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| Abstract | Plastic pollution is a widespread environmental problem that is currently one of the most discussed issues by scientists, policymakers and society at large. The potential ecotoxicological effects of plastic particles in a wide range of organisms have been investigated in a growing number of exposure studies over the past years. Nonetheless, many questions still remain regarding the overall effects of microplastics and nanoplastics on organisms from different ecosystem compartments, as well as the underlying mechanisms behind the observed toxicity. This chapter provides a comprehensive literature review on the ecotoxicological impacts of microplastics and nanoplastics in terrestrial and aquatic organisms in the context of particle characteristics, interactive toxicological effects, taxonomic gradients and with a focus on synergies with associated chemicals. Overall, a total of 220 references were reviewed for their fulfilment of specific quality criteria (e.g. experimental design, particle characteristics, ecotoxicological endpoints and findings), after which 175 were included in our assessment. The analysis of the reviewed studies revealed that organisms' responses were overall influenced by the physicochemical heterogeneity of the plastic particles used, for which distinct differences were attributed to polymer type, size, morphology and surface alterations. On the other hand, little attention has been paid to the role of additive chemicals in the overall toxicity. There is still little consistency regarding the biological impacts posed by plastic particles, with observed ecotoxicological effects being highly dependent on the environmental compartment assessed and specific morphological, physiological and behavioural traits of the species used. Nonetheless, evidence exists of impacts across successive levels of biological organization, covering effects from the subcellular level up to the ecosystem level. This review presents the important research gaps concerning the ecotoxicological impacts of plastic particles in different taxonomical groups, as well as recommendations on future research priorities needed to better understand the ecological risks of plastic particles in terrestrial and aquatic environments. |

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# Chapter 7
# Ecotoxicological Impacts of Micro- and Nanoplastics in Terrestrial and Aquatic Environments

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**Abstract** Plastic pollution is a widespread environmental problem that is currently one of the most discussed issues by scientists, policymakers and society at large. The potential ecotoxicological effects of plastic particles in a wide range of organisms have been investigated in a growing number of exposure studies over the past years. Nonetheless, many questions still remain regarding the overall effects of microplastics and nanoplastics on organisms from different ecosystem compartments, as well as the underlying mechanisms behind the observed toxicity. This chapter provides a comprehensive literature review on the ecotoxicological impacts of microplastics and nanoplastics in terrestrial and aquatic organisms in the context of particle characteristics, interactive toxicological effects, taxonomic gradients and with a focus on synergies with associated chemicals. Overall, a total of 220 references were reviewed for their fulfilment of specific quality criteria (e.g. experimental design, particle characteristics, ecotoxicological endpoints and findings), after which 175 were included in our assessment. The analysis of the reviewed studies

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revealed that organisms' responses were overall influenced by the physicochemical heterogeneity of the plastic particles used, for which distinct differences were attributed to polymer type, size, morphology and surface alterations. On the other hand, little attention has been paid to the role of additive chemicals in the overall toxicity. There is still little consistency regarding the biological impacts posed by plastic particles, with observed ecotoxicological effects being highly dependent on the environmental compartment assessed and specific morphological, physiological and behavioural traits of the species used. Nonetheless, evidence exists of impacts across successive levels of biological organization, covering effects from the sub-cellular level up to the ecosystem level. This review presents the important research gaps concerning the ecotoxicological impacts of plastic particles in different taxonomical groups, as well as recommendations on future research priorities needed to better understand the ecological risks of plastic particles in terrestrial and aquatic environments.

## 7.1 Introduction

Plastic particles are a widespread environmental problem and possibly an important human health issue that has recently garnered significant interest from scientists, policymakers, natural resource managers, media entities and the public (Prata et al. 2021; Thompson et al. 2004). The complexity of plastic pollution follows a dynamic environmental cycle (Bank and Hansson 2019, 2021), which involves bidirectional fluxes across different ecosystem compartments including the atmosphere, hydrosphere, biosphere as well as terrestrial environments (Vince and Hardesty 2017; Windsor et al. 2019). There has been an outburst of research into plastic pollution in recent years, with research focusing on sources, presence and transport in the environment (as presented in other chapters in this volume – e.g. Bank and Hansson 2021; Kallenbach et al. 2021; Lundebye et al. 2021). Despite this, many questions remain regarding the ecotoxicology of plastic particles and their overall effect on wild populations of biota from different ecosystem compartments (de Sá et al. 2018; Galloway et al. 2017; GESAMP 2020; Law and Thompson 2014; Prakash et al. 2020; VKM 2019).

Many of the challenges related to understanding the ecotoxicological consequences of plastic particles are inherently linked to their complex nature as environmental contaminants (Rochman et al. 2019). Microplastics are made up of different polymers and additives which can influence their impact on living organisms. Furthermore, microplastics can originate from many different sources. Some are specifically designed (primary microplastics), whereas others are formed through the breakdown of larger plastics (secondary microplastics) (Cole et al. 2011). The terminologies used to describe plastic particles can also hold significant weight in terms of how data is interpreted. Microplastics are most commonly defined by their size, being less than 5 mm (GESAMP 2019), although definitions used across different research fields does introduce inconsistencies, especially with reference to their lower size limit (Hartmann et al. 2019). For the purpose of this chapter, we kept the definitions of microplastics as <5 mm in size (GESAMP 2019), even though much of the ecotoxicological data presented involved particles <1 mm in size. The lower size limit of microplastics is here defined as 1 μm, following the definition set by Hartmann et al. (2019) in reference to nanoplastics (1–1000 nm).

A wide array of impacts and toxic effects have been reported for both microplastics and nanoplastics, and as a brief example, several studies have examined the direct and indirect effects of a broad range of size fractions on a range of different species. Effects observed include impacts on reproduction, population dynamics, oxidative stress, ingestion, physiology, feeding behaviour, metabolic and hepatic functions as well as interactions with other contaminants (e.g. Anbumani and Kakkar 2018; Haegerbaeumer et al. 2019; Kögel et al. 2020). However, the extent to which the available data is useful to interpreting consequences across different biological levels (cellular-organ-individual-population; Galloway et al. 2017) has been called into question (VKM 2019).

The potential risks of micro- and nanoplastics to the environment and biota health have been the subject of several recent reviews and risk assessments by international authorities including (i) the European Food Safety Authority (EFSA), Panel on Contaminants in the Food Chain (CONTAM) on the presence of nano- and microplastics in food (EFSA CONTAM Panel 2016); (ii) a technical paper from the Food and Agriculture Organization of the United Nations (FAO) on the status of knowledge on microplastics related to fisheries and aquaculture (Lusher et al. 2017); (iii) a scientific perspective on microplastics in nature and society (SAPEA 2019); (iv) an updated knowledge summary built on the foundations of the previous three reports (VKM 2019); and (v) an ecological and human health risk assessment conducted by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP 2020). During the VKM systematic assessment (VKM 2019), publications were judged based on a set of criteria to assess their quality, and those with poor quality were excluded. The accepted papers were used to attempt conceptual human and environmental risk assessments; however, many uncertainties and knowledge gaps were identified. One of the most significant limitations was that nano- and microplastics were treated as one entity, ignoring their physicochemical heterogeneity (Rochman et al. 2019). There was also a disproportionate representation between different species and different environmental compartments (marine, brackish, freshwater, terrestrial), which hampered the understanding of impacts in specific ecosystems. Much of the information available focused on species which are routinely used in standard test guidelines developed by the Organization for Economic Cooperation and Development (OECD) and the International Organization for Standardization (ISO).

