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Review

Micro- and nanoplastic toxicity on aquatic life: Determining factors

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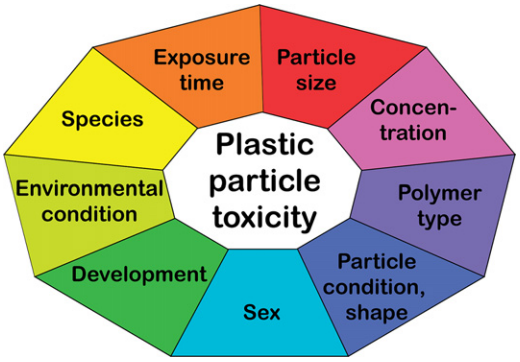
HIGHLIGHTS

- Plastic particle toxicity (PPT) factors: concentration, particle size, exposure time
- PPT co-factors: Contaminants, food availability, species, developmental stage
- PPT: Reduced growth, energy, movement. Stress, inflammation, aberrant development
- More PPT reported below particle size 10 μm, as compared to above 10 μm
- Amount of plastic <10 μm unquantified in all environmental niches

GRAPHICAL ABSTRACT

Main plastic particle toxicity (PPT) determining factors, as published until including 2018. These nine factors were the most mentioned determining factors for PPT in the combined publications included in this review.



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ABSTRACT

Plastic pollution has become a major environmental concern due to its omnipresence and degradation to smaller particles. The potential toxicological effects of micro- and nanoplastic on biota have been investigated in a growing number of exposure studies. We have performed a comprehensive review of the main determining factors for plastic particle toxicity in the relevant exposure systems, from publications until including the year 2018. For a focused scope, effects of additives or other pollutants accumulated by the plastic particles are not included. In summary, current literature suggests that plastic particle toxicity depends on concentration, particle size, exposure time, particle condition, shape and polymer type. Furthermore, contaminant background, food availability, species, developmental stage and sex have major influence on the outcome of plastic particles exposures. Frequently reported effects were on body and population growth, energy metabolism, feeding, movement activity, physiological stress, oxidative stress, inflammation, the immune system, hormonal regulation, aberrant development, cell death, general toxicity and altered lipid metabolism. Several times reported were increased growth and food consumption, neuro-, liver- or kidney pathology and intestinal damage. Photosynthesis disruption was reported in studies investigating effects on phytoplankton. For the currently unquantified plastic particles below 10 μm, more toxic effects were reported in all aquatic life, as compared to plastic particles of larger size.

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Contents

1. Introduction

Today, plastic comprises a large fraction of marine litter globally (Barnes et al., 2009). A recent assessment of marine litter in the upper 60 meters (m) of the supposedly pristine Barents Sea found that plastic comprises 85% of the recordings in that niche (Grosvik et al., 2018). Generally, nearly all investigated species are found to have encountered plastic, counting 529 species, and rising (Gall and Thompson, 2015; Secretariat of the Convention on Biological Diversity, 2016, cited in Lusher et al., 2017). Macroplastic can be detrimental for large animals, by clogging the digestive system or by entanglement, as shown by increasing counts of affected or killed sea mammals and birds. Adding on to this obvious problem, large plastic pieces are degraded to less- or invisible micro- and nanoplastic particles (MNPs) by various mechanisms, such as abrasion during the time the product is in use, mechanical wear such as wave action, photo-oxidation and biological degradation (Cozar et al., 2014; Gigault et al., 2016; Lambert et al., 2013; Lambert and Wagner, 2016; O'Brine and Thompson, 2010). MNPs also enter the water systems through wastewater and runoff from urban areas. Even if treated in state-of-the-art waste water treatment plants, which are reducing the microplastic (MP) concentration of effluent water by >98%, an estimated 65 million MNPs were still released into the receiving water daily in a study (Murphy et al., 2016). Sewage sludge, produced in waste water treatment plants, is commonly applied to agricultural land as fertilizer, and has been reported to contain synthetic clothing fibers, shown to persist five years post-application (Zubris and Richards, 2005). From these and other sources, MNPs are also distributed by air and snow (Bergmann et al., 2019; Catarino et al., 2018; Dris et al., 2015) and recently the recognition of the plastic pollution problem as a biogeochemical cycle was launched (Bank and Hansson, 2019). With such omnipresence, it is time to quantify MNPs occurrence and assess the risk of their plastic particle toxicity (PPT) in the environment.

To extract the *status quo* of PPT research in aquatic biota, we have reviewed 114 exposure study publications until including the year 2018. Several of the studies are backed up by repeated and extended versions of the same exposure regimes in the same model systems (Cedervall et al., 2012; Mattsson et al., 2015; Mattsson et al., 2017; Rochman et al., 2013; Rochman et al., 2014b; Canesi et al., 2015; Canesi et al., 2016; Jeong et al., 2018; Jeong et al., 2016; Biquand et al., 2017; Okubo et al., 2018; Redondo-Hasselerharm et al., 2018a; Redondo-Hasselerharm et al., 2018b). 66 of 73 publications investigating MNPs below 10 μm, show that they can have detrimental effects on aquatic and shoreline organisms, and that the size of the particles is a crucial factor in determining uptake, retention and effects. However, particle amounts of the size fraction below 10 μm are completely unquantified in all environmental niches, including biota and humans. Therefore, the concentration regimes of exposure studies on small plastic particles cannot be anchored in environmental studies at present. Additionally, for those publications not assessing or providing information on the lower particle size limit of exposure material, and for those environmental quantifications that do not assess or provide their lower particle size detection limits, the information necessary for anchoring toxicological exposure studies is lacking. The comparison of quantitative measurements of plastic particles in different matrices is further hampered by different sampling protocols, extraction procedures and measurement methods, and by reporting in different categories, both per shape, polymer type and particles size. The applied categories in different studies often prove incomparable. When ignoring these categories in meta-analysis, the results will contain limited information and will be biased to an extent that could be interpreted as deceit.

It is therefore important to increase stakeholder's awareness of the role the plastic particle size plays in toxicity, and of lower size detection limit of various quantification methods. Furthermore, there is a need for standardization of the applied methods and standardization in the reporting of the results for comparability. These measures are important to facilitate meaningful guidelines for monitoring of plastic contamination and to improve decision-making on all levels towards streamlined future efforts. This was the motivation for the compilation of this review, in which we categorize and summarize reported PPT of MNPs on aquatic life with respect to their main reported determining factors. The long-term motivation is to enable risk assessment, as human health is both affected directly by MNP exposure through air, water and food, as well as indirectly by impact on ecosystems (Barboza et al., 2018), thereby also influencing food security. Therefore, MNP contamination will be of concern for food

safety authorities, environmental agencies and food security stakeholders such as UN organs.

2. Material and methods

2.1. Investigated parameters

We analyzed the currently published literature and extracted the following factors to play a determining role in PPT on aquatic and shoreline biota: 1) concentration, 2) particle size, 3) exposure time, 4) particle condition, 5) environmental condition, 6) polymer type, 7) species, 8) developmental stage, and 9) sex (see Graphical abstract). Most categories are self-explanatory. Particle condition includes shape, surface modifications and weathering. Environmental condition includes food availability, and stress factors such as load of other contaminants, predators, temperature, pH and salinity. To maintain a manageable scope of this review, publications on both MNPs and other contaminants were only included if also effects from MNPs alone were reported. Additives and contaminants, even though likely important factors, were disregarded here as discrete factors, and were included into the factor group of "environmental condition". Along the same line of thinking, chemical polymeric or degradation-wise diversity and modifications, were not included separately, but mentioned in the supplementary tables where clear differences could be stated. However, often several factors were compared at the same time, rendering it impossible to conclude, whether the effect was due to the polymer/modification, or due to another factor, such as shape or size. Plausible factors in this respect were reviewed elsewhere (Andrady, 2017). Studies solely describing ingestion and egestion of MNPs without reporting toxic effects, were not reviewed systematically and only included to illustrate uptake and retention thresholds. With these guidelines, we have conducted an extensive literature study on all published articles (114) we are aware of until including the year 2018, for the analysis of PPT on aquatic life. Introduction and discussion also include some later publications. Electronic keyword searches were performed using Web of Science, Google Scholar, PubMed, and references within references. Keywords used included nanoplastic*, microplastic*, plastic* 'specific polymers' (such as PA (polyamide), polycarbonate (PC), PS (polystyrene), PE (polyethylene), PET (polyethylene terephthalate), PP (polypropylene), PVC (polyvinyl chloride), PLA (polylactic acid), ABS (acrylonitrile butadiene styrene), PMMA (poly(methyl methacrylate)), latex, nylon, Teflon, rubber, biodegradable), specific phylum/sub-phylum (specified in Supplemental Tables 1–5), specific exposure target organisms (e.g. *Daphnia magna*), classes (English; bird, fish, zooplankton, phytoplankton). We have also used combinations of the above key words, e.g. fish and microplastic*, or fish and nanoplastic*. To assess if the severity of the impact of MNPs ingestion varied according to our suspected determining factors (see introduction), information was summarized and compiled in Supplemental Tables 1–5 (Sup. 1–5), per the terms: Biota group, organism, exposure with MNPs: Type of polymer, particle size, concentration, exposure duration. Toxic effect or no effect was emphasized by two background colors red and green, respectively. In rare cases we could not clearly assign either of those categories and left the background white. Polymer types were assigned background greyscales and duration of exposure was emphasized by background bluescales. Special awareness was paid to the role of the size of the particles. We have organized toxic effects to four size categories: Nanometer range (1–999 nm), small micrometer range (1–9 μm), medium micrometer range (10–500 μm) and larger than 500 μm. To achieve an easier, concise overview, we have divided the data of the main text, including main tables, into only two categories, below and above 10 μm. We set the differentiation threshold at 10 μm as this is the current methodological threshold where semi-quantification is possible in larger environmental samples (see list of publications in Small particles are largely unquantified). Second, intestinal M-cells, located to Peyer's patches in mammals, have been shown to sample and transport particles up to 10 μm. These cells have a high transcytotic activity and potentially transport MPs over the intestinal barrier (reviewed in Wright and Kelly, 2017). Determining factors (Table 1) and reported effect categories (Table 2) were summarized in the tables in the main text. Effect groups included the following reported effects: Red, reduced body growth or energy: Reduced body growth, mass, glycogen, energy, food intake, assimilation, filtering, valve opening, digestive enzyme activity, increased metabolic rate, glycolysis, serum glucose, respiration, feeding time. Magenta, reduced population growth or survival: Reduced population growth, survival, reproduction, mortality. Black, reduced activity: Immobilization, abnormal swimming, lower distance or area covered, reduced emergence, fewer casts, faster settlement, reduced respiration, byssus production. Brown, physiological stress, hormonal dysregulation: Oxidative stress, reactive oxygen species (ROS) production, endocrine disruption, inflammation indications, stressed immune system, degranulation, stress gene expression. Blue, aberrant development: Aberrant development, malformations. Grey-cyan, cell death, general toxicity: Cell death, necrosis, apoptotic alterations, nuclear abnormalities, DNA strand breaks, lysosomal membrane stability, toxicity, reduced detoxification. Plum-yellow, altered lipid metabolism: Affected fat storage, vacuolization, metabolism, lipid peroxidation, elevated serum cholesterol. Dark green, increased body growth or food consumption: Faster feeding time, increased food consumption, increased body growth, length, mass or condition factor. Yellow, neuropathology: Decreased nerve signals, acetyl-choline esterase (AChE), neurotoxicity. Red-brown, liver or kidney pathology: Liver or kidney histopathology, hepatic stress, hepatopancreas swelling. Violet, intestinal damage: Intestinal damage, damaged microvilli, increased volume of mucus, changed microbiota. Light green, affected photosynthesis, chlorophyll. Grey, other: Edema, imbalance of water in the brain, fluffy, whiter and swollen brain, increased or delayed molting (crustaceans), decreased gastric milling capacity (krill), increased sugar (phytoplankton), infectivity suppressed (phytoplankton symbiont), increased protein content, suppressed symbiosis (cnidaria/dinoflagellata), tubular dilation (bivalve), increased ammonium production (bivalve), decreased species richness, different muscle texture, increase in heart rate, increase in pericardial sack size, organ homeostasis. Numbers of publications reporting determining factors (Table 1; Graphical abstract) and effect groups (Table 2) are provided, sorted by aquatic biota group and divided into the subgroups above and below 10 μm. Table 3 reports publication numbers for the particle polymer type employed (Table 3). If exposed with particle mixtures including both particles below 10 μm and above 10 μm, for the table, the effects were exclusively assigned to the group below 10 μm, to avoid double counting. As the currently published literature points towards a larger effect and uptake of his group, this size fraction is more likely to be causing the effects. If plastic particles from both size groups were applied and assessed separately, the study was counted in both size groups. If several publications from the same research team, using the same model system with the same range of size and concentration only using slightly different conditions, that group of publications was counted as one study.

3. Evidence for physiological effects of plastic particles in different species groups

3.1. Crustaceans (Sup. 1)

We have evaluated 48 publications, grouped into 46 studies, on crustaceans, of which 17 investigated the water flea *Daphnia magna*, and further two different *Daphnia* species. Otherwise, copepods, rotifers and amphipods dominate the field, however larger crustaceans ranging from krill to Norway lobster are also represented. The polymer types used in these exposure studies were PS (28 studies), PE (13), PET (4), PP (3), PMMA (2), PA (2), car tire particles (1), unidentified polymers (1) or mixes (1) in sizes from 20 nm to 5 mm. The three different shapes

Table 1
Nine determining factors for PPT.

