mg/L greatly exceed those likely to be found in the environment since the concentration of MPs is estimated to be between 0.4 and 34 ng/L in surface waters in the United States and Europe and the highest concentration estimate for marine waters is 0.51 mg/L (Koelmans et al., 2015). There is a concern as these unrealistically high concentrations of MPs in laboratory exposures may lead to misleading conclusions about the impacts of plastic particles on aquatic ecosystems (de Sá et al., 2018). The use of these plastic

microbeads is increasingly discussed because they are not representative of plastics particles encountered in the natural environment (Gigault et al., 2018, 2016; Phuong et al., 2016). To better reflect the behaviour and effects of plastics found in the environment, different research strategies have been implemented which depend on plastics characteristics: shape, biofouling and contaminants. In addition, plastics extracted from everyday products or collected in the environment were used.

Shape

In environmental samples, microplastics come in a variety of shapes: fibers, fragments, films, foams and pellets (Rezania et al., 2018). Fibers and fragments are the most common debris in seawater samples (Desforges et al., 2014; Ivar do Sul et al., 2013; Lusher et al., 2014; Phuong et al., 2016; Suaria et al., 2020) and fragments, fibers, foams and films in freshwater ecosystems (Strungaru et al., 2019). These irregular shapes sometimes have sharp edges which may increase the physical damages in the gills, intestine and skin (Strungaru et al., 2019) when compared to beads. In the same way, plastics sampled in many aquatic organisms are also dominated by fibers and fragments (Desforges et al., 2015; Phuong et al., 2016; Rezania et al., 2018). To get closer to the plastics found in the aquatic environment, some studies have been carried out to better understand the impacts caused by fibers on aquatic organisms (Table 1). The vast majority of these studies focused on the effects of fibers and reported greater damage induced by fibers in comparison to other shapes (Au et al., 2015; Jemec et al., 2016; Qiao et al., 2019; Watts et al., 2015; Welden and Cowie, 2016; Ziajahromi et al., 2017). For instance, the freshwater zooplankton *Ceriodaphnia dubia* developed carapace and antenna deformities after exposure to polyester (PL) fibers whereas no such effects were observed after exposure to polyethylene (PE) beads (Ziajahromi et al., 2017). Although both types of plastics induced reduction of growth and reproductive output, the effects of the fibers were greater than those of the beads at environmentally relevant concentrations (Ziajahromi et al., 2017). Higher toxicity of fibers was reported on the freshwater amphipod *Hyalella azteca* (Au et al., 2015). In this study, the residence time was shown to be longer for polypropylene (PP) fibers than for PE beads in the gut (Au et al., 2015). Studies related to fiber ingestion also showed that organisms can ingest large number of microfibers and particles much longer than the commercial beads generally used in the laboratory (Hu et al., 2020; Jemec et al., 2016; Vendel et al., 2017; Watts et al., 2015). Jemec et al. (2016) reported that *Daphnia magna* was able to ingest very large twisted fibers measuring 1400 μm (Jemec et al., 2016). Concerning

this organism, fibers have been associated with increased mortality. Long fibers were also observed in the crab *Carcinus maenas,* inducing a reduction of food consumption and a reduction of the energy available for growth (Watts et al., 2015). Interestingly, in this study, authors reported knotting of plastic fibers due to the action of gastric mills (Watts et al., 2015). This balling of plastic fibers has previously been observed in the lobster *N. norvegicus* (Murray and Cowie, 2011). This means that plastic fibers could stay longer in the gut than the beads or even block it depending on the organism's ability to evacuate them. It was also shown that the shrimp *Palaemonetes varians* ingested fibers and beads along with food (Saborowski et al., 2019). Interestingly, while the beads and shortest fibers (up to 100 mm) were egested, the longest fibers (100-4400 mm) were regurgitated. Although a study reported that corals *M. cavernosa* and *O. faveolata* have the ability to recognize and expel both microbeads and microfibers (Hankins et al., 2018), bleached anemones were shown to be less effective at rejecting microfiber plastic than symbiotic anemones (Romanó de Orte et al., 2019). This implies that symbiotic organisms could be particularly vulnerable to plastic fibers when combined with other stressors. Moreover, a study conducted on the impact of microfibers on anemones also revealed that these organisms can react to chemical clues when they are stimulated by the presence of a prey. In this case, while sea anemones only ingest nylon (NY) microfibers in the absence of their prey, they ingest NY, PE and PP microfibers in the presence of their prey (Romanó de Orte et al., 2019). Although plastic fragments have been found in aquatic organisms (Boerger et al., 2010; Rochman et al., 2015; Tanaka and Takada, 2016), studies on the impacts of this form of plastic are rare. However, it has been shown that plastic fragments can be ingested by zooplankton, echinoderms and sea cucumbers (Graham and Thompson, 2009; Vroom et al., 2017). When ingested, fragment aggregates were shown to fill 30-90% of the gut of the copepod *Calanus finmarchicus* (Vroom et al., 2017). In addition, four species of sea cucumbers exhibited ingestion of PVC and NY fragments which was significantly greater than expected (Graham and Thompson, 2009). It was also shown that the number of fragments ingested by the shrimp *P. pugio* was significantly higher than the number of fibers (Gray and Weinstein, 2017). Since it has been shown that the shape of plastics can impact their residence time in organisms and induce various negative effects, there is a strong need to diversify studies on the impacts of plastic particles, including fragments, foam, fibers, films and pellets.

Weathered/biofouled plastics

Another notable difference between microbeads and environmental plastics concerns the surface. The latter is homogeneous and smooth on beads whereas plastics have "aged" in seawater. Indeed, abrasion of the surface occurs because of photodegradation, wave, microorganism and contact with sedimenting particles. Microbial biofilms may develop on the floating plastic in the marine environment as to rapidly cover the surface (Andrady, 2011; Zettler et al., 2013). These biofilms can then lead to the colonization of algae and invertebrates on the surface of the plastic particles (Andrady, 2011; Muthukumar et al., 2011), inducing significant changes in the physicochemical properties of plastics (Lobelle and Cunliffe, 2011). The biological communities present on microplastics can make them more attractive to grazing species and facilitate their ingestion (Vroom et al., 2017). In order to take into account the effect of biofilms, some researchers have soaked plastics in sea water or weathered plastics to enable biofilm formation before offering them to marine organisms (Allen et al., 2017; Bråte et al., 2018; Kaposi et al., 2014; Vroom et al., 2017) (Table 1). Biofouled PS beads were shown to be ingested by higher proportions of copepods *C. finmarchicus* (male and female) and of *A. longiremis* (female) than pristine PS beads (Vroom et al., 2017). In the same way, Bråte et al. (2018) reported higher ingestion of PE biofouled particles than virgin ones in mussel *M. galloprovincialis* (Bråte et al., 2018). Both plastics induced histopathological changes in the gills and digestive gland and necrosis in the mantle with no difference in the degree of tissue alteration between virgin and biofouled particles (Bråte et al., 2018). Conversely, ingestion rate was shown to be higher for unfouled plastics compared to biofouled ones for a sea urchin larvae (Kaposi et al., 2014) and a coral (Allen et al., 2017). Kaposi et al. (2014) hypothesized that biofouling increased the size of the beads and gave rise to the formation of aggregates. Larger sizes of the biofouled beads could reduce sea urchin larvae's attraction to microplastics since larval feeding is closely linked to particle size (Kaposi et al., 2014). Allen et al. (2017) put forward several hypotheses to explain the lower ingestion of biofouled plastics by coral *A. poculata*: the elevated concentration of biofouled plastics compared to unfouled ones may have impacted the ingestion, and/or phagostimulants present on the biofouled plastics may have leached during the preparation of the biofouling before they were offered to the coral (Allen et al., 2017). Another study reported no impact of both PE microbeads and weathered PP particles on embryo development and larval settlement of the coral *A. tenuis* (Berry et al., 2019). The impacts of aged plastics are thus still poorly understood and require further studies. A limitation to previous studies is the lack of characterization of biofilms formed on plastics. Although Bråte et al. (2018) have characterised the presence of a biofilm by FT-IR spectra,

239 they did not observe it by SEM imaging (Bråte et al., 2018). Future studies should verify the
240 presence of the biofilms on plastics and require in-depth characterization. This could be
241 achieved by DNA analysis or through flow cytometry (Sgier et al., 2016).

242 *Plastics associated with contaminants*

243 Microplastics may be potential vectors of chemical contaminants, representing a major
244 concern for the health of aquatic organisms (Brennecke et al., 2016; Cole et al., 2011; Ivar Do
245 Sul and Costa, 2014). Indeed, MPs found in the environment have been shown to efficiently
246 adsorb heavy metals and POPs such as families of polycyclic aromatic hydrocarbons,
247 polychlorinated biphenyls and nonylphenols (Antunes et al., 2013; Endo et al., 2005; Mato et
248 al., 2001). In addition, NPs have a high surface area and may be related to stronger sorption
249 affinities for toxic chemicals and heavy metals than MPs (Bergmann et al., 2015; da Costa et
250 al., 2016; Velzeboer et al., 2014). Several studies have therefore focused on the transfer of
251 contaminants and/or additives into marine organisms (Table 1). They revealed elevated
252 bioavailability of chemicals after the ingestion of MPs, transfer of the pollutants and
253 chemicals to gut tissues and toxicological effects (Avio et al., 2015a; Browne et al., 2013;
254 Gomiero et al., 2018; Guilhermino et al., 2018; Oliveira et al., 2013; Rainieri et al., 2018;
255 Wardrop et al., 2016). Indeed, MP-adsorbed benzo[a]pyrene (BaP) and perfluorooctane
256 sulfonic acid (PFOS) have been shown to induce higher damage in the digestive gland of the
257 clam *Scrobicularia plana* compared to virgin MPs (O'Donovan et al., 2018). In addition,
258 accumulation of persistent, bioaccumulative and toxic substances (PBTs) was reported from
259 fish exposed to a mixture of PE MPs with chemical pollutants sorbed from the marine
260 environment (Rochman et al., 2013). This exposure led to the transfer of pollutants to the
261 liver, inducing toxicity and pathology (Rochman et al., 2013). Research on chronic exposure
262 to polyethylene pellets that had been aged in seawater for several months may have impacted
263 the endocrine system of fish (Rochman et al., 2014). Ultimately, this perturbation may impair
264 reproductive success and threaten wild populations (Rochman et al., 2014). At the opposite,
265 some studies have also reported almost no effects, with little to no contribution of MPs to the
266 bioaccumulation of chemicals (Ašmonaitė et al., 2018; Besseling et al., 2017; Brennecke et
267 al., 2016; Caruso et al., 2018; Devriese et al., 2017; Magara et al., 2018; Paul-Pont et al.,
268 2016; Sıkdokur et al., 2020; Sleight et al., 2017). For instance, exposures of lobsters *N.*
269 *norvegicus* to PE and PS with a mixture of polychlorinated biphenyl (PCB) congeners did not
270 lead to bioaccumulation of the chemicals (Devriese et al., 2017). Paul-Pont et al. (2016)

reported that the transfer of fluoranthene in mussels' tissues was more due to water and food exposure than microplastics exposure in laboratory conditions (Paul-Pont et al., 2016).

The hypothesis that MPs and NPs act as vectors of contaminants has recently been questioned. Indeed, it is difficult to separate the effects attributed to the plastic particles from those due to the baseline contamination levels of seawater and organisms. Moreover, MPs and NPs contributions are very low compared to natural exposure sources such as organic matter, plankton and detritus whose abundance greatly exceeds that of MPs and NPs in the environment (Paul-Pont et al., 2016; Rodrigues et al., 2019). As a matter of fact, the potential role of MPs and NPs as vectors for contaminants after ingestion is far from understood. It is currently difficult, if not impossible, to distinguish *in situ* the effects attributed to pollutants on microplastics from those from other sources. Therefore, laboratories studies should be continued with exposure of key species to plastic type, composition, size, shape and concentrations similar to those found in the environment using adequate controls to assess the toxicity of reference toxicants (Rodrigues et al., 2019).