Here we provide an overview and synthesis of microplastic and nanoplastic ecotoxicology (2012- August 2019) in the context of particle characteristics (e.g. polymer type, morphology, size fractions), interactive toxicological effects, taxonomic gradients and with a focus on other potential synergies with associated chemical compounds. The specific objectives of this chapter are to (1) synthesize the literature and scientific consensus regarding the ecotoxicity of microplastics and nanoplastics and their potential relationships with other chemical compounds; (2) evaluate the effects of microplastic and nanoplastic concentrations, polymer type, size and morphology, experimental design, exposure time and pathways on ecotoxicological endpoints; (3) identify critical data and knowledge gaps in microplastic and nanoplastic toxicity research; and (4) suggest approaches and guidelines for addressing the most pressing questions and for advancing microplastic and nanoplastic ecotoxicity research.

## 7.2 Methods Used for Review Process

### *7.2.1 Overall Review Process*

A comprehensive assessment of available published peer-reviewed literature was conducted up to August 2019 using the Web of Science, ScienceDirect, Scopus, PubMed and Google Scholar databases. The search was based on a combination of keyword terms, such as microplastic, nanoplastic, effects, toxicity, specific phylum/sub-phylum and specific target organisms (e.g. fish, crustaceans, bivalves, etc.), in any topic, title or keywords. Additional targeted searches were conducted from references included in relevant peer-reviewed articles (including review papers), as well as relevant reports overlooked by the search engines used. Of the identified references, only those focusing on studies reporting ecotoxicological effects were retained for further analysis. Studies only describing ingestion and egestion of plastic particles without reporting toxicity assessment were excluded from the collected literature. The ingestion of nano- and microplastics by biota has been described in previous review articles (e.g. Collard et al. 2019; Wang et al. 2019b, 2020). Particles >5 mm were not included in this assessment. An overview of the review process can be found in Fig. 7.1.

### *7.2.2 Extraction and Compilation of Data*

A total of 220 references containing relevant ecotoxicity data were selected for review, after which the following information was extracted and compiled in an EXCEL spreadsheet for subsequent analysis: (i) experimental design, (ii) group of organisms, (iii) particles used, (iv) ecotoxicological endpoints and (v) publication information.

In terms of experimental design, the information extracted was categorized according to (i) exposure time, as described by authors and converted into days; (ii) particle concentration, in mass and/or particle number; (iii) exposure regime, static, semi-static or flow-through; (iv) replication, as number of independent replicate experiments or number of replicate exposure vessels; (v) use of controls, negative

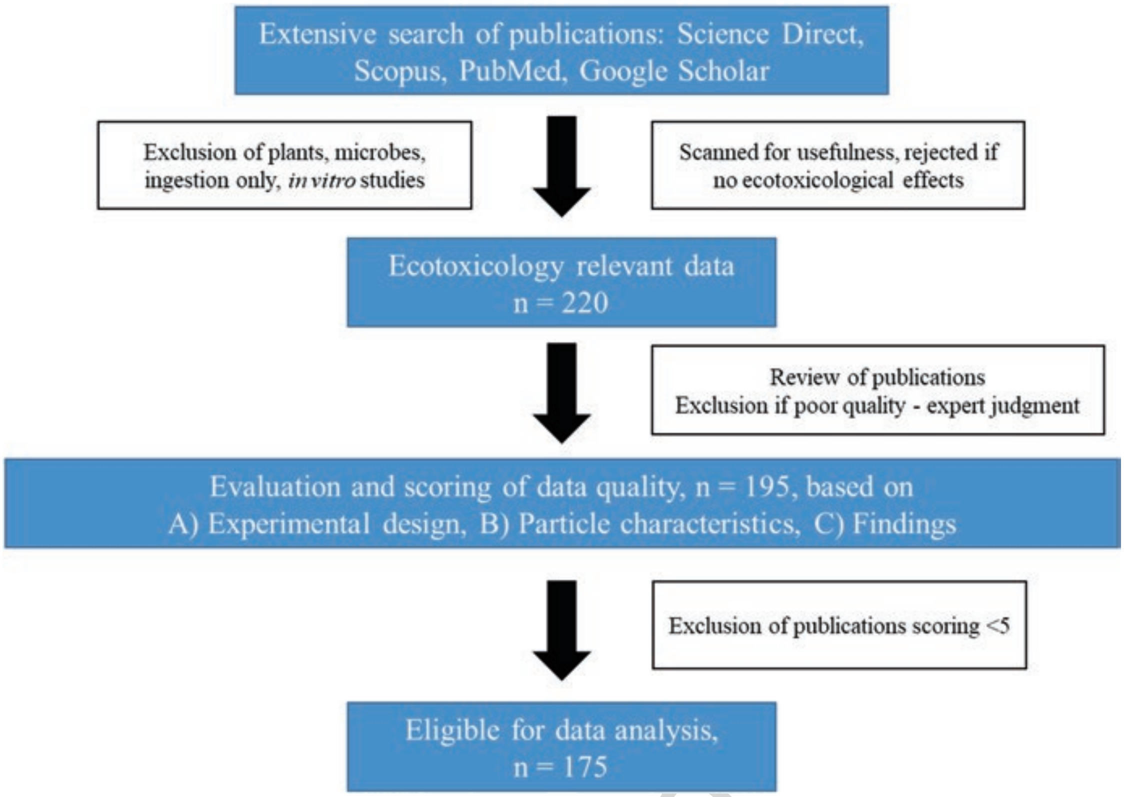


**Fig. 7.1** Schematics on the literature review search of references containing relevant ecotoxicity data regarding micro- and nanoplastics



control (no plastic, only exposure media), additive/preservative control (e.g. tween 20, $NaNO_3$), particle control (kaolin, clay, etc.) or chemical control (co-exposure with other contaminants); (vi) confirmation of test concentration, nominal versus measured; (vii) exposure route, water, sediment/soil, food (e.g. inert pellets), prey (food chain) or others; and (viii) additional information, not included in the previous categories.

The types of organisms used in the studies reviewed were divided into the following taxonomic groups: Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata, Mollusca, Nematoda, Phytoplankton and Rotifera. For each group, the following information was extracted: (i) taxonomic class; (ii) species, full Latin name; (iii) developmental stage, egg, embryo, larvae, juvenile, adult and others; (iv) feeding strategy, filter feeder, deposit feeder, scavenger, suspension feeder, predator or others; (v) supply of food during exposure; (vi) environmental compartment, freshwater, seawater or soil/sediment; (vii) replication, number of organisms per endpoint determination; and (viii) ingestion, checked, yes or no. Toxicity studies on higher plants, bacteria and in vitro were not included in this review.

For information on the particles used, the following categories were chosen as the most representative in terms of physicochemical characteristics: (i) polymer type; (ii) particle morphology, spheres, fibres, fragments (same as irregular), pellets or others if missing; (iii) surface modification, plain, COOH, $NH_2$, others or not specified; (iv) particle size; (v) co-exposure/mixture, yes or no in case of spiking with chemicals; (vi) chemical details, chemical name and concentration used; (vii) characterization, only by the supplier and/or additional by the authors; and (viii) others, additional information on particles, e.g. fluorescence, density, etc. In terms of particle type, the following list of polymer types was used to classify the particles used in the selected studies, which include the main groups of polymer materials reported in PlasticsEurope (2019): polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP), polyvinylchloride (PVC), polyamide (PA), acrylonitrile butadiene styrene (ABS), nylon, polycarbonate (PC), polyhydroxy butyrate (PHB), polylactic acid (PLA), polymethylmethacrylate (PMMA), polyoxymethylene (POM), styrene acrylonitrile (SAN), phenylurea-formaldehyde (PUF), proprietary polymer as well as not specified (NS). High- and low-density PE were not differentiated but included in an overall PE group. To assess the impact of particle size (i.e. nanoplastic versus microplastic), one or more of the following size categories were used: < 0.05 µm, 0.05–0.099 µm, 0.1–0.99 µm, 1–9 µm, 10–19 µm, 20–49 µm, 50–99 µm, 100–199 µm, 200–500 µm and > 500 µm.