Dependencies	Concentration	Particle size	Exposure time	Particle condition	Environmental condition	Polymer type	Species	Developmental stage	Sex
<10 µm									
Crustaceans	18	12	8	6	3	2	3	1	
Gastropoda	9	1	2	1	2	2	1	1	
Fishes	5	6	2	2	4			1	1
Animals, other	6		4	3		1			
Phytoplankton	10	4	1	2			1		
sum	48	23	17	14	9	5	5	3	1
≥10 µm									
Crustaceans	3	3	4	1	1		1	1	
Gastropoda	1	1		1	1	1			
Fishes	5	4	2	1	2				
Animals, other	3						1		
Phytoplankton				1		1			
sum	12	8	6	4	4	2	2	1	

Number of studies reporting dependencies on nine factors for PPT. See cross-references to the original publications in Supplemental Tables 1–5. Increasing red, higher number. Description of the factors, section Material and methods.

used in the experimental set up were beads, fragments and fibers. The crustaceans were exposed for 30 min to six weeks either through water, sediment or feed, with concentrations ranging between 0.001 mg/l and 1000 mg/l (water), 0.1–40% (sediment), 29–100,000,000 p/l (sediment), 12,000–120,000 p/g feed, 0.3 mg/g feed or 0.4–80% of feed dry and wet weight.

All exposure studies using NPs reported PPT and the smaller NPs had more severe effects on the exposed crustaceans. One major limitation for the studies comparing the effect of different particle sizes was, that the doses were usually based on mass, leading to higher particle number concentrations for the smaller particle sizes. Nevertheless, the one exposure study on copepods, using increasing mass with increasing size from 50 nm to 6 µm, still found a higher PPT for the smaller particles (Lee et al., 2013). The same study described effects on the survival and development of the next generation. The surface chemistry played a role, as aminated PS particles (PS-NH_2) of 40–50 nm were toxic within two days exposure, in contrast to carboxylated ones (PS-COOH), which showed no effect under the given exposure conditions (Manfra et al.,

Table 2
Reported PPT effects.

Effects	Reduced body growth or energy	Reduced population growth or survival	Reduced activity	Physiological stress, hormonal disregulation	Cell death, general toxicity	Aberrant development	Altered lipid metabolism	Increased body growth or food consumption	Neuropathology	Liver or kidney pathology	Intestinal damage	Affected photosynthesis, chlorophyll	Other
<10 µm													
Crustaceans	9	14	9	4	4	4	1		2		1		3
Gastropoda	8	3	2	6	4	2	1	2	1				1
Fishes	5	1	8	5	3		5	2	2	4	1		3
Animals, other	4	1	1	3	2	4							1
Phytoplankton		7		3	1	1						5	1
sum	26	26	20	21	14	11	7	4	5	4	2	5	9
≥10 µm													
Crustaceans	5	7	3	1			1	2		1			
Gastropoda	2				1	1							2
Fishes	4	1	5	5	1		1	2	2	1	4		
Animals, other	3		1		1	2	1						2
Phytoplankton								1					1
sum	14	8	9	6	3	3	3	5	2	2	4	0	5

Number of studies reporting classified effects. See color coded cross-references to original publications in Supplemental Tables 1–5. Increasing red, higher number. Description of the effect groups, section Material and methods.

Table 3
Numbers of exposure studies according to polymer type used.

Number of studies with NMPs <10 μm								
Particle type	PS	PE	PVC	PET	PP	PA	Other	Sum
Crustaceans	22	6		1		1	3	33
Gastropoda	12	4	1				1	18
Fishes	11	4					3	18
Animals, other	6	4	2				2	
Phytoplankton	11	1	1				1	38
Sum	62	19	4	1		1	10	97
%	63.9	19.6	4.1	1.0	0.0	1.0	10.3	
Cumulated %	63.9	83.5	87.6	88.7	88.7	89.7	100.0	

Number of studies with MPs ≥10 μm								
Particle type	PS	PE	PVC	PET	PP	PA	Other	Sum
Crustaceans	8	11		4	3	2	2	30
Gastropoda	6	4	1	1				12
Fishes	4	11	6	2	1	2	1	27
Animals, other	6	3	1				2	
Phytoplankton		3	1		1			5
Sum	24	32	9	7	5	4	5	86
%	27.9	37.2	10.5	8.1	5.8	4.7	5.8	
Cumulated %	27.9	65.1	75.6	83.7	89.5	94.2	100.0	

Number of studies employing different polymer types. See cross-references in supplemental tables to original publications. Increasing red, higher number. For the sum in the column to the right, every polymer has been considered an own study, even though sometimes several polymers were investigated in one publication. Therefore, these sums are higher than the number of publications in the supplemental data tables.

2017; Mattsson et al., 2017). Aminated 50 nm PS also had larger effects as compared to carboxylated 40 nm PS on molting, which was increased, and on mortality (Bergami et al., 2016; Bergami et al., 2017). Based on these observations, interference with the exoskeleton may play a role for the negative impact of plastic on crustaceans. However, carboxylation does not render the particles without any effect. In a different study with extended observation time, 2 μm carboxylated PS increased mortality and reduced growth, at similar concentrations (Aljaibachi and Callaghan, 2018). Another study reported that 200 nm PS immobilized *Daphnia* concentration-dependently (1–80 mg/l). For this endpoint, carboxylated PS had a larger effect than aminated PS (Kim et al., 2017). Ample food alleviated the effects (Aljaibachi and Callaghan, 2018; Rist et al., 2017). For particles between 1 and 9 μm, 60% of the evaluated studies reported effects on the experimental animals, while for the larger particles (>10 μm), the number was 44%. We observed that studies using fibers had a stronger impact on crustaceans as compared with exposure designs using beads or fragments.

One study comparing PE beads of the sizes below and above 100 μm found increased mortality of shrimps exposed to the smaller particles, but not to the larger particles (Gray and Weinstein, 2017). Gray and Weinstein (2017) reported that grass shrimp mortality was higher when exposed to the large fibers (PP) as compared with PS or PE beads of 30–75 μm at 50 particles/ml (Gray and Weinstein, 2017). Spherical particles below 50 μm, did also not induce acute toxicity in Antarctic krill after ten days feeding (Dawson et al., 2018). Furthermore, in water flea, PET fibers of up to 1 mm were not ingested, but caused abnormal swimming behavior and carapace and antenna deformities, by interaction from the outside (Ziajahromi et al., 2017) with concentrations within an order of magnitude of reported environmental levels (Ogonowski et al., 2016). Male Norway lobster (*Nephrops norvegicus*) was fed PP fibers for eight months, and reduced feeding rates, body mass and lipid storage were reported (Welden and Cowie, 2016). In contrast to that, the marine isopod *Idotea emarginata* exposed to PA fibers for 1.5 months, did not show any distinct adverse effects on survival, intermolt duration or growth (Hamer et al., 2014), while Blarer and Burkhardt-Holm (2016) reported reduced assimilation efficiency in amphipods fed PA fibers for 28 days (Blarer and Burkhardt-Holm, 2016). Together, the data points towards that larger fibers have a negative impact on the exoskeleton, but not under all conditions.

To summarize the main reported adverse effects of MNPs on zooplankton crustaceans, they comprise abnormal embryonal development (Jeong et al., 2017; Lee et al., 2013), decreased lipid droplet storage (Cui et al., 2017), decreased feeding rates (Cole et al., 2015; Cole et al., 2013; Ogonowski et al., 2016; Rist et al., 2017), energy depletion (Cole et al., 2015), decreased survival (Au et al., 2015; Manfra et al., 2017), reduced growth (Aljaibachi and Callaghan, 2018; Au et al., 2015; Besseling et al., 2014; Jeong et al., 2016; Redondo-Hasselerharm et al., 2018b; Ziajahromi et al., 2018), altered reproduction (Au et al., 2015; Besseling et al., 2014; Cole et al., 2015; Cui et al., 2017; Jeong et al., 2017; Jeong et al., 2016; Lee et al., 2013; Ogonowski et al., 2016; Ziajahromi et al., 2017), malformations (Besseling et al., 2014), delay in molting (Jeong et al., 2017), abnormal swimming behavior (Rehse et al., 2016; Ziajahromi et al., 2017) and damaged intestinal microvilli (Chae et al., 2018).

Impacts on beach hoppers included reduced jump height and survival, when exposed to salt water exposed PE of 38–45 μm (Tosetto et al., 2016), while sand hoppers exposed to PE of a similar size did not display effects (Ugolini et al., 2013). However, the studies are hardly comparable, as the PE of the second study was not exposed to seawater, the exposure time was shorter, and the exposure was via food, as compared to sediment. Amphipods were shown to react with reduced growth, reproduction (Au et al., 2015; Redondo-Hasselerharm et al., 2018b), assimilation efficiency (Blarer and Burkhardt-Holm, 2016) and a mortality increase that was exponential to the exposure dose (Au et al., 2015). Those studies exposed for extended periods of one month or more to PP or PA fibers or PS beads. Another study exposed with tire particles for 1 month with tire particle and did not find such effects (Redondo-Hasselerharm et al., 2018a). However, the maximum doses were 10% of the sediment. Also, exposure to PET did not cause negative effect, but the exposure time in this study was only two days. Together this might point towards exposure time playing a crucial role in Blarer et al. (2016), where only the longer exposure with PA fibers exerted an effect.

The main impacts of MNPs on larger crustaceans were increased mortality, molting, toxicity and altered swimming behavior for shrimp (Bergami et al., 2017; Casado et al., 2013; Gambardella et al., 2017; Gray and Weinstein, 2017), decreased weight gain with decreased growth rate and hepatosomatic index for crab (Yu et al., 2018), and reduced body mass, feeding rates, hepatosomatic index and metabolic rates, catabolism of stored lipids and water in the hepatopancreas for Norway lobster (Welden and Cowie, 2016).

Some of the reviewed exposure studies found no impact of MNPs on the experimental crustaceans. However, negative impacts might have been overlooked in those studies, as: a) endpoints analyzed were unaffected but different from endpoints affected in other studies (Rosenkranz et al., 2009), b) few endpoints on PPT were assessed due to a different focus of the study (Mattsson et al., 2015), c) only larger MPs (15 μm, 63–75 μm) were fed (Canniff and Hoang, 2018; Vroom et al., 2017), while in other studies smaller MNPs had effects, d) particles were co-fed with an abundance of algae that might counteract the PPT (Vroom et al., 2017), or e) it was exposed only with low particle numbers (Weber et al., 2018) compared to similar studies finding effects (Au et al., 2015).

One crustaceans study investigated the important question if natural particles of similar size might have different effects from plastic particles. *D. magna* was fed with both, and results show that MNPs had a stronger adverse effect on feeding compared to the investigated naturally occurring mineral particles of kaolin (Ogonowski et al., 2016). Another study demonstrated different response effects in shrimp and barnacle larvae exposed to the same PS particle of 100 nm on swimming activity and excretion. Species dependency of toxic effects of MNPs was demonstrated in three crustacean studies (Beiras et al., 2018; Gambardella et al., 2017; Redondo-Hasselerharm et al., 2018b).

Potentially contributing to the breakdown of MPs in the ocean, Antarctic krill (*Euphausia superba*) was shown to mill down 31.5 μm large MPs to NPs when exposed to concentrations within the order of magnitude observed in pelagic systems of the North Pacific Subtropical Gyre (Dawson et al., 2018). The animal's milling capacity decreased with higher doses and exposure times. Besides the effect on the animals themselves, this could contribute to the biodegradation of MPs in the ocean towards more harmful size classes.

Based on the reviewed literature on crustaceans with the observation that several of studies reported impact on the exoskeleton, such as swimming behavior and molting and late irreversible biological responses such as decreased growth, survival and assimilation, we suggest that crustaceans may be at high risk for ecosystem disadvantage by MNP contamination loads.

3.2. Gastropoda (Sup. 2)

We have evaluated 22 relevant publications (21 studies) on gastropods, with the main experimental animals being the blue mussel *Mytilus* spp., i.e. *M. edulis* and *M. galloprovincialis*, in addition to three species of oysters and clams, green muscle, snail and slipper limpet. The investigated polymer types in these exposure studies were PS (15), PE (7), PVC (2), PET (1) and PLA (1) in sizes from 30 nm to 704 μm as beads or fragments from cosmetic products. The concentrations used ranged from 0.8 μg/l to 2 g/l, or 1000–1,800,000 p/l and 0.0003% of plastic in water. The concentration used when co-fed with algae was 0.2–70% plastic per algae and when suspended in the sediment 0.045 particles/g sediment. Exposure times ranged from 3 h to three months and all studies reported minor or adverse physiological/toxic impact on the experimental gastropods, except two studies (Browne et al., 2008; Cole and Galloway, 2015).

Generally, for gastropods, there seemed to be size-related uptake thresholds and retention times (Browne et al., 2008; Farrell and Nelson, 2013; Sussarellu et al., 2016; Van Cauwenberghe et al., 2015), which also depended on developmental stages (Cole and Galloway, 2015). However, not in all studies the smallest particles were preferred for uptake. There seem to be preferred size ranges instead. Furthermore, studies showed that uptake was enhanced by seawater (Brate et al., 2018), that aminated PS was preferred over carboxylated PS (Cole and Galloway, 2015), that different polymer types had different effects (Avio et al., 2015; Rochman et al., 2017), and the dose played a significant role (Gardon et al., 2018; Rist et al., 2016; Rochman et al., 2017).

