



Understanding How Microplastics Affect Marine Biota on the Cellular Level Is Important for Assessing Ecosystem Function: A Review

Natalie Prinz and Špela Korez

Abstract

Plastic has become indispensable for human life. When plastic debris is discarded into waterways, these items can interact with organisms. Of particular concern are microscopic plastic particles (microplastics) which are subject to ingestion by several taxa. This review summarizes the results of cutting-edge research about the interactions between a range of aquatic species and microplastics, including effects on biota physiology and secondary ingestion. Uptake pathways via digestive or ventilatory systems are discussed, including (1) the physical penetration of microplastic particles into cellular structures, (2) leaching of chemical additives or adsorbed persistent organic pollutants (POPs), and (3) consequences of bacterial or viral microbiota contamination associated with microplastic ingestion. Following uptake, a number of individual-level effects have been observed, including reduction of feeding activities, reduced growth and reproduction through cellular modifications, and oxidative stress. Microplastic-associated effects on marine biota have become increasingly investigated with growing concerns regarding human health through trophic transfer. We argue that research on the cellular interactions with microplastics provide an understanding of their impact to the organisms' fitness and, therefore, its ability to sustain their functional role in the ecosystem. The review summarizes information from 236 scientific publications. Of

those, only 4.6% extrapolate their research of microplastic intake on individual species to the impact on ecosystem functioning. We emphasize the need for risk evaluation from organismal effects to an ecosystem level to effectively evaluate the effect of microplastic pollution on marine environments. Further studies are encouraged to investigate sublethal effects in the context of environmentally relevant microplastic pollution conditions.

Keywords

Plastics · Tissue level · Chemical contamination · Oxidative stress · Sublethal effects · Ecosystem function

6.1 Introduction

Plastic pollution is ubiquitous in the global environment. The Industrial Revolution paved the way for the rapid development in manufacturing of long-lasting plastic materials. Consequently, the volume of plastic waste produced has increased. Our reliance on this man-made material has led to what some call “the plastic age” (Thompson et al. 2009a). Worldwide ~348 million tons of plastics were produced in 2017, of which approximately 42% was used for single-use packaging (Geyer et al. 2017; Plastics Europe 2018). Littering, ineffective recycling management practices, weather events, etc. have all been linked to the release of plastics into the environment. It has been estimated that between 4.4 and 12.7 million tons of plastic enter the marine environment annually (Jambeck et al. 2015). Over time, in the environment and exposed to weathering, sunlight, and mechanical degradation, large plastics will become brittle and break down to secondary microplastics (<5 mm) and nanoplastics (<100 nm) (MSFD Technical Group on Marine Litter 2013). Secondary microplastics also include microfibers that are washed out of synthetic

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clothes (Browne et al. 2011). Primary microplastics are small particles designed to be used for manufacturing large plastic items, including virgin resin pellets and microbeads (from cosmetics and personal care products) (Andrady 2017). Many fibers and microbeads are too small to be removed by filters used in sewage systems and will be flushed into the sea (Carr et al. 2016; Lebreton et al. 2017). This makes the issue of marine plastics more pressing for the coming centuries due to a consistent increment in microplastic abundance (Browne et al. 2007). Nowadays, microplastics are omnipresent, in rivers, estuaries, on shorelines, the ocean surface or in the water column, and on the seafloor (GESAMP 2015). The ubiquitous nature of microplastics in the environment means that biota can, and will, interact with them from the surface waters of the ocean to the deep sea. The bioavailability of microplastics depends on their size, density, abundance, shape, and color (Wright et al. 2013a). Over 1401 marine species are known to interact with marine plastic debris in different ways (Ocean Plastics Lab 2018). However, entanglement and ingestion are the most common types of interaction between biota and plastics (Gregory 2009). Fouling of bacteria on plastic particles may promote the ingestion of plastic materials by biota (Zettler et al. 2013; Vroom et al. 2017). Microplastic ingestion has been described for many taxa of animals including plankton, invertebrates, fish, sea turtles, and marine mammals (Cole et al. 2013; Foekema et al. 2013; Schuyler et al. 2013; Hämer et al. 2014; Lusher et al. 2015; Scherer et al. 2018). Current research efforts focus on the effects of microplastics entering and being channeled up aquatic food chains. It is still being investigated which species are more susceptible to the encounter and uptake, and which mechanisms are simultaneously affected (Rochman et al. 2015). Many species have been observed to directly take up plastics, either by selective targeting of plastic items, or accidental ingestion by filtration or predation (Lusher 2015).

Most organisms are constantly confronted with inert particles of different sizes, shapes, and materials throughout their life. Seif et al. (2018) highlighted that, apart from plastic, metal, glass, and building materials were also found in the intestines of gulls. Microplastics are often similar in size to sediment particles or may resemble a grain of sand. Therefore, it is not surprising that animals in coastal areas, particularly filter feeders, consistently encounter natural particles as well as particles generated by human activity like microplastics (Van Cauwenberghe and Janssen 2014; Weber et al. 2018). Usually, if an animal is not able to digest an item, it egests it after some time (Garrett et al. 2012; Santana et al. 2017). Plastic particles represent foremost foreign bodies inside an organism; nevertheless, their charge, chemical

composition, and contamination are of particular interest. In many cases, added chemicals in plastic manufacturing and persistent organic pollutants seem to be the actual threat. Increasingly, studies focus on physiological effects of microplastics on animals on an individual scale (Lusher 2015), as microplastics potentially cause cryptic sublethal effects that have to date rarely been investigated (Koelmans 2015). The effects include pathological stress, reproductive complications, changes in enzymes activities, reduced growth rate, and oxidative stress (Besseling et al. 2014; Sutton et al. 2016). Smaller particles (<100 nm) may have greater consequences upon ingestion, because they may end up in the tissues or even inside the cells (Lusher 2015). The time a particle spends inside the body (i.e., the retention time) is crucial for estimating chemical exchanges within the body. Many studies investigate the occurrence of plastic within the intestinal tract of an organism without discussing an impact on the animal itself (Boerger et al. 2010; Lusher et al. 2013; Battaglia et al. 2016; Rummel et al. 2016; Baalkhuyur et al. 2018). Yet, a wealth of studies identify effects of microplastic with artificial concentrations that are far beyond natural levels as currently encountered in the ocean (Pedà et al. 2016; Lusher et al. 2017; Critchell and Hoogenboom 2018). Nevertheless, findings provide evidence that plastic particles can cause internal wounds, lesions, or blockage of the digestive tract, which can promote a feeling of satiation that can lead to starvation, depletion of strength, and even death (Gregory 2009, Jovanović 2018).

It is important to disentangle the risks associated with ingested particles in an ecologically relevant context (Koelmans et al. 2017a). In a future of ever smaller particles, many organisms will be confronted with them, regardless of the size of the organism (Mattsson et al. 2017; Vendel et al. 2017; Critchell and Hoogenboom 2018).

This review evaluates the consequences of microplastic ingestion by summarizing the pathways of ingested microplastics and their subsequent effect on marine species, with some examples from freshwater species. The specific aims were to (i) collect results from current research of microplastic-derived impacts of organismal physiology and (ii) highlight the urgent need for embedding research on microbiological functioning of internal structures into the impact on ecosystem functioning. Further, this review aims to (iii) highlight the gaps of research that elaborate the sublethal effects of microplastics on an ecosystem function approach. An extensive literature review of 236 scientific publications resulted in this synthesized review. The percentage of articles discussing impacts on ecosystem function were calculated.