The effects reported were categorized following the levels of biological organization as suggested by Galloway et al. (2017): subcellular (e.g. enzyme activity, gene expression, oxidative damage), cellular (e.g. apoptosis, membrane stability), organ (e.g. histology, energetic reserves), individual (e.g. mortality, growth), population (e.g. reproduction, larval development) and ecosystem (e.g. behaviour, ecosystem function, community shifts). In cases where a large amount of data was generated in a specific study, detailed information on biological endpoints was also recorded, such as gene and protein expression data, enzymatic activities, histopathology effects, etc. Presence or absence of significant effects were recorded as yes or no, followed by the direction of the effect recorded as up (induction) and down (inhibition). Whenever disclosed, the ECx (concentration showing a x% effect), NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values were also recorded.

Within the selected references, descriptions of experiments using different experimental conditions (e.g. time of exposure and concentration), two or more species (e.g. life stages and route of exposure) or particles with different characteristics (e.g. polymer type, size, morphology) were considered as individual records and added as separate entries in the data matrix. For example, whenever the size distribution for a given particle spanned more than one of the defined size categories, multiple entries were recorded, each corresponding to a size category. If a study included more than one species, a separate record was added for each species, each one with multiple entries dependent of the varying treatments used by the authors. Accordingly, the number of studies and corresponding entries presented in the results section represent the number of interactions of the classification criteria recorded for each reference, and not the total number of publications reviewed.

After revision of the 220 references collected, 25 were excluded due to poor quality in one or more of the classification criteria used. Examples were poor experimental design, lack of information on particles used or particle characterization, inadequate data representation or conclusions not supported by data. The exclusion of these 25 references was based on expert judgement, and data entries pertaining to these references were removed from the data matrix. The data matrix can be made available upon demand.

### *7.2.3 Evaluation and Scoring of Data Quality*

The 195 references considered of acceptable quality were further evaluated and given a quality score based on the criteria presented in earlier publications. This was to ensure that the highest quality data generated through ecotoxicological studies was also the data that had the most impact in this analysis. Evaluation criteria were divided in three groups, experimental design, particle characterization and findings, as detailed in Table 7.1 (based on Connors et al. 2017; VKM 2019). Specifically:

– "Experimental design" included the use of reference controls and chemical controls, as well as replication within the test system. Maximum score = 3.

**Table 7.1** Evaluation criteria used to score data quality of reviewed references (based on Connors et al. 2017; VKM 2019)

| Criteria | Description | Scoring definition |
|---|---|---|
| Experimental design (0–3) | Use of reference controls | Use of reference particles other than plastic (e.g. kaolin, sand, etc.) |
| | Use of chemical controls | Applies to vector studies only, where the particles are spiked with one or more chemicals, or when further characterization was carried out and results indicate the presence of chemicals on the particles. Otherwise, 1 point should be automatically attributed |
| | Replication in test system | Exposure replication of minimum 3; total number of individuals: Depends on the endpoint |
| Characterization (0–5) | Particle size | Concentration range of particles used determined by authors (e.g. DLS, particle counter, etc.) |
| | Particle charge | Applies for nanoparticles only. If microparticles are used, 1 point should be automatically attributed |
| | Polymer confirmation | Confirmation of polymer used in exposure system (e.g. FT-IR) |
| | Chemical characterization | Applies for studies using spiked particles, particles obtained from the grinding of consumer goods, deployed particles, industrial particles (e.g. nurdles). Only in the case of particles obtained from a "trusted" supplier (e.g. Cospheric, sigma, etc.) and said to be "pristine", 1 point should be automatically attributed |
| | Test concentration confirmation | Test concentration measured in exposure system and not nominal concentration used |
| Findings (0–1) | Conclusions supported by the results | Accurate interpretation of the results without conjecture beyond experimental design |

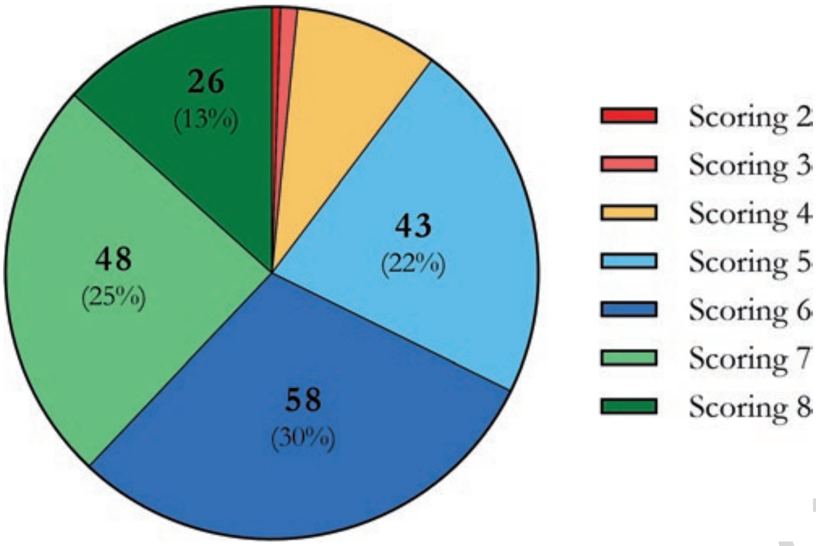


**Fig. 7.2** Scoring of the 195 reviewed references. The number and % of references are only presented for those scored with 5 or more points

- "Particle characterization" included the reporting of particle size, particle charge, polymer confirmation, chemical characterization and confirmation of the test concentration. Maximum score = 5.
- "Findings" included the assessment of whether the conclusions were supported by the results. Maximum score = 1.

For each time a criterion was met, 1 point was attributed, and references were categorized based on a quality score out of 9. References that scored 4 or less were excluded from further analysis and corresponding data entries removed from the data matrix. Of the 195 references scored, 20 were eliminated due to low score, in which 17 papers scored 4 points, 2 papers scored 3 points and 1 paper scored 2 points. None of the papers scored either 1 or 9 points (Fig. 7.2).

### *7.2.4 Treatment of Extracted Data*

Species sensitivity distributions (SSDs) were fitted for three relevant exposure routes: water exposure, sediment/soil exposure and food exposure. Ecotoxicity data for terrestrial, freshwater and marine compartments and species were extracted and summarized for use in the SSD model fitting. Information on polymer types and size classes were combined, and for this reason, studies using fibres were excluded from the SSDs. Ecotoxicity endpoints were limited to individual and population levels (Burns and Boxall 2018; Connors et al. 2017), and only NOECs and $EC_{50}$ values were included. When only acute NOEC or $EC_{50}$ data was available, chronic NOEC values were extrapolated as proposed by Posthuma et al. (2019). When multiple NOEC values were available for the same species, the geometric mean of the NOECs was used to summarize the information. To allow the comparison of ecotoxicological data from studies reporting different dose metrics, mass-based concentrations were converted to mg per litre (mg/L) and particle-based concentrations converted to particles per litre (particles/L). In the case of studies where particles were added via sediment/soil or via food, concentrations were converted to mg per kg (mg/kg) of sediment/soil or food and particles per kg (particle/kg) of sediment or food. As several studies only reported concentrations in either mass or particle number, two SSDs were created per exposure route. Studies where none of the above dose metrics were employed were excluded from the SSD fitting. The SSDs were realized as Bayesian distributional regression models assuming a log-normal data distribution (Ott 1990). All modelling was performed using statistical programming language R (R Core Team 2020) and its add-on package brms (Bürkner 2017, 2018). A total of 10,000 posterior draws were used to characterize each SSD. Where applicable, the value indicating the concentration at which 5% of the species are affected (hazard concentration, $HC_5$) was extracted from the posterior draws and summarized as average and 95% credible interval.