Plastics collected in the environment

Finally, some pioneering studies aimed to understand the effects of plastics sampled in the aquatic environment (Baudrimont et al., 2019; Gandara e Silva et al., 2016; Nobre et al., 2015; Pannetier et al., 2020) (Table 1). The toxicity of leachates from virgin and beached plastic pellets towards the embryonic development of the brown mussel *P. perna* both induced negative effects (Gandara e Silva et al., 2016). However, embryos exposed to leachates from beached pellets showed a significantly higher proportion of abnormal or dead individuals compared to the virgin pellets. The authors suggested that the beached pellets caused high toxicity because of their additives and adsorbed contaminants whereas virgin pellets only contain additives. In the same way, MPs collected in the environment were shown to induce adverse effects on fish medaka larvae and rainbow trout cell line, such as an increase of DNA damage (Pannetier et al., 2020, 2019a) and modulation of EROD activity induction at environmental concentrations (Pannetier et al., 2020). This toxicity is certainly due to the presence of additives and adsorbed contaminants on the plastics (Pannetier et al., 2020). Another study evaluated the toxicity of virgin and beached plastic pellets on the development of embryos of sea urchin *Lytechinus variegatus* (Nobre et al., 2015). Virgin pellets were shown to cause higher anomalous larval development than beached pellets. In this case, the additives present on the virgin pellets may be more harmful than the pollutants

adsorbed on the beached pellets, supporting the work of Browne et al. (2013). Pellets entering the marine environment could thus pose a significant risk to organisms soon after their introduction (Nobre et al., 2015). In the previous studies with divergent results, the plastic consisted of a mixture sampled in the environment which had not been characterised. The composition of the plastics, the presence of additives and/or contaminants must have had a strong impact on the results and must be characterized. To minimize the effects of different types of plastic, a strategy implemented by Baudrimont et al. (2019) was to prepare nanometric plastic particles, from a single type of plastic, PE, from virgin reference material and from microplastic debris sampled in the North Atlantic gyre (Baudrimont et al., 2019). Environmental NPs were shown to cause higher growth inhibition of the freshwater algae *S. subspicatus* than reference NPs under the same experimental conditions (Baudrimont et al., 2019). In parallel, a characterization of trace metals on the two types of NPs revealed a higher concentration for various metals in environmental plastics compared to those of reference. The presence of metals and other contaminants on environmental NPs could be the cause of the reduction in algae growth. In this study, both types of nanoplastics were also offered to the bivalve *Corbicula fluminea* (Baudrimont et al., 2019). They did not impact their filtration activity. However, feces and pseudofeces production increased after the exposure to nanoplastics collected in the environment and could be a mechanism to discard plastic particles (Baudrimont et al., 2019).

In light of these studies, it is essential to continue exposures of organisms to both micro and nanoplastics sampled in the environment and to focus on the precise characterization of the composition and surface of these plastics as well as on their absorbed pollutants. In addition, further research should be carried out at the interface between freshwater and seawater where environmental conditions are changing and for which data are scarce. Indeed, the major part of plastic debris entering the ocean comes from land (Jambeck et al., 2015; Sadri and Thompson, 2014; Wagner et al., 2014) as rivers represent one of the dominant input of plastics into oceans (Lebreton et al., 2017; Moore et al., 2011). Passing through estuaries, plastics are subjected to significant variations in salinity, temperature or pH which can modify their structure and composition. For instance, salinity and pH have been shown to modify the adsorptive properties of plastics toward trace metals under estuarine conditions (Holmes et al., 2014). The adsorption of trace metals originates from the short-term adsorption of organic matter and from the long-term plastic ageing process (Holmes et al., 2014). Indeed, plastic particles may be degraded by biotic and abiotic processes, resulting in the modification of

their surface (da Costa et al., 2016; Lambert et al., 2014). Among abiotic processes, UV-radiation, changing temperatures and salinity typically observed in estuaries, impact the surface of plastic particles leading to erosion, cracked surface and glazed surface (Bråte et al., 2018; ter Halle et al., 2017). These surface irregularities could enhance the sorption of pollutants (Veerasingam et al., 2016) and biofouling (Acosta-Coley and Olivero-Verbel, 2015) which can in turn modify the toxicity of plastic particles for estuarine organisms.

2. Relevance of investigating effects of micro and nanoplastics through a freshwater-marine continuum: the importance of estuaries

The body of knowledge on the accumulation and the effects of plastics in freshwater and terrestrial systems is much less studied than in marine systems (Thompson et al., 2004; Wagner et al., 2014). The distribution of micro and nano sized plastics within fresh and brackish water is thus poorly known, with the presence of microplastics recently demonstrated in lakes (Sarijan et al., 2020), rivers (Eerkes-Medrano et al., 2015) and estuaries (Morritt et al., 2014; Sadri and Thompson, 2014). Despite the great importance of estuaries in the transfer of plastics from the continent to the marine environment, there are very few studies on the impacts of plastic particles in this environment.

Estuaries form dynamic transition zones between river (freshwater) and ocean (saltwater) environments under the influence of tidal oscillations (Wolanski and Elliott, 2015). They can be divided into three sections: lower, middle and upper estuaries with different environmental characteristics according to a salinity gradient (Barletta et al., 2017). Each of these sections forms a habitat that can move horizontally depending on the variation of seasonal precipitations or human intervention (Barletta et al., 2008). Estuaries are ecologically important since they provide habitat, protection, food, reproduction sites and recruitment areas for highly diverse species including marine, estuarine and freshwater organisms. Most plastic particles being produced on land and transported to the oceans by estuaries, species living on the estuaries are at the forefront of plastic pollution. In addition, estuarine organisms and especially freshwater ones are particularly vulnerable to MPs and NPs since they are generally unable to extend their range contrary to marine organisms (Prokić et al., 2019). Estuarine species are tolerant to variations in temperature, salinity and organic matter typical of estuaries. They are also exposed to high quantities of plastic particles which can be

369 modified due to the environmental variations and yet the impacts of MPs and NPs on these
370 species are still poorly understood (Vendel et al., 2017).
371
372 In recent years, MPs have been identified in various estuarine systems around the world
373 through quantification in different tissues in organisms (Table 2): In Europe (Portugal:
374 Montego and Tagus estuaries (Bessa et al., 2018; Vandermeersch et al., 2015; Wójcik-
375 Fudalewska et al., 2016); Italy and Spain: the Po and Ebro estuary respectively
376 (Vandermeersch et al., 2015); England: Thames estuary and three estuaries in the eastern
377 English Channel (Kazour et al., 2018; Mcgoran et al., 2018), in the Persian Gulf Musa estuary
378 (Abbasi et al., 2018)), in Asia: Yangtze and Pearl River estuary (H. X. Li et al., 2018; Su et
379 al., 2018); in America: United States: Florida, Georgia, Charleston harbor and Chollas Creek
380 (Keisling et al., 2019; Payton et al., 2020; Sinicrope Talley et al., 2020; Waite et al., 2018);
381 Brazil: Goiana estuary and Laguna Estuarine System (Dantas et al., 2019; Ferreira et al.,
382 2018, 2016; Possatto et al., 2011; Silva et al., 2018), Amazon estuary (Pegado et al., 2018),
383 Paraiba and Mamanguape estuary (Vendel et al., 2017); Argentina: La Plata and Bahía Blanca
384 estuary (Arias et al., 2019; Fernández et al., 2019; Pazos et al., 2017; Villagran et al., 2019);
385 in Colombia: Ciénaga Grande de Santa Marta estuary (Calderon et al., 2019); in Australia
386 (Halstead et al., 2018); in India (Dowarah et al., 2020) and in South Africa (Naidoo et al.,
387 2019). MPs detected in these studies are of various origins coming from both primary and
388 secondary sources, of different shapes (filaments, fibers, nylon, particles, films) and have
389 sizes ranging from 1 µm to less than 5 mm. The sources of MPs can be identified either by the
390 nature or the relative abundance of plastics. For example, raw plastics (fragment and hard
391 plastics) have been found in the Goiana estuary where fisheries are responsible for a
392 significant portion of marine debris (Possatto et al., 2011). The absence of primary MPs but
393 the abundance of secondary fragments in other estuaries suggests that the origin comes from
394 the decomposition of household items. These particles are particularly present in wastewater
395 discharges (Browne et al., 2011) and in manufacturing plant effluents (Hays and Cormons,
396 1974). Rivers are important vectors of these plastic particles to the ocean. Therefore, the role
397 of freshwater and brackish (estuary) water systems as transport pathways must be considered
398 in plastic pollution studies. The link between marine pollution and estuaries is evident for
399 several types of pollutants, both from land-based sources and stormwater. One of the few
400 studies looking at plastic flows into and out of an estuary suggests that the Tamar River (UK),
401 during the spring and summer period, was neither a source nor a sink, with identical particle
402 input and output (Sadri and Thompson, 2014). It should be noted that the Thames Estuary is

not densely populated, and estuaries receiving inputs from highly industrialized and/or densely populated watersheds can be expected to contribute to high densities of MPs to the ocean.

Field studies related to estuaries showed that MPs contaminate various estuarine species (Table 2). In the fish studies, between 4.9-100% of the individuals ingested MPs. The crustacean studies showed that the individuals collected contained approximately 1.5 MP per gram for the shrimp *P. semisulcatus* (Abbasi et al., 2018) and 4.2 pieces/individual for the crab *P. herbstii* (Waite et al., 2018). In addition, some crustaceans ingest MPs of varying sizes, ranging from 0.5 to 5mm and < 0.2mm to 1.5 mm for the crabs *Eriocheir sinensis* an *Neohelice granulata* respectively (Villagran et al., 2019; Wójcik-Fudalewska et al., 2016). Finally, MPs were also observed in zooplankton and different species of bivalve (Dowarah et al., 2020; Fernández et al., 2019; Keisling et al., 2019; H. X. Li et al., 2018; Payton et al., 2020). In the vast majority of these studies the MPs were extracted from the digestive system (Table 2). However, the transfer of plastic particles in the digestive system or plastic particles adhering to other tissues such as the gills should be considered external to the body (Triebskorn et al., 2019). Some studies reported the translocation of MPs from the gut to the liver and the kidney in different aquatic species such as crustaceans, mollusks, sea urchin and fish while others demonstrated that after ingestion, plastic particles pass only through the digestive system before being eliminated (Abbasi et al., 2018; Avio et al., 2015b; Browne et al., 2008; Elizalde-Velázquez et al., 2020; Jovanović et al., 2018). It therefore appears important to study the rates of ingestion and excretion of plastic particles concomitantly in order to provide a relevant interpretation of the presence of these particles in organisms (Burns and Boxall, 2018). In addition, identifying the presence of MPs in other tissues inside organisms would provide a better understanding of the mechanisms underlying the translocation of plastic particles and its effects. At present, there are only a few studies on the exposure of estuarine species to plastic particles. Most of these studies were based on the quantification of MPs *in situ* or in the contents of the digestive systems. Although there are methodological issues to quantify NPs, future studies should be conducted to quantify them, at least in different tissues of estuarine species.