Three types of consequences of microplastics uptake through the digestive tract or the respiratory system have

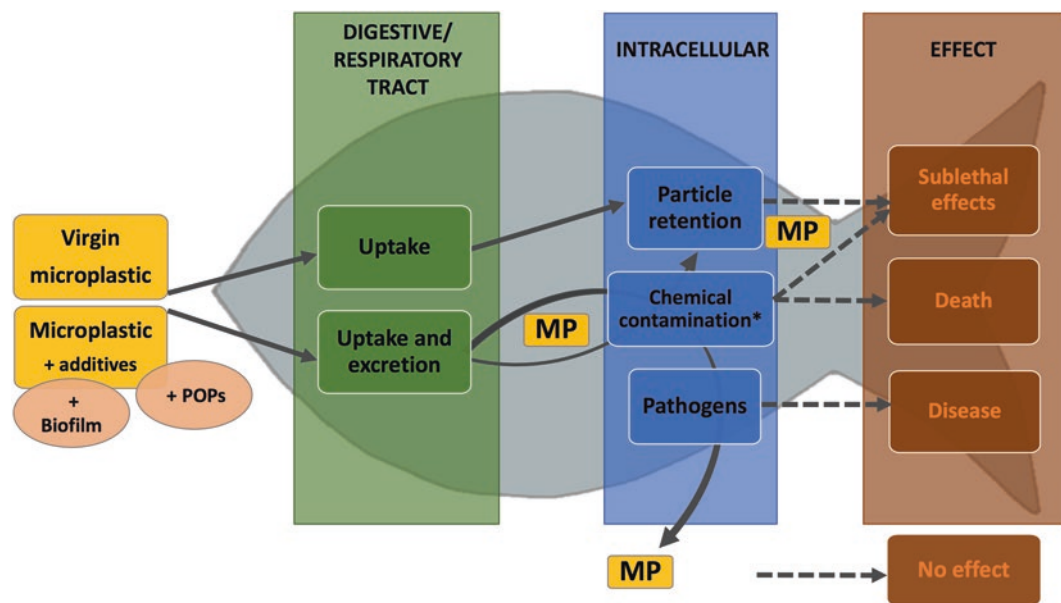


Fig. 6.1 Transfer pathways of microplastic particles and associated contaminants in the body of an organism (MP, microplastics; POPs, persistent organic pollutants). *Chemical impacts are graphically explored in more detail in Anbumani and Kakkar (2018)

been identified: (1) physical penetration of microplastic particles into cellular structures, (2) leaching of chemical additives or persistent organic pollutants (POPs) into the body, or (3) infecting eukaryotic and bacterial microbiota from the surface of ingested microplastics (Fig. 6.1). First, availability of microplastics to different biota will be discussed (Sect. 6.2). This entails the interactions of flora and fauna with microplastics. Further, known consequences of plastic particles in the tissues and cells are summarized (Sect. 6.3) with an evaluation on how cellular biomarkers are used (Sect. 6.4). Finally, the interactions between chemical pollutants and structures in the body are evaluated (Sect. 6.5) leading to a discussion about trophic cascading (Sect. 6.6) and human health (Sect. 6.7). Finally, this review discusses pathways of microplastic particle interaction with biota on the cellular level and concludes with suggestions for concrete research foci (Sect. 6.8).

6.2 Interactions of Different Organisms with Microplastics

6.2.1 Microplastic Interaction with Aquatic Primary Producers

Effects on algae are often neglected to be considered. Bhattacharya et al. (2010) reported that nanosized plastic beads can be adsorbed by a green alga (*Scenedesmus*

spp.), hindering the photosynthetic activity. This occurrence was attributed to the physical chemistry of the particles when positively charged. Photosynthesis of a marine diatom (*Thalassiosira pseudonana*) and marine flagellate (*Dunaliella tertiolecta*) was not affected, although at high concentrations and decreasing particle size of uncharged polystyrene particles, growth was reduced (Sjollem et al. 2016). Microplastics can form aggregates with some phytoplankton species. The phytoplankton *Rhodomonas salina* has a tendency to incorporate more microplastic to the aggregate compared to *Chaetoceros neogracile* (Long et al. 2015). More concerning effects are addressed in a recent study by Kalčíková et al. (2017) with a freshwater species. Sharp polyethylene microplastics from exfoliating cosmetic products are reducing the viability of the root cells of the duckweed (*Lemna minor*), which detrimentally affects their growth. A similar phenomenon was observed in moss (*Sphagnum palustre*) where small aggregates of microplastics entered into the hyalocyte cells of the leaf. Bigger aggregates of microplastic adsorbed on the moss' surface (Capozzi et al. 2018). Adsorption was also observed in the colonial green alga *Scenedesmus* or seaweed *Fucus vesiculosus* (Bhattacharya et al. 2010; Gutow et al. 2016). Such results address the significance of primary producers interacting with microplastic (Yokota et al. 2017). Green et al. (2016) concluded that a reduction of macroalgal biomass can be responsible for the overall primary productivity of a sandy bottom ecosystem. This clearly alludes to further

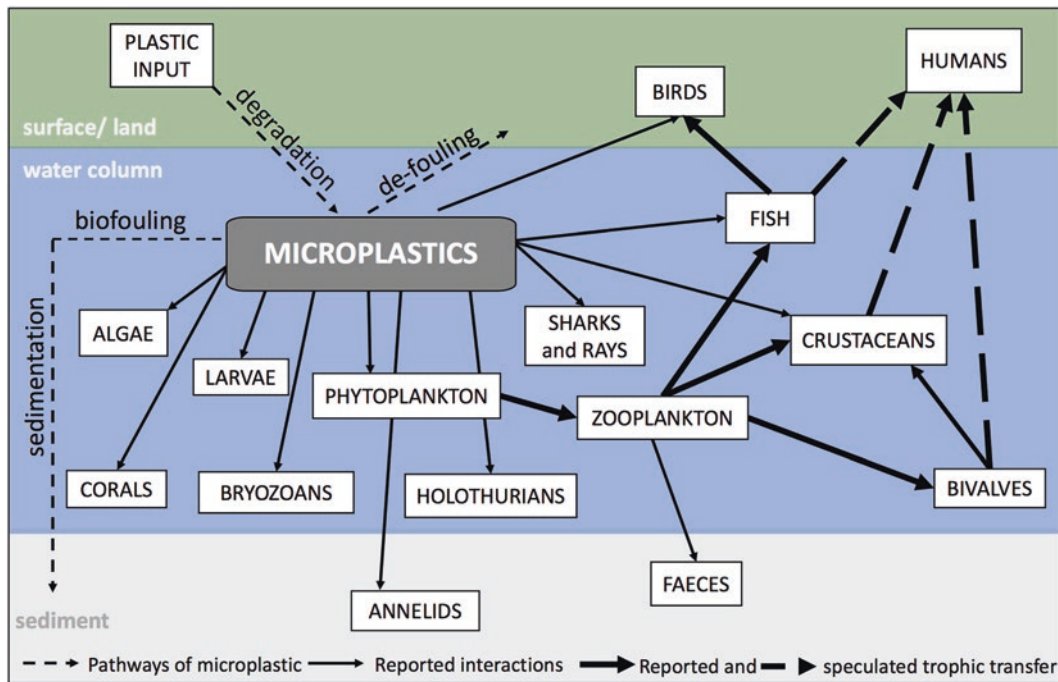


Fig. 6.2 Schematic presentation of microplastic interaction with different organisms in the food web. (Based on Wright et al. 2013a; Lusher 2015; Tosetto et al. 2017)

studies that quantify the effect of microplastics on the function that primary producers exhibit in the marine environment (Troost et al. 2018).

6.2.2 Microplastic Interactions with Invertebrates

The bioavailability of microplastic allows biological interactions with organisms of different feeding types (Fig. 6.2). Availability of microplastic is sometimes dependent on the organisms itself, as for Antarctic krill (*Euphausia superba*) which can biologically fragment microplastic into smaller nanoparticles upon ingestion (Dawson et al. 2018). Apart from the direct ingestion from the water, microplastic can be ingested through their prey (Watts et al. 2014; Green et al. 2015) or through adherence on the organs that are primarily not involved in digestion (Kolandhasamy et al. 2018). The latter was observed in blue mussels with microplastic presence in the gonad, mantle, adductor, visceral, and foot (Kolandhasamy et al. 2018). Here, the digestive gland contained the highest levels of microplastics; however, a clearance experiment showed the retention of microplastics also in other organs.