## 7.3 Results and Discussion

A key issue in understanding how microplastics and nanoplastics interact with the surrounding environment is their dynamic nature. The physicochemical properties of the parent material, including density, morphology, charge and size, are likely to influence particles' physical behaviour in the environment, fate (e.g. presence in the water column or in sediments), potential to adsorb environmental contaminants (e.g. Trojan horse effect), bioavailability and potential toxicological impacts on organism health (e.g. de Sá et al. 2018; Galloway et al. 2017; Haegerbaeumer et al. 2019; Kögel et al. 2020). The extensive literature review carried out showed that the responses of organisms to particle exposure were mostly dependent on particle characteristics as polymer type, size, morphology and surface alterations. However, it is possible that other factors were driving the observed impacts, as, for example, the presence of additive chemicals associated with the plastic particles, which are rarely considered in studies. A special emphasis has therefore been given to particle size, with a higher consensus in terms of increased internalization for smaller sized particles than larger ones and thus higher potential for toxic effects. A variety of experimental designs have been used to evaluate the effects of nanoplastics and microplastics in organisms, in which exposure time and particle concentration seem to be determinant for the induction of toxicity. Nonetheless, the observed effects were highly dependent on the environmental compartment assessed, in combination with specific morphological, physiological and behavioural traits of the species used, as, for example, developmental stage, trophic level and feeding strategy.

In terms of ecotoxicological effects, there is still little consensus regarding the biological impacts posed by plastic particles, as well as a limited understanding on the underlying toxic mechanisms causing the observed effects. This limited knowledge on mechanistic toxicity data also makes it difficult to understand and distinguish physical from chemical toxicological effects of plastic particles. And even though it is quite clear from wider literature that large particles (e.g. macroplastics) cause visible effects at the organism level (Kühn et al. 2015; Rochman 2015), the direct and indirect physiological effects of the smaller plastic particles remain elusive. Based on this review, effects were found at different levels of biological organization in a range of organisms. However, many of these studies used standard ecotoxicity approaches based on OECD or ISO guidelines that do not consider effects at the lower levels of biological organization such as cellular or subcellular mechanisms, which may be more sensitive and have a higher impact on the physiological traits of organisms, especially in the long term. To a small degree, some of the reviewed studies highlighted that the combination of nanoplastics and microplastics with organic and inorganic contaminants also modify and potentiate their toxicity towards biological systems. Nonetheless, the effects of chemical additives present in plastic particles are also understudied, and it is still not clear if the presence of these additives rather than the polymeric composition of particles are the main driver of the adverse effects reported in organisms. Based on the 175 publications reviewed, a more general and detailed report of the main factors influencing particle toxicity towards the different groups of organisms are presented in the sections below.

## *7.3.1 General Overview of Information Extracted from Reviewed Publications*

### 7.3.1.1 Polymer Type, Morphology, Surface and Size

Within the 175 reviewed publications, the most commonly used polymer type was PS (90 studies, 51%), followed by PE (62 studies, 35%), PVC (17 studies, 10%) and PET (11 studies, 6%). The remaining polymer types (acrylonitrile butadiene styrene [ABS], nylon, polyamide [PA], polycarbonate [PC], polyhydroxybutyrate [PHB], polylactic acid [PLA], poly(methyl methacrylate) [PMMA], polyoxymethylene [POM], polypropylene [PP], styrene acrylonitrile resin [SAN]) were used in less than 5% in the reviewed studies. The use of PS and PE as polymers of choice in exposure studies is consistent with the most commonly found polymers in the environment, as PS, PE and PP are typically retrieved from surface waters and sediments (e.g. Koelmans et al. 2019 and references therein). Given that polymer type can influence the fate and behaviour of particles within test systems, in particular density and presence of chemical additives (e.g. Gallo et al. 2018), other polymers should be comprehensively assessed in order to build up knowledge regarding how their composition influence toxicity towards organisms.

Despite the prevalence of fragments, fibres and films in environmental samples due to degradation of larger pieces of plastic (see Burns and Boxall 2018; Kooi and Koelmans 2019; Phuong et al. 2016), the majority of studies focused on spherical particles (106 studies, 61%), with only 40 studies looking at the impacts of fragments/irregular particles (23%) and even less focusing on the effects of fibres (13 studies, 7%). The main reason for the use of spherical particles is that they are easier to produce than the other morphological types (e.g. fibres, fragments, foams), especially in terms of sufficient quantity within a certain size range. The irregular and non-standardized morphology of these particles also make them more difficult to characterize and track during exposure experiments, which results in poorly comparable ecotoxicity data. Nonetheless, irregularly shaped particles resulting from the fragmentation of larger plastic items or materials containing synthetic polymers as fibres have a higher environmental relevance and should be used more often in effects studies, especially in terms of increasing ecological relevance for advancing quantitative data to assess environmental risks.

Among the reported surface alterations, plain/pristine particles were used in 163 publications out of the 175 (93%) studies reviewed. Of all the particles reported with surface alterations, the majority was for PS, with PS-COOH and PS-$NH_2$ in the nano-size range being the most commonly used (10% and 9%, respectively). Particle surface chemistry, i.e. chemical groups and surface charge, was one of the main properties driving the behaviour of particles in the aquatic environment – this is particularly true for smaller sized particles – especially when it comes to stability, aggregation, mobility and sedimentation (e.g. Mudunkotuwa and Grassian 2011). In fact, particle surface charge, more so than polymer composition, has been suggested as the main driver behind behaviour and consequent toxicity of smaller sized plastics (Lowry et al. 2012; Nel et al. 2009). Even though functionalized particles are commonly used as surrogates for naturally altered particles, their prevalence in the environment has been questioned. The presence of negatively charged PS-COOH has been suggested as widespread in the environment, although there is very little information on its fate in different environmental compartments. Similarly, the presence of PS-$NH_2$ as a plastic degradation product in the environment has not yet been fully recognized/determined (Besseling et al. 2014).

An overview of the number of studies per particle type and size class is presented in Fig. 7.3. Of the size classes tested, most studies used particles smaller than those that can be detected with confidence in environmental matrices (<100 μm, e.g. (de Ruijter et al. 2020). Sixty-five of the reviewed studies used particles with sizes in the range 1–9 μm (37%), followed by 43 studies with size in the range 20–49 μm (25%), 36 studies with sizes in the range 50–99 μm (21%) and 34 studies with sizes in the range 10–19 μm (19%). As for smaller size ranges, 39% of the reviewed publications used particles <1 μm (total 69 studies), with a predominance of particles within 0.1–0.99 μm. Regarding fibres, the size ranges used were between 362 and 3000 μm in length and 41 and 3000 μm in diameter. In terms of size distribution per polymer type, for PS and PMMA a higher focus has been given to particles <10 μm, especially for PS in the nano-range size, as seen in Fig. 7.3. This is the opposite of PE, as well as the remaining polymers reported, where most particles used have a size range > 1 μm. Most of the studies comparing the effects of both nanoplastics and microplastics of the same polymer composition reported size-dependent effects, with an increase in toxicity with decreasing particle size (e.g. Jeong et al. 2016, 2017; Lee et al. 2013; Lei et al. 2018a; Snell and Hicks 2011). Nonetheless, this