In the field of estuarine water, very few plastic exposures have been carried out in the laboratory to better characterize the impacts of MPs and NPs on organisms (Table 3). Early investigations demonstrated various negative effects such as a change in sodium and calcium

ions regulation, a reduction of the predatory performance and a decrease in coelomocytes viability in crab, fish or ragworm (de Sá et al., 2015; Miranda et al., 2019; Oliveira et al., 2013; Revel et al., 2020; Watts et al., 2016). In the study from Oliveira et al. (2013), an inhibition of AChE activity was measured in the estuarine fish *P. microps* exposed to MPs at a concentration of 18.4 µg/L which is consistent with the concentration measured in estuary surface water which is around 51 µg/L (Zhao et al., 2014). Current studies are generally carried out for a single salinity and a single temperature which often correspond to the salinity and temperature of the natural environment at the time of the sampling of the organisms (Table 3). However, plastic particles react with the aquatic environment. Water surface area, local topography, depth, wind, currents and particle density are all factors that determine the transport and fate of particles in aquatic systems (Eriksen et al., 2013; Vermeiren et al., 2016). Indeed, the behavior of plastic particles in environmental matrices is complex and may be influenced by the surrounding environment (Handy et al., 2008). This may be particularly important at the junction between the marine and freshwater parts. Some studies focused on the effects of water chemistry on plastic particles (Bakir et al., 2014; Guo et al., 2019; Rodrigues et al., 2019; Zuo et al., 2019). For instance, salinity can accelerate the aggregation of different types of NPs (Wu et al., 2019). These aggregates are less mobile but may be uptaken by organisms living on the sediment and by filter feeders (Farré et al., 2009). Thus, the behavior of MPs and NPs is likely to be different in marine and freshwater environments (Handy et al., 2008) and therefore in the different dynamic zones forming estuaries. Studies also revealed that environmental parameters could modulate the relationship between plastics and contaminants, especially temperature, pH and salinity (Hartmann et al., 2017; Rodrigues et al., 2019). For instance, the sorption of phenanthrene decreased for four different MPs as dissolved organic matter increased (Zuo et al., 2019). Moreover, the sorption capacity of sulfamethoxazole has been shown to decrease with the increase of pH and salinity for six different types of MPs (Guo et al., 2019). In the same line, the sorption of perfluorooctanesulfonate (PFOS) was shown to be favored in seawater (Wang et al., 2015). In this last study, authors also pointed out that the sorption of two perfluorochemical was stronger for PE and PVC than PS (Wang et al., 2015). This means that the sorption process seems to be highly dependent on both the properties of MPs and NPs and the water chemistry. This subject requires further studies given that the contamination of estuaries by pollutants is generally higher than in the open sea (Dauvin, 2008; Kennish, 2002). Moreover, authors demonstrated that toxicological interactions between MPs and pollutants (pyrene and chromium) induced a decrease of energy, an increase of oxidative damages and a reduction of

the predatory performance for estuarine species (Table 3, (Luís et al., 2015; Oliveira et al., 2013). Interestingly, environmental conditions may also influence the predatory performances (de Sá et al., 2015). In this study, the authors hypothesized that differences of environmental conditions during the developmental phases of fish from two distinct estuaries led to differences in predatory performance and efficiency. Fish sampled in an estuary with higher pollution level showed a reduced capability in discriminating MPs from their natural prey in laboratory compared to fish from a less polluted estuary (de Sá et al., 2015).

From these studies, it appears urgent to continue research efforts related to the impact of MPs and NPs on estuarine organisms. In order to assess these impacts more accurately, one approach would consist in exposing individuals to variations of salinity, temperature, pH and dissolved organic matter corresponding to that encountered along an estuary. Moreover, estuarine ecosystems support juvenile forms of many coastal species and complex fish populations resulting from a combination of freshwater and marine species. To improve knowledge of the impacts of plastics on the freshwater-seawater continuum while conducting more realistic studies from an environmental point of view, several avenues must be considered: increase the number of plastic exposures on species living in brackish water in parallel to the ones living in marine or freshwaters, carry out plastic exposures on the same species on gradients of environmental parameters similar to that found in estuaries which condition the behavior and bioavailability of plastics and conduct studies including different stages of development of species and particularly juveniles which are numerous in estuaries which could be more sensitive to plastics than adults.

3. Relevance of investigating the effects of micro and nanoplastics through a trophic continuum

Plastic particles can be ingested either directly by organisms, by filtration, by adsorption or by the consumption of preys already contaminated by plastics (Setälä et al., 2018). The feeding strategy and the trophic level of a species can greatly influence the consumption of MPs and NPs.

Aquatic ecosystems support numerous food webs that are composed of a large variety of organisms with different feeding strategies, occupying different ecological niches and having distinct roles in food web dynamics. In order to assess the impact of environmental

505 contamination by plastic particles, it is necessary to understand how these particles are
506 transferred between species within an ecosystem. In fact, trophic transfer has been shown for
507 other types of contaminants, such as POPs. Primary species may be directly exposed to
508 environmental contamination, thus integrating these pollutants and transferring them to their
509 natural predators, cascading through the trophic chain and even biomagnifying (Fisk et al.,
510 2001; Kelly et al., 2007; Verhaert et al., 2013). Therefore, direct exposure of a single species
511 to plastics in the laboratory cannot properly represent the exposure in the environment. The
512 latter is the sum of many potential sources, correlated with the complexity of trophic
513 interactions. Direct absorption of plastic has been studied and reported for a wide range of
514 aquatic species (Hays and Cormons, 1974; Jeong et al., 2017; Watts et al., 2016), but indirect
515 absorption by trophic transfer is still poorly understood. It becomes particularly relevant to
516 better understand this process since Nelms et al. (2018) have shown empirical evidence of
517 trophic transfer of MPs from fish to a top marine predator. In this perspective, recent studies
518 have described the trophic transfer of plastic particles quantitatively and/or qualitatively
519 (Table 4). These studies are related in particular to the transfer of MPs and NPs through
520 simple food chains, which is the first step to understand the role of trophic transfer in the
521 plastic contamination of an ecosystem. In these experiments on simple food chains, the
522 transfer is evaluated on one predator after the ingestion of prey exposed to plastic particles.
523 According to these studies, what is generally observed is that MPs pass through the intestine
524 of the exposed prey and are mostly excreted within 72 hours. Although trophic transfer has
525 been demonstrated, this type of study does not make plastic particles good candidates for the
526 measurement of bioaccumulation (Batel et al., 2016; Farrell and Nelson, 2013). However, the
527 duration of these studies is currently discussed because the exposure of organisms *in situ*
528 takes place over longer periods. Thus, plastic particles would be available in the intestine and
529 therefore potentially transferable to the upper trophic level. Studies carried out *in situ* support
530 this hypothesis and have shown that species in higher trophic levels present plastic particles in
531 their digestive tracts which were very probably accumulated during their life (Vendel et al.,
532 2017; F. Zhang et al., 2019). In addition, other transfer pathways for plastic particles may
533 exist such as the one recently highlighted between a seaweed and a marine herbivore (Gutow
534 et al., 2015). Authors showed that the seaweed *F. vesiculosus* can retain suspended MPs on its
535 surface, with the number of MPs that adhered to the algae correlated with the concentrations
536 of suspended particles in the water (Gutow et al., 2015). Moreover, they observed MPs in the
537 stomach and in the gut of the herbivore gastropod *Littorina littorea* after feeding on these
538 contaminated algae. They notably observed that *L. littorea* did not discriminate a

contaminated seaweed from a pristine one, meaning that this may be a significant transfer pathway of MPs from water to numerous marine benthic herbivores. The authors also observed that the majority of particles were egested within a week, which would potentially represent a low risk of accumulation of plastic particles in these organisms. However, this raises an interesting question about coprophage species, which may be particularly exposed to high concentrations of plastic particles inside feces. These species could represent a significant vector of plastic particles which remains to be explored. These examples show the diversity of sources of transfer of plastic particles in ecosystems, arguing for an approach based on more complex food chains.

There are currently very few studies taking more than two trophic levels into account while providing crucial knowledge. Santana et al. (Santana et al., 2017) were one of the first to demonstrate a transfer of microplastics (10 µm) between a prey and two predator species (mussel *Perna perna*, crab *Callinectes ornatus* and fish *Spheoeroides greeleyi*). Although a transfer of MPs from the prey to both predators was observed, the particles did not persist in tissues and gut cavity after a depuration time of 10 days. Particles only remained in the faeces of both predators. However, in this study, when offered to predators, preys had already cleared their guts and the MPs were only present in the hemolymph. The authors thus point out that the impact of plastic particles could be more harmful when predators ingest the prey immediately after their contamination by MPs (Santana et al., 2017). Another major criterion to consider is the size of plastic particles used for the experiments. Elizalde-Velázquez et al. (2020) studied the trophic transfer of microplastics in a food chain composed of three levels (Elizalde-Velázquez et al., 2020). The first level, green algae *Raphidocelis subcapitata* (8-10 µm), and the MPs that were used (6 µm) were similar in size so the absorption of MPs did not occur. Trophic transfer was evidenced between the two other levels represented by zooplankton *Daphnia magna* and fathead minnow *Pimephales promelas*. *D. magna* were exposed to a mixture of algae + MPs for 3 hours to reach two concentrations: 20 and 20,000 particles.mL^{-1}. Fathead minnow were then fed once a day during 5 days with *D. magna* previously exposed to MPs. This highlights the usefulness of testing a size continuum from nano to microplastics in these complex food chains. Indeed, the first trophic levels, because of their size, will not absorb the same plastic particles as the higher trophic levels. They absorb NPs and most likely transfer them to higher trophic levels but this is still very little understood and requires further investigation. In that respect, Chae et al. (2018) observed a transfer through four trophic levels using polystyrene NPs (51nm). Interestingly, they

observed an uptake from the first trophic level (alga *Chlamydomonas reinhardtii*) and a transfer at each level until the last trophic level (fish *Zacco temminckii*) of their food chain. Furthermore, they reported major toxic effects such as behavior disruption and histopathological changes in the liver of *Z. temminckii*, suggesting a biomagnification through the experimental food chain. Although tested NPs concentration (50 mg/L) was higher than those encountered in the natural environment, the authors hypothesized an accumulation of NPs in organisms that are exposed to them for a long time in the environment (Chae and An, 2017). Finally, another pathway of plastic particles transfer has recently been highlighted with the transformation of microplastics into nanoplastics by digestive fragmentation within the Antarctic krill *Euphausia superba* (Dawson et al., 2018). Authors compared the size distribution of particles from their stock suspension to the particles size in the krill and in the egested faecal pellets. They found that the mean particle size in the krill was on average 78% smaller than in the stock suspension. This transformation could impact the bioavailability and biomagnification of these triturated particles.