When microplastics aggregate with marine snow (Summers et al. 2018) or phytoplankton (Long et al. 2015)

they are especially attainable for small and large filter feeders (Setälä et al. 2016, Besseling et al. 2015), and zooplankton (Cole et al. 2013). Over time, microplastic is introduced to the sediment habitat. Together with sediment particles or feces, it can be consumed by benthic suspension or deposit feeders and detritivores, such as annelids (Besseling et al. 2013). Cole et al. (2015) observed that microplastics encapsulated within the fecal pellets can be transferred between coprophagous copepod species. Furthermore, floating microplastics that wash onto the shore are available to invertebrates in the intertidal (Lourenço et al. 2017). Unsurprisingly, microplastic is not only ingested by marine invertebrates. Studies report about representative freshwater organisms such as zooplankton (*Daphnia magna*), amphipods (*Hyalella azteca*, *Gammarus pulex*), and sponges (*Hydra attenuate*) to be affected as well (Au et al. 2015; Rehse et al. 2016, 2018; Murphy and Quinn 2018; Weber et al. 2018).

6.2.3 Microplastic Interactions with Vertebrates

Predatory vertebrate species can ingest microplastic unintentionally, when misidentifying synthetic microparticles for prey. This is especially common when the actual prey is of distinctive color, like in the case of the family of fish

Gerreidae and blue copepods (Ory et al. 2017). In addition to fish (Ramos et al. 2012; Choy and Drazen 2013), microplastic was also reported in predators such as sea birds (Kühn and van Franeker 2012), sea turtles (Schuyler et al. 2013, Yagmour et al. 2018), and marine mammals (Lusher et al. 2018). Vertebrates that ingested microplastic can also promote trophic transfer by ingesting microplastic-containing invertebrates (i.e., bivalves, amphipods, barnacles, polychaetes) or even while scraping on biofilm (Ramos et al. 2012; Reisser et al. 2014, Hodgson et al. 2018). Once the microplastic-containing organisms, such as fish, crustaceans or polychaetes egest feces, microplastic can be available to coprophagous organisms (Cole et al. 2016).

The level of plastic uptake of an organism is accommodated by several factors, such as foraging location, feeding strategies, life stage, and type of plastic in the environment. For example, the location of foraging plays an important role in what is ingested. Interestingly, oceanic juvenile and adult turtles ingested more debris than coastal foragers (Schuyler et al. 2013 and literature cited therein). Feeding mode seems to be correlated to the amount of plastic ingested by fish (Anastasopoulou et al. 2013; Romeo et al. 2015; Battaglia et al. 2016). Early life stages of fish are suggested to be increasingly confronted with microplastic, as they dwell close to the ocean surface where floating microplastic concentrate (N. Prinz, unpubl. data), or in the water column where particles become masked by microbial communities. Understanding differences in exposure conditions in the wild is of major importance to investigate how different species cope with exposure to microplastic in experimental set-ups (Rochman and Boxall 2014).

6.3 The Physical Aspect: Consequences of Microplastic Uptake

To quantify the interactions and effects of microplastic uptake in biota, laboratory exposure experiments are used on key species, resistant to versatile laboratory conditions (Devriese et al. 2015). To increase the probability of microplastic uptake, the concentrations often exceed environmental levels by several orders of magnitude. Studies provide us with the future scenario without appropriate current representation of the microplastic pollution (Rochman and Boxall 2014; Paul-Pont et al. 2018). Therefore, caution needs to be taken when interpreting the results. Furthermore, studies need to clearly disentangle consequences of exaggerated microplastic uptake from those likely encountered in the wild.

Physical effects of microplastics can be observed on individual or population level (Galloway and Lewis 2016). However, from 236 scientific publications reviewed herein, only 11 extrapolate results to the impact on ecosystem func-

tion (4.6%), with only three studies mentioning ecosystem function in the title (1.3%) (Table 6.1).

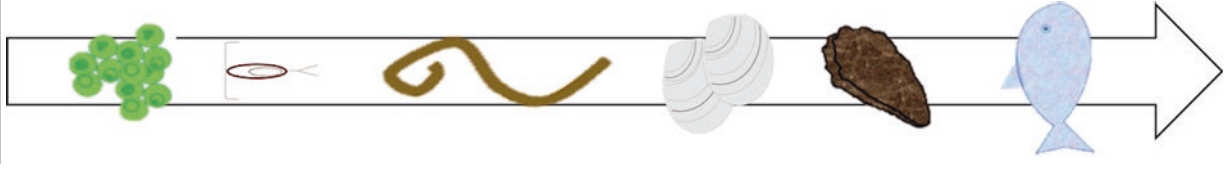
The effects of microplastics on specific specimen are mostly investigated in marine species. However, some already report the effects on freshwater organisms. Mattsson et al. (2017) reported that the uptake of microplastics by freshwater *Daphnia magna* positively correlated with microplastic concentrations. This was also observed in the marine species such as bivalves (*Macoma baltica*, *Mytilus trossulus*), mysids, and in the fiddler crab, *Uca rapax* (Brennecke et al. 2015; Setälä et al. 2016). Upon ingestion, microplastics are either retained in the organism, accumulated, egested, translocated into the tissue (Browne et al. 2008), or rejected. Rejection was observed in larvae of the sea urchin (*Tripneustes gratilla*). The ingestion of microplastic was thus reduced as the larvae actively discriminated between edible and inedible particles (Kaposi et al. 2014). Moreover, zebrafish showed spitting behavior in laboratory conditions as an identification mechanism of ingested but inedible microplastics (Kim et al. 2019). A similar mechanism of selection due to low nutritional content of the microplastic was observed also in blue mussels, where particles were excreted as pseudofeces (Wegner et al. 2012; Farrel and Nelson 2013).

Yet, possibly not all animals have the ability of rejection, and microplastics are likely retained. The effect of the retained microplastics depends on the particle size (Wright et al. 2013a) and seems to be affecting organisms in several ways. Some organisms like the Atlantic Sea scallop (*Placopecten magellanicus*) retain bigger beads longer, as they are probably transferred to the digestive gland for digestion. Smaller particles are trapped in the rejection grooves on the sorting tracts and egested (Brillant and MacDonald 2000). Wright et al. (2013b) attribute longer retention in lugworm to the low nutritional value of the particles and their extensive and energetically costly digestion. Similarly, in corals, the particles moved deep into their polyps, wrapped in their mesenterial tissue. Since the tissue is responsible for the digestion, this raises concerns of the ability to ingest natural food (Hall et al. 2015; Allen et al. 2017). Research on corals is still scarce, but some negative impacts on the health of stony corals were documented with the potential to be sublethal in the long term (Reichert et al. 2018; Tang et al. 2018). In addition to size, the shape of the microplastics is influential. Irregularly shaped microplastic can cause histopathological damages, as observed in the intestine of adult zebrafish (Duis and Coors 2016; Horton et al. 2017; Lei et al. 2018) and European sea bass (Pedà et al. 2016).

The ingestion, retention, and egestion can impair the nutritional health of the organisms. The lungworm (*Arenicola marina*) is used in several studies as an indicator species and ecosystem engineer (Green et al. 2016). Besseling et al.

Table 6.1 Selected studies investigating microplastic effects on marine and freshwater biota with regard to impacts on the organisms' function ordered after trophic level from algae to fish. Studies in bold mention ecosystem function in their title