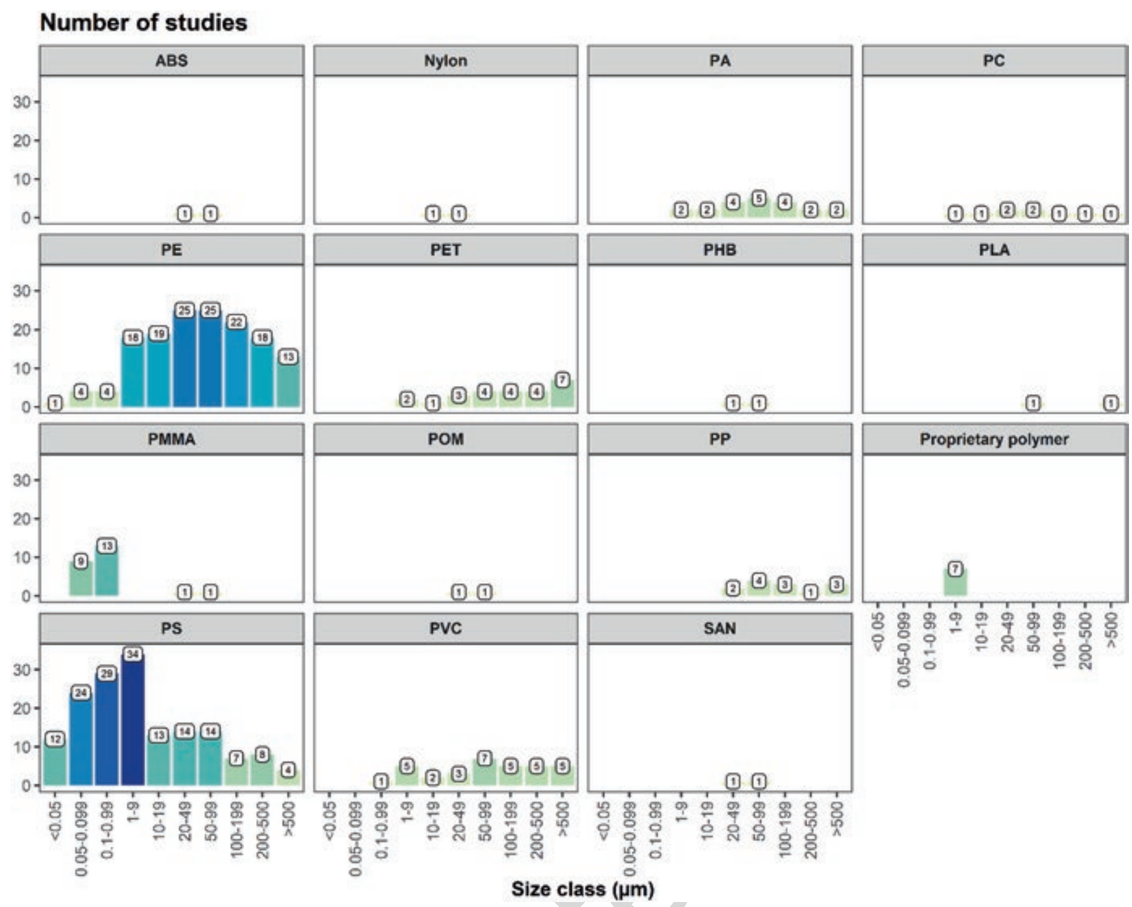


**Fig. 7.3** Overview of the number of studies per particle type and size class. Note: There can be more than one size class within a study for a specific particle. See Material and Methods section for more information on how particle size was categorized. *ABS* acrylonitrile butadiene styrene, *PA* polyamide, *PC* polycarbonate, *PE* polyethylene, *PET* polyethylene terephthalate, *PHB* polyhydroxy butyrate, *PLA* polylactic acid, *PMMA* polymethylmethacrylate, *POM* polyoxymethylene, *PP* polypropylene, *PS* polystyrene, *PVC* polyvinylchloride, *SAN* styrene acrylonitrile AU1

size-toxicity correlation seems to be species and phyla dependent. Irrespective of the potentially higher adverse effects imposed by smaller sized particles in organisms, their detection in different environmental compartments and resulting uncertainties in terms of natural concentrations remain an ongoing analytical challenge. Nonetheless, their presence in the environment as a consequence of fragmentation and degradation of plastic debris is widely accepted, having been proven under laboratory conditions (e.g. Lambert and Wagner 2016) and where their occurrence in the North Atlantic subtropical gyre has also been suggested (Ter Halle et al. 2017).

Even though particle ingestion and egestion were not considered in this review chapter, the selective size ingestion of micro- and nanoplastics has been reported for a range of aquatic organisms (e.g. bivalves, Ward et al. 2019). Accordingly, the size distribution of microplastics and nanoplastics used in ecotoxicological studies need to be appropriate for the species used, as this may influence exposure and particle-organism interactions.

#### 7.3.1.2 Experimental Conditions

Standard test protocol guidelines commonly used in toxicity testing of chemicals are not always suitable for testing of particles (e.g. Hermsen et al. 2018). Accordingly, ecotoxicity testing of nano- and microplastics often require modifications in experimental design to address specific particle behaviour and/or characteristics, leading to a lack of standardization. The lack of standardized test protocols for plastic particles results in a multiplicity of experimental conditions, which limits consistency and result comparison and interpretation (Connors et al. 2017; VKM 2019).

Considering the absence of consistent particle quantification in the environment in size ranges as small as those commonly used in ecotoxicological studies (Paul-Pont et al. 2018), the use of the so-called environmentally relevant doses of plastic particles also remains a challenge. Concentration range and units expressed in either mass or particle number are two of the main issues that have been highlighted related to the dosing of plastic particles in exposure systems. More than half of the publications reviewed reported particle concentrations in mass (minimum $7x10^{-7}$ mg/L to maximum 12,500 mg/L), with the most commonly used concentration range of 1–100 mg/L (organisms exposed via water, 72% of studies). As for particle mass used in exposures via food (17% of studies) or sediment/soil (10% and 7% of studies, respectively), concentrations varied from $7x10^{5}$ to 100 mg/kg food (most common 4000, 12,000, 100,000 mg/kg food) and $4x10^{5}$ to 1 mg/kg sediment/soil (most common 1000 to 50,000 mg/kg sediment/soil). Few studies reported concentrations in terms of particle number, with concentrations ranging from 1 to $8x10^{15}$ particles/L, 16 to $23x10^{7}$ particles/kg sediment/soil and $3x10^{5}$ to $1x10^{8}$ particles/kg food. Therefore, it seems that the nano- and microplastics used in the reviewed publications have been tested in numbers several orders of magnitude higher than those currently detected in the natural environment. This is particularly true for the small sized plastics within a wide range of polymer types, where realistic concentrations are rarely available for sizes >10 μm and not available for sizes <10 μm (for more information on environmental data on plastic contamination, check Litter Database webpage: http://litterbase.awi.de/litter). In addition, the failure to provide particle concentrations in both mass and number complicates the comparison of effect data across published studies, confounding the ability to reach precise conclusions over exposure and risk.

Exposure time is another important aspect related to varying experimental conditions used in nano- and microplastic ecotoxicological studies. The most commonly used exposure times in the reviewed studies were 48 h (27% studies), 24 h (18% studies), 96 h (17% studies) and 72 h (14% studies). These exposure durations are within those recommended in ecotoxicity guidelines for acute testing (e.g. OECD and ISO). In these tests, model organisms are normally exposed to high concentrations of a test compound over a short period of time, after which effect endpoints such as mortality or development are commonly assessed. Even though several of these studies showed evidence of deleterious effects at high concentrations, there are still knowledge gaps – which are hidden by the present focus in acute ecotoxicological testing, relating to limited environmental relevance. As exposure concentration and duration are two major parameters influencing toxicity, results based on short-term and high exposure concentrations make it difficult to extrapolate data to a more realistic scenario of exposure to low concentrations over a long period of time. One of the main gaps in the reviewed studies was the underrepresentation of long-term exposures at environmentally relevant concentrations and their consequent long-term effects at the organism and ecosystem levels (e.g. chronic exposure, whole life cycle, multi-generational effects). Long-term (or chronic) studies on the effects of nano- and microplastics were mostly carried out for 28 and 21 days (11% studies each), followed by 14 days (10% studies). Only a very small percentage of studies have used an exposure period higher than 28 days, with only 4 studies looking at ecotoxicological effects above 3 months of exposure (maximum 240 days, i.e. 8 months). Long-term exposures carried out over more than 1 life stage or whole organism's lifespan allow to focus on population-relevant adverse endpoints (e.g. reproduction), as well as other sublethal effects that might constitute more reliable endpoints for risk assessment and are therefore urgently needed.