Finally, a concern has recently emerged regarding the impact of pollutants adsorbed on plastic particles which could be transferred within food webs. The property of plastic particles to adsorb pollutants such as POPs has been demonstrated. In addition, POPs are known to be responsible for bioaccumulation and biomagnification within ecosystems (Kelly et al., 2007). This could represent a key aspect of the impact of plastic contamination on ecosystems requiring further investigation in the future. Some studies have already tackled this perspective such as Batel et al. (2016) (Batel et al., 2016) which showed that benzo[a]pyrene (BaP) adhering to microplastic particles (polymer of undisclosed composition and PE of size 1-20µm, BaP 252µg/L) was transferred within an artificial food chain from the zooplankton *Artemia nauplii* to the zebrafish *Danio rerio.* The majority of the particles passed through the intestine and were excreted after 5h to 6h. BaP was shown to desorb from microplastics and then to transfer to *Artemia* and further to the zebrafish. Consistent with these results, Athey *et al* .(2020) (Athey et al., 2020) have shown a transfer of DDT from unicellular tintinnid (*Favella spp.*) to larval inland silversides (*Menidia beryllina*) through microplastics. Interestingly, they observed that silverside larvae exposed directly to MPs ingested fewer MPs than those exposed to contaminated preys. Moreover, silverside larvae ingested more ciliates exposed to DDT-treated MPs than ciliates exposed to untreated MPs. This could be explained by a disturbance of prey behavior caused by DDT, making them more sensitive to predation and thus enhancing plastic trophic transfer. Similarly, another study reported

607 significantly lower predation rates of *L. balthica* larvae by the cockle *Cerastoderma edule* for
608 prey contaminated with MPs (PS, 100,000 MPs/mL, one-hour incubation) than those that had
609 not ingested MPs (Van Colen et al., 2020). A disruption in larvae swimming behavior from
610 MPs ingestion may reduce their filtration risk (Van Colen et al., 2020). These behavioral
611 disturbances complicate the understanding of plastic trophic transfer and may differ
612 depending on the species and the properties of MPs or NPs. Additional research is needed to
613 elucidate the effects of plastic particles and their contaminants on prey-predator interactions.
614
615 Thus, at present, there are very few studies on the trophic transfer of plastic particles. In
616 general, as with direct plastic exposures to organisms, most studies focus on the impact of a
617 single type and size of plastic particles, which is not representative of the diversity of plastics
618 sampled in the environment (Gray et al., 2018; Zhao et al., 2015). In fact, different types and
619 sizes of plastic particles have been shown to produce different deleterious effects on
620 organisms. Depending on their properties, the plastic particles could be transferred *via*
621 different mechanisms and would not accumulate in the same way in organisms. For instance,
622 it has been demonstrated, under the same laboratory conditions, that the size of the plastic
623 particles influence their ingestion and
624 egestion for the crustacean *Daphnia magna* (Kennish, 2002), with the nanometer-sized
625 particles potentially more hazardous than the micrometric ones. In addition, to our
626 knowledge, there is only one study evaluating a trophic transfer on the nanometric scale,
627 demonstrating a strong potential for biomagnification and toxic effects. It therefore appears
628 necessary to develop future studies on plastic trophic transfer using a size continuum, from
629 nano to microparticles. NPs could notably enter the food chain from the first levels and play a
630 major role in the trophic transfer of plastics. Moreover, current experiments are usually
631 conducted for a very limited time (not more than 21 days), which may prevent observing the
632 effects of chronic exposure and long-term accumulation. This is particularly true in the
633 context of a food web since biomagnification is most likely to be a long-term environmental
634 process, occurring under chronic exposure. Finally, the trophic transfer of plastic particles is
635 mainly studied through simple food chains which are not representative of the complexity of
636 the trophic networks observed in the aquatic environment. Future studies related to plastic
637 trophic transfer should mimic more closely *in situ* conditions, in particular by using complex
638 food chains and plastic particles representative of those found in the field. In addition,
639 samples could be collected directly *in situ* from organisms of different trophic levels,

associated with a fine characterization of the plastics found in tissues and the study of environmental factors determining their bioavailability.

Conclusion

There is an urgent need to conduct more realistic experiments from an ecological point of view to better assess the risk of MPs and NPs in aquatic environments. It would be helpful to use plastic particles, from nano to micro size, sampled in natural environments and to characterize these particles (composition, pollutants, additives, biofilms…). Many investigations must be continued, in particular concerning the toxicity of plastic particles in brackish water environments such as estuaries and the trophic transfer of plastic particles in complex food chains. To this aim, Barletta et al. (2020) proposed a methodology to better understand the relationships between MPs contamination, the seasonal patterns of habitats by species belonging to different trophic levels and their feeding ecology along a riverine-estuarine-coastal food web (Barletta et al., 2020). They describe sampling strategies and protocols that could provide a framework for future studies.

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Table 1. Plastic exposures aiming at better reflect the behavior and effects of plastics found in the environment (MPs: microplastics, PE: polyethylene, PP: polypropylene, PL: polyester, PET: polyethylene terephthalate, NY: nylon, PVC: polyvinyl chloride, PS: polystyrene)

	Organisms	Species	Micro/nano plastics type	Micro/nano plastics concentration	Major(s) finding(s)	References
Shape	Amphipod	*Hyalella azteca*	PE beads, 10 to 27 µm diameter PP rope fibers, 20 to 75 µm length	Acute exposure : 0 – 100,000 MPs particles/mL Chronic exposure : 0 – 20,000 MPs particles/mL	PP fibers more toxic than PE beads Reduced growth with PP fibers Longer residence times for the PP fibers than PE beads in the gut	(Au et al., 2015)
	Crab	*Carcinus maenas*	PP rope fibers, 1−5 mm length	Plastic microfibers added to the feed: 0.3% (0.6 mg), 0.6% (1.,2 mg) and 1% (2 mg) added to 2 g of the feed. Animals were placed in individual tanks of 2L.	Reduced food consumption Reduction in energy available for growth Balling of fibers in the foregut	(Watts et al., 2015)
	Crustacean	*Daphnia magma*	PET textile fibers, 62-1400 µm length	A test with a range of concentration 12.5 – 100 mg/L A test with four repetitions of the highest concentration 100 mg/L	Ingestion of long fibers up to 1400 µm Increased mortality	(Jemec et al., 2016)
	Crustacean	*Nephrops norvegicus*	Fibers (3-5 mm in length and approximately 0.2 mm in diameter).	Five fibers per feeding during 8 months: potential exposition to 360 fibers over the experimental period.	Reduction in feeding rate, body mass, and metabolic rate as well as catabolism of stored lipids in plastic contaminated animals in comparison to the fed and unfed groups.	(Welden and Cowie, 2016)
	Shrimp	*Palaemonetes pugio*	11 sizes of plastic; spheres (30, 35, 59, 75, 83, 116, and 165 µm), fragments (34 and 93 µm), and fibers (34 and 93 µm)	50,000 particles/L for three hours	The number of fragments (22.23±9.57 particles/shrimp) within the gut was significantly higher than the spheres and fibers, and the number of spheres within the gut (9.0±13.55 particles/shrimp) was significantly higher than the number of fibers (4.12±6.27 particles/shrimp) Mortality was significantly higher at 93 µm fibers	(Gray and Weinstein, 2017)

				than other sizes tested	
Fish	*Danio rerio*	Pristine low-density polyethylene (LDPE) fragments	5, 50, or 500 µg/L for 10 or 20 days	No significant changes were observed in any of the selected biomarkers across MP concentrations at days 10 or 20.	(Karami et al., 2017)
Crustacean	*Ceriodaphnia dubia*	PE beads, 1-4 µm diameter PL textile fibers, 25 to 1150 µm length	Single acute exposure: animals exposed to a concentration range of 0.5 to 16 mg/L of PE beads and 0.125 to 4 mg/L of PE fibers.	Greater toxicity of PL fibers compared to PE beads. Carapace and antenna deformities with PL fibers.	(Ziajahromi et al., 2017)
Coral	*Montastraea cavernosa* *Orbicella faveolata*	PE beads, 425–500 µm diameter PL fibers, 3–5mm length	30 mg/L	No difference in ingestion or retention times between microbeads and microfibers	(Hankins et al., 2018)
Fish	*Barbodes gonionotus*	PVC fragments Approximately 10% of the particle size was less than 40 mm, 50% less than 140 mm, and 90% less than 310 mm	0.2, 0.5 and 1.0 mg/L for 96 h	No histopathological damage to internal tissue or gills after 96 h. MPs found in the proximal and distal intestine, and in fish exposed to 0.5–1.0 and 1.0 mg/L. Identified MPs associated with localized thickening of the mucosal epithelium	(Romano et al., 2018)
Mussel	*Mytilus edulis*	PET fibers	From 3 to 30 microfibers/mL	Filtration rates was significantly higher for mussels that were not exposed to fibers than mussels that were exposed to PET fibers. No significant difference in the condition index among mussels exposed to different microfibers concentrations or between exposed versus non-exposed mussels.	(Woods et al., 2018)
Copepod	*Calanus finmarchicus*	NL MPs granules (10–30 µm) or fibers (10 × 30 µm)	~50 MPs/L	Exposure to NY fibers: a nonsignificant 40% decrease in algal ingestion rates Exposure to NY granules: nonsignificant lipid accumulation	(Cole et al., 2019)
Copepod	*Calanus helgolandicus*	PE microspheres NY and PET fibers NY fragments	100 MPs/mL	Exposure to NY fibers induced a 6% decrease in ingestion of similar shaped algae Exposure to NY fragments led to an 8% decrease in ingestion of an algae similar in shape and size	(Coppock et al., 2019)

Clam	*Corbicula fluminea*	Experiment 1: microfibers from six different polymers: black polyester-amide (PEA), red polyester (PET), black acrylic (AC), blue polyamide (PA), red rayon (RA) and white polyvinyl alcohol (PVA). Experiment 2: PET fibers of different size classes	100 and 1000 items/L	Experiment 1: organisms ingest more PET (4.1 items/g) than other polymers. Experiment 2: The fibers extracted from the clams were more likely to be curved than fibers before exposure The uptake of fibers belonging to any size class was greater in the 1000 items/L exposure treatments than that in the 100 items/L exposure treatments	(Li et al., 2019)
Fish	*Danio rerio*	Fluorescent and pristine PS MPs beads Pristine microplastic fragments Pristine PS MPs fiberts	Exposure to 20 mg/L pristine MPs of three different shapes and the control group	Shape-dependent accumulation of MPs in the gut was observed with the order of fibers > fragments > beads. Fibers induced more severe intestinal toxicity than fragments and beads.	(Qiao et al., 2019)
Sea anemone	*Aiptasia pallida*	NY, PL and PP rope fibers, 50-1000 µm length	10 mg microfibers/L	Higher percentage of nylon ingestion compared to the other polymers in the absence of chemical cues of prey 80% of all plastic types ingested in the presence of prey	(Romanó de Orte et al., 2019)
Fish	*Carassius auratus*	PE fragments, films, and filaments 0.5–2 mm	100 items/L with 0.3 g fish feed for one hour	In the presence of food, significantly higher amounts of microplastic films were ingested compared with fragments and filaments.	(Xiong et al., 2019)
Crab	*Emerita analoga*	PP rope fibers	Three MPs fibers/L	Microfibers increased adult crab mortality, and decreased retention of egg clutches, causing variability in embryonic development rates.	(Horn et al., 2020)
Fish	*Oryzias latipes*	Commercially dyed green PL fibers (PES, 10–20 µm diameter) and transparent PP fibers (50–60 µm diameter)	PES or PP microfibers (10,000 MPs/L) for 21 days.	No changes in body condition, gonadosomatic- or hepatosomatic indices. PES exposure: no reproductive changes. PP exposure: females produced more eggs over time. Microfibers exposure did not affect embryonic mortality, development, or hatching	(Hu et al., 2020)

	Sea cucumber	*Apostichopus japonicus*	PL microfibers	Exposure to microfibers from the water: 0.003 g/L and 0.006 g/L Exposure from the ingested sediment: concentrations of 0.6 MFs/g,1.2 MFs/g and 10 MFs/g	Water exposure: organisms ingested the MPs along with water during respiration. The MPs got stuck in the branches of the respiratory tree. At 72 h post-transfer to the sand-filtered water, the MPs were persisted in the coelomic fluid. Sediment exposure: organisms ingested the feed with MPs but the MPs were not found to transfer from the water to the coelomic fluid even up to 60 days of exposure.	(Mohsen et al., 2020)
Weathered/ biofouled plastic	Sea urchin	*Tripneustes gratilla* larvae	PE beads fouled by biofilm, 10–45 μm diameter	500 beads/mL	Ingestion rates reduced by biological fouling and in the presence of phytoplankton food	(Kaposi et al., 2014)
	Crab	*Uca rapax*	PS pellets submersed at a polluted and a pristine site near Niterói, Brazil, for 2 weeks. Crabs were exposed for 2 months to fragments (180-250 μm) derived from these pellets	Lagoon sediment (ls) + 108 mg of fragments from pellets per kg dry sediment previously deployed in the pristine site; ls + 108 mg of fragments from pellets previously deployed in the polluted site; ls + 1000 mg of fragments from pellets previously deployed the polluted site; (iv) ls without fragments.	MPs observed in the stomach, gills and hepatopancreas. No difference in fragment retention between the sites and the quantity of MPs in the sediment.	(Brennecke et al., 2016)
	Coral	*Astrangia poculata*	Microbe-free standard HDPE and LDPE pellets, pre-production pellets of PP, PET, PC, PVC and PS, 500–1000 μm diameter Mixture of weathered (sunlight, aride climate and occasional rain) pre-production pellets of PS, LDPE and HDPE: unfouled vs bio-fouled,	500 mg of either unfouled or bio-fouled plastic in a feeding chamber filled with 1.62 L of aged seawater and containing 13–17 colonies of *A. poculata*	Ingestion of all plastic types Very low response of cnidocytes and very low consumption for organic-free sand compared to plastics Higher ingestion rate of unfouled plastics compared to bio-fouled ones	(Allen et al., 2017)