Common name	Species name	Plastics	Chemicals	Tissue effects	Ecologically relevant effects	References
Freshwater algae	<i>Chorella vulgaris</i>	Nano polystyrene (PS)	Positively charged	Adsorption of positively charged particles	Increased ROS, decreased chlorophyll concentration, photosynthesis rate of algae	Bhattacharya et al. (2010)
Marine and freshwater microalgae	<i>Dunaliella tertiolecta</i> <i>Thalassiosira pseudonana</i> <i>Chlorella vulgaris</i>	Micro Polystyrene (PS)	negatively charged carboxylated PS	Growth was negatively affected by uncharged particles	Altered growth of algae	Sjollema et al. (2016)
Copepod	<i>Calanus helgolandicus</i>	Micro Polystyrene (PS)	n/a	Reductions in ingested carbon biomass owing to a subtle shift in the size of algal prey consumed	Reduced feeding on prey of copepods, energy depletion	Cole et al. (2015)
Lugworm	<i>Arenicola marina</i>	Micro polyvinyl chloride (PVC)	Nonylphenol, phenanthrene, Triclosan and PBDE-47	Higher susceptibility to oxidative stress	Diminished ability to engineer sediment burrows and remove pathogenic bacteria, mortality, ecophysiological function	Browne et al. (2013)
Lugworm	<i>Arenicola marina</i>	Micro un-plasticised polyvinylchloride (UPVC)	n/a	Long residence time in gut, inflammation, feeding apparatus affected	Reduced feeding activity, energy assimilation, seabed engineering and bioturbation affected	Wright et al. (2013b)
Lugworm	<i>Arenicola marina</i>	Micro polystyrene (PS)	12 PCBs	Accumulation of PCBs in tissue	Weight loss, reduced feeding activity	Bessling et al. (2013)
Peppery furrow shell clam	<i>Scrobicularia plana</i>	Micro low-density polyethylene (LDPE)	benzo[a]pyrene (BaP) and perfluorooctane sulfonic acid (PFOS)	Neurotoxicity, mechanical injury to gills, MPs-adsorbed BaP and PFOS exerting a negative influence over the assessed biomarkers in this tissue	Bioindicator for evaluating the health status of coastal and estuarine ecosystems, playing a key role in their structure and functioning	O'Donovan et al. (2018)
European flat oyster, Blue mussel	<i>Ostrea edulis</i> , <i>Mytilus edulis</i>	Micro polylactic acid (PLA), high density polyethylene (HDPE)	n/a	Reduced filtration rates	Altered filtration rates affecting concentrations and fluxes of benthic inorganic nitrogen	Green et al. (2017)
Crucian carp, Bleak, Rudd Tench pike and Atlantic salmon	<i>Carassius carassius</i> , <i>Alburnus alburnus</i> , <i>Scardinius erythrophthalmus</i> , <i>Tinca tinca</i> , <i>Esox esox</i> , <i>Salmo salar</i>	Nano Virgin polystyrene (PS)	n/a	Triglycerides:cholesterol ratio in blood serum, nanoparticles bind to apolipoprotein A-I in fish serum in-vitro, restraining them from properly utilizing their fat reserves	Consumption of nanoparticle-containing zooplankton affects the feeding behavior of the fish	Cedervall et al. (2012)
Freshwater zooplankter, Crucian carp	<i>Daphnia magna</i> , <i>Carassius carassius</i>	Nano polystyrene (PS)	Positively charged	Direct interactions between plastic nanoparticles and brain tissue of fish	Reduce survival of aquatic zooplankter and penetrate the blood-to-brain barrier in a top consumer fish and cause behavioral disorders	Mattsson et al. (2017)
Beach hoppers, Frillfin goby	<i>Platorchestia smithi</i> , <i>Bathygobius krefftii</i>	Micro polyethylene (PE)	Sorbed PAHs	n/a	No effect on boldness and exploration variables in fish behavior	Tosetto et al. (2017)



(2013) and Wright et al. (2013b) observed reduced feeding rates and weight loss in the lugworm upon feeding on polystyrene microplastics. The authors observed reduced growth, maturity, reproduction, and somatic maintenance due to depleted energy reserves (Wright et al. 2013b). Langoustine (*Nephrops norvegicus*) lost body mass due to microplastic retention, which resulted in lower growth rates (Welden and Cowie 2016). Sea urchin larvae had reduced their body width, which was again related to reduced feeding efficiencies (Kaposi et al. 2014). Upon microplastic ingestion, Sussarellu et al. (2016) report that Pacific oysters (*Crassostrea gigas*) reallocated energy for reproduction to structural growth and maintenance. However, microplastic is differently affecting the nutritional health of the freshwater organisms. Weber et al. (2018) namely observed no significant effect on survival, development, metabolism (glycogen, lipid storage), or feeding activity of the freshwater amphipod *Gammarus pulex* upon microplastics ingestion. This is likely attributed to this species being a detritivore and adapted to non-digestible material.

Interestingly, the predatory performance of blue discus (*Symphysodon aequifasciatus*) juveniles and the common goby (*Pomatoschistus microps*) were negatively affected after microplastic exposure (de Sá et al. 2015; Fonte et al. 2016; Wen et al. 2018). Reduced performance raises concerns for survival of the organism as it diminishes the chances of capturing prey or escaping the predators. This might have subsequent effects on the population level if the levels of offspring are reduced on account of starvation, reduced growth, reproductive failure, and mortality (Ferreira et al. 2016; Galloway and Lewis 2016). In the studies by Lee et al. (2013) and Mazurais et al. (2015) mortality correlated with microplastic abundance in nauplii and copepodite stages of copepods and in the larvae of the European sea bass (*Dicentrarchus labrax*). Microplastic intake had lethal effects on fish larvae (Mazurais et al. 2015), forming a possible bottleneck in population dynamics which would lead into decrease of fish stocks (Steer et al. 2017; N. Prinz, unpubl. data).

Effects of microplastic were observed also on population level. Green and collaborators have shown a holistic effect of plastic on the function of bivalve-dominated sandy bottom ecosystems through measuring animal-mediated biogeochemical processes and abundance of different biota (Green et al. 2015, 2016, 2017). Only a few studies conclude with possible impacts on ecosystem function by microplastics that induced reduction of intracellular metabolic and endocrine functioning.

Galloway et al. (2017) described the potential impacts of microplastic exposure from the subcellular to the ecosystem level. This emphasizes again that effects on enzyme activity, oxidative damage, or gene expression can lead to sublethal pathological responses in the cells and organs, eventually harming entire populations through reduced fitness. The con-

sequence of behavioral changes or community shifts can affect the ecosystem as we know it.

6.4 The Cellular Aspect: When Microplastic Particles Translocate into the Tissue

Current scientific efforts focus on more invasive effects of microplastics on organisms. Microplastic is not just affecting organisms when passing through the digestive system, but it can enter into the cells of the digestive tissue, be found in the blood and translocate between tissues (Volkheimer 1975, 1977). Browne et al. (2008) first showed the translocation of microplastic from the gut to the circulatory system of the blue mussels (*Mytilus edulis*) in 3 days. The particles stayed there for almost 50 days. The translocation to the hemocytes was not particle size-dependent, as both 3 μm and 9.6 μm small microspheres translocated. Nevertheless, the smaller particles showed a higher probability of entering into the hemolymph. Translocated microplastics were also found in the laboratory experiments with the shore crab (*Carcinus maenas*). After 1 h the 0.5 μm polystyrene microspheres were found in the stomach, hepatopancreas, ovary, gills, and hemolymph (Farrel and Nelson 2013). The experiments with bigger microparticles (10 μm) failed to show translocations to other organs (Watts et al. 2014), suggesting a size-dependent translocation in organisms. Similar observations of microplastic presence in the hepatopancreas, the stomach, and the gills were made in the laboratory experiments with the fiddler crab, *Uca rapax* (Brennecke et al. 2015). Microplastics were observed in the endocytotic vacuoles of digestive epithelial cells of blue mussels, in their intestine and in the lumina of their primary and secondary ducts of the digestive gland. Epithelial cells of ducts and tubuli were eliminating microplastics, which were phagocytosed into the tissue, forming granulocytomas, an inflammatory response against the foreign particles (von Moos et al. 2012). The translocation of microplastic can sometimes be specific. In held mullet (*Mugil cephalus*), in zebrafish and in European Anchovies (*Engraulis encrasicolus*) microplastic translocated to their liver (Avio et al. 2015; Lu et al. 2016; Collard et al. 2017). Once translocated, microplastics can either cause oxidative stress (von Moos et al. 2012; Lu et al. 2016) or remain inert (Oliveira et al. 2013; Alomara et al. 2017).

6.4.1 Biomarkers Revealing the Effects of Microplastic on the Cellular Level

The direct impacts of microplastics on signaling pathways in the tissue are of interest to increase the knowledge on cellular effects. To investigate this, biomarkers are used as these

biochemical tools measure an organisms' response to environmental contaminants (Monteiro et al. 2005). Many studies measured the activities of digestive enzymes as biomarkers. Microplastics affected, namely, digestive enzyme activities in the digestive system of isopods (*Idotea emarginata*), freshwater blue discus (*Symphysodon aequifasciatus*), silver barb (*Barbodes gonionotus*), and common carp (*Cyprinus carpio*) (Haghi and Banaee 2017; Romano et al. 2017; Wen et al. 2018; Š. Korez, unpubl. data). The affected activities of enzymes, such as lipase, esterase, trypsin, amylase, or alkaline phosphatase, show some kind of physiological challenge to the organism upon microplastic ingestion.