#### 7.3.1.3 Organisms Used in Ecotoxicological Studies

When it comes to environmental compartments, most test organisms used were from the marine environment (61%), followed by freshwater (31%) and terrestrial (8%) compartments, as presented in Fig. 7.4. Only 1 study reported the use of brackish organisms (1%). This highlights that the effects of nano- and microplastics on terrestrial and freshwater ecosystems have been understudied and deserve further attention (e.g. Adam et al. 2019; Haegerbaeumer et al. 2019; Horton et al. 2017; Strungaru et al. 2019). These knowledge gaps are of particular concern, especially when terrestrial and freshwater environments are considered the main sources and transport pathways of plastic particles to the marine environment. Given that many

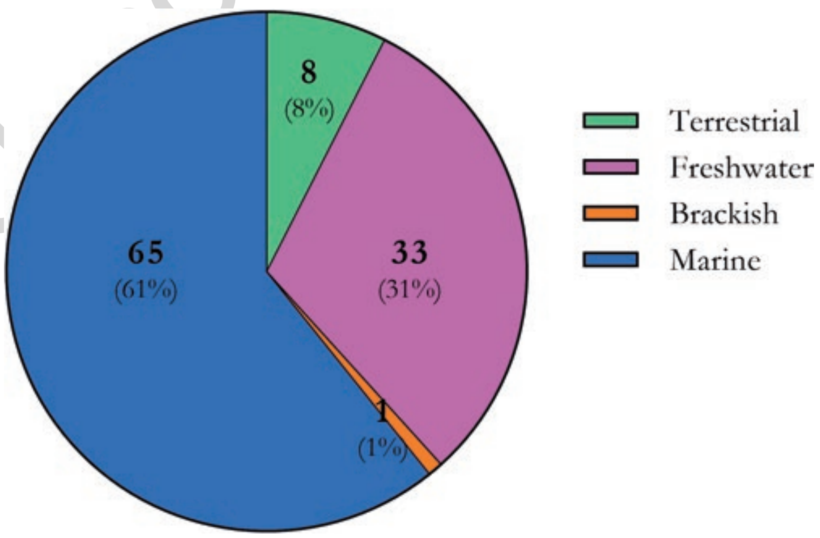


**Fig. 7.4** Number of species (total of 107) from each environmental compartment used in the reviewed references

plastic particles are used and disposed on land, terrestrial environments will be subject to extensive pollution by particles of varying characteristics at high concentrations, making terrestrial organisms at high risk of exposure. As for freshwater organisms, these will be directly affected by terrestrial runoff and other anthropogenic sources (e.g. wastewater treatment discharge, sewage sludge application), potentially containing high levels of plastic particles, as well as other associated contaminants (Adam et al. 2019; Horton et al. 2017 and references therein).

At the phylum level, Arthropoda was the most studied (34%, 59 publications), followed by Chordata (23%, 41 publications), Mollusca (21%, 36 publications), Phytoplankton (14%, 25 publications), Annelida (9%, 16 publications), Cnidaria and Echinodermata (2% each, 4 publications), Rotifera (2%, 3 publications) and finally Nematoda (1%, 1 publication). The freshwater crustacean *Daphnia magna* (17% overall studies) was the most studied species, followed by the marine mussel *Mytilus galloprovincialis* and the freshwater zebrafish *Danio rerio* (both with 6% of overall studies). In terms of developmental stage, most of the studies assessed effects in adult organisms (42%, 73 studies total) and a small percentage used juveniles or neonates (both with 14%, 25 studies). Very few studies have looked at whole cycle assessments, 3% of the total of reviewed publications, and those that did were only directed towards arthropods. In terms of feeding strategy, 32% of the species used were filter feeders, followed by photosynthetic organisms (21%), predators (17%), detritivores (10%), grazers (9%), scavengers (8%) and deposit feeders (5%). Only one herbivore and one microbivore were used.

Even though the organisms used in the reviewed publications have different roles in terrestrial and aquatic food webs, there is still a lack of studies conducted on organisms other than fish, small crustaceans and bivalves. Specifically, more studies on the effects of nano- and microplastics on organisms that are the basis of aquatic food chains should be conducted (e.g. planktonic species). These species have critical roles in ecosystem balance and might be at highest risk of exposure due to their feeding strategies and relative position in the water column. Moreover, small plastic particles are easily confused as food and ingested by planktonic species, thus serving as a route of transfer to secondary and tertiary consumers in food chains (Botterell et al. 2018). In addition, soil- and sediment-dwelling organisms are of major importance, as soil/sediment is considered the main sink for contaminants in the environment, increasing the likelihood of synergistic effects of plastic particles with other environmental contaminants (Adam et al. 2019; Horton et al. 2017 and references therein). Furthermore, targeted studies on species other than those commonly used in OECD and ISO guidelines should also be conducted, as the toxicological and mechanistic effect data on these species might not provide sufficient information into impacts on other ecologically relevant species. The same can be said in terms of transferring knowledge from marine to freshwater or terrestrial environment. Given the differences in habitat, physiological traits and feeding mechanisms, it is not clear as to what extent ecotoxicological effects on marine organisms can be applied to freshwater and terrestrial species within the same taxonomical group and vice versa.

#### 7.3.1.4 Levels of Biological Organization

Most of the reviewed studies focused on the effects of nano- and microplastics at the individual level (133 studies, 40%), followed by the subcellular level (78 studies, 23%). The population level has been addressed in 45 studies (14%), ecosystem in 33 (10%), closely followed by the organ level with 30 studies (9%). Only 13 studies (4%) analysed effects at the cellular level. Within the individual endpoints, growth and mortality were the most studied (74 and 73 studies, respectively), while at the subcellular level, effects looking at alterations in gene expression (41 studies) were the most frequent, followed by oxidative stress (26 studies) and enzymatic activities (24 studies). Within population-related endpoints, the most determined were reproduction (21 studies) and larval development (16 studies). Within ecosystem, 29 studies looked at behaviour and 22 looked at community shifts. As for organ level, most studies (17) looked at histopathological alterations, followed by nine studies looking at energy reserves. At the cellular level, eight studies looked at membrane stability, five at cell size and four at both cell number and cell complexity. When looking at the number of studies categorized by environmental compartment (Fig. 7.5), the majority of the studies for both freshwater and marine environments