		125–1000 µm length			
Zooplankton	*Calanus finmarchicus* *Pseudocalanus spp.* *Acartia longiremis* Decapod larvae (indet.)	PS beads, 15 and 30 µm diameter PS fragments (pulverization of granules on beads), <30 µm length Aged PS microbeads (3 weeks in filtered seawater to enable biofilm formation), 15 µm diameter	Ingestion of MPs fragments: approximately 100 fragments/mL Effect of aging on MPs ingestion: 100 particles/mL (0.19 mg/L) for *C. finmarchicus* and 200 particles/mL (0.38 mg/L) for *A. longiremis*	Ingestion of PS beads dependent on taxon and plastic size Ingestion of PS fragments by *Calanus finmarchicus* Aged PS beads ingested by higher proportions of *Calanus finmarchicus* (male and female) and of *Acartia longiremis* (female) than pristine PS beads.	(Vroom et al., 2017)
Mussel	*Mytilus galloprovincialis*	PE extracted from a toothpaste Virgin PE, 50 – 590 µm length Weathered PE (simulator basin with continuous intake of sea water for 21 days), 50–590 µm length	0.01 mg/mL	Higher ingestion of weathered particles than virgin considering PE dosed by weight but not regarding particles number. Tissue alteration cause by both plastic types. No difference in the degree of tissue alteration between virgin and weathered plastics	(Bråte et al., 2018)
European sea bass adult	*Dicentrarchus labrax*	Pre-production PVC pellets in the form of native microplastics (MPV) and after being deployed for 3 months in the Milazzo harbor (MPI)	90 days with three different treatments: a control food containing no added MPs (CTRL), and two food experimental treatments, containing 0.1% (w/w) of native microplastics (MPV) and weathered PVC microplastics (MPI), respectively.	Particulate organic matter was lower in MPI than MPV and CRTL. No severe affection of bacterial metabolism by MPs although enzymatic activities decreased and microbes utilized a lower number of carbon substrates in MPI than MPV and CTRL.	(Caruso et al., 2018)
Coral gametes and larvae	*Acropora tenuis*	PE beads: 1 µm and 6 µm Weathered PP particles: 0.5-2 mm^2	Beads: 25, 100 and 200 microbeads L^{-1} Weathered Particles: 5, 15 or 50	Fertilisation negatively affected by the 2 mm^2 weathered MPs. No impact of both MPs type on embryo development and larval settlement	(Berry et al., 2019)

				polypropylene pieces/L		
Plastics associated with contaminants	Lungworm	*Arenicola marina*	Clean sand Sand with MPs that was presorbed with pollutants (nonylphenol and phenanthrene) and additive chemicals (Triclosan and PBDE-47)	Sand with 5% MPs	Pollutants and additives transfer via desorption from both sand and MPs to the tissues Extent and rate of desorption was much greater from sand than from plastic Reduced survival (Triclosan), reduced feeding (Triclosan and PBDE), reduced immunity (nonylphenol), and reduced antioxidant capacity (PVC)	(Browne et al., 2013)
	Fish	*Pomatoschistus microps* juveniles	Exposure to pyrene with or without polyethylene microspheres, 1-5 µm	Exposition to pyrene (20 and 200 µg/L) in the absence and presence of MPs (0, 18.4 and 184 µg/L).	MPs: delay pyrene-induced death and increase the pyrene metabolites concentration in the fish bile. Pyrene–MPs combination: reduced IDH activity, not observed following exposure to the two stressors separately	(Oliveira et al., 2013)
	Fish	*Oryzias latipes*	Virgin LDPE fragments, < 0.5 mm Virgin LDPE fragments deployed in the southern part of San Diego Bay for 3 months, < 0.5 mm	Negative control treatment diet contained 0% plastic and the virgin- and marine- plastic treatment diets contained 10% plastic	Severe glycogen depletion, fatty vacuolation, cellular necrosis, and lesions from virgin and marine-plastic fragments PE ingestion is a vector for the bioaccumulation of PBTs in fish Toxicity results from the sorbed contaminants and plastics.	(Rochman et al., 2013)
	Fish	*Oryzias latipes*	Control: no plastic Virgin LDPE fragments, < 0.5 mm Virgin LDPE fragments deployed in the southern part of San Diego Bay for 3 months, < 0.5 mm	Negative control diet: 0% plastic Diets containing plastic: 10% plastic by weight	Marine-plastic treatment: down-regulation of Chg H in males and abnormal proliferation of germ cells (one fish) Plastic treatment: down-regulation of Vtg I, Chg H and ERα in females	(Rochman et al., 2014)
	Amphipod	*Allorchestes Compressa*	Exposition to different type of PBDEs in the absence or presence of PE particles, 11-700 µm	0.1 g/mL Concentration of PBDEs on MPs: 50 and 500 ng PBDEs/g of MPs	Lower concentrations of some PDBE congeners in the presence of PE plastic particles compared to those without PE particles	(Chua et al., 2014)
	Mussel	*Mytilus galloprovincialis*	PE and PS microplastics associated to pyrene, <100 mm	MPs 1.5 g/L Pyrene 50 mg/L	Concentrations on exposed microplastics increased with a time- and dose-dependent trend, for both PE and PS Transfer of the pyrene adsorbed on microplastics to organisms and accumulation in the haemolymph,	(Avio et al., 2015a)

				gills and digestive tissues Toxicological effects of contaminated microplastics: immunological responses, antioxidant system, neurotoxic effects, genotoxicity…	
		PS NPs (70nm) and MPs (5 μm and 20 μm)			
Mussel	*Mytilus edulis* *Mytilus galloprovincialis*	PS alone or in combination with fluoranthene, 2-6 mm	MPs 2 mm (1800 microbeads/mL/ day) and 6 mm (200 microbeads/mL/day, obtaining a final concentration of 2000 microbeads/mL/day, corresponding to a mass concentration of 32 µg PS/L/day FLU: 30 µg/L/day	PS with fluoranthene: no modification of fluoranthene bioaccumulation Highest histopathological damages and levels of anti-oxidant markers PS alone: increase in hemocyte mortality, increase in reactive oxygen species production in hemocytes and enhancement of anti-oxidant and glutathione-related enzymes in mussel tissues	(Paul-Pont et al., 2016)
Fish	*Melanotaenia fluviatilis*	PE MPs from personal care products (10-700 µm in length) PBDEs (BDE-28, -47, -99, -153, -154, -183 200 ng g-1; BDE-209 2000 ng/g)	Food only Food with clean-MPs Food with PBDE spiked MPs (exposure group)	PBDEs sorbed to MPs accumulated in fish tissue after particles were ingested. Less-brominated congeners with lower octanol-water partition coefficients more readily desorbed and accumulated in fish compared to higher congeners which may be too strongly sorbed to MBs to readily partition	(Wardrop et al., 2016)
Lungworm	*Arenicola marina*	PE, 10 – 180 µm, added to sediment. Sediment-plastic mixture was spiked with the PCB congeners and mix	0.05 % PE in sediment + PCB ≈ 7 µg/kg DW sediment mixture 0.5% PE treatment + PCB ≈ 23 µg/kg DW sediment mixture	PE ingestion contributed marginally to bioaccumulation.	(Besseling et al., 2017)
Fish	*Danio rerio*	PS NPs Bisphenol A	BPA alone: 0.78 µg/L and 1 µg/L NPs alone: 1 mg/L Co-exposure:	Highest BPA tissue concentrations were in viscera, followed by gill, head and muscle. NPs and BPA uptake in different tissues followed the same order with viscera > gill > head > muscle Co-exposure treatment induced neurotoxic effects	(Chen et al., 2017)

			mixture of BPA (1 µg/L) and NPs (1 mg/L)	in both central nervous system and dopaminergic system.	
Lobster	*Nephrops norvegicus*	PE, 500-600 µm PS, 500-600 µm or 6 µm with and without loading a mixture of ten PCB congeners	Gelatin + PCBs Gelatin + 155 mg clean MPs Gelatin + 155 mg PCB-loaded MPs Gelatin + PCB+155 mg clean MPs	No significant bioaccumulation of the chemicals in the exposed organisms. Limited uptake of PCBs in tail tissue after ingestion of PCB-loaded PE Almost no PCBs in animals exposed to PCB-loaded PS	(Devriese et al., 2017)
Fish	*Danio rerio*	PVC MPs (200–250 µm) phenanthrene (Phe) 17α-ethinylestradiol (EE2),	MPs 400 mg/L Phe 0.5mg/L EE2 1 µg/L MPs and contaminant mixed for 5 days (MP + Contaminant) MPs removed (filtration) after mixing (as to investigate the level of expression induced (bioavailability) by contaminant left in solution (MP Filtered).	Both Phe and EE2 sorbed to MPs,which reduced bioavailability by a maximum of 33% and 48% respectively. Contaminated MPs settled to the bottom and did not lead to increased bioavailability of Phe or EE2	(Sleight et al., 2017)
Fish	*Oncorhynchus mykiss*	PS MPs (100–400 µm) PS MPs exposed to sewage or harbor effluent	Control Food containing virgin PS MPs or PS MPs exposed to sewage or harbor effluent. Estimated intake of MPs in plastic-containing diets varied from 500–700 to 2226–2411 particles/fish/day	Virgin PS MPs did not induce adverse changes in hepatic biomarker responses, suggesting low chemical toxicity of commercial virgin PS MPs PS MPs sorb environmental contaminants (PAHs, nonylphenol and alcohol ethoxylates and others) but did not induce adverse hepatic stress in fish liver and did not affect lipid peroxidation or rancid odor development,	(Ašmonaitė et al., 2018)
Fish	*Dicentrarchus labrax*	MPs (1–5 µm diameter) Mercury	Mercury (0.010 and 0.016 mg/L), MPs	MPs ncreased the concentration of mercury in gills and liver.	(Barboza et al., 2018)