Microplastics enter into the cells through endocytosis or permeate through the lipid membrane when smaller than 50 nm (Fig. 6.3) (von Moos et al. 2012; Pinsino et al. 2015; Jeong et al. 2017). One biomarker used to estimate the health of an animal is the lysosomal membrane stability (LMS) which is sensitive to environmental pollutants (Moore et al. 2006). Lysosomes are single membrane organelles in the cell cytoplasm and are sensitive to environmental pollutants. Their function is cell-specific, however they are responsible for digesting the material taken into the cell (Martínez-Gómez et al. 2015). Microplastics were found in the lysosomes of blue mussels and caused the lysosomal membrane to destabilize, indicating that mussels were affected by the presence of these particles (von Moos et al. 2012; Avio et al. 2015).

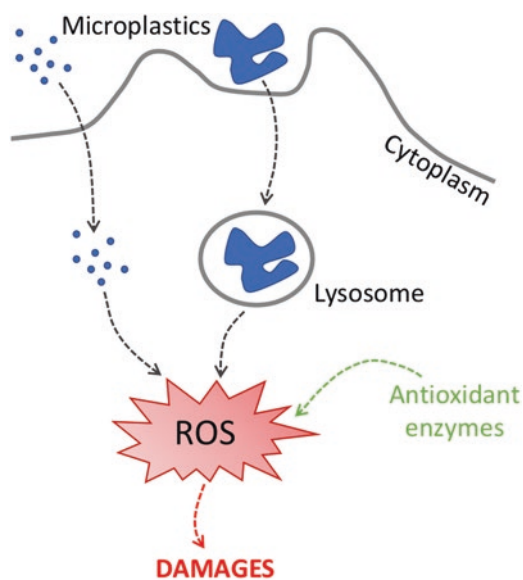


Fig. 6.3 Simplified schematic presentation of events upon microplastic entering the cell. Reactive oxygen species (ROS) are produced as a response to the foreign particle. The antioxidant enzymes are protecting the cell against ROS. In case of constant exposition to ROS, oxidative damages occur that target biomolecules. (Adapted after Wakamatsu et al. 2008)

Once in the cell, microplastic can induce oxidative stress due to a generation of reactive oxygen species (ROS). These are generated when particles are recognized as foreign particles by inflammatory cells, which generate an oxidative response (Miller et al. 2012). Through antioxidants, such as vitamins and enzymes, cells are usually appropriately protected (Lushchak 2011). Enzymes regulate the level of ROS in the cell but in the case of continuous exposure to microplastic can cause oxidative damage (Fig. 6.3) (Sureda et al. 2006).

There are a few studies concerning the biological effects of microplastics that use oxidative stress as a biomarker. Elevated ROS levels were observed in mussels (*Mytilus* spp.), monogonont rotifers (*Brachionus koreanus*), the labrid fish *Coris julis*, and the zebrafish *Danio rerio* after exposure to microplastic (Sureda et al. 2006; Paul-Pont et al. 2016; Jeong et al. 2016; Lu et al. 2016). Overall, microplastic toxicity generally increases with the decreasing particle size (Pan et al. 2007; Choi and Hu 2008; Jeong et al. 2016, 2017). Specifically, a negative correlation between ROS levels and decreasing microparticle sizes was shown in copepods, rotifers, and zebrafish (Jeong et al. 2016, 2017; Lu et al. 2016). The corresponding enzymatic defense mechanisms against elevated ROS follow the same trend of microplastic-size dependence. Rotifers (*B. koreanus*) and copepods (*Paracyclopsina nana*) showed increased defense enzyme activities with decreasing microplastic size (Jeong et al. 2016, 2017). Endocytosis of nanoscale microplastics was not observed to induce oxidative stress responses in red mullet *Mullus surmuletus*. However, the increase in the activity of glutathione-S-transferase (GSF) was observed, suggesting activation of detoxification systems (Alomara et al. 2017). Above listed species experienced no cellular changes, increase in enzyme activity, or oxidative damage even though the organisms ingested microplastics.

ROS can have detrimental effects on biomolecules such as lipids when there are insufficient amounts of antioxidants present (Lushchak 2011). Lipid droplets in the liver of zebrafish confirmed that microplastics affect lipid metabolism (Lu et al. 2016). However, the trend was not universal in all organisms, as the lipid peroxidation levels remain unchanged in the labrid fish *Coris julis*, in the common goby and mussels (*Mytilus* spp.) after microplastics exposure (Sureda et al. 2006; Oliveira et al. 2013; Paul-Pont et al. 2016). The time of microplastic exposure plays a significant role, as short-term exposures showed no effect on lipid metabolism (von Moos et al. 2012; Avio et al. 2015). In addition to lipids, ROS can oxidate proteins and induce gene expression of specific metabolic pathways (Jeong et al. 2016, 2017). In copepods (*P. nana*) and rotifers (*B. koreanus*), kinase proteins were activated, indicating cell death (Jeong et al. 2016, 2017). In the copepod, *P. nana* (Jeong et al.

2017), the nematode, *Caenorhabditis elegans* (Lei et al. 2018), and *Mytilus galloprovincialis* microplastics up-regulated genes of cellular and immune defense pathways and enhanced the energy production (Avio et al. 2015; Détrée and Gallardo-Escárte 2017). The scleractinian coral (*Pocillopora damicornis*) showed induced antioxidant enzymes and detoxifying and immune enzyme activities were repressed (Tang et al. 2018). In *M. galloprovincialis* microplastic caused DNA damages (Avio et al. 2015).

6.5 The Chemical Aspect: Uptake of Leachates from Microplastics into the Body

Microplastics do not solely have consequences as a foreign body. If microplastics are ingested, they can act as a vector for the transfer of chemical contaminants to individuals. Given the diversity of contaminants in aquatic environments as well as the complex chemical structure of plastic polymers, a multitude of different chemical exchanges may occur inside the body of organisms upon ingestion (Karami et al. 2016a; Karami 2017). Also in this regard, the retention time of microplastic particles within the body is especially crucial for possible chemical exchanges into the cells (Welden and Cowie 2016). Some plastic polymers are considered biologically inert (Rist et al. 2018). Therefore, environmentally sorbed contaminants are of particular interest, as these chemicals can leach from the particle into the organism and affect metabolic pathways (Rochman 2015). An exact evaluation of the pollutants and their concentrations on the particle is needed to draw solid conclusions about the impact of microplastics on biota.

6.5.1 Leaching Additives and Persistent Organic Pollutants: The Real Threat?

Plastics are synthesized from monomers, which are polymerized to form macromolecular chains (Galloway 2015). Microplastics, in particular, can act as a vector for compounds that are added during plastic production and may be toxic to organisms (Browne et al. 2008; Hermabessiere et al. 2017). The final plastic polymers often include initiators, catalysts, solvents, stabilizers, plasticizers, flame retardants, pigments, and fillers (Crompton 2007; Galloway 2015). Because of their low molecular weight, toxic compounds, such as nonylphenol (NP) and bisphenol A (BPA), leach out of the plastic polymer, as they can naturally break down and release into the surrounding environment (Flint et al. 2012; Galloway 2015). Based on biodynamic modeling, microplastic-exposed animals, like lugworm and cod, are

threatened by already low concentrations of NP and BPA (Koelmans et al. 2014, Bakir et al. 2016). Other evidence suggests BPA to cause reproductive toxicity in breeding zebrafish (*Danio rerio*) (Laing et al. 2016).