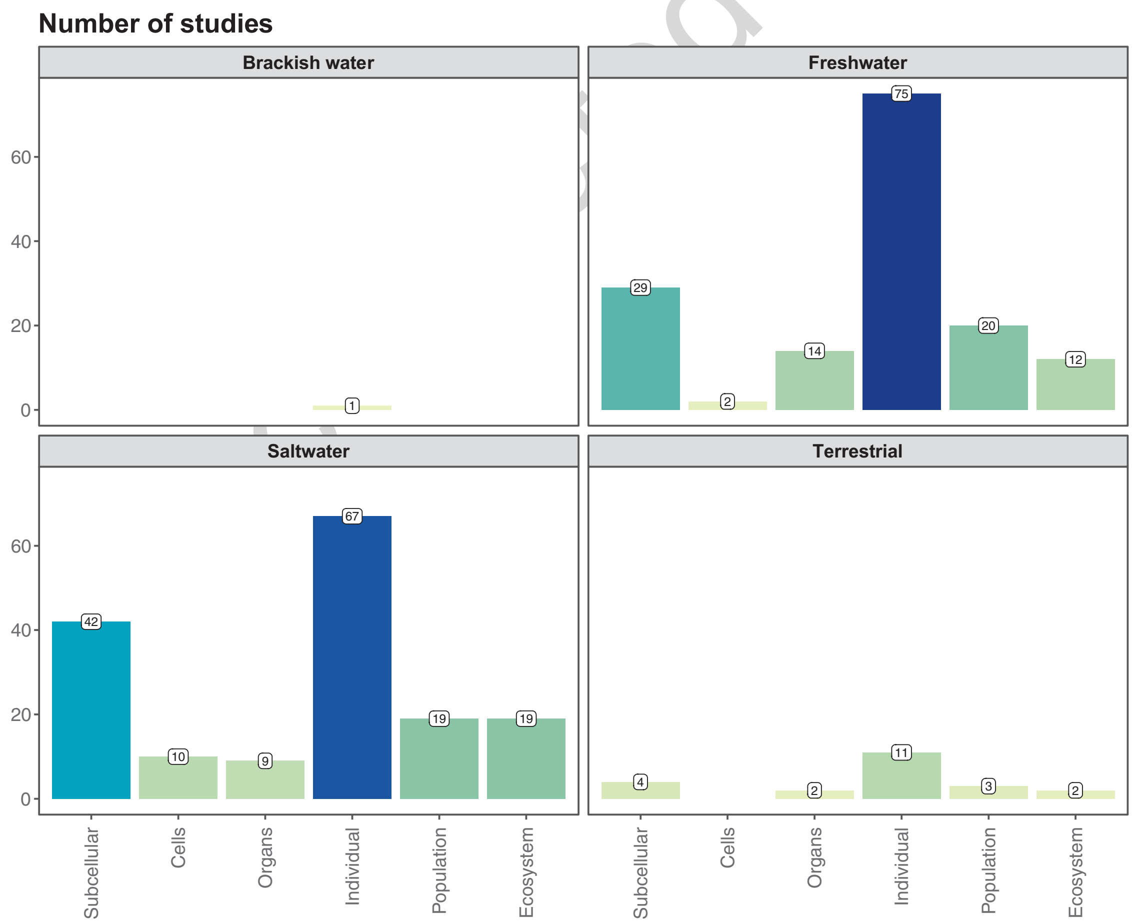


**Fig. 7.5** Number of studies categorized by level of biological organization per environmental compartment

covered endpoints at the individual level (75 and 72 studies, respectively), followed by effects at the subcellular level (29 and 42 studies, respectively). Impacts at the individual and cellular levels were also the most determined in terrestrial organisms (ten and 4 studies, respectively), while only one study covered individual endpoints in the brackish environment. Studies on effects at the cellular level were less common in freshwater and marine environments (two and ten studies, respectively), while no studies addressed this level of biological organization in terrestrial and brackish environments.

## *7.3.2 Ecotoxicological Effects*

While a range of ecotoxicological effects caused by plastic particle exposure have been documented across several groups of organisms, there are still distinct research gaps concerning effects of both nano- and microplastics in specific taxonomical groups. In the following paragraphs, particle characteristics, exposure conditions and consequent ecotoxicological effects will be described for each taxonomical group considered in the present review: Phytoplankton, Cnidaria, Nematoda, Rotifera, Arthropoda, Annelida, Mollusca, Echinodermata and Chordata.

### 7.3.2.1 Phytoplankton

Phytoplankton include unicellular organisms such as microalgae that are at the bottom of the aquatic food chain. Small disruptions of microalgae populations due to exposure to plastic particles may lead to serious repercussions at the ecosystem level, being thus imperative to characterize the risks/effects of plastic particles on this taxonomical group (Prata et al. 2019). Phytoplankton were evenly represented from marine and freshwater environments in the reviewed studies (12 and 13 studies, respectively). Exposure studies included 21 different species belonging to 8 different classes (Bacillariophyceae, Chlorodendrophyceae, Chlorophyceae, Coscinodiscophyceae, *Cyanophyceae*, Dinophyceae, Prymnesiophyceae and Trebouxiophyceae). The most used class was Chlorophyceae (14 studies). *Raphidocelis subcapitata*, previously named as *Pseudokirchneriella subcapitata*, was the most used species with four studies. Six other species (*Chaetoceros neogracile*, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa*, *Dunaliella tertiolecta*, *Scenedesmus obliquus* and *Skeletonema costatum*) had two studies each, while the remaining had only one publication.

A total of 7 different polymers were used across the 25 reviewed studies, with PS as the most studied polymer (15 studies). Five studies used PE, four used PVC, two used PP, while PMMA, proprietary polymer and PUF were represented by one study each. Most studied PS spheres (n = 12), while only two used PVC spheres. Regarding size, eight studies used PS particles ranging between 0.05 and 0.099 µm, and four used PS particles between 1 to 9 µm and 0.1 to 0.99 µm. There were two studies on PE particles between 50 and 99 μm and PVC particles between 1 and 9 μm. In terms of particle surfaces, plain PS particles (n = 7 studies) were the most used, followed by PS-COOH (n = 6) and PS-$NH_2$ (n = 5).

All phytoplankton publications addressed effects at the individual level, with 60% reporting effects. Growth was the most common endpoint (24 studies, 21 with effects), followed by pigment content (9 studies, 7 with observed effects), photosynthesis and photosynthetic performance (8 studies, 7 with effects) and chlorophyll a content (1 study with significant effects) (Baudrimont et al. 2020; Bellingeri et al. 2019; Bergami et al. 2017; Besseling et al. 2014; Bhargava et al. 2018; Canniff and Hoang 2018; Casado et al. 2013; Chae et al. 2018; Gambardella et al. 2018; Garrido et al. 2019; González-Fernández et al. 2019; Lagarde et al. 2016; Liu et al. 2019; Long et al. 2017; Luo et al. 2019; Mao et al. 2018; Nolte et al. 2017; Prata et al. 2018; Sendra et al. 2019; Seoane et al. 2019; Thiagarajan et al. 2019; Zhang et al. 2017; Zhao et al. 2019; Zhu et al. 2019). At the cellular level, effects on membrane stability (four studies, three with effects), cell complexity (three studies, all with effects) and cell size (four studies, three with effects) were addressed in marine and freshwater species (González-Fernández et al. 2019; Liu et al. 2019; Mao et al. 2018; Sendra et al. 2019; Seoane et al. 2019). Nine studies looked at several effects at the subcellular level, including oxidative stress (six studies, all observing effects), lipid peroxidation (three studies, two with effects), reactive oxygen species (ROS) formation (one study, no effects), neutral lipid content (one study with effects), protein content (two studies with effects), DNA damage (one study with effects) and gene expression (one study with effects) (Bellingeri et al. 2019; González-Fernández et al. 2019; Lagarde et al. 2016; Liu et al. 2019; Mao et al. 2018; Sendra et al. 2019; Seoane et al. 2019; Thiagarajan et al. 2019; Zhu et al. 2019). Only one publication studied effects at the ecosystem level, such as bacteria concentration and community shifts, with effects only reported for the latter (González-Fernández et al. 2019).

Overall, phytoplankton growth does not seem to be greatly impacted by micro- or nanoplastic exposure, for which little or no effects were reported for both freshwater and marine species. However, deleterious effects were seen at concentrations considered high. The lowest concentration at which effects on growth were reported was 0.001 mg/L for *D. tertiolecta* exposed to PS spheres (72 hrs, size range 0.1 to 0.99 μm), even though complete growth inhibition was not achieved (Gambardella et al. 2018). In this study, a dose-dependent growth inhibition was observed in exposed microalgae and associated with the use of energy sources in detoxification processes, such as the generation of extracellular polysaccharides (Gambardella et al. 2018). Of the 25 reviewed studies, only 2 reported $EC_{50}$ values for PS nanoplastics: an $EC_{50}$ value of 12.97 mg/L was recorded for the marine microalgae *D. tertiolecta* (size range 0.05–0.099 μm) (Bergami et al. 2017), while $EC_{50}$ of 0.58 mg/L and 0.54 mg/L were obtained for freshwater microalga *P. subcapitata* (polyethyleneimine PS with different size ranges of 0.05–0.099 and 0.1–0.99 μm, respectively) (Casado et al. 2013). For sublethal effects, the consensus is that toxicity in microalgae was influenced by size and surface chemistry of particles, with nanoplastics exerting stronger impairment than their micro-sized counterparts (e.g.