Six of the reviewed studies either exposed with or did not exclude that NPs were present in the exposure material, and all found PPT on the investigated species, i.e. blue mussel, periwinkle, peppery furrow shell clam, European flat oyster using either PS, PE or PLA NPs. The observed effects included decreased lysosomal membrane stability (Avio et al., 2015; von Moos et al., 2012), formation of granulocytomas (von Moos et al., 2012), decreased peroxisomal proliferation (Avio et al., 2015), enhancement of DNA strand breaks in hemocytes (Avio et al., 2015), cytotoxicity (Canesi et al., 2015; Canesi et al., 2016), developmental arrest (Balbi et al., 2017), inflammation (Avio et al., 2015), and increased ROS (Canesi et al., 2015; Canesi et al., 2016) and decreased filtering and increased pseudofeces (Wegner et al., 2012).

Nine studies investigated particles in the size range between 1 μm and 9 μm, and nine studies used larger particles than that. Of those, all but two studies reported PPT. One of those studies exposed blue mussels to PS (2–16 μm) for 3–12 h before observation for 1.5 months and reported no significant reduction of oxidative status of hemolymph, phagocytic activity or filter feeding activity. The exposure time in this study was very short (Browne et al., 2008), as compared to other, later studies finding effects. There were also no effects on feeding and growth of pacific oyster larvae using comparatively low particle numbers of 100,000 particles/l (Cole and Galloway, 2015). In the different MNP exposure studies on Gastropoda, PPT was observed as a decrease in periods of valve opening, byssus production (Rist et al., 2016), settlement time (Lo and Chan, 2018), gametogenesis (Gardon et al., 2018), survival (Rist et al., 2016), oocyte number and diameter, sperm velocity and development (Sussarellu et al., 2016), growth (Gardon et al., 2018), assimilation efficiency (Gardon et al., 2018), detoxification, lipid peroxidation (Paul-Pont et al., 2016), and an increase in glycolysis, hemocyte-/granulocyte mortality, anti-oxidant/glutathione-related enzymes (Paul-Pont et al., 2016), tubular dilation, abnormalities, tissue necrosis, energy/food consumption (Sussarellu et al., 2016; Van Cauwenberghe et al., 2015) and endocrine disruption (Sussarellu et al., 2016). Respiration was found to be increased (Green et al., 2016) or decreased (Rist et al., 2016) in two studies with incomparable design.

Interestingly, increased ammonium concentration was observed in the overlaying water of Manila clams when exposed to PE smaller than 33 μm (Cluzard et al., 2015). Increased ammonium production may be linked to an interruption of the nitrification, which may lead to eutrophication and the authors suggested that this could be used as a response biomarker in other ecological studies on MNP.

Rochman et al. (2017) examined whether environmentally relevant concentrations of different types of MPs (PET, PVC, PS, PE sized 12–704 mm) with or without PCBs, directly affected Asian clams and indirectly affected white sturgeon. They reported tubular dilations and tripled rates of abnormalities, which were aggravated by additional exposure to PCB in the Asian clams, especially for PVC and PS. This finding points towards polymer specific effects when investigating the impact of other contaminants.

Based on the reviewed literature on gastropods with the observation that several of studies reported decreased feeding and assimilation efficiency with increased respiration and energy consumption, and late irreversible biological responses such as decreased growth, survival and offspring development, we suggest that gastropods may be at high risk for ecosystem disadvantage by MNP contamination loads.

3.3. Fishes (Sup. 3)

We have reviewed 35 exposure publications (29 studies) using fishes. The main experimental fish were zebrafish, Japanese medaka, common goby, crucian carp and sea bass and the polymers used in the exposure studies were PE (17), PS (15), PVC (6), PET (2), PA (2), PP (1) and PC (1), or unidentified plastic (2) in sizes from 24 nm to 2 mm. The particle types ranged from pristine pellets or fragments, to environmentally exposed polymers, surface modified or fluorescently labelled polymers. The exposure studies with fish were either designed as water exposure, feeding studies, or feeding via the food chain. The concentrations of the plastic polymers in the water exposure studies ranged from 0.001 to 500 mg/l with durations from three to 50 days. For the dietary studies the concentrations of the polymers were given either as percentage of the feed (0.1–40%), mg/kg (100–500) or particles/kg diet (10,000–100,000). For the food chain studies, only the first link (algae) was exposed to the MNPs resulting in an uncertain dose arriving in the exposed fish (Cedervall et al., 2012, Mattsson et al., 2015, Mattsson et al., 2017). The duration of the feeding studies in our data material ranged from seven days to three months in Krefft's frillgobies and sea bass, respectively.

Except for three studies, one food chain study on Krefft's frillgobies and feeding studies on sea bream and rainbow trout (Jovanovic et al., 2018; Manabe et al., 2011; Rummel et al., 2016; Tosetto et al., 2017), all studies concluded with one or more PPT effects in the investigated fish species. Of the 15 fish exposure studies with MNPs up to 10 μm, all found negative effects, such as decreased survival (Manabe et al., 2011), decreased activity of a neurotransmission biomarker, AChE (de Sa et al., 2015; Ferreira et al., 2016; Luis et al., 2015; Oliveira et al., 2013), decreased energy storage of glycogen (Rochman et al., 2014a; Rochman et al., 2013), aberration of liver energy metabolism (Karami et al., 2016), effects on heart and lipid tissues (Lu et al., 2016; Ravit et al., 2017), effects on heart rate (Pitt et al., 2018), increased feeding

time (Cedervall et al., 2012; Mattsson et al., 2015; Mattsson et al., 2017), inflammation (Lu et al., 2016), oxidative damage (Chen et al., 2017), necrosis (Chen et al., 2017), effects on body length (Chen et al., 2017), intestinal bacterial composition (Jin et al., 2018) and texture of brain and muscle including impact on the water balance in the brain (Cedervall et al., 2012; Mattsson et al., 2015; Mattsson et al., 2017). Of the 17 studies employing MPs larger than 10 μm, only three reported no impact on the experimental fish. In the fish behavioral study by Tosetto et al., 2017, feeding Krefft's frillgobies PE exposed beachhoppers, not reporting adverse behavioral effects, the exposure dose was not quantified, which lead to a high degree of uncertainty when it comes to the knowledge that could be extracted from the study.

Some evidence suggests that species might react differently to exposure to MPs: While PET <300 μm fed to reef fish (Critchell and Hoogenboom, 2018) and PET, PVC, PS or PE fed to white sturgeon changed their condition (Rochman et al., 2017), the comparable size class of PE fed to rainbow trout did not (Rummel et al., 2016). No effects of growth performance were reported for seabream and rainbow trout exposed to 212–250 μm and 25–112 μm respectively (Jovanovic et al., 2018; Rummel et al., 2016). However, another sea bass exposure study 300–500 μm particles and a seambream exposure study with 40–150 μm particles (Espinosa et al., 2017; Peda et al., 2016), with relatively similar size ranges of MPS, investigating early response biomarkers, reported intestinal damage, inflammatory changes, loss of storage fat, liver and kidney damage and decrease in serum glucose (Espinosa et al., 2017). The different end points that were analyzed may explain the discrepancies that may seem to be there. The end point evaluation of biological responses in the organisms are of major importance in all exposure studies and may range from late responses such as effects on growth to early responses such as effects at the cellular level. Although not unique for exposure studies using MNPs, the two seabream studies are two examples, in which the study design do assess different endpoints resulting in two different conclusions: no effect or major effects of MNPs exposure. Hence, when comparing between studies, maybe especially investigating “novel” undesirables such as MNPs, results must be interpreted carefully, and the question needs to be risen whether relevant endpoints were assessed.

Another effect observed with particles larger than 10 μm were decreased predatory performance (de Sa et al., 2015; Wen et al., 2018).

Effects on body length were ambiguous, as both reduced body length (Chen et al., 2017) and increased body length (Peda et al., 2016) or condition factors (Rochman et al., 2017) were reported. MNP size may play a role here, but the data are too few to be conclusive. Direct comparisons of effects caused by differently sized particles found particle size dependency, but were often biased as the concentration of the MNPs were normalized by mass, not particle number or surface area (Chen et al., 2017; Critchell and Hoogenboom, 2018; Jin et al., 2018; Lu et al., 2016), which leads to the unresolved question whether it was the increased particle number or the decreased particle size, which was responsible for the observed effects. The possibility to draw solid conclusions from this data is limited, as different model systems, polymers, concentrations, exposure times and -routes were applied.

Several studies point towards enhanced toxicological effects of the combination of MNPs and sorbed contaminants (Barboza et al., 2018; Greven et al., 2016; Karami et al., 2016; Oliveira et al., 2013). One suggested possible mechanism is inhibition of multixenobiotic resistance, as shown for rotifers (Jeong et al., 2018). However, lack of an enhancement of contaminant effects by plastic has also been demonstrated (Ferreira et al., 2016). This effect might be contaminant-specific, as the same research group, in the same model system, a goby, reported an effect of the same size of MP on pyrene and Cr toxicity, but not when examining Au toxicity. Contrasting this, alleviation of contaminant effects by MPs was also reported for fish that came from a high contamination background, but not for fish that came from a low contamination background (Luis et al., 2015). In that respect, MPs might act as a sorbing medication with side effects – only desirable at contaminated conditions. That way, plastic particles potentially modulate the toxicity of other contaminants dependent on the background environmental conditions. Another crucial factor might be the influence of environmental conditions during the critical phases of the development of the organisms (de Sa et al., 2015).

Based on the reviewed literature on fishes with the observation that several of studies reported decreased feeding or predatory performance, disturbed energy metabolism and inflammation in different organs, we suggest that fishes may be at risk for ecosystem disadvantage by MNP contamination loads. It may also be considered to evaluate plastic contamination in the context of animal welfare.

3.4. Other animals (Sup. 4)

Seven publications reported physiological or toxicological effects on sea urchins, of those, five studies reported effects on *Paracentrotus lividus* and one each on *Lytechinus variegatus* and *Tripneustes gratilla*. All studies but one found negative impact on the organisms. The polymer types in the exposure studies were PS (5) and PE (3), in sizes ranging from 40 nm to 80 μm, or not indicated, as pellets or fragments, pristine or from beaches, aged, surface modified or with protein corona. The concentrations ranged from 0.005 to 5000 mg/l and 1–100,000,000 p/l, and some of the studies did not provide amounts. The group of animals in this section was exposed from one day to one week. Three regimes exposed the animals in the nm range and used concentrations in the low mg/l range and found severe PPT including malformations, oxidative stress and induction of apoptosis (Della Torre et al., 2014; Marques-Santos et al., 2018; Pinsino et al., 2017). Three studies exposed in the μm range and the one study not reporting any adverse effects used the lowest concentration with maximally 300,000 p/l, corresponding to approximately 20 mg/l (Kaposi et al., 2014). The studies that reported significant effects generally used MP concentrations in the g/l range, reconciling the different results. Reported effects included reduced growth and fertility and malformations/abnormal larval development (Martinez-Gomez et al., 2017; Messinetti et al., 2018; Nobre et al., 2015).

Five publications reported physiological effects on lugworms, by exposing the worms to PVC (3) and PS (2). One of the latter studies compared PVC to PE (1) and PLA (1). Particle sizes varied from 1.4 μm to 1.3 mm or were not provided, and concentrations were 100,000 p/l or between 0.074 and 7.4% of sediment, 1–100 g/l, or 0.5–5% of weight. Observation times given for the investigated studies were from ten days to one month. All studies reported physiological effects on the experimental animals, but one of the studies with lugworm only reported increased whole body protein content (Van Cauwenberghe et al., 2015). The only study with exposure conditions including particles below 10 μm employed no specific MP size, but a range from 1.4 to 478 μm (Green et al., 2016) and another study might have included such small particles, but no lower size limit was provided (Browne et al., 2013). With such a wide range, size specific toxicological effects cannot be pinpointed. Further effects of exposure of lugworms with MNPs were reduced feeding activity, fewer casts and bodyweight decrease, and increased metabolic rate and depleted energy reserves (Besseling et al., 2017; Browne et al., 2013; Green et al., 2016; Wright et al., 2013). All studies in this category point towards an effect on the worms' overall energy budget, with one exception (Van Cauwenberghe et al., 2015) that operated with lower doses than high amounts found in the environment, i.e. 0.5% weight of sediment (Wright et al., 2013).

Two publications on blackworm did not observe effects on growth or number of worms with exposure with 10–586 μm MPs (Redondo-Hasselerharm et al., 2018a; Redondo-Hasselerharm et al., 2018b).

Two coral studies reported suppressed symbiosis (Okubo et al., 2018) and bleaching and tissue necrosis (Reichert et al., 2018) using 3–17 μm PS, face wash or 37–163 μm PE particles. Bleaching

and tissue necrosis were species dependent, but highly prevalent, as detected in five out of six species. The freshwater cnidarian *Hydra attenuate* was exposed to <400 μm PE flakes of unknown lower size limit and lower feeding rates and morphology scores were reported (Murphy and Quinn, 2018). Furthermore, in ascidians exposed to 10 μm PS, slowed down the metamorphosis was observed within one week (Messinetti et al., 2018). In tadpoles 3 μm PS had no effect on body growth or swimming activity, however, the length of the exposure time was not provided, and during the first exposure timespan, the mouth opening was not developed yet, and the animals lived of their egg-reserves (De Felice et al., 2018). As the only study on birds included here, white chinned petrel fed with 40 pellets of undisclosed size, displayed no significant effects on stomach lining or assimilation efficiency (Ryan and Jackson, 1987).