			(0.26 and 0.69 mg/L), and mixtures of the two substances (same concentrations)	MPs and mercury (alone and combined) caused oxidative stress in gills and liver	
Mussel	*Mytilus galloprovincialis*	PS NPs (diameter of 110 ± 6.9 nm) Carbamazepine (Chz)	Concentration range of PS (from 0.05 up to 50 mg/L) Cbz (6.3 µg/L) alone Mixture of PS + Cbz (0.05 mg/L+ 6.3 µg/L)	Increase of the oxidative status (digestive gland) and peroxidative damage (digestive gland) caused by PS NPs. Possible neurotoxic effect of PS alone (decrease of ChE activity) Co-exposure appeared to alleviate the toxicity of Cbz even if it induced specific responses in mRNA levels	(Brandts et al., 2018)
Worm	*Hediste diversicolor*	PVC MPs (250.0 ± 2.5µm) Benzo(a)pyrene	Uncontaminated sediments, Sediments spiked with B[a]P, sediments spiked only with virgin PVC at 200 particles/kg of sediment (LC-MPS) and 2,000 particles/kg (HC-MPS) as well as similar concentrations for B[a]P spiked MPs particles	PVC MPs were shown to adsorb benzo(a)pyrene with a time and dose-dependent relationship. Highest adverse responses were observed in organisms exposed to sediments spiked with PVC MPs pre-incubated with B[a]P when compared against sediments spiked with B[a]P and plastic microparticles separately	(Gomiero et al., 2018)
Bivalve	*Corbicula fluminea*	MPs (1–5 µm diameter) Florfenicol	Control (test medium only) Solvent-control (test medium containing 0.9 ml/l v/v of acetone, similar to the acetone concentration in the treatment containing the highest concentration of florfenicol); 1.8 mg/l of florfenicol;	MPs found in various tissues. Florfenicol alone: significant inhibition of cholinesterase (ChE) activity (~32%). MPs alone: exposition to 0.2 mg/l induced ChE activity inhibition (31%), Mixture: feeding inhibition (57–83%), significant ChE inhibition (44–57%) and of isocitrate dehydrogenase activity, and increased anti-oxidant enzymes activity and lipid peroxidation levels. Mixtures containing florfenicol and MPs more toxic than florfenicol and MPs alone.	(Guilhermino et al., 2018)

			7.1 mg/l of florfenicol; 0.2 mg/l of MPs; 0.7 mg/l of MPs; 1.8 mg/l of florfenicol +0.2 mg/l of MPs; 1.8 mg/l of florfenicol +0.7 mg/l of MPs; 7.1mg/l of florfenicol+0.2mg/l of MPs; 7.1 mg/l of florfenicol +0.7 mg/l of MPs 96h		
Fish	*Dicentrarchus labrax*	LD-PE MPs (125–250 μm) PCBs and brominated flame retardants (BFRs)	Control feed containing only background contamination Contaminated feeds of - contaminants sorbed to microplastics before incorporation of 2% into pellets, - contaminants without microplastics, coated with oil onto feed pellets and - contaminants and clean microplastics.	Co-exposure: significantly higher accumulation of PCBs and BFRs. Feed containing contaminants sorbed to MPs: greatest effects on seabass toxicokinetics Contaminants' bioavailability, lipid distribution and contaminant concentrations influenced by MPs. MPs inhibit or induce detoxification in the liver.	(Granby et al., 2018)
Fish	*Danio rerio*	PS MPs ((5-μm diameter) Cadmium (Cd)	Control group Cd group (10 μg/L); Cd + MPs (L) group (10 μg/L Cd + 20 μg/L MPs); Cd + MPs (H) group (10 μg/L Cd + 200 μg/L MPs) 3 weeks	Co-exposure: MPs increased the accumulation of Cd in livers (46% and 184%), guts (10% and 25%) and gills (9% and 46%). Oxidative damage and inflammation in tissues. MPs enhanced the toxicity of Cd (biochemical biomarkers, histopathological observation and gene expression).	(Lu et al., 2018)
Mussel	*Mytilus edulis*	PE MPs (size range: 10–90 μm)	Flu-only MPs-only	Individual contaminant exposures to Flu or MP led to varying responses but coexposures and incubated	(Magara et al., 2018)

			Coexposure MPs-Flu Fluincubated MPs. Each treatment was conducted at a low and high concentration. For Flu, mussels were exposed to 50 µg/L and 100 µg/L, while for MP to 100 MP/ mL or 1000 MP/mL	exposures did not result in additive or synergistic effects. MP-only exposure induced effects on the oxidative stress system (activities of CAT and GPx).	
Clam	*Scrobicularia plana*	Virgin LDPE microparticles, 11–13 µm LDPE microparticles with adsorbed contaminants benzo[a]pyrene—BaP and perfluorooctane sulfonic acid—PFOS	MPs 1 mg/L BaP: 16.87 ± 0.22 µg/g of MPs and PFOS: 70.22 ± 12.41 µg/g of MPs	Oxidative damage observed in all microplastics, with adsorbed BaP and PFOS, treatments in digestive gland and gill tissues Digestive gland less affected by virgin LDPE exposure. MP-adsorbed BaP and PFOS: negative influence over the biomarkers in digestive gland	(O'Donovan et al., 2018)
Fish	*Danio rerio*	LDPE plastic particles, 125–250 µm 2% LDPE to which a mixture of PCBs, BFRs, PFCs and methylmercury were sorbed Mixture of contaminants only	Basic feed without any added contaminant but with background contaminant levels Basic feed to which 4% of clean MPs was added Basic feed with 2% of MPs that had previously been immersed overnight in a solution of chemical contaminants to simulate environmental conditions.	No significant effect of microplastics alone Greater level of vacuolization with plastics and sorbed contaminants compared to only microplastics and only contaminants White formations in the liver for 60% of fish exposed to microplastics and sorbed contaminants. Absence of these formations for other conditions.	(Rainieri et al., 2018)
Fish	*Symphysodon aequifasciatus*	PS MPs (32-40 µm diameter) Cadmium (Cd)	MPs (0, 50 or 500 mg/L) combined with two levels of Cd (0 or 50 mg/L) for 30 days.	MPs and Cd: no adverse effects on growth and survival Accumulation of Cd in the body of fish decreased with increasing MP concentrations. Activities of superoxide dismutase and glutathione	(Wen et al., 2018)

				peroxidase increased with MPs but decreased with Cd. MPs, Cd or the mixture increased catalase activity. Glutathione levels increased when exposed to high MP concentrations but decreased when co-exposed to Cd In the presence of MPs reduction of Cd accumulation	
Fish	*Cyprinus carpio*	PE microbeads Cadmium chloride (Cd)	Control, Cd alone (100 and 200 mg/L. MPs alone (250 and 500 mg/L). Combined dose of 100 mg/L Cd and 250 mg/L MPs. Combined dose of 100 mg/L Cd and 500 mg/L MPs. Combined dose of 200 mg/L Cd and 250 mg/L MPs. Combined dose of 200 mg/L Cd and 500 mg/L MPs.	Exposure to Cd or MPs alone is toxic to fish altering the biochemical and immunological parameters. Alterations are greater for combined dose of Cd and MPs. Possible synergistic effects.	(Banaee et al., 2019)
Fish	*Danio rerio*	PS NPs 44 nm Elizabeth River Sediment Extract (ERSE): environmental mixture of water and suspended solids with a total PAH content of 5073 ng/mL PAHs as result of 36 different analyzed PAHs	Nano-PS (0.1, 1, or 10 ppm), ERSE (0.1%, 0.5%, 1%, 2%, and 5%), or a combination of both Nano-PS and ERSE (10 ppm of Nano-PS + 1%, 2% or 5% ERSE).	Nano-PS alone: no developmental defects. ERSE (2−5%) alone: PAH toxicity (heart malformation and deformities in the jaw, fin, and tail). ERSE (5%) also impaired vascular development in the brain. Co-exposition: Nano-PS decreased the developmental deformities and impaired vascular development caused by ERSE.	(Trevisan et al., 2019)
Crustacean	*Daphnia magna*	PS NPs (1-µm and 10-µm) Roxithromycin (ROX)	0.1 mg/L 1-µm PS, 0.1 mg/L 10-µm PS, 0.01 mg/L ROX, and the mixed exposure treatments	Exposure to PS and ROX alone or in combination altered the oxidative stress Co-exposure: lower GPx and GST activities Co-exposure to 1-µm PS and ROX induced the strongest stress effect followed by ROX alone, 1-µm PS alone, 10-µm PS and ROX mixture, and 10-µm PS alone.	(P. Zhang et al., 2019)

	Mussel	*Mytilus edulis*	PVC MPs (1 to 75 μm) CdCl2	control, CdCl2 (200 μg/L Cd2+), PVC (20 particles/mL) and CdCl2 + PVC (200 μg/L Cd2+ and 20 particles/mL PVC	No significant increase of Cd uptake with PVC MPs compared with Cd alone treatment	(Li et al., 2020)
	Clam	*Ruditapes philippinarum*	PE MPs 10-45 μm	Control Hg alone: 10 mg/L MPs alone: 25 mg/L Co-Exposure: 25 mg/L MPs and 10 mg/L Hg Co-exposure: 25 mg/L MPs contaminated with Hg exposure.	MPs ingested and translocated to various tissues. Contaminated MPs: negligible vector role in terms of mercury bioaccumulation. Both pollutants affected the immune system of the organisms. Histological alterations were determined in the gill and digestive gland tissues but no effect on oxidative stress biomarkers.	(Sıkdokur et al., 2020)
	Bivalve	*Tegillarca granosa*	PS MPs at nominal sizes of 30 μm and 500 nm benzo[a]pyrene (B[a]P) and 17β-estradiol (E2)	1 mg/L microplastics at sizes of 30 μm or 500 nm 5 or 50 μg/L B[a]P or 0.1 or 1 μg/L E2	Toxicity of POPs was generally aggravated by smaller microplastics (500 nm) and mitigated by larger ones (30 μm). Potential size dependent interactions between microplastics and POPs.	(Tang et al., 2020)
Plastics collected in the environment	Sea urchin	*Lytechinus variegatus* embryos	Virgin pellets Beached pellets	Pellet–water interface: 2 mL of plastic pellets (virgin or beach-collected) in 8 mL of seawater Elutriate treatment: 200 mL of pellets placed in 1 L beakers filled with 800 mL of filtered seawater.	Virgin pellets induced anomalous embryonic development in the pellet–water interface and elutriate assays Lower toxicity of beached pellets than virgin pellets, only for pellet–water interface assay	(Nobre et al., 2015)

Mussel	*Perna perna* embryos	Virgin PP pellets Beached plastic pellets	Zero (control), 0.5 mL, 1 mL and 2mL of pellets mixed with seawater (8 mL in the test tube and 10 mL in the beaker procedure) + 500 fertilized embryos	Virgin and beached pellets impacted embryo development Higher toxicity of beached pellets than that of virgin pellets	(Gandara e Silva et al., 2016)
Fish cell line	Cell line derived from the liver of adult of rainbow trout *Oncorhynchus mykiss*	Plastic collected from different sandy beaches	Cells to different concentrations of organic MPs extract. Comet assay: two concentrations tested 0.01% and 0.1% of MPs extract	DNA damage observed after exposure to four microplastics samples on the six tested.	(Pannetier et al., 2019a)
Fish	Embryos and prolarvae of Japanese medaka	Plastic collected from different sandy beaches	Virgin MPs: mixture of 40% of LDPE, 25% of HDPE, 25% of PP and 10% of PS Environmental MPs collected from different beaches. Virgin and environmental MPs < 600 µm	Larvae swimming speed was not impacted by contamination by environmental MPs extracts. Significant lower speed for prolarvae exposed to B(a)P coated MPs (Cþ) than prolarvae exposed to DMSO and some environmental MP extracts.	(Pannetier et al., 2019b)
Algae Clam	*Scenedemus subspicatus* *Thalassiosira weissiflogii* *Corbicula fluminea*	NP polyethylene made from virgin polyethylene beads or polyethylene collected in the North Atlantic subtropical gyre, < 0.45 µm diameter	1, 10, 100, 1000, and 10,000 µg/L	No influence of both types of NP on the cell growth of *T. weissiflogii* Negative response on *S. subspicatus* growth was greater to NP from the environment than to the reference. No influence on the filtration rate of *C. fluminea* but increase of feces and pseudofeces production.	(Baudrimont et al., 2019)
Fish	*Orizias latipes* larvae and juveniles	Virgin plastic: LDPE, HDPE, PP and PS, 90% < 960 µm Beached plastics sampled from three locations, composed of mostly PP and PE. Presence of additional NY or PS for	Larvae and juveniles of Japanese Medaka were fed for 30 days with three doses of MPs (0.01, 0.1 and 1% w/w in fish food)	MP from the environment increased DNA damage and modulated of EROD activity	(Pannetier et al., 2020)

two locations.