Alternatively, chemicals dissolved in the surrounding seawater can adsorb on the microplastic's surface. A multitude of factors influence the sorption-desorption of persistent pollutants (PPs) from the seawater onto microplastics, including shape, size, type of polymer, fouling, pH, temperature, PP concentration, and K_{ow} (n-Octanol/Water Partition Coefficient) of PPs (Teuten et al. 2007; Wang et al. 2016). Some persistent pollutants (PPs) that sorb onto microplastics are polycyclic aromatic hydrocarbons (PAHs), pesticides (dichlorodiphenyl trichloroethane, DDTs), polychlorinated biphenyls (PCBs), metals, and other endocrine disrupting chemicals (Ng and Obbard 2006; Cole et al. 2011; Bakir et al. 2014; Avio et al. 2015; Llorca et al. 2018). The importance of chemical exchange not only in the water column but in the sediment cannot be underestimated, as heavy metals from antifouling paints, fuel combustion, and industrial waste in sediments can sorb onto microplastics (Deheyn and Latz 2006; Holmes et al. 2012; Rochman et al. 2013; Khan et al. 2015; Brennecke et al. 2016). The global concentration of POPs in marine plastic pellets was estimated to be 1 – 10,000 ng g⁻¹ (Ogata et al. 2009; Hirai et al. 2011).

Additives and pollutants sorbed onto microplastics are bioavailable to marine microorganisms which can metabolize them (Chua et al. 2014; Avio et al. 2015; Wardrop et al. 2016; Auta et al. 2017). Laboratory studies artificially spike microplastics to quantify in how far digestion is an important process in the so-called leaching or desorption of POPs. When particles containing adsorbed chemicals are ingested by an organism, the change in surrounding conditions can promote the release of pollutants (e.g., Besseling et al. 2013; Browne et al. 2013; Batel et al. 2016). Desorption rates of some contaminants in gut surfactants are up to 30 times faster than in the surrounding seawater (Bakir et al. 2014). These desorption rates are influenced by many factors such as pH and body temperature (Hollman et al. 2013; Bakir et al. 2014). For instance, PCBs may leach into fat tissue due to their hydrophobic properties (Hollman et al. 2013). In short-tailed shearwaters (*Puffinus tenuirostris*) from the field, chemical tracers were identified in the blubber tissue and the same tracers were isolated from plastics found in their stomachs (Tanaka et al. 2013). This is particularly interesting, as most studies up-to-date only investigate the digestive tract and draw conclusions from there. Some other important factors for leaching processes, like the constituent polymer, shapes, sizes, and buoyancy differences, are to be considered in bioassay protocols and microplastic toxicity testing (Karami et al. 2016b).

Biomarker responses in organisms like fish can provide insights in specific chemical interactions (Rudneva 2013). Measuring biomarkers such as the activity of enzymes is not only used for the effect of the inert particles on internal metabolism (Sect. 6.4.1) but also to elucidate the effect of chemical contaminants such as pesticides in the body (Ferreira et al. 2016). Plastic-associated chemicals can bind to specific cell receptors, which activate signaling pathways. In the common goby, virgin plastic particles did not induce acute toxicity of chromium (Luís et al. 2015). PAH, Benzo[a]pyrene, with which plastics were spiked, sorb into the intestine in adult zebrafish (Batel et al. 2016). The decrease in enzyme activity leads to a loss of energy (Oliveira et al. 2013), which can result in movement and vision difficulties and consequently influence predatory performance of the organisms (Ferreira et al. 2016; Fonte et al. 2016; Wen et al. 2018). This, in turn, could be investigated further to estimate the effect on the function of an organism in the ecosystem.

Chemical contaminants can have a wide range of harmful effects such as causing cancer and endocrine disruption, hepatic stress, birth defects, immune system problems, and early development issues (Teuten et al. 2009; GESAMP 2015; Rochman et al. 2013; Setälä et al. 2016; Auta et al. 2017). Bioaccumulation has been found in animal as well as in plant tissue with the consequence of ecotoxicity (Chua et al. 2014; Chae and An 2017; Smith 2018). Toxicity can already occur by simple attachment of contaminated microplastics on epithelia of zebrafish, with serious effects of waterborne toxic substances on early life stages (Batel et al. 2018). This shows that adherence rather than ingestion led to the accumulation of microplastics and associated toxicity (Batel et al. 2018). Furthermore, it is suggested that freshwater species suffer a higher risk, as the presence of salts in the water decrease the tendency of some chemicals to be sorbed onto plastic surfaces (Llorca et al. 2018).

Koelmans et al. (2016) suggested that microplastics ingestion by marine biota does not increase their exposure to hydrophobic organic compounds but could have a “cleaning effect”, i.e., adsorption of bioaccumulated POPs onto microplastics, while being ingested. This theoretical explanation is supported by Rehse et al. (2018), who concluded that the presence of ingested microplastic particles can actually reduce the effects of BPA from surrounding water in freshwater zooplankton by a decreased body burden of the environmental pollutant. Kleinteich et al. (2018) found a similar result where a lower bioavailability of PAHs was found when they were sorbed to microplastics. As virgin particles not loaded with POPs did not cause any observable physical harm in zebrafish and clams (Batel et al. 2016; O’Donovan et al. 2018), there is evidence that chemical contamination is the key to understanding the exact impact of microplastic on marine biota (Hermabessiere et al. 2017).

Another line of evidence suggests that the combined effect of microplastics and sorbed contaminants altered organs homeostasis in a greater manner than the contaminants alone (Rainieri et al. 2018). This can only be further evaluated with controlled laboratory exposures to facilitate monitoring of the uptake, movement, and distribution of chemical compounds in whole organisms and excised tissues such as gills, intestinal tract, and liver (Lusher et al. 2017). Yet, little is known about the effects and influence of microplastic-associated toxins on the functionality of an organisms’ body, and consequently associated altered ecosystem function (Table 1).

6.5.2 Microplastics as a Vector for Pathogens

A variety of biotic and abiotic particles can serve as vectors for pathogens, yet due to the persistence of plastic in the marine environment, microplastics are likely to travel farther and for longer periods of time than other types of foulable particles (Dobretsov 2010; Harrison et al. 2014). Contaminated microplastics within the marine environment may be transported between ocean basins and may contribute to the transfer of contaminants between ecosystems (Zarfl and Matthies 2010). This transfer is not limited to chemical contaminants, but also includes the transport of microbial communities consisting of “epiplastic” diatoms, coccolithophores, bryozoans, barnacles, dinoflagellates, invertebrate eggs, cyanobacteria, fungi, and bacteria (Zettler et al. 2013; Reisser et al. 2014; De Tender et al. 2015; Eich et al. 2015; Quero and Luna 2017). Bacterial communities associated with microplastics can potentially modify presently unpolluted habitats (Kleinteich et al. 2018).

Microplastics can serve as a substrate for microbiota as they offer a surface, the so-called *plastisphere* for attachment and settlement (Zettler et al. 2013). Microplastics can thus become a vector for non-ciliate pathogens, such as viruses (Masó et al. 2003; Pham et al. 2012) and pathogenic bacteria (Viršek et al. 2017). Studies in temperate and coral reef environments have investigated how pathogens on microplastic may trigger disease outbreaks in organisms. For example, Lamb et al. (2018) found that the likelihood of disease in corals increases from 4% to 89% when they are in contact with plastic, and, Goldstein et al. (2014) reported the transmission of the coral pathogen *Halofolliculina* spp. on plastic debris. Polypropylene marine debris is dominated by the genus *Vibrio* (Zettler et al. 2013), which are opportunistic pathogenic bacteria that can cause coral disease (Bourne et al. 2015). The microbial biofilm on microplastics, i.e., ecocorona (Lynch et al. 2014) can not only transport pathogens but influence the physical properties of the particle itself. A thick ecocorona reduces the ultraviolet (UV) light,

reaching the surface of polyethylene particles by 90% (O'Brine and Thompson 2010) and makes the particle more hydrophilic (Lobelle and Cunliffe 2011). This increases a particle's sinking velocity (Li and Yuan 2002), which may influence their bioavailability by exposing organisms in other parts of the marine environment to microplastics and associated chemicals (Bråte et al. 2018).

Microplastic biofilms appear distinct compared to those on other marine substrata and are shaped by spatial and seasonal factors (Oberbeckmann et al. 2015). Foulon et al. (2016) summarize that the colonization of microplastics by the oyster-infecting *Vibrio crassostrea* is enhanced when the microplastic was already coated by a layer of primary marine aggregates. These secondary colonizers show a chemical attraction to the particle surface indicating a layering of colonizers in the ecocorona (Galloway et al. 2017).