3.5. Phytoplankton (Sup. 5)

For phytoplankton, we have reviewed 17 relevant publications, including ten marine species and nine freshwater species. The polymer types used in the exposure studies were either pristine PS (11), PE (3), PP (1), PVC (1) or unidentified beads (1) in sizes from 40 nm to 1 mm and concentrations of 0.001–2000 mg/l, or 150,000,000 p/l for 30 min to 2.5 months.

Eight of ten studies exposing phytoplankton to particles in the nm range reported negative impact. When no effects were found, either mortality, effective toxicity (Chae et al., 2018) or photosynthesis (Sjollema et al., 2016) were used as endpoints, in contrast to the less severe endpoint growth inhibition, which consistently was reported to be impacted (Bergami et al., 2017; Besseling et al., 2014; Gambardella et al., 2018; Mao et al., 2018; Sjollema et al., 2016). This size-class was exclusively investigated with PS. For particles of 1–9 μm, five of eight publications reported effects, with PS, PVC and unidentified particles isolated from face wash. Of the studies reporting no effects in this size class (Long et al., 2017; Sjollema et al., 2016), one operated with rather low concentrations up to 0.04 mg/l (Long et al., 2017). In comparison, those finding effects employed 1.5–250 mg/l (Davarpanah and Guilhermino, 2015; Mao et al., 2018; Zhang et al., 2017), including the study providing particles/ml (based on a calculated mass estimation by us; Biquand et al., 2017; Okubo et al., 2018). Studies exposing to MPs larger than 10 μm found either no effects (Zhang et al., 2017), increased growth without an effect on the investigated growth or stress regulating genes (Canniff and Hoang, 2018; Lagarde et al., 2016), or increased sugar production (Lagarde et al., 2016), where PP and PE had different effects.

One algae study directly comparing the effects of exposure to three different size classes of plastic particles, did not report any effects for the largest MP class, while effects for the lower size classes were found for only one of three algae species (Sjollema et al., 2016). In the study by Sjollema et al., 2016, the applied concentrations were normalized per mass, leading to three orders of magnitude differing particle numbers. Therefore, it cannot be determined whether the size or the concentration was the determining factor. However, PVC exposure inhibited growth and damaged cell walls of the marine algae (*Skeletonema costatum*) at 1 μm particle size, but not at 1 mm size, with adjusted concentrations for particle size (Zhang et al., 2017), providing an indication that size might be a relevant determining factor for PPT, also on phytoplankton, at least for large size differences. The clearest trend, backed up by six studies, is that PPT of particles up to 1 μm on phytoplankton is concentration dependent over a large range.

We also would like to highlight one study using Duckweed (*Lemna minor*) that reported mechanical blockage of root growth specific for sharp PE particles, as compared to smooth edged PE particles, thereby pointing towards a role of the shape of the particles (Kalcikova et al., 2017).

4. Flaws and conclusions from the current literature

4.1. Size dependency of MNP uptake, retention and PPT

Ample evidence points towards the tendency of larger negative impact by smaller plastic particles compared to larger ones, from the nm to triple-digit μm size range (Chen et al., 2017; Cole et al., 2013; Critchell and Hoogenboom, 2018; Jeong et al., 2017; Jeong et al., 2018; Jeong et al., 2016; Jin et al., 2018; Lee et al., 2013; Lu et al., 2016; Ma et al., 2016; Mattsson et al., 2015; Mattsson et al., 2017; Rehse et al., 2016; Rist et al., 2017; Sjollema et al., 2016). However, most studies adjusted the exposure amount for the different conditions of the size comparisons by mass, leading to three orders of magnitude higher particle numbers for each order of magnitude smaller particle diameter. Thereby, for most of the study cases, it is impossible to conclude whether it is the size or the sheer particle number that has the largest impact on the effect.

It is reasonable to expect that size dependent effects should also depend on the compartments that are reached by and retain the particles. A common assumption is that MPs in fish remain in the gastrointestinal tract, supported by findings that particles of >63 μm and 150 μm, in goldfish and sea bass, respectively, were not absorbed through the gut, but expelled with the feces (Grigorakis et al., 2017; Peda et al., 2016). On their way, they could damage the intestinal wall (Peda et al., 2016). However, there is also evidence speaking against the assumption that plastic remains in the gastrointestinal tract: More plastic was found in filets, as compared to the gastrointestinal tract, in two of four investigated species in one study (Karami et al., 2017). Furthermore, considerable amounts of relatively large plastic particles (124–438 μm) were detected in the liver of three fish species (Collard et al., 2017), and in feet of mussel (1–5 mm) (Kolandhasamy et al., 2018). It is interesting to note that the authors of the latter study suspect entry through adherence rather than through ingestion. Uptake- and retention rates of MNPs, and location of MPs in organisms, are related to the MNP size. The number of particles reaching and being retained in several compartments is shown to increase with decreasing particle size (Browne et al., 2008; Critchell and Hoogenboom, 2018; Jani et al., 1992; Jeong et al., 2016; Kashiwada, 2006), as reviewed previously (Wright and Kelly, 2017), possibly with an exponential relation (Kokalj et al., 2018). Different organs might have different entry thresholds. Entry into or through the intestinal wall was observed for particles in the nm range in waterflea *Daphnia* (Rosenkranz et al., 2009) and zebrafish embryos (van Pomeren et al., 2017).

In exposure studies, PE particles of up to 20 μm possibly entered epithelial cells of the intestinal wall of zebrafish (Batel et al., 2016) and 1 μm and 20 nm PS crossed the epithelial intestinal barrier of waterflea (Rosenkranz et al., 2009). In the waterflea study, the uptake of the 20 nm particles, as compared to one μm particles, was lower in terms of mass, but equal or greater in terms of surface area or particle number. In the same study, depuration was rapid for one μm beads (>90%/4 h), while it was less effective for 20 nm beads (40%/4 h) (Rosenkranz et al., 2009). However, another research group could not replicate the study due to fluorescence leaching from the beads (Schur et al., 2019). Mechanisms transporting MNPs through physiological barriers might be greatly influenced by physiological conditions of the animal, such as diabetes, which was shown to reduce NP transport in a model system (McMinn et al., 1996). The uptake of MNPs, and the modification of such uptake by physiological conditions in mammals is warranting further investigation of the impact of MNP contamination in humans.

When it comes to other organs, reaching the lymph was shown for 3.0 and 9.6 μm PS (Browne et al., 2008) and <100 μm particles (Avio et al., 2015) in blue mussels. Reports on uptake of plastic particles from mixtures below 80 or 100 μm into epithelial digestive tubule cells or hemolymph of blue mussel, deliver unfortunately no information on the upper size limit for uptake, as no size distribution of the particle uptake was provided (Avio et al., 2015; von Moos et al., 2012). It

cannot be excluded that only the smaller particles were taken up. Into liver, MNPs from 39 nm to 5 μm were shown to enter in studies with medaka and zebrafish, respectively (Kashiwada, 2006; Lu et al., 2016; Pitt et al., 2018). The aforementioned study of wild fishes mentioned the size range of 5–250 μm (Collard et al., 2017). Finally, uptake studies with mitten crabs reported PS particles of 5 μm and below 1000 μm, respectively, in the gills (Brennecke et al., 2015; Yu et al., 2018).

Several factors seem to play a role for the MNP size limits for uptake into tissues: There is evidence for a species dependency, as study on blue mussel showed a higher uptake into hemolymph and hemocytes of 3 μm PS than of 9.6 μm PS (Browne et al., 2008), while 30 and 90 μm PS were not taken up at all. For lugworms, however, also PS up to 30 μm was found inside the animal (Van Cauwenberghe et al., 2015). Furthermore, the condition of the particles plays a major role for uptake into tissues, as PE MPs of 50–570 μm that were pre-exposed to ocean water, were shown to be taken up to a larger extent into blue mussels than PE MPs that were not pre-exposed to ocean water (Brate et al., 2018). Also for zooplankton, uptake rates for 15 and 30 μm particles are size-dependent, and enhanced by seawater (Vroom et al., 2017). Finally, the developmental stage of the organisms plays a role for uptake into tissues, as shown for oyster larvae at different developmental stages, were younger larvae were taking up particles from 160 nm to 7.3 μm, while older larvae also took up larger particles of 20.3 μm (Cole and Galloway, 2015). Furthermore, some organisms, such as tadpoles, need to reach a certain developmental stage before developing a mouth, thus excluding MP uptake via this pathway at an early life stage (De Felice et al., 2018).

We would also like to highlight the potential unintentional production of smaller size fractions by the experimental setup. Of two studies investigating the effects of PS in lugworms, one found an increase in protein content by 10–30 μm PS (Van Cauwenberghe et al., 2015), the other found a decrease in weight and reduced feeding activity by feeding 400 μm to 1.3 mm large particles (Besseling et al., 2013). This would be difficult to reconcile with the observation that lugworms take up 30 μm MP, but not 90 μm MP (Van Cauwenberghe et al., 2015). However, Besseling et al. (2013) had mixed the plastic particles with sediment on a roller for six weeks, which might have led to the production of smaller plastic particles via abrasion by the rolling with the sediment. This opens for a possible hypothesis, that the smaller particles induced an effect. Additionally, the study by Besseling et al. (2013) had exposed the animals for four weeks with sorbed PCBs (Besseling et al., 2013), as compared to two weeks and pristine PS (Van Cauwenberghe et al., 2015). A similar bias might have occurred during a study on sea urchins, which did not indicate the size range employed. They report more malformations with the elutriate, that was rinsed off the particles of a larger size, and it is not excluded that the elutriate might contain plastic nanoparticles (Nobre et al., 2015).

In contrast to MPs, which seem to have restricted access to different parts of the organisms, NPs were shown to enter a wide range of organs and have rather long retention times. For example, 500 nm PS was shown to enter hemolymph, stomach, hepatopancreas, ovary and gills, with a clearance time exceeding three weeks in a model system demonstrating transfer from blue mussels to crabs (Farrell and Nelson, 2013). In copepods, it was shown that 6 μm and 500 nm particles remained in the digestive tract, while 50 nm particles were dispersed throughout the body (Jeong et al., 2017; Jeong et al., 2016). Generally, these numbers cannot be regarded as limits, as datasets are still too scarce. NPs have been shown to enter pancreas, gallbladder, heart, brain and into eggs of zebrafish (Pitt et al., 2018), blood, gallbladder, testis and brain of Japanese medaka (Kashiwada, 2006), the eye of zebrafish (van Pomeren et al., 2017), the ovary of water flea (Cui et al., 2017), the brain of crucian carp (Mattsson et al., 2017), the yolk sac of Chinese rice fish (Chae et al., 2018), and to accumulate in oil droplets to the chorion of medaka eggs, from where they shifted to the yolk and gallbladder during embryonic development (Kashiwada, 2006).

There is some evidence promoting particle size as an independent factor not only for retention and uptake but also determining PPT. In a study with waterflea, 2 μm PS particles did not decrease water flea feeding rates when fed at 0.1–1 mg/l for three weeks, while 100 nm PS particles did (Rist et al., 2017). One study on copepods adjusted for increasing mass with increasing size from 50 nm to 6 μm and still found a higher PPT for the smaller particles (Lee et al., 2013). Another study on water flea that compared 1 μm particles with larger ones (Rehse et al., 2016) reported a similar outcome as the study by Lee et al. (2013). However, a study on sediment dwellers, comparing the effects of similar sized particles (1–4 μm, 10–27 μm and larger PE), normalized by particle number, found that the middle size fraction was most harmful (Ziajahromi et al., 2018). These studies did not contradict each other, as a fraction comparable to that middle size employed by Ziajahromi et al. (2018) was not investigated by Rhese et al. (2016), also using different experimental setups such as a different organism and different endpoints. MNPS of different sizes may not necessarily cause impact with directional correlation to their size, but exert different effects in different physiological niches, depending on their location and interactions.

Regarding mammalian models, a study by Volkheimer (1975) showed that large doses of particles of 5–110 μm, administered to an array of mammals, were recovered in a multitude of organs, including placenta, cerebrospinal fluid and milk. However, the recovery in the organs was only reported as numbers of particles, and not particles size (Volkheimer, 1975). Therefore, the particles that were recovered in this study might have been the smallest ones of the administered range, and the upper limit for the particle translocations in terms of particle size cannot be determined and might have been only 5 μm. Of starch particles that were administered to humans in a similar setup, as a non-toxic alternative, up to 40 μm large particles were recovered in human blood and therefore should have passed the intestinal wall (Volkheimer, 1975). Dogs fed with 200 g of PVC transported the powder to the blood, and to the brain (Volkheimer, 1975). Later on, uptake of PS of 1.8 μm was shown to enter the liver, spleen and mesentery (Jani et al., 1990; Jani et al., 1992; McMinn et al., 1996) and enterocytes of rats (McMinn et al., 1996; Walczak et al., 2015). Particles in the nanometer range were shown to cross the human placental barrier ex vivo (Wick et al., 2010), and to enter blood (Jani et al., 1990), heart, kidney (Walczak et al., 2015), bone marrow (Jani et al., 1990) and enterocytes in rats (McMinn et al., 1996; Walczak et al., 2015).