Table 2. Sampling and characterization studies of microplastics in estuarine organisms

Estuary	Plastic particle size,	Taxa, species	Organs in which plastic particles have been	Major(s) finding(s)	References

	composition		extracted		
Musa (Persian Gulf)	Filamentous fragments, 100 and 250 µm or 250 to 500 µm	Demersal and pelagic fish (*Platycephalus indicus*, *Saurida tumbil*, *Sillago sihama*, *Cynoglossus abbreviatus*) and tiger prawn, *Penaeus semisulcatus*	Guts, skin, muscle, gills and liver of fish Exoskeleton and muscle of the prawn	A total of 828 MPs were detected in the guts (gastrointestinal tracts), skin, muscle, gills, skeleton and liver. MPs ranged from 0.16 g-1 for *C. abbreviatus* to 1.5 g-1 for *P. semisulcatus*	(Abbasi et al., 2018)
Bahía Blanca (Argentina)	Fibers, pellets, fragments and laminas 0.98 to 5 mm.	Fish *Micropogonias furnieri*	Gastrointestinal tract	100% of fish contained MPs MPs abundance ranged from 9 to 14 particles per gastrointestinal tract on average MPs were categorized as fibers (60.8%), pellets (28.9%), fragments (8.6%) and laminas (1.4%),	(Arias et al., 2019)
Mondego (Portugal)	Fibers and fragment, size not indicated	The sea bass (*Dicentrarchus labrax*), the common two-banded seabream (*Diplodus vulgaris*) and the European flounder (*Platichthys flesus*)	Gastrointestinal tract	A total of 157 MPs was extracted, with an average of 1.67 ± 0.27 particles/fish. Polymer types found were polyethylene (6%), polypropylene (14%), polyester (31%), nylon (5%) and rayon (30%)	(Bessa et al., 2018)
Ciénaga Grande de Santa Marta (Colombia)	Fibers and fragments.	Fish *Mugil incilis*, *Caranx hippos*, *Caquetaia kraussii* and *Eugerres plumieri*	Digestive tract	12.1% of the 140 fish contained MPs. The MPs were composed of fibers (89.5%) and fragments (10.5%). PL and PE were the most common fibers.	(Calderon et al., 2019)
Laguna Estuarine System (Brazil)	Fragments	Fish *Genidens genidens*	Gut	No significant differences of the mean size of MPs ingested by juveniles (2.16 mm ± 1.95) and adults (2.07 mm± 3.02)	(Dantas et al., 2019)
Three estuaries in Pondicherry (India)	Fragments	Bivalve species *Perna viridis* and *Meretrix meretrix*	Soft tissues	*Meretrix meretrix*, MPs were in the range of 21.06 µm to 1.5 mm (mean = 157.84 ± 18.89 µm) *Perna viridis*, majority (77.42%) of the particles were smaller than 100 µm	(Dowarah et al., 2020)
Bahía Blanca (Argentina)	Fibers, fragments, pellets and beads 0.17 - 5 mm	Oyster *Crassostrea virginica*	Gut	MPs in oysters are mostly fibers (91%)	(Fernández et al., 2019)

Goiana (Brazil)	Filaments < 5 mm	Fish *Cynoscion acoupa*	Digestive tract	97% of the debris was plastic filaments. MPs were more frequently ingested than any other food item Across the ontogenetic phases of *C. acoupa*, 64.4% of juveniles, 50% of sub-adults and 100% of adults ingested MPs.	(Ferreira et al., 2016)
Goiana (Brazil)	Filaments < 5 mm	Fish *Cynoscion acoupa*	Digestive tract	Over the 552 individuals that were analysed, 51% of guts were contaminated by plastic particles. The average number of particles ingested was 3.03 ± 4.06 particles/digestive tract, ranging from 0 to 63 particles per individual. The majority of MPs was filaments	(Ferreira et al., 2018)
Sydney harbour (Australia)	Shape and size of MPs not indicated	Fish *Acanthopagrus australis*, *Mugil cephalus* and *Gerres subfasciatus*	Digestive tract	Synthetic microplastics made up 55% of identified debris in *M. cephalus* and 36% in *A. australis* were synthetic MPs. Analysis in *G. subfasciatus* was limited (3 of the 5 debris were lost during sampling)	(Halstead et al., 2018)
Three estuaries in the eastern English Channel (UK)	Fibers 70 µm - 4510 µm Fragments 5 µm - 66 µm.	Wild and caged juvenile European flounder *Platichthys flesus*	Digestive tract	An average of 2.04 ± 1.93 MP items was ingested by wild individuals and 1.67 ± 1.43 by caged individuals. The majority of plastic collected were fibers and blue in color.	(Kazour et al., 2018)
A rural estuary in Georgia (USA)	> 8 µm.	Oyster *Crassostrea virginica*	Soft tissues	0.72 MP/individual (0.18 MP/gram wet mass)	(Keisling et al., 2019)

Thames (UK)	Film, synthetic fiber, sphere and irregularly shaped fragment.	21 estuarine species	Digestive tract	Pelagic species ingested more plastic than flatfish and shrimp Average number of ingested MPs: pelagic species (3.2), flatfish (2.9) and shrimp (1)	(Mcgoran et al., 2018)
Amazon (Brazil)	Size ranging from 0.38 to 4.16 mm (pellets, sheets, fragments and threads)	Fishes from the Amazon River (189 fish specimens representing 46 species from 22 families)	Gastrointestinal tract	228 MP particles were removed of 26 specimens representing 14 species. Positive correlation between fish standard length and number of particles found in gastrointestinal tracts. The main polymers identified were polyamide, rayon and polyethylene.	(Pegado et al., 2018)
Pearl River (China)	Fibers; 20 to 5000 mm and 83.9% < 100 mm	Bivalve *Saccostrea cucullata*	Soft tissues	The MPs abundances in oysters ranged from 1.4 to 7.0 items per individual. The oysters near urban areas contained significantly more MPs than those near rural areas.	(H. X. Li et al., 2018)
Four estuaries in KwaZulu-Natal (South Africa)	0.1 – 4.8 mm	Fish *Oreochromis mossambicus, Terapon jarbua, Ambassis dussumieri* and *Mugil sp.*	Whole organism	Fibers (68%) and fragments (21%) were the dominant shapes found. 52% of the 174 fish sampled contained MPs with 0.79 ± 1.00 particles per fish.	(Naidoo et al., 2019)
Mouths of the Ashley and Cooper Rivers in Charleston Harbor (USA)	43-104 µm	Zooplankton	Gut	Zooplankton from the Cooper River ingested MPs but not zooplankton from the Ashley River 1% of the zooplankton ingested MPs suggesting selective feeding	(Payton et al., 2020)
La Plata (Argentina)	Fibers; 0.06 and 4.7 mm	87 fish belonging to 11 species	Gut content	Presence of MPs was verified in the 100% of fish. The number of MPs in gut contents was higher close to sewage discharge	(Pazos et al., 2017)
Goiana (Brazil)	Millimeter scale, nylon fragments and hard plastic	Catfish, juvenile, sub-adult, and adult, *Cathorops spixii*, *Cathorops agassizii*, *Sciades herzbergii*	Stomach content	Between 17 and 33 % of individuals across all species ingested plastic. All size classes ingested plastic. Size classes differed in number of ingested fragments.	(Possatto et al., 2011)

Goiana (Brazil)	Fragments < 5 mm	Fish *Pomadasys ramosus* and *Haemulopsis corvinaeformis*	Stomach	*P. ramosus*: 100% of juveniles in the lower estuary contained MPs. Frequency of occurrence of MPs varied between 50 and 100% for sub-adults and adults in the upper and middle estuary. *H. corvinaeformis*: highest averages of ingestion of microfilaments in number by adults (1.25 MPs/stomach) occurred in the lower estuary in the late dry season.	(Silva et al., 2018)
Yangtze (China)	>20 µm; fragments (type not indicated)	13 fish species of from coastal areas of China	Gut and gills	MPs were detected 22 %-89 % of total individuals. MPs in gut varied from 0.3 to 5.3 items/ind. and varied from 0.3 to 2.6 items/ind in gills.	(Su et al., 2018)
Chollas Creek (USA)	< 5mm	Fish	Gut	Almost one quarter of fish examined contained small plastics with 12% of California killifish (7 of 61) and 32% of sailfin molly (24 of 75) having consumed plastic. California killifish and sailfin molly each consumed 10–11 different types of plastic items, mostly consisting of fibers and hard pieces.	(Sinicrope Talley et al., 2020)
Tagus (Portugal)	Fibers, particles; size not indicated	Bivalve *Mytilus galloprovincialis*	Soft tissues	0.13 +/- 0.14 total MPs g^{-1} was recorded in commercial species from 5 countries Acid mix Method: 0.18 +/- 0.14 g^{-1} Nitric acid method = 0.12+/- 0.14 g^{-1}	(Vandermeersch et al., 2015)
Paraiba and Mamanguape (Brazil)	Fibers, films and fragments; size not indicated	2233 fish from two estuaries (69 species)	Stomach and intestine contents	9% of the individuals (24 species) had MPs in their gut contents. MPs ingestion occurred irrespective of fish size and functional group.	(Vendel et al., 2017)
Bahía Blanca (Argentina)	Fibers: 500–1500 µm Fragments < 200 µm	Crab *Neohelice granulata*	Gills and digestive tract	In gills and digestive tract: 60% of the MPs were fibers and 40% were fragments. Pellets almost insignificant. Most abundant color in both tissues was blue corresponding to fibers.	(Villagran et al., 2019)
Florida (USA)	Fibers, mostly royal/dark blue in color 63.3 - 3.6 mm	Eastern oysters (*Crassostrea virginica*) and Atlantic mud crabs (*Panopeus herbstii*)	Oyster: soft tissues Crab: digestive tract and gills	One-liter water samples had an average of 23.1 MP pieces Crabs had an of 4.2 pieces in tissues/ind Adult oysters had of 16.5 MP pieces/individual	(Waite et al., 2018)
Tagus (Portugal)	Plastic balls and strands ranged from 0.5 to 5 mm	Crab *Eriocheir sinensis*	Stomach	No difference in the number of specimens containing plastic between males and females No difference in the number of specimens	(Wójcik-Fudalewska et al., 2016)

containing plastic between size classes of individuals

Table 3. Plastic exposures of estuarine species in laboratory

Organisms	Species	Micro/nano plastics type	Micro/nano plastics concentration	Temperature (°C)	Salinity (PSU)	Major(s) finding(s)	References
Fish	*Pomatoschistus microps*	Polyethylene MP spheres (3 types) - 420- 500 µm Exposure: 96 h	Seven prey types treatments in 300 mL of artificial salt water : 30 *Artemia franciscana nauplii* (Art) alone (controls); 30 MPs (white, red or black) alone; 15 Art in combination with 15 MPs (white, red or black).	21	18	All the MPs types were ingested, suggesting confusion with food Under simultaneous exposure to MPs and *Artemia*, fish from one estuary showed reduction of the predatory performance (65%) and efficiency (up to 50%) while fish from another estuary did not.	(de Sá et al., 2015)
Fish	*Pomatoschistus microps,* juveniles	Polyethylene fluorescent microspheres) 1-5 µm Potassium dichromate was used as Cr (VI)	Effects of Cr(VI) alone : 0 (control), 5.6, 8.4, 12.6, 18.9 and 28.4 mg/L MPs and Cr(VI): control (water only); MPs alone (0.184 mg/L);	20	18	Cr (VI) alone induced mortality and significantly decreased fish predatory performance. Cr (VI) and MP, a significant decrease of the predatory performance and an inhibition of AChE activity. MP alone caused an AChE inhibition. Mixture treatments containing Cr (VI) concentration increased LPO levels in L-est fish. Long-term influenced the sensitivity and	(Luís et al., 2015)