These "camouflaged" plastic particles can be ingested by organisms such as zooplankton (Eich et al. 2015; Vroom et al. 2017) and even larger organisms. Some laboratory experiments concluded that bioavailability of plastics seems to be enhanced by particles that have been exposed to natural seawater for some time (Bråte et al. 2018). Yet, Allen et al. (2017) suggest that plastic contains phagostimulants that promote ingestion by corals. Interestingly, corals ingested more virgin plastic than plastics covered in microbial biofilm. Both lines of evidence highlight the likelihood of microplastic being ingested by different organisms for different reasons which needs to be better understood in a future with likely increasing amounts of microplastics in the ocean (Harrison et al. 2011; Allen et al. 2017).

Microorganisms in coastal sediments represent a key category of life with reference to understanding and mitigating the potential effects of microplastics, due to their role as drivers of the global functioning of the marine biosphere (Harrison et al. 2011). This is of particular interest with regards to their ability to biodegrade plastic-associated additives, contaminants, or even the plastics themselves (Harrison et al. 2011).

6.6 Trophic Cascade

It has been hypothesized that microplastics transfer within the marine food web from prey to predator (Fig. 6.2). The real extent to which trophic transfer occurs in the wild, however, remains largely unknown, although, laboratory studies have tried to investigate this (Nobre et al. 2015; Setälä et al. 2016). These studies demonstrated trophic transfer for low trophic level food chains, such as *Artemia* sp., crabs and fish (Murray and Cowie 2011; Farrell and Nelson 2013; Setälä et al. 2014; Watts et al. 2014; Batel et al. 2016). Observations of whole prey demonstrate trophic transfer from sand eels (*Ammodytes tobianus*) to plaice (*Pleuronectes platessa*) in

the wild. The lack of significant difference in microplastic abundance between predator and prey however suggests that microplastic is not retained by *P. platessa* (Welden et al. 2018). The likelihood of secondary ingestion is limited, as retention times and transit of particles through the gut of a prey organism can be relatively fast.

Interestingly, transfer of microplastics can occur from prey to predators, without evidences of microplastics persisting in their tissues after 10 days of exposure (Santana et al. 2017). Higher concentrations of microplastics were found in a predatory shellfish from the Persian Gulf, which lead the authors to suggest trophic transfer of microplastics in the food web without quantification in the prey (Naji et al. 2018). Seabird fecal pellets contained a similar composition of fibers to those which were identified in their macroinvertebrate prey which suggests that trophic transfer may be occurring (Lourenço et al. 2017). All predatory marine organisms are susceptible to ingest microplastic through their prey. Toothed marine mammals may be more likely to experience trophic transfer as primary route of microplastic ingestion than through direct intake (Lusher et al. 2016, Hocking et al. 2017). Feces of grey, harbor and fur seals or regurgitated fulmar remains of skuas suggest trophic transfer as these species are known to ingest whole prey (Eriksson and Burton 2003; Rebolledo et al. 2013; Hammer et al. 2016, Nelms et al. 2018). The contamination of microplastics appears to be transported into the deep ocean, not only by the change in density by fouling (Sect. 6.5.2) but through sinking of animal carcasses where it becomes available for scavengers (Clark et al. 2016).

A study by Mattsson et al. (2017) describes how plastic nanoparticles are transferred up through a freshwater algae-daphnia-fish food chain and enter the brain of the top consumer. The damaging effect on the brain leads to a disruption of the fish's natural behavior. In contrast, marine Krefft's frill gobies (*Bathygobius krefftii*) (Tosetto et al. 2017) and an indo-pacific planktivore (*Acanthochromis polyacanthus*) (Critchell and Hoogenboom 2018) did not show altered behavior. Studies investigating animal's behavior are of extreme importance to draw conclusions about potential effects on ecosystem function. There are many relevant species for ecosystem function that need scientific attention (Rochman 2016; Wiczorek et al. 2018), such as different functional groups of fishes (Vendel et al. 2017).

An outdoor mesocosm experiment in sediment cores evaluated the potential effect of microplastics on the functioning of an ecosystem by quantifying the filtration rates of European flat oysters (*Ostrea edulis*) and blue mussels (*Mytilus edulis*) and the entire sedimentary community (Green et al. 2017). Filtration rates significantly decreased in *M. edulis* but increased in *O. edulis* when exposed to microplastics, affecting porewater ammonium. A decrease in biomass of benthic cyanobacteria and polychaetes emphasized

the potential of microplastics to impact the functioning and structure of the sediment environment. Here, not only trophic transfer but the simultaneous effect of microplastic on the function biota in an ecosystem was stressed.

If trophic transfer occurs in the wild, this may also be a route for the transfer of any associated chemicals on the plastics. For example, laboratory experiments on simple artificial food chains, such as with nauplii and zebrafish, have estimated that a transfer of associated POPs occurs (Zhu et al. 2010). Bioaccumulation and biomagnification of chemical contaminants, such as PCBs and organochlorine pesticides (OCPs), are known to occur at higher trophic levels, particularly affecting marine top predators (Tsygankov et al. 2015; Jepson et al. 2016). Whether or not this chemical accumulation is connected to plastic-associated leaching remains unknown. It has been shown, however, that microplastic-associated chemicals can cause toxicity not only in marine animals (Choy and Drazen 2013; Rochman et al. 2015; Rummel et al. 2016; Karami et al. 2018) but also in humans (Hecht et al. 2010).

6.7 Microplastics and Human Health

Concerns of marine organism-derived microplastic and human health were extensively reviewed when microplastics began emerging as a potential threat to ecosystems (Thompson et al. 2009b; Talsness et al. 2009). Microplastic-induced toxicity and the evaluation of consequences for human health have been the focus of current literature (Revel et al. 2018). These concerns are magnified due to the presence of microplastic particles in food items worldwide. Research into the abundance of plastics in food has focused on seafood caught or cultured for human consumption (Van Cauwenberghe and Janssen 2014; Rochman et al. 2015; Naji et al. 2018). In dried fish the eviscerated flesh contained higher microplastic loads than the excised organs, which highlights that removing the digestive tract does not eliminate the risk of microplastic intake by consumers (Karami et al. 2017). When consuming an average portion of filter feeders like mussels, consumers can ingest up to 90 microplastic particles (Lusher et al. 2017). It was estimated that a European shellfish consumer annually ingests between 1800 and 11,000 microplastics (Van Cauwenberghe and Janssen 2014), with the potential for increased concentrations in farmed shellfish (Murphy 2018). Still, studies conclude that the low prevalence of often inert microplastics might indicate limited health risks as suggested by investigations of microplastic loads in canned fish (Karami et al. 2018). Particle uptake in the human body depends on the particle's size, surface charge and functionalization, hydrophobicity, and protein corona (Wright and Kelly 2017). The uptake of

inert particles across the gut has been widely studied (O'Hagan 1996). Nanopolymers can be taken up across the gut into the circulation and be redistributed to the liver and spleen (Galloway 2015). In theory, all organs may be at risk following chronic exposure to nanopolymers. This includes the brain, testis, and reproductive organs, prior to their eventual excretion in urine and feces as evidenced in recent laboratory studies in invertebrates and fish (Jani et al. 1996; Garrett et al. 2012).

In fact, recent media has featured research on microplastics in other non-aquatic consumables such as bottled water, sugar, salt, beer, and honey (see EFSA 2016; Karami et al. 2017; Schymanski et al. 2017; Rist et al. 2018). Carbery et al. (2018) reviewed that there is no robust evidence for the transfer of microplastics and associated contaminants from seafood to humans and the implications for human health. Microplastic uptake through seafood consumption may be minimal when compared to other routes of human exposure, for example, fibers settling on consumables, or dust in the household (Catarino et al. 2018). Food items packaged in plastic may lead daily exposure to different plastic-associated chemicals up to 250 $\mu\text{g kg}^{-1}$ body weight (EFSA 2011; Muncke 2011). Rist et al. (2018) describe that according to a comparison of two studies, exposure to microplastic ingestion from packaging is higher to a magnitude of 40 million compared to the exposure from shellfish. Prata (2018) summarized diseases originating from airborne microplastics and the consequences to human health; a person's lungs could be exposed to between 26 and 130 airborne microplastics per day. The continuous daily interaction with plastic items already leads to the presence of plastic and associated chemicals in the human body (Galloway 2015). Plastic additives, such as BPA, are a risk factor to human health (Srivastava and Godara 2017). Lithner et al. (2011) conducted a comprehensive ranking of plastic polymers, identifying physical, environmental, and health risks. The quantification of plastic particles in food is suggested to be included as one of the components of food safety management systems (Karami et al. 2018).