Size dependency of uptake was reported in Sprague Dawley rats, where 50 and 100 nm PS particles were reaching the liver, spleen, blood and bone marrow. The 50 nm particles were absorbed to 34%, while the 100 nm PS were absorbed to a lesser extent, 26% (Jani et al., 1990). 50 nm particles entered the liver earlier than larger particles (Jani et al., 1992). Furthermore, Jani et al. also reported that particles larger than 100 nm did not reach the bone marrow and particles larger than 300 nm were absent from the blood (Jani et al., 1990). Later, the same research group investigated the location of the uptake in the intestine. The uptake in the small intestine occurred to 60% through the Peyer's patches conforming earlier observations by Volkheimer, but a significant amount of the total uptake was also shown to occur in the lymphoid section of the large intestine (Hillery et al., 1994). Five days after oral gavage, 10% of the administered dose was recovered from the entire gastrointestinal tract (Hillery et al., 1994). Finally, McMinn et al. investigated which organs in rats are reached, when feeding different doses of 1.8 μm MPs: 6000 p/g reached to the lymph nodes, 80 p/g to the liver and <10 p/g to the spleen within 30 min. In the small intestine, most tissue-associated particles were within the epithelial layer, predominantly within enterocytes, but also entry to the mesenteric lymph nodes was shown (McMinn et al., 1996). A PPT study with mice (Deng et al., 2017) indicated that ingestion of 0.5 mg per day of PS of 5 and 20 μm lead to accumulation of the MPs in liver, kidney and gut. They concluded that tissue-accumulation kinetics and distribution pattern was related to the MPs particle size. The difference in the size

of the particles that entered organs between the study by McMinn et al. (1996) and Deng et al. (2017) might be due to the different species used or the different exposure concentrations.

4.2. Potential PPT of MNPs in humans

For the evaluation of human health risk posed by MNPs, there is a large body of evidence for translocation of plastic particles from artificial body parts to lymph nodes and other body, intentionally administered plastic particles to transport medical treatment, and for occupational hazard upon inhalation, previously reviewed in (Lusher et al., 2017). There is, however, little data on the exposure of humans to plastic through food, neither directly, nor in model systems. However, several exposure studies in mammalian model systems point towards a potential uptake of MNPs into a variety of organs, as described in the section about size dependency.

Hallab et al., 2012 reported polymer type and size-dependent cytotoxicity (Hallab et al., 2012) in human macrophages. In this study 20 particles per cell of PE and a PEK (polyetherketone), in two different sizes, 700 nm and 10 μm, caused cytotoxicity, where PE and the smaller particles were more toxic than the larger ones or PEK. This in vitro study with human macrophages provides evidence that the commonly observed size-dependency of PPT is not only caused by larger particle numbers or surface area, but by the size of the particles themselves (Hallab et al., 2012). In human cerebral and epithelial cell lines a mixture of 40–250 nm PS, 10 μm PS and 100–600 nm PE and 3–16 μm PE, caused a dose-dependent increase in ROS (Schirinzi et al., 2017). Furthermore, blood platelets were reported to be activated by in vitro addition of aminated PS (Nemmar et al., 2003).

There is also some evidence of PPT in mammalian model exposure studies. In a study on mice, daily oral gavage of 5 and 20 μm fluorescent polystyrene MP particles resulted in accumulation of both types of particles in the liver and kidney (Deng et al., 2017), confirming the early studies of Volkheimer, mentioned above. The very high doses (from 2×10^4 to 150×10^4 items per animal per day) induced liver inflammation, and changes in metabolic profiles suggesting effects on energy and lipid metabolism, oxidative stress and neurotoxic effects (Deng et al., 2017). Mice exposed orally to 1 mg/l of 500 nm and 50 μm large PS particles (Lu et al., 2018) showed decreased body, liver and lipid weights after five weeks. In addition, the mice exhibited increased mucus secretion, effects on the relative microbiota abundance and impact on key genes related to lipogenesis and triglyceride synthesis in the liver. Hepatic triglyceride and total cholesterol levels decreased. Correspondingly, the relative mRNA levels of some key genes related to lipogenesis and triglyceride synthesis decreased in the liver and epididymal fat (Lu et al., 2018).

Based on the investigations mentioned above, we suggest that exposure to MNPs to humans through food or other routes, could have negative implications for human health. However, it is not clear to which extent humans are exposed to such particles, as most effects have been demonstrated for particles in the size range below 10 μm, and this size class is unquantified in both food and the environment due to the present limitations in methodology.

4.3. Small particles are largely unquantified

Most of the present exposure studies investigate pristine spherical, either heavily colored or fluorescent, MPs. In combination with translucent species or life-stages, this enables the researchers to follow the particles throughout the body, using optical, electron or fluorescent microscopy down to the micro- or nanoscale (Hüffer, Praetorius et al. 2017). Tracing particles using nuclear isotope techniques to investigate the fate of plastic particles including their accumulation, translocation and trophic transfer has also potential (Lanctot et al., 2018). However, in environmental compartments such as the ocean, the plastic particles are not pre-labelled, and plastic cannot be differentiated from natural material by normal imaging. Additionally, polymer composition plays a role for toxicity. Hence, chemical identification is necessary. Chemical imaging methods for MNPs have typically larger particle size detection limits for single particles as compared to fluorescent or electron microscopy, ranging between below one μm for Raman technologies and around 10–20 μm for μFTIR imaging (Huffer et al., 2017; Mintenig et al., 2017; Simon et al., 2018). The quantification of particles in air and food is challenging, as pointed out in a recent review (Cox et al., 2019). We suggest that such accounts are hugely biased by the lack of comparability due to different, and rather high detection limits related to the particle size, combined with non-reported/assessed measurement uncertainties. Currently published occurrence data, in terms of contaminated percentages of both species and individuals, are therefore possibly a vast underestimation. The reason for that is that most studies that provide numbers estimating contamination rates, only include larger MPs, and often only in the intestinal tract. Conkle et al. (2018) reviewed aquatic surveys and found that ~80% of 1655 articles did not account for MNPs below 300 μm. Indeed, very few studies quantify MNPs below 20 μm particle size, which is about the size where uptake into organs is expected (see section above). This is a major lack of data, as there is evidence that the composition of small particles cannot be extrapolated from the occurrence of larger particles both from the nano- to the micrometer range and from the small micrometer to the large micrometer range (Haave et al., 2019; Ter Halle et al., 2017). For example, filtering seawater through either 300 or 100 μm meshes yields a particle number difference by four orders of magnitude (Covernton et al., 2019). There should be increasing numbers of MNPs with decreasing size in the environment (Bergmann et al., 2017; Lambert and Wagner, 2016), if they are not degraded very rapidly after reaching a point of no return towards complete mineralization or metabolizable entities. The latter is still a possible mechanism and working hypothesis, possibly accounting for a part of the unaccounted-for bulk of lost plastic (Eunomia, 2016). We may also be facing increasing accumulation and sequestering of MNPs in diverse matrices such as sediment and biota. Currently, MNP concentrations remain to be quantified in all environmental compartments. Despite the ubiquitous distribution, MNPs are commonly not quantified in environmental studies due to high detection limits related to particle size or restriction of investigations to the intestinal content, omitting other tissues and internal organs. Plastic occurrence is often reported as “encounters” or frequency of occurance (FO), as to how many percent of a population have taken up plastic. Following the line of thought in this section, such reports are likely to be generally underestimating the situation.

Recently, a number of review articles and white papers (Conkle et al., 2018; Lusher et al., 2017; Revel et al., 2018; Wright and Kelly, 2017) have pointed out the necessity to take into account particle sizes, for both monitoring and evaluation of physiological impact. There are only few articles quantifying environmental samples for plastic particles down to 10 μm, and most of them were published very recently: in fish stomach (Fischer and Scholz-Bottcher, 2017; Pellini et al., 2018), Barents Sea sediments (Bergmann et al., 2017), German and Danish waste water treatment plant effluent (Mintenig et al., 2017; Simon et al., 2018), Arctic sea ice (Peeken et al., 2018), sediments of a Norwegian urban fjord in Bergen (Haave et al., 2019), German river Rhine sediments (Mani et al., 2019), salt water and sediments (Fischer and Scholz-Bottcher, 2019), storm water retention ponds (Liu et al., 2019; Olesen et al., 2019), indoor airborne MPs (Vianello et al., 2019) and snow (Bergmann et al., 2019). Below 10 μm, quantitative data is essentially a white area on the map, with one semiquantitative attempt in North Atlantic water (Ter Halle et al., 2017) and a pilot experiment on NP quantification in food (Correia and Loeschner, 2018).

Although it is evident that NPs are present in the environment (Ter Halle et al., 2017) and can be generated from larger plastic pieces by physical (Lambert and Wagner, 2016) and biological influence (Dawson et al., 2018), quantitative data on their occurrence in the environment or in food are lacking. Extrapolating existing

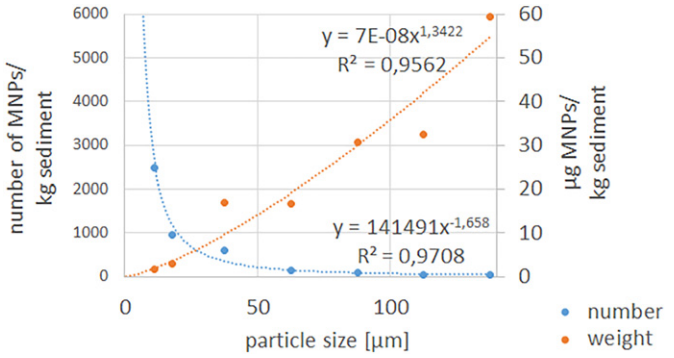

Fig. 1. Extrapolated plastic concentrations based on Bergmann et al. (2017). Abundance of MPs in Arctic deep-sea sediments from the HAUSGARTEN observatory extrapolated into the <11 µm region based on (Bergmann et al., 2017) (blue) and the weight of the respective size fractions (orange). Data points for particle numbers were generated by using the average number of particles per kg sediment per station and the arithmetic mean of the particle size fraction. The weight of the corresponding fractions was calculated assuming spherical particles with a density of 1 g/cm^3. Trend lines were generated in Microsoft® Excel using the power function and a forward forecast set to zero. Please note that extrapolation of microplastic findings in Arctic sea ice (Peeken et al., 2018) delivers a comparable trend, however, with slightly different total numbers.

datasets point towards a possible large number of invisible small MPs and NPs (Fig. 1).

Based on the above-mentioned relationships, the increasing evidence for negative impact of MNPs from exposure studies cannot be related to exposure levels. Based on the data from the previous section, it can be assumed that even more lifeforms and in higher percentages are contaminated with NPs than with MPs. This leads to the pressing question whether exposure to *relevant* concentrations of small plastic particles is physiologically harmful, and where the toxicological concentration thresholds are, both for environmental and health-related concerns. We cannot answer those questions without quantifying concentrations of small plastic particles. Underlying reasons for the lack of such data are related to the difficulties of the extraction of the MNPs from tissue and sediments, without losing or destroying the MNPs. In conclusion, to perform a risk assessment, more, and better quantitative and qualitative chemical analysis data are needed. Only then, toxicological tests can be designed with appropriate exposure regimes.

4.4. Comparing several factors

Preventing the possibility to pinpoint the determining factor for PPT in the experimental studies due to the comparison of too many factors at once without a proper multifactorial design, is a recurring flaw in this research field: Particle shapes were compared, but additionally, the particles had different sizes, concentrations, were pristine as compared to secondary, or were composed of different polymers (Au et al., 2015; Biquand et al., 2017; Blarer and Burkhardt-Holm, 2016; Ogonowski et al., 2016; Okubo et al., 2018; Ziajahromi et al., 2017). That way, it cannot be concluded if the size or the shape or the chemical identity were the determining factors for the observed effects. Illustrating the same principle, Aljaibachi and Callaghan (2018) compared an acute test on neonates with a long-term test on adults (Aljaibachi and Callaghan, 2018). This experimental setup precludes determining if the short exposure time or the developmental stage led to the difference in mortality. Generally, the multitude of polymers, shapes, sizes, conditions, exposure times, -routes and -concentration ranges renders it currently difficult to compare the impact of different determining factors for PPT. Keeping several factors stable, while varying a few, or large-scale multifactorial studies with proper design are necessary to gain knowledge here.

4.5. Lack of controls

A study exposing mussels to PE isolated from toothpaste and reporting tissue necrosis (Brate et al., 2018), did not include a control for toothpaste components that might have had diffused into the particles. Based on the data given here, it cannot be concluded if the effect was caused by plastic or by sorbed substances. The same accounted for exposure of clams to particles smaller than 33 µm, increasing the ammonium concentration in overlaying water, where the authors unfortunately do not exclude the possibility of an effect of antibacterial agents, as the PE particles were isolated from face scrub (Cluzard et al., 2015). This common flaw should be avoided in future studies.

4.6. Environmentally relevant concentrations and conditions

From the literature, it is described that exposure ranges are often higher, but sometimes in the environmental range. For example, Rochman et al. (2017), in an Asian clam exposure study, claimed that 3–4 mg/l were comparable to concentrations of MPs that have been recorded in freshwater environments. Others mention environmentally relevant doses of 10,000 p/l, but at the same time point out that quantitative measurement of environmental concentrations of particles in the small µm range is not published yet (Lo and Chan, 2018). Besseling et al., 2014, observed malformations in *Daphnia magna* neonates starting at 30 mg/l using 70 nm PS. According to the authors this is a much higher concentration than in marine water but could be realistic concentrations in sediment pore water in highly polluted areas. We would like to emphasize that environmentally relevant concentrations cannot be extrapolated from a different size class than the one investigated, and, NPs are not quantified in any environmental niche yet. In a different approach, five rather large fibers of 3–5 mm per feeding were fed over eight months to Norway lobster with levels that are comparable to those in the Clyde Sea close to Scotland (Welden and Cowie, 2016), in a size class that is accessible for quantification with contemporary methods. It can only be claimed that an exposure regime is environmentally relevant, if quantification of the amount of the relevant size class, including chemical identity, are available. Therefore, high quality quantification studies should be prioritized.