			5.6 mg/L of Cr(VI) + 0.184 mg/L of MPs; 8.4 mg/L of Cr(VI) + 0.184 mg/L of MPs; 12.6 mg/L of Cr(VI) + 0.184 mg/L of MPs; 18.9 mg/L of Cr(VI) + 0.184 mg/L of MPs; 28.4 mg/L of Cr(VI) + 0.184 mg/L of MPs			responses of juveniles to Cr (VI)	
Fish	*Pomatoschistus microps* juveniles	PE microspheres (1–5 µm)	Control, MPs (0.18 mg/L), 3 mg/L of Cd; 6 mg/L of Cd; 13 mg/L of Cd; 25 mg/L of Cd; 50 mg/L of Cd; microplastic-cadmium (MPs-Cd) mixtures containing each one the Cd concentrations previously indicated and 0.18 mg/L of MPs.	20-24	18	Juveniles sampled in the estuaries of Minho and Lima Rivers have comparable sensitivity to Cd and MPs did not influence the Cd-induced mortality. Toxicological interaction between Cd and MPs for AChE activity. No differences in the mean predatory performance between control group and juveniles from Minho estuary exposed to 0.14 mg/L of MPs alone Lima fish exposed to MPs alone had significantly decreased (54%) predatory performance compared to the control group	(Miranda et al., 2019)
Fish	*Pomatoschistus microps*	PE microspheres (1–5 µm) Exposure = 96 h to pyrene (20 and 200 g L−1) in the absence	Control; solvent (acetone)	20	15	MP delayed pyrene-induced fish mortality and increased the concentration of bile pyrene metabolites.	(Oliveira et al., 2013)

		and presence of microplastics (0, 18.4 and 184 g/L)	control; 20 µg/L pyrene; 200 µg/L pyrene; 18.4 µg/L MP; 184 µg/L MP; 20 µg/L pyrene + 184 µg/L MPs; 200 µg/L pyrene + 18.4 µg/L MPs; 200 µg/L pyrene + 184 µg/L MPs			MPs, alone or in combination with pyrene, reduced AChE activity, an effect also observed for pyrene alone. MP combined to pyrene decreased isocitrate dehydrogenase activity.	
Ragworm	*Hediste diversicolor*	PE and PP, Size distribution between 0.4 and 400 µm	Control, Water phase: 10 µg of MPs/L, and 100 µg of MPs/L. Sediment: 10 and 50 mg of MPs/kg	16		Water exposure: average number of MP/worm ranging from 0 to 2.5 and from 1 to 36 identified in ragworms exposed to 10 and 100 µg of MPs/L respectively. Sediment: less than 1 MP/worm MPs exposure induced a decrease in coelomocytes viability but no effect on phenoloxidase, acid phosphatase and phagocytosis activity.	(Revel et al., 2020)
Crab	*Carcinus maenas*	Polystyrene microspheres (8µm)	106 and 107 microspheres/ L	14.5	33	MPs inhaled into the gill chamber had a small but significant dose dependent effect on oxygen consumption. A small but decrease in hemolymph sodium ions and an increase in calcium ions	(Watts et al., 2016)

Table 4. Studies on the trophic transfer of plastic particles

	Organisms	*Species*	*Micro/nano plastics type and duration of exposure*	*Micro/nano plastics concentration*	*Major(s) finding(s)*	*References*
Simple food chain	Zooplankton Fish	*Artemia sp. n*auplii *Danio rerio*	1-5 µm and 10- 20µm + BaP co exposition 14 days	MPs : $1.2x10^6$ particles/L per 20 000 *Artemia* nauplii BaP : 252µg/L	BaP transfer over plastic transfer, advocating for a most likely vector role of plastic particles	(Batel et al., 2016)
	Mussel Crab	*Mytilus edulis* *Carcinus maenas*	0.5µm 21 days	0.125µL/mL	Plastic particles trophic transfer shown for one of the first times, small amount though	(Farrell and Nelson, 2013)
	Periwinkle Algae	*Littorina littorea* *Fucus vesiculosus*	Beads: 10µm Fragments: 1−100 µm Fibers: 90 to 2200 µm 1-3 days	Beads: 1.39-55.65 mg/L Fragments: 1.10-56.95 mg/L Fibers: 0.004-0.027 mg/L	Assessing plastic particles adherence to seaweeds as a pathway for trophic transfer, but poor bioaccumulative potential shown	(Gutow et al., 2015)
	Mussel Crab Pufferfish	*Perna perna* *Callinectes ornatus* *Spheoeroides greeleyi*	0.1-1µm 10 days	0.5 g/L	Transferof MPs from prey to predators shown but without evidences of particle persistence in their tissues after 10 days of exposure. Low likelihood of trophic cascading of plastic particles	(Santana et al., 2017)
	Crustacean Fish	*Platorchestia smithi* *Bathygobius krefftii*	38-45µm + adsorbed PAHs co	Addition of contaminated MPs in the sediment at	No behavior disruption for fishes after plastic trophic	(Tosetto et al., 2017)

		exposition 72h	3.8% (dry weight) MPs contained 0.007 mg/g of PAHs	transfer	
Cockle Mollusc	*Cerastoderma edule* *Limecola balthica*	4.8µm 4 days	0-10000 particles/mL	Trophic transfer of MPs and predator-prey interaction disruption	(Van Colen et al., 2020)
Crab Mussel	*Carcinus maenas* *Mytilus edulis*	8-10µm 21 days	4.0×10^4–9.4×10^5 MPs/L	Particles retention (14 days) shown after trophic transfer, even more (21 days) following inspiration across the gills	(Watts et al., 2014)
Larval inland silversides Unicellular tintinnid	*Menidia beryllina* *Favella spp.*	10-20µm +DDT co exposition 16 days	5×10^5 MPs/L DDT concentration on the MPs: 0.21 mg/g of MP	Plastic particles vector role shown and DDT trophic transfer assessed, with behavior disruption enhancing the contaminated uptake, suggesting a strong biomagnification potential	(Athey et al., 2020)
Mussel Crab	*Mytilus edulis* *Necora puber*	0.5 µm PS 21 days	$4.1x10^6$ in 400 mL of aerated seawater for each mussel Crab were fed one or three dosed mussels during 21 days	Trophic transfer of MPs in stomach, gills, testes and brains. Significant increase in the number of MPs with the number of mussels consumed in the stomach	(Crooks et al., 2019)
Mussel Shrimp	*Mytilus edulis* *Litopenaeus vannamei*	44 nm PS 21 days	50 µg/mL Each shrimp was fed one unexposed or exposed mussel per day for 21 days	No significant differences between changes in the physical characteristic of control and exposed shrimp. Significant decreases in amino acids and fatty acids in shrimp exposed to NPS	(Chae et al., 2019)

					compared to control shrimp.	
Complex food chain	Mesozooplankton Macrozooplankton Crustacean Algae	Zooplankton, *Mysis relicta, Mysis mixta* *Neomysis integer* *Fucus vesiculosus*	10µm 3h	1000-10000 MPs/mL	Direct uptake by zooplankton and trophic transfer shown, but quick egestion (<12h)	(Setälä et al., 2014)
	Algae Crustacean Fish	*Raphidocelis subcapitata* *Daphnia magna* *Pimephales promelas*	6µm 5 days	20-2000 particles/mL	Trophic transfer shown on small amount, with very low bioconcentration and bioaccumulation factors calculated (<0.1)	(Elizalde-Velázquez et al., 2020)
	Algae Crustacean Fish Fish	*Chlamydomonas reinhardtii* *Daphnia magna* *Oryzias sinensis* *Zacco temminckii*	51 nm 7 days	50 mg/L	NPs transfer shown all along the modeled trophic chain, with adverse effects on higher trophic level, suggesting a biomagnification potential	(Chae et al., 2018)

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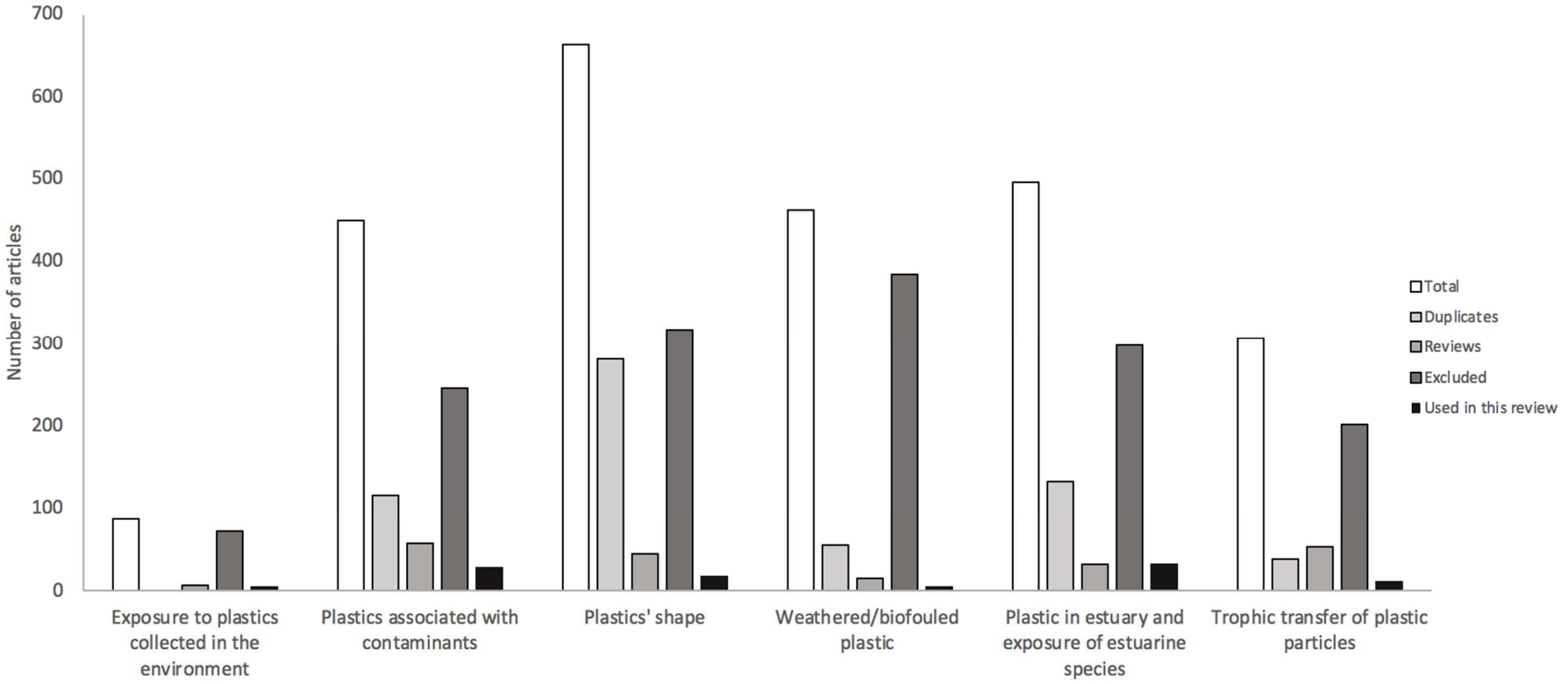

Figure 1. Results of the literature search. For each section of the review, we started by eliminating duplicates. Then, reviews were also eliminated. We reviewed the title and abstract of the remaining articles and excluded those that were not relevant to our topic of interest.