Given the long-term persistence of plastics within extensive variety of polymer types and additive composition, more research is required to adequately assess the risks that accumulation of micro- and nanoplastics in the body may pose and the true potential to induce pathology (Galloway 2015; Prata 2018). Furthermore, exposure to nanoplastics cannot be precisely estimated yet due to a lack of technological means (EFSA 2016). Despite the focus on human health being a major driving force to increase the investigation of marine biota and plastic interactions because of the economic value of marine protein, the diminished ecosystem service that some species might provide for humans should be highlighted.

6.8 Research Gaps and Future Work

In spite of almost a decade of research, microplastic research is still in its infancy, and it is still very difficult to estimate the cumulative risks of chronic exposure to plastics and their additives. This is due to the limited information available about rates of degradation and fragmentation, leaching of chemicals into environmental matrices, and entry into the food chain (Hermabessiere et al. 2017). Additionally, biological responses of microplastic on the molecular level are difficult to interpret, as the particles' chemical structure is complex and versatile. It can be concluded that current plastic use is not sustainable (Thompson et al. 2009a), which calls for an immediate change in plastic production, consumption, and human behavior, to reduce the amount of microplastics present in the environment. Mendenhall (2018) highlights the large-scale impacts of plastic debris on ecosystem function as a major knowledge-gap.

Although the informative review by Anbumani and Kakkar (2018) summarized different "ecological impacts" of microplastics on aquatic biota and the potential for ecological niche imbalance, an organism's role in ecosystem function is not discussed. Auta et al. (2017) also elaborate on the effects and fate of microplastic ingested by biota and suggest remedies such as microbial activity against microplastic contamination in the environment. Galloway et al. (2017) reviewed current literature and considered microplastic debris to become a planetary boundary threat through its effects on crucial processes exhibited by biota. Chae and An (2017) discuss different global concentrations in freshwater and marine environments, as well as the intrinsic and complex toxicological effects on biota. It is mentioned that research studying effects on generational and ecological effects is important, but no specific references are given.

There is a possibility that organisms may adapt to certain conditions, especially when they are exposed to low concentrations of contaminants for a longer period of time (Sureda et al. 2006). One could even propose that animals will evolutionarily adapt to microplastic concentrations in the environment, which in the future would not affect their fitness. Such suggestion could only apply to the organisms in water column or water surface habitats where the microplastic concentration is mostly stable. Organisms in the sediment or in the intertidal may however be exposed to an ever-increasing microplastic concentration in the near future (Lobelle and Cuncliffe 2011; Green et al. 2017). It is, therefore, critical to continuously evaluate removal rates from the water column towards the sediment or deep sea, as intended by analytical model approaches (Koelmans et al. 2017b). Reduced functionality is correlated to the disappearance of animals (Lusher et al. 2017). If biological processes at the base of ecosystems are altered because of the

presence of microplastics, biologically mediated disruption to the long-term storage of carbon could occur (Villarrubia-Gómez et al. 2017). Despite attempts to model whether microplastics can affect the overall productivity of a marine ecosystem, no clear conclusion can be drawn yet (Troost et al. 2018).

Upon reviewing 222 journal articles, 9 book chapters, two reports, two dissertations and one exhibition, the following 9 research foci need to be especially considered in the future:

1. Laboratory studies should focus on experiments with environmentally relevant quantities and sizes of microplastic and contaminants to estimate actual impacts. Therefore, for instance, studies should include plastic particles, fouled in natural seawater to estimate the role of fouling and/or investigate the degree of chemical contamination from a certain area in the sea in the laboratory.
2. Studies suggest that toxicity of virgin microplastics, spiked microplastics, additives, or contaminants affect the organisms differently (Karami 2017). Further studies are needed to elucidate and distinguish these effects on different organisms and with regards to varying availability of plastic debris and POPs in different ecosystems.
3. Usually the digestive system is investigated for microplastic presence and their effects. Other tissues such as muscle tissue and fat (blubber) should be collected and analyzed for the presence of microplastic tracers and further compared to stomach analysis results (Tanaka et al. 2013; Lusher et al. 2015).
4. More studies on the base of the food chain and the subcellular level are necessary to conclude effects on the individual or population level. For this, we suggest microbiome studies and genetic tools.
5. Limited studies relate the effect on the ecological function of marine organisms after being influenced by microplastics and associated contaminants (Mattsson et al. 2017) (Table 1). Different feeding strategies need to be considered.
6. More research is needed to understand the potential impact of micro- and nanoplastics on primary production and food web interactions.
7. There is a necessity to develop techniques to identify bacterial communities on microplastics.
8. A special focus should be put on freshwater species as they may be at higher risk of some chemicals to be sorbed onto plastic surfaces (Llorca et al. 2018).
9. Many indigestible materials apart from plastic, such as wood, metal, glass and building materials, that are found in the nature that need to be considered. Therefore, other natural and anthropogenic materials should be considered as a comparison, when analyzing the effects of microplastics.

6.9 Summary

Along with ever-increasing plastic production, the amount of plastic waste that enters the oceans is also on the rise. The breakdown of larger debris into microplastic pieces is of high scientific concern as it can become bioavailable. In recent years, aquatic flora and fauna have been found to be affected in different ways when coming into contact with microplastics. This review summarizes that microplastics can attach or get physically ingested by almost all aquatic taxa or affect biota via leachates or pathogens from the microplastic surface. Some studies highlighted that under environmentally relevant levels, microplastic may not necessarily pose risks to the organisms, as particles are often inert. However, other lines of evidence found adverse physiological effects of microplastic in organisms, either through tissue damage, through cellular uptake, or through chemical contamination of leachates from the microplastics. In addition, microplastics can be a vector of pathogens into the tissue of organisms. Often, these effects do not cause death but a sublethal alteration of body functions. The consequences result in reduced primary productivity, compromised energy allocation, reduced growth, changed feeding efficiency, or altered predatory performance. Combined with other environmental stressors, this can lead to alterations of the ecological function of a species in the ecosystem. Only 4% of studies reviewed here investigated how reduced physiological processes, caused by microplastic, are linked with the ecological role, an organism and its population play. There is a general consensus that both the microplastic size and their concentration is critical to understand the impact on an organism. This review emphasized the importance that decreasing particle size and chemical contamination can affect organisms to the extent that critical body functions are impaired. This, in turn, can influence the functional role the organism fulfills in the ecosystem. Since microplastic is bioavailable to the smallest of organisms, secondary ingestion can occur, which may be channeled through the food web. Particular concern arises when microplastic is found in species for human consumption. Nevertheless, we argue that the uptake of plastic and plastic-associated chemicals occurs more through everyday sources in the urban environment, rather than seafood consumption and highlight the need to investigate the importance of impacted ecological functionality of species regarding ecosystem services for humans.

This review summarized cutting-edge research to understand some hazard potentials for different species and research gaps that still need to be examined. This particular field of science is necessary as reliable risk assessments are crucial, contributing to current environmental and societal discussions, and future perspectives concerning microplastic pollution. The focus should be set on the elucidation of

microscopic impacts of plastics on biota for the sake of understanding the impact these small particles can have on populations and functionality of an entire ecosystem that needs to be protected.

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Appendix

This article is related to the YOUMARES 9 conference session no. 7: “Submerged in Plastic: impacts of plastic pollution on marine biota”. The original Call for Abstracts and the abstracts of the presentations within this session can be found in the Appendix “Conference Sessions and Abstracts”, Chapter “6 Submerged in Plastic: Impacts of Plastic Pollution on Marine Biota”, of this book.

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