There is also a considerable number of studies investigating the role of surface modifications for MNP uptake and PPT. Several of the reviewed studies compare the effects of aminated and carboxylated PS NPs. Studies with sea urchin show that PS-COOH forms microaggregates and triggers the upregulation of the gene coding for the abcd efflux pump, while PS-NH_2 forms nanoaggregates and is seems to be more toxic (Della Torre et al., 2014). Similar findings were reported in phytoplankton were authors concluded lower effects on growth inhibition with carboxylated PS as compared to aminated or uncharged PS (Bergami et al., 2017; Bhattacharya et al., 2010). Aminated particles in the size range of 40–50 nm were shown to be toxic to rotifers and waterfleas after two days of exposure, in contrast to carboxylated ones (Manfra et al., 2017; Mattsson et al., 2017). Aminated PS particles of 50 nm also resulted in increased mortality and induced molting in shrimp as compared with carboxylated 40 nm PS (Bergami et al., 2016; Bergami et al., 2017). However, carboxylation does not render the particles effectless, as in a different study with waterflea with extended observation time exceeding one week, 2 µm carboxylated PS beads increased mortality and reduced growth (Aljaibachi and Callaghan, 2018). Furthermore, 200 nm PS-COOH particles immobilized *Daphnia*, concentration dependently (1–80 mg/l), with a larger effect than for the aminated particles (Kim et al., 2017). However, the relevance of these surface modifications for environmental evaluations is questionable, since such modifications are not expected to be part of the natural aging process of MNPs.

4.7. Mechanistic insight

An increasing number of studies provide the first mechanistic insight for PPT. In adult *Daphnia magna*, differential expression of various genes related to stress responses (i.e. heat shock proteins HSP60, HSP70& GST) as well as of genes involved in body function and body composition (i.e. SERCA) were observed already 48 h after exposure to mixed MPs of 40 μm size (Imhof et al., 2017). In the copepod *Paracyclopina nana*, exposure to 50 nm sized PS lead to an increase in oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms such as increase in antioxidant related enzymes (Jeong et al., 2017). In brine shrimp, aminated nano-PS induced *clap* and *cstb* genes, possibly explaining the increase in molting observed in this study (Bergami et al., 2017). Chinese mitten crab exposed to 5 μm PS for three weeks, showed a decreased activity of AChE and GPT activity with increasing concentration of PS (Yu et al., 2018). In the same study, the activity of superoxide dismutase (SOD), aspartate transaminase (GOT), glutathione (GSH), and glutathione peroxidase (GPx) increased at 40 and 400 μg/l and decreased at higher concentrations of 4000 and 40,000 μg/l. Yu et al. (2018) also reported that the activities of acetylcholinesterase, catalase (CAT), and alanine aminotransferase were lower at all concentrations as compared with the control group. In blue mussel exposed to 2 and 6 μm PS, decreased fluoranthene detoxification and lipid-peroxidation and increased hemocyte-/granulocyte mortality, reactive oxygen species in hemocytes, anti-oxidant/glutathione-related enzymes in tissues and glycolysis was found (Paul-Pont et al., 2016). In sea urchins exposed to PS NPs, aminated nano-PS induced the cas8 gene cascade, suggesting triggering of the apoptotic pathway, and carboxylated nano-PS upregulated the abcb1 gene–efflux pump (Della Torre et al., 2014). For fish, NPs and MPe were shown to cause a decrease in AChE, trypsin activity, being antagonistic to amylase, affect alkaline phosphatase activity (Wen et al., 2018), affect choriogenin (Chg H) gene expression in males and vitellogenin (Vtg I), choriogenin H (Chg H) and estrogen receptor (ERα) gene expression in females (pointing towards a possibility for reduced fecundity, Rochman et al., 2014b), AchE activity (Chen et al., 2017; Ferreira et al., 2016; Luis et al., 2015; Oliveira et al., 2013), and lipid peroxidation levels (Ferreira et al., 2016), peroxidase activity and IgM levels in mucus (Espinosa et al., 2017), levels of IL1a, IL1b and IFN in the gut (indicating inflammation; Jin et al., 2018), activity of superoxide dismutase and catalase, increased fatty acids (Lu et al., 2016), gfap, α1-tubulin, zfrho and zfblue gene expression (Chen et al., 2017), cytochrome-P450-1A1 (cyp1a1) (Mazurais et al., 2015), citrate synthase and cytochrome *c* oxidase (Wen et al., 2018), globulin (Espinosa et al., 2017), degranulation of primary granules and neutrophil extracellular trap release (Greven et al., 2016). Espinosa et al. (2017) reported a decrease of prdx5 and hsp90 and increase of prdx1, prdx3 and ucp1 gene expression suggesting major gene transcript effects, indicating stressed head kidney and liver (Espinosa et al., 2017).

There is evidence that points towards that the mechanism of the internalization of negatively charged PS NPs is via clathrin- and dynamin-dependent endocytosis; the uptake of carboxylated PS NPs by macrophages was inhibited by the presence of onodansyl cadaverine and dynasore. Onodansyl cadaverine and dynasore are inhibitors of clathrin-mediated endocytosis and dynamin-dependent endocytosis, respectively. Alternatively, positively charged PS NPs are internalized through micropinocytosis. Surface chemistry, not charge alone, has been found to have a greater influence on translocation, as there was a 30-fold difference in uptake between two types of negatively charged PS NPs (Walczak et al., 2015).

5. Conclusions and future recommendations

As summarized in this review, MNPs are causing a wide range of negative impact on marine and fresh water associated organisms in exposure studies. For a better overview, we have summarized nine main determining factors for PPT of MNPs, which are of overall importance (Table 1) and have structured the toxicological effects into groups (Table 2). However, we want to highlight that at the current time point, the statistics on physiological and toxicological effects is prone to large biases, since cherry-picked biological response pathways were investigated, rather than screening approaches employed.

With the pinpointed nine factors (Table 1) in mind, some seemingly contradicting results of the research field, such as the presence or absence of measurable negative effects, are reconcilable, as described in the sections above. However, at present it is essentially unknown to which extent the polymer type, its dose and the environmental conditions that were applied in the exposure studies reflect the conditions in the environment, as those are scarcely assessed and generally unknown. Especially for plastic particles below 10 μm, quantitative studies lack entirely, while most toxic effects are reported on this size class (Table 3).

In conclusion, existing data point towards a potential, but so far not quantified, risk for PPT through environmental exposure for all life forms. We conclude that the multitude and severity of the reported effects of MNPs on aquatic life justifies efforts to quantify the relevant size fractions in the environment, biota and humans, to determine realistic environmental conditions. This should be followed by standardized toxicity studies under those conditions, to enable future risk assessments.

We suggest assessing the environment according to those nine PPT factors, illustrated in the Graphical abstract and Table 1. Thereby, conclusions on the extent of a potential threat of the current and extrapolated future amount of environmental MNPs could be drawn in an orderly fashion. Different action/lack of action scenarios of political, industry- and community driven measures could be envisioned. Sustainability goals could then be based on those assessments. Of crucial importance are the MNP concentrations for different size classes, particle conditions, such as particle shape, aging and surface modifications, and polymer types. Interestingly, 50% of all studies have used PS in their experimental setup, which is not even the most common polymer in production today (https://www.plasticseurope.org/en). Our data sets show that 63.9% of the exposure studies using plastic particles below 10 μm and 27.9% above 10 μm have used PS particles. Just four polymer types comprise 83% of all exposure studies on MPs above 10 μm, and two polymer types for all exposure studies on MNPs below 10 μm (Table 3).

When considering the total number of polymers used in these exposure studies three polymers, PE (ca. 30%), PET (ca. 7%) and PVC (ca. 7%),

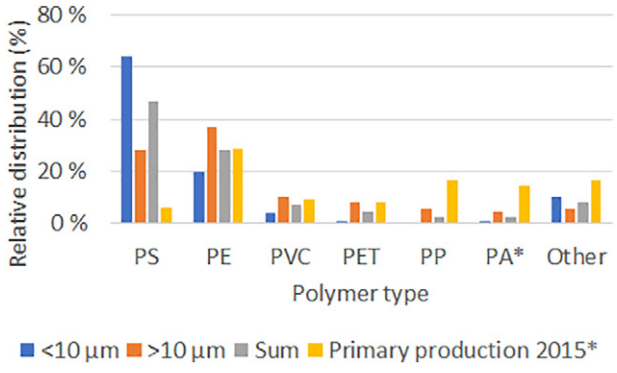

Fig. 2. Relative polymer use in exposure studies as compared to primary production. Relative polymer use in exposure studies as compared to primary production as published in Geyer et al. (2017), based on million cubic tons. Percentage of exposure studies on the effects of the respective polymer types (x-axis) for particles below 10 μm (blue), above 10 μm (orange), in total (grey), compared to primary production (yellow). PS, polystyrene; PE, polyethylene; PVC, polyvinylchloride; PET, polyethylene terephthalate; PP, polypropylene; PA, polyamide and Polyacrylate combined.

are used proportionally comparable to the relative amounts of these polymers produced as primary waste (Geyer et al., 2017; Fig. 2). Opposite, the toxicity testing of PA and PP in exposure studies, ca. 2% and ca. 3%, are underrepresented when we consider the relative amounts of these polymers produced as primary waste.

Evidence has been provided that different particle sizes exert effects through different physiological pathways (see section Size dependency of MNP uptake, retention and PPT). To assess the full risk imposed by plastic debris, these size classes need to be analyzed quantitatively and qualitatively in different species of different developmental stage and in different tissues. To reconcile the toxicological data with the environmental status quo, which is necessary to perform risk assessments for the environment and human health, monitoring efforts need to be directed towards the smaller size fractions, despite the analytical challenges. At least, the analyzed size-range needs to be provided, preferably divided into standardized sub-ranges. Conclusions from comparing uptake amounts from different studies applying different size ranges are invalid. Due to the complexity of the subject, the establishment of indicator species seems unavoidable.

Furthermore, to provide a sound basis for risk assessment, experimental designs must not only be able to disentangle the effects of food limitation and PPT, but also demonstrate whether MNPs cause impacts that differ from those induced by natural particles (Ogonowski et al., 2016).

Monitoring should not only include the gastrointestinal tract, but also muscle and liver, and possibly the kidney, as (rat/dogs) experiments show that liver and kidney are the recipients of MNPs, which cross the intestinal barrier and enter the blood stream.

After these parameters are empirically analyzed in the environment, a new series of exposure experiments should be conducted. This new series, based on these realistic environmental plastic loads, should consider exposure times, environmental conditions such as food availability and load margin of exposure of other contaminants, species, developmental stage and sex. Based on such datasets, tolerable intake/exposure doses in relation to the determining factors could be established to allow future risk assessment and the determination of meaningful sustainable levels of plastic use.

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Author contribution

TK: Conceptualization, methodology, original draft, visualization and project administration; data curation; TK, BT, ØB, AB and MS: Formal analysis, investigation, writing, validation, review and editing; TK and MS: Funding acquisition.

Declaration of competing interest

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

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References

Aljaibachi, R., Callaghan, A., 2018. Impact of polystyrene microplastics on Daphnia magna mortality and reproduction in relation to food availability. Peerj 6.
Andrady, A.L., 2017. The plastic in microplastics: a review. Mar. Pollut. Bull. 119, 12–22.
Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of Hyalella azteca to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572.
Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., et al., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.
Balbi, T., Camisassi, G., Montagna, M., Fabbri, R., Franzellitti, S., Carbone, C., et al., 2017. Impact of cationic polystyrene nanoparticles (PS-NH2) on early embryo development of Mytilus galloprovincialis: effects on shell formation. Chemosphere 186, 1–9.
Bank, M.S., Hansson, S.V., 2019. The plastic cycle: a novel and holistic paradigm for the Anthropocene. Environmental Science & Technology 53, 7177–7179.
Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018. Single and combined effects of microplastics and mercury on juveniles of the European seabass (*Dicentrarchus labrax*): changes in behavioural responses and reduction of swimming velocity and resistance time. Environ. Pollut. 236, 1014–1019.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B-Biological Sciences 364, 1985–1998.
Batel, A., Linti, F., Scherer, M., Erdinger, L., Braunbeck, T., 2016. Transfer of benzo[a]pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environ. Toxicol. Chem. 35, 1656–1666.
Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., et al., 2018. Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. J. Hazard. Mater. 360, 452–460.
Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., et al., 2016. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp Artemia franciscana larvae. Ecotoxicol. Environ. Saf. 123, 18–25.
Bergami, E., Pugnalini, S., Vannuccini, M.L., Manfra, L., Faleri, C., Savorelli, F., et al., 2017. Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species Dunaliella tertiolecta and Artemia franciscana. Aquat. Toxicol. 189, 159–169.
Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., et al., 2017. High quantities of microplastic in Arctic deep-sea sediments from the HAUSGARTEN observatory. Environmental science & technology 51, 11000–11010.
Bergmann, M., Mutzel, S., Primpke, S., Tekman, M.B., Trachsel, J., Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci. Adv. 5.
Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). Environmental science & technology 47, 593–600.
Besseling, E., Wang, B., Lurling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of S. obliquus and reproduction of D. magna (vol 48, pg 12336, 2014). Environmental science & technology 48, 14065.
Besseling E, Foekema EM, van den Heuvel-Greve MJ, Koelmans AA. The effect of microplastic on the uptake of chemicals by the lugworm Arenicola marina (L.) under environmentally relevant exposure conditions. Environmental Science & Technology 2017; 51: 8795–8804.
Bhattacharya, P., Lin, S.J., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C 114, 16556–16561.
Biquand, E., Okubo, N., Aihara, Y., Rolland, V., Hayward, D.C., Hatta, M., et al., 2017. Acceptable symbiont cell size differs among cnidarian species and may limit symbiont diversity. ISME J. 11, 1702–1712.
Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod Gammarus fossarum. Environ. Sci. Pollut. Res. 23, 23522–23532. https://doi.org/10.1007/s11356-016-7584-2.
Brate, I.L.N., Blazquez, M., Brooks, S.J., Thomas, K.V., 2018. Weathering impacts the uptake of polyethylene microparticles from toothpaste in Mediterranean mussels (M-galloprovincialis). Sci. Total Environ. 626, 1310–1318.
Brennecke, D., Ferreira, E.C., Costa, T.M., Appel, D., da Gama, B.A., Lenz, M., 2015. Ingested microplastics (>100 μm) are translocated to organs of the tropical fiddler crab Uca rapax. Mar. Pollut. Bull. 96, 491–495.
Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L). Environmental science & technology 42, 5026–5031.
Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392.
Canesi, L., Ciacci, C., Bergami, E., Monopoli, M.P., Dawson, K.A., Papa, S., et al., 2015. Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene nanoparticles in the hemocytes of the marine bivalve Mytilus. Mar. Environ. Res. 111, 34–40.
Canesi, L., Ciacci, C., Fabbri, R., Balbi, T., Salis, A., Damonte, G., et al., 2016. Interactions of cationic polystyrene nanoparticles with marine bivalve hemocytes in a physiological environment: role of soluble hemolymph proteins. Environ. Res. 150, 73–81.
Canniff, P.M., Hoang, T.C., 2018. Microplastic ingestion by Daphnia magna and its enhancement on algal growth. Sci. Total Environ. 633, 500–507.
Casado, M.P., Macken, A., Byrne, H.J., 2013. Ecotoxicological assessment of silica and polystyrene nanoparticles assessed by a multitrophic test battery. Environ. Int. 51, 97–105. https://doi.org/10.1016/j.envint.2012.11.001.
Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ. Pollut. 237, 675–684.
Cedervall, T., Hansson, L.A., Lard, M., Frohm, B., Linse, S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS One 7.
Chae, Y., Kim, D., Kim, S.W., An, Y.J., 2018. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. Sci. Rep. 8, 284.
Chen, Q.Q., Gundlach, M., Yang, S.Y., Jiang, J., Velki, M., Yin, D.Q., et al., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584, 1022–1031.

Cluzard, M., Kazmiruk, T.N., Kazmiruk, V.D., Bendell, L.I., 2015. Intertidal concentrations of microplastics and their influence on ammonium cycling as related to the shellfish industry. Arch. Environ. Contam. Toxicol. 69, 310–319.
Cole, M., Galloway, T.S., 2015. Ingestion of nanoplastics and microplastics by Pacific oyster larvae. Environmental science & technology 49, 14625–14632.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., et al., 2013. Microplastic ingestion by zooplankton. Environmental science & technology 47, 6646–6655.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. Environmental science & technology 49, 1130–1137.
Collard, F., Gilbert, B., Compere, P., Eppe, G., Das, K., Jauniaux, T., et al., 2017. Microplastics in livers of European anchovies (Engraulis encrasicolus, L.). Environ. Pollut. 229, 1000–1005.
Conkle, J.L., Del Valle, C.D.B., Turner, J.W., 2018. Are we underestimating microplastic contamination in aquatic environments? Environ. Manag. 61, 1–8.
Correia, M., Loeschner, K., 2018. Detection of nanoplastics in food by asymmetric flow field-flow fractionation coupled to multi-angle light scattering: possibilities, challenges and analytical limitations. Anal. Bioanal. Chem. 410, 5603–5615.
Covernton, G.A., Pearce, C.M., Gurney-Smith, H.J., Chastain, S.G., Ross, P.S., Dower, J.F., et al., 2019. Size and shape matter: a preliminary analysis of microplastic sampling technique in seawater studies with implications for ecological risk assessment. Sci. Total Environ. 667, 124–132.
Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E., 2019. Human consumption of microplastics. Environ Sci Technol 53, 7068–7074.
Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez-Leon, S., et al., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. U. S. A. 111, 10239–10244.
Critchell, K., Hoogenboom, M.O., 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus). PLoS One 13.
Cui, R., Kim, S.W., An, Y.J., 2017. Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean Daphnia galeata. Sci. Rep. 7.
Davarpanah, E., Guilhermino, L., 2015. Single and combined effects of microplastics and copper on the population growth of the marine microalgae Tetraselmis chuii. Estuarine Coastal and Shelf Science 167, 269–275.
Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M., et al., 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nat. Commun. 9.
De Felice, B., Bacchetta, R., Santo, N., Tremolada, P., Parolini, M., 2018. Polystyrene microplastics did not affect body growth and swimming activity in Xenopus laevis tadpoles. Environ. Sci. Pollut. Res. 25, 34644–34651.
de Sa, L.C., Luis, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362.
Della Torre, C., Bergami, E., Salvati, A., Faleri, C., Cirino, P., Dawson, K.A., et al., 2014. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos Paracentrotus lividus. Environmental Science & Technology 48, 12302–12311.
Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7, 46687.
Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. 12, 592–599.
Espinosa, C., Cuesta, A., Esteban, M.A., 2017. Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (Sparus aurata L.). Fish & Shellfish Immunology 68, 251–259.
Eunomia, 2016. Plastics in the Marine Environment.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177 (1–3).
Ferreira, P., Fonte, E., Soares, M.E., Carvalho, F., Guilhermino, L., 2016. Effects of multistressors on juveniles of the marine fish Pomatoschistus microps: gold nanoparticles, microplastics and temperature. Aquat. Toxicol. 170, 89–103.
Fischer, M., Scholz-Bottcher, B.M., 2017. Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography-mass spectrometry. Environmental science & technology 51, 5052–5060.
Fischer, M., Scholz-Bottcher, B.M., 2019. Microplastics analysis in environmental samples - recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data. Anal. Methods 11, 2489–2497.
Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92, 170–179.
Gambardella, C., Morgana, S., Ferrando, S., Bramini, M., Piazza, V., Costa, E., et al., 2017. Effects of polystyrene microbeads in marine planktonic crustaceans. Ecotoxicol. Environ. Saf. 145, 250–257.
Gambardella, C., Morgana, S., Bramini, M., Rotini, A., Manfra, L., Migliore, L., et al., 2018. Ecotoxicological effects of polystyrene microbeads in a battery of marine organisms belonging to different trophic levels. Mar. Environ. Res. 141, 313–321.
Gardon, T., Reisser, C., Soyez, C., Quillien, V., Le Moullac, G., 2018. Microplastics affect energy balance and gametogenesis in the pearl oyster Pinctada margaritifera. Environmental science & technology 52, 5277–5286.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3.
Gigault, J., Pedrono, B., Maxit, B., Ter Halle, A., 2016. Marine plastic litter: the unanalyzed nano-fraction. Environmental Science-Nano 3, 346–350.
Gray, A.D., Weinstein, J.E., 2017. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). Environ. Toxicol. Chem. 36, 3074–3080.
Green, D.S., Boots, B., Sigwart, J., Jiang, S., Rocha, C., 2016. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (Arenicola marina) and sediment nutrient cycling. Environ. Pollut. 208, 426–434.
Greven, A.C., Merk, T., Karagoz, F., Mohr, K., Klapper, M., Jovanovic, B., et al., 2016. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 35, 3093–3100.
Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (Carassius auratus). Chemosphere 169, 233–238.
Grosvik, B.E., Prokhorova, T., Eriksen, E., Krivosheya, P., Homeland, P.A., Prozorkevich, D., 2018. Assessment of marine litter in the Barents Sea, a part of the joint Norwegian Russian Ecosystem Survey. Front. Mar. Sci. 5.
Haave, M., Lorenz, C., Primpke, S., Gerdts, G., 2019. Different stories told by small and large microplastics in sediment - first report of microplastic concentrations in an urban recipient in Norway. Mar. Pollut. Bull. 141, 501–513.
Hallab, N.J., McAllister, K., Brady, M., Jarman-Smith, M., 2012. Macrophage reactivity to different polymers demonstrates particle size- and material-specific reactivity: PEEK-OPTIMA® particles versus UHMWPE particles in the submicron, micron, and 10 micron size ranges. Journal of Biomedical Materials Research Part B-Applied Biomaterials 100B, 480–492.
Hamer, J., Gutow, L., Kohler, A., Saborowski, R., 2014. Fate of microplastics in the marine Isopod Idotea emarginata. Environmental Science & Technology 48, 13451–13458.
Hillery, A.M., Jani, P.U., Florence, A.T., 1994. Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. J. Drug Target. 2, 151–156.
Huffer, T., PraetoriusP'll, A., Wagner, S., von der Kammer, F., Hofiliannte, T., 2017. Microplastic exposure assessment in aquatic environments: learning from similarities and differences to engineered nanoparticles. Environ. Sci. Technol. 51, 2499–2507. https://doi.org/10.1021/acs.est.6b04054.
Imhof, H.K., Rusek, J., Thiel, M., Wolinska, J., Laforsch, C., 2017. Do microplastic particles affect Daphnia magna at the morphological, life history and molecular level? PLoS One 12.
Jani, P., Halbert, G.W., Langridge, J., Florence, A.T., 1990. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J. Pharm. Pharmacol. 42, 821–826.
Jani, P.U., Mccarthy, D.E., Florence, A.T., 1992. Nanosphere and microsphere uptake via Peyer patches - observation of the rate of uptake in the rat after a single oral dose. Int. J. Pharm. 86, 239–246.
Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., et al., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (Brachionus koreanus). Environmental science & technology 50, 8849–8857.
Jeong, C.B., Kang, H.M., Lee, M.C., Kim, D.H., Han, J., Hwang, D.S., et al., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci. Rep. 7.
Jeong, C.B., Kang, H.M., Lee, Y.H., Kim, M.S., Lee, J.S., Seo, J.S., et al., 2018. Nanoplastic ingestion enhances toxicity of persistent organic pollutants (POPs) in the monogonont rotifer Brachionus koreanus via multixenobiotic resistance (MXR) disruption. Environmental Science & Technology 52, 11411–11418.
Jin, Y.X., Xia, J.Z., Pan, Z.H., Yang, J.J., Wang, W.C., Fu, Z.W., 2018. Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ. Pollut. 235, 322–329.
Jovanovic, B., Gokdag, K., Guven, O., Emre, Y., Whitley, E.M., Kideys, A.E., 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Mar. Pollut. Bull. 130, 123–131.
Kalcikova, G., Gotvajn, A.Z., Kladnik, A., Jemec, A., 2017. Impact of polyethylene microbeads on the floating freshwater plant duckweed Lemna minor. Environ. Pollut. 230, 1108–1115.
Kaposi, K.L., Mos, B., Kelaher, B.P., Dworjanyn, S.A., 2014. Ingestion of microplastic has limited impact on a marine larva. Environmental science & technology 48, 1638–1645.
Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (Clarias gariepinus). Environ. Res. 151, 58–70.
Karami, A., Golieskardi, A., Ho, Y.B., Larat, V., Salamatinia, B., 2017. Microplastics in eviscerated flesh and excised organs of dried fish. Sci. Rep. 7.
Kashiwada, S., 2006. Distribution of nanoparticles in the see-through medaka (Oryzias latipes). Environ. Health Perspect. 114, 1697–1702.
Kim, D., Chae, Y., An, Y.J., 2017. Mixture toxicity of nickel and microplastics with different functional groups on Daphnia magna. Environmental science & technology 51, 12852–12858.
Kokalj, A.J., Kunej, U., Skalar, T., 2018. Screening study of four environmentally relevant microplastic pollutants: uptake and effects on Daphnia magna and Artemia franciscana. Chemosphere 208, 522–529.
Kolandhasamy, P., Su, L., Li, J.N., Qu, X.Y., Jabeen, K., Shi, H.H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610, 635–640.
Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. 215, 331–339.
Lambert, S., Wagner, M., 2016. Characterisation of nanoplastics during the degradation of polystyrene. Chemosphere 145, 265–268.

Lambert, S., Sinclair, C.J., Bradley, E.L., Boxall, A.B., 2013. Effects of environmental conditions on latex degradation in aquatic systems. Sci. Total Environ. 447, 225–234.

Lanctot, C.M., Al-Sid-Cheikh, M., Catarino, A.I., Cresswell, T., Danis, B., Karapanagioti, H.K., et al., 2018. Application of nuclear techniques to environmental plastics research. J. Environ. Radioact. 192, 368–375.

Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod Tigriopus japonicus. Environmental science & technology 47, 11278–11283.

Liu, F., Olesen, K.B., Borregaard, A.R., Vollertsen, J., 2019. Microplastics in urban and highway stormwater retention ponds. Sci. Total Environ. 671, 992–1000.

Lo, H.K.A., Chan, K.Y.K., 2018. Negative effects of microplastic exposure on growth and development of Crepidula onyx. Environ. Pollut. 233, 588–595.

Long, M., Paul-Pont, I., Hegaret, H., Moriceau, B., Lambert, C., Huvet, A., et al., 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. 228, 454–463.

Lu, Y.F., Zhang, Y., Deng, Y.F., Jiang, W., Zhao, Y.P., Geng, J.J., et al., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environmental science & technology 50, 4054–4060.

Lu, L., Wan, Z.Q., Luo, T., Fu, Z.W., Jin, Y.X., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Sci. Total Environ. 631-632, 449–458.

Luis, L.G., Ferreira, P., Fonte, E., Oliveira, M., Guilhermino, L., 2015. Does the presence of microplastics influence the acute toxicity of chromium(VI) to early juveniles of the common goby (Pomatoschistus microps)? A study with juveniles from two wild estuarine populations. Aquat. Toxicol. 164, 163–174.

Lusher, A., Hollman, P., Mendoza-Hill, J., 2017. Microplastics in fisheries and aquaculture - status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper 615.

Ma, Y.N., Huang, A.N., Cao, S.Q., Sun, F.F., Wang, L.H., Guo, H.Y., et al., 2016. Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ. Pollut. 219, 166–173.

Manabe, M., Tatarazako, N., Kinoshita, M., 2011. Uptake, excretion and toxicity of nano-sized latex particles on medaka (Oryzias latipes) embryos and larvae. Aquat. Toxicol. 105, 576–581.

Manfra, L., Rotini, A., Bergami, E., Grassi, G., Faleri, C., Corsi, I., 2017. Comparative ecotoxicity of polystyrene nanoparticles in natural seawater and reconstituted seawater using the rotifer Brachionus plicatilis. Ecotoxicol. Environ. Saf. 145, 557–563.

Mani, T., Primpke, S., Lorenz, C., Gerdts, G., Burkhardt-Holm, P., 2019. Microplastic pollution in benthic mid-stream sediments of the Rhine River. Environ Sci Technol 53 (10), 6053–6062.

Mao, Y.F., Ai, H.N., Chen, Y., Zhang, Z.Y., Zeng, P., Kang, L., et al., 2018. Phytoplankton response to polystyrene microplastics: perspective from an entire growth period. Chemosphere 208, 59–68.

Marques-Santos, L.F., Grassi, G., Bergami, E., Faleri, C., Balbi, T., Salis, A., et al., 2018. Cationic polystyrene nanoparticle and the sea urchin immune system: biocorona formation, cell toxicity, and multixenobiotic resistance phenotype. Nanotoxicology 12, 847–867.

Martinez-Gomez, C., Leon, V.M., Calles, S., Gomariz-Olcina, M., Vethaak, A.D., 2017. The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. Mar. Environ. Res. 130, 69–76.

Mattsson, K., Ekvall, M.T., Hansson, L.A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environmental Science & Technology 49, 553–561.

Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 7.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., et al., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Mar. Environ. Res. 112, 78–85.

McMinn, L.H., Hodges, G.M., Carr, K.E., 1996. Gastrointestinal uptake and translocation of microparticles in the streptozotocin-diabetic rat. J. Anat. 189, 553–559.

Messinetti, S., Mercurio, S., Parolini, M., Sugni, M., Pennati, R., 2018. Effects of polystyrene microplastics on early stages of two marine invertebrates with different feeding strategies. Environ. Pollut. 237, 1080–1087.

Mintenig, S.M., Int-Veen, I., Loder, M.G.J., Primpke, S., Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 108, 365–372.

Murphy, F., Quinn, B., 2018. The effects of microplastic on freshwater Hydra attenuata feeding, morphology & reproduction. Environ. Pollut. 234, 487–494.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ Sci Technol 50, 5800–5808.

Nemmar, A., Hoylaerts, M.F., Hoet, P.H.M., Vermylen, J., Nemery, B., 2003. Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis. Toxicol. Appl. Pharmacol. 186, 38–45.

Nobre, C.R., Santana, M.F.M., Maluf, A., Cortez, F.S., Cesar, A., Pereira, C.D.S., et al., 2015. Assessment of microplastic toxicity to embryonic development of the sea urchin Lytechinus variegatus (Echinodermata: Echinoidea). Mar. Pollut. Bull. 92, 99–104.

O'Brine, T., Thompson, R.C., 2010. Degradation of plastic carrier bags in the marine environment. Mar. Pollut. Bull. 60, 2279–2283.

Ogonowski, M., Schur, C., Jarsen, A., Gorokhova, E., 2016. The effects of natural and anthropogenic microparticles on individual fitness in Daphnia magna. PLoS One 11.

Okubo, N., Takahashi, S., Nakano, Y., 2018. Microplastics disturb the anthozoan-algae symbiotic relationship. Mar. Pollut. Bull. 135, 83–89.

Olesen, K.B., Stephansen, D.A., van Alst, N., Vollertsen, J., 2019. Microplastics in a stormwater pond. Water 11.

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby Pomatoschistus microps (Teleostei, Gobiidae). Ecol. Indic. 34, 641–647.

Paul-Pont, I., Lacroix, C., Fernandez, C.G., Hegaret, H., Lambert, C., Le Goic, N., et al., 2016. Exposure of marine mussels Mytilus spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737.

Peda, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., et al., 2016. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: preliminary results. Environ. Pollut. 212, 251–256.

Peeken, I., Primpke, S., Beyer, B., Gutermann, J., Katlein, C., Krumpen, T., et al., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9.

Pellini, G., Gomiero, A., Fortibuoni, T., Ferra, C., Grati, F., Tassetti, A.N., et al., 2018. Characterization of microplastic litter in the gastrointestinal tract of Solea solea from the Adriatic Sea. Environ. Pollut. 234, 943–952.

Pinsino, A., Bergami, E., Della Torre, C., Vannuccini, M.L., Addis, P., Secci, M., et al., 2017. Amino-modified polystyrene nanoparticles affect signalling pathways of the sea urchin (Paracentrotus lividus) embryos. Nanotoxicology 11, 201–209.

Pitt, J.A., Kozal, J.S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., et al., 2018. Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (Danio rerio). Aquat. Toxicol. 194, 185–194.

Ravit, B., Cooper, K., Moreno, G., Buckley, B., Yang, I., Deshpande, A., et al., 2017. Microplastics in urban New Jersey freshwaters: distribution, chemical identification, and biological affects. Aims Environmental Science 4, 809–826.

Redondo-Hasselerharm, P.E., de Ruijter, V.N., Mintenig, S.M., Verschoor, A., Koelmans, A.A., 2018a. Ingestion and chronic effects of car tire tread particles on freshwater benthic macroinvertebrates. Environmental Science & Technology 52, 13986–13994.

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018b. Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environmental science & technology 52, 2278–2286.

Rehse, S., Kloas, W., Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna. Chemosphere 153, 91–99. https://doi.org/10.1016/j.chemosphere.2016.02.133.

Reichert, J., Schellenberg, J., Schubert, P., Wilke, T., 2018. Responses of reef building corals to microplastic exposure. Environ. Pollut. 237, 955–960.

Revel, M., C, A., Mouneyrac, C., 2018. Micro(nano)plastics: a threat to human health? Environmental Science & Health 1, 17–23.

Rehse, S., Kloas, W., Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna. Chemosphere 153, 91–99. https://doi.org/10.1016/j.chemosphere.2016.02.133.

Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., et al., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel Perna viridis. Mar. Pollut. Bull. 111, 213–220.

Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in Daphnia magna - quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398–407.

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.

Rochman, C.M., Hentschel, B.T., Teh, S.J., 2014a. Long-term sorption of metals is similar among plastic types: implications for plastic debris in aquatic environments. PLoS One 9, e85433.

Rochman, C.M., Lewison, R.L., Eriksen, M., Allen, H., Cook, A.M., Teh, S.J., 2014b. Polybrominated diphenyl ethers (PBDEs) in fish tissue may be an indicator of plastic contamination in marine habitats. Sci. Total Environ. 476-477, 622–633.

Rochman, C.M., Parnis, J.M., Browne, M.A., Serrato, S., Reiner, E.J., Robson, M., et al., 2017. Direct and indirect effects of different types of microplastics on freshwater prey (Corbicula fluminea) and their predator (Acipenser transmontanus). PLoS One 12.

Rosenkranz, P., Chaudhry, Q., Stone, V., Fernandes, T.F., 2009. A comparison of nanoparticle and fine particle uptake by Daphnia magna. Environ. Toxicol. Chem. 28, 2142–2149.

Rummel, C.D., Adolfsson-Erici, M., Jahnke, A., MacLeod, M., 2016. No measurable "cleaning" of polychlorinated biphenyls from Rainbow Trout in a 9 week depuration study with dietary exposure to 40% polyethylene microspheres. Environmental Science-Processes & Impacts 18, 788–795.

Ryan, P., Jackson, S., 1987. The lifespan of ingested plastic particles in seabirds and their effect on digestive efficiency. Mar. Pollut. Bull. 18, 217–219.

Schirinzi, G.F., Perez-Pomeda, I., Sanchis, J., Rossini, C., Farre, M., Barcelo, D., 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ. Res. 159, 579–587.

Schur, C., Rist, S., Baun, A., Mayer, P., Hartmann, N.B., Wagner, M., 2019. When fluorescence is not a particle: the tissue translocation of microplastics in Daphnia magna seems an artifact. Environ. Toxicol. Chem. 38, 1495–1503.

Simon, M., van Alst, N., Vollertsen, J., 2018. Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. Water Res. 142, 1–9.

Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261. https://doi.org/10.1016/j.aquatox.2015.12.002.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., et al., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. U. S. A. 113, 2430–2435.

Ter Halle, A., Jeanneau, L., Martignac, M., Jarde, E., Pedrono, B., Brach, L., et al., 2017. Nanoplastic in the North Atlantic Subtropical Gyre. Environmental science & technology 51, 13689–13697.

Tosetto, L., Williamson, J.E., Brown, C., 2017. Trophic transfer of microplastics does not affect fish personality. Anim. Behav. 123, 159–167.

Tosetto, L., Browne, C., Willamson, J.E., 2016. Microplastics on beaches: ingestion and behavioural consequences for beachhoppers. Mar. Biol. 163 (10), 163–199. https://doi.org/10.1007/s00227-016-2973-0.

Ugolini, A., Ungherese, G., Ciofini, M., Lapucci, A., Camaiti, M., 2013. Microplastic debris in sandhoppers. Estuarine Coastal and Shelf Science 129, 19–22.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environ. Pollut. 199, 10–17.

van Pomeren, M., Brun, N.R., Peijnenburg, W., Vijver, M.G., 2017. Exploring uptake and biodistribution of polystyrene (nano)particles in zebrafish embryos at different developmental stages. Aquat. Toxicol. 190, 40–45.

Vianello, A., Jensen, R.L., Liu, L., Vollertsen, J., 2019. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci. Rep. 9.

Volkheimer, G., 1975. Hematogenous dissemination of ingested polyvinyl-chloride particles. Ann. N. Y. Acad. Sci. 246, 164–171.

von Moos, N., Burkhardt-Holm, P., Kohler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. Environmental science & technology 46, 11327–11335.

Vroom, R.J.E., Koelmans, A.A., Besseling, E., Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. Environ. Pollut. 231, 987–996.

Walczak, A.P., Hendriksen, P.J.M., Woutersen, R.A., van der Zande, M., Undas, A.K., Helsdingen, R., et al., 2015. Bioavailability and biodistribution of differently charged polystyrene nanoparticles upon oral exposure in rats. J. Nanopart. Res. 17.

Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate Gammarus pulex. Environ. Pollut. 234, 181–189.

Welden, N.A.C., Cowie, P.R., 2016. Long-term microplastic retention causes reduced body condition in the langoustine, Nephrops norvegicus. Environ. Pollut. 218, 895–900.

Wen, B., Zhang, N., Jin, S.R., Chen, Z.Z., Gao, J.Z., Liu, Y., et al., 2018. Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. Aquat. Toxicol. 195, 67–76.

Wegner, A., Besseling, E., Foekema, E.M, Kamermans, P., Koelmans, A.A, 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (Mytilus edulis L). Environ. Toxicol. and Chem. 31, 2490–2497. https://doi.org/10.1002/etc.1984.

Wick, P., Malek, A., Manser, P., Meili, D., Maeder-Althaus, X., Diener, L., et al., 2010. Barrier capacity of human placenta for nanosized materials. Environ. Health Perspect. 118, 432–436.

Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? Environmental science & technology 51, 6634–6647.

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Current biology: CB 23, R1031–R1033.

Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36.

Zhang, C., Chen, X.H., Wang, J.T., Tan, L.J., 2017. Toxic effects of microplastic on marine microalgae Skeletonema costatum: interactions between microplastic and algae. Environ. Pollut. 220, 1282–1288.

Ziajahromi, S., Kumar, A., Neale, P.A., Leusch, F.D.L., 2017. Impact of microplastic beads and fibers on waterflea (Ceriodaphnia dubia) survival, growth, and reproduction: implications of single and mixture exposures. Environmental science & technology 51, 13397–13406.

Ziajahromi, S., Kumar, A., Neale, P.A., Leusch, F.D.L., 2018. Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. Environ. Pollut. 236, 425–431.

Zubris, K.A.V., Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. Environ. Pollut. 138, 201–211.