Bergami et al. 2017; Seoane et al. 2019; Zhang et al. 2017). PS nanoplastics, size range 0.05–0.99 μm, were found to induce oxidative stress in the form of ROS formation (PS-$NH_2$ and plain PS (González-Fernández et al. 2019; Sendra et al. 2019)), result in effects on protein and neutral lipid content, affect membrane stability, cause DNA damage (plain PS (Sendra et al. 2019)), decrease pigment content including chlorophyll a (PS, PS-$NH_2$ and PS-COOH (Besseling et al. 2014; González-Fernández et al. 2019; Sendra et al. 2019)), alter cell size and complexity (PS-$NH_2$ and plain PS (González-Fernández et al. 2019; Sendra et al. 2019)) as well as cause community shifts (PS-$NH_2$ (González-Fernández et al. 2019)) in both freshwater and marine microalgae. Furthermore, positively charged PS-$NH_2$ have been shown to have higher interaction and toxicity than negatively charged PS-COOH and plain PS due to increased adhesion onto algal surfaces, with particle charge being recognized as the cause for the increased severity (Bergami et al. 2017; Chae et al. 2018; Nolte et al. 2017).

Overall, ecotoxicological data obtained for microalgae demonstrated that exposure to nano- or microplastics caused a variety of cellular and biochemical effects, from altered expression of genes involved in metabolic pathways, to photosynthetic impairment and growth inhibition (e.g. Lagarde et al. 2016; Mao et al. 2018). The toxicity observed to microalgae seems to be dependent of many factors including particle size (Zhang et al. 2017), polymer type (Lagarde et al. 2016), surface chemistry (González-Fernández et al. 2019; Seoane et al. 2019), particle concentration (Mao et al. 2018), exposure time as well as targeted species (Long et al. 2017). Nonetheless, the environmental relevance and toxicity mechanisms of nano- and microplastics in microalgae remain unclear. This is mostly due to the determination of growth inhibition as the most common toxicological endpoint, in which the exposure duration is too short, and it is not possible to clearly discriminate between direct toxic effects and indirect physical effects caused by particles. Limitations in the use of this method have also been highlighted in studies using nanomaterials, mostly related to particle interference with algal growth quantification techniques (i.e. measurement chlorophyll a fluorescence) due to a shading effect (Handy et al. 2012). The presence of particles in suspension can cause shading either by reducing the access of algae to light or by obstructing the fluorescence signal from the algae to the fluorescent detector. This shading effect will impact the accuracy of the measured fluorescence response, leading to an underestimation of chlorophyll a quantification, thereby overestimating the overall toxic effect (Farkas and Booth 2017). In view of the important role that phytoplankton have in aquatic food webs, there is a need to develop better toxicological assays/endpoints with increased sensitivity that are able to reveal underlying toxic effects of plastic particles.

#### 7.3.2.2 Cnidaria

The group Cnidaria is composed of aquatic organisms with basic body forms, swimming medusae or sessile polyps, that inhabit both the freshwater and the marine environments, even though more predominant in the latter. Examples of cnidarians are sea anemones, corals and jellyfish. The cnidarians used in the reviewed publications were all coral species and exclusively from the marine environment. Nine species were represented across four studies, all from the class Anthozoa. *Pocillopora damicornis* was the only species used in more than one study. The eight other species (*Acropora formosa*, *A. humilis*, *A. millepora*, *Montastraea cavernosa*, *Orbicella faveolata*, *Pocillopora verrucosa*, *Porites cylindrica*, *P. lutea*) were all used in single studies. All Cnidaria species investigated were filter feeders and were exposed to particles via water. Most studies were carried out on polyps (two studies).

Four studies have been carried out on Cnidaria investigating irregular fragments and beads composed of two polymer types. PE was used in three of the four studies (Hankins et al. 2018; Reichert et al. 2018; Syakti et al. 2019), while only one study used PS (Tang et al. 2018). Two studies used PE fragments (Reichert et al. 2018; Syakti et al. 2019), one used PE beads (Hankins et al. 2018), and the remaining study did not specify the morphology of PS particles used (Tang et al. 2018). In terms of size, one study focused on the smallest size category, 0.1 to 0.99 μm (Jia Tang et al. 2018); PE fragments were studied in the size range 20–49 μm (Reichert et al. 2018), 50–99 μm (Reichert et al. 2018; Syakti et al. 2019) and 100–199 μm (Reichert et al. 2018; Syakti et al. 2019); and one study used the size range 200–500 μm (Syakti et al. 2019). PE beads were investigated in the size ranges 50–99 μm, 100–199 μm, 200–500 μm and > 500 μm during a single study (Hankins et al. 2018).

The subcellular level was studied in one publication reporting effects on enzymatic activity and gene expression (Tang et al. 2018). At the individual level, two studies investigated and reported bleaching (Reichert et al. 2018; Syakti et al. 2019); one study investigated and reported effects on mucus production, tissue necrosis and growth (Reichert et al. 2018); one study investigated and reported mortality and tissue necrosis (Syakti et al. 2019); and one study investigated calcification but did not observe any effects (Hankins et al. 2018). Only one publication studied community shifts, although no effects were observed on symbiont density or symbiont chlorophyll content (Tang et al. 2018). Bleaching was the most common endpoint, with both studies detecting effects. No studies were found at the population level.

Regarding concentrations and particle size, only a single concentration (50 mg/L) and size (1–9 μm) was used to investigate subcellular-level effects (Tang et al. 2018). The effects of PS on enzymatic activity were investigated, where alterations in superoxide dismutase, alkaline phosphatase, catalase and glutathione S-transferase activity were observed throughout exposure. No effects were observed for phenoloxidase activity.

The reported effects at the individual level ranged from exposure to 50 mg/L to 150 mg/L. Exposure to PE fragments increased mortality, bleaching and necrosis in *A. formosa* after 2 days of exposure at 50, 100 and 150 mg/L (size range 50 to 500 μm (Syakti et al. 2019)), as well as in *A. humilis*, *A. millepora*, *P. cylindrica*, *A. humilis*, *P. verrucosa* and *P. damicornis* after 28-day exposure at 100 mg/L (size range 20 to 100 μm (Reichert et al. 2018)). Growth was also impaired across these species, but this was dependent on the size of particles used in the exposure. Mucus production only appeared to be affected in *P. lutea* also exposed to PE fragments

(100 mg/L, size range 20–100 μm) (Reichert et al. 2018). At the ecosystem level, the only observed effect was a community shift in chlorophyll content symbiont at 12-hr exposure to PS 50 mg/L (Tang et al. 2018).

#### 7.3.2.3 Nematoda

Nematodes, also called roundworms, are unsegmented worms found in almost every terrestrial and aquatic habitat. Only a single study addressed the effect of microplastics on nematodes (Judy et al. 2019). The nematode *Caenorhabditis elegans*, which lives in the pore water of soils, was exposed to fragments larger than 500 μm, produced by shredding consumer products (Judy et al. 2019). The exposure scenarios used organisms at the adult stage, exposed through contact with the soil solution, implying both dermal and trophic exposure to microplastics.

The effects of a single high concentration (5 g/kg soil dry weight) of three polymer types (PE, PET, PVC) were assessed at the individual level (mortality and reproduction), after various contact time between soil and plastics (0, 3 and 9 months). Increased mortality was only observed for PET incubated in soil for 3 months, while decreased reproduction was only observed for PVC incubated in soil for 9 months (Judy et al. 2019).

#### 7.3.2.4 Rotifera

Rotifers are organisms that are bilaterally symmetrical and have a microscopic size and unsegmented soft body, with a common distribution in both the freshwater and marine environments. As main components of zooplankton, these small organisms have an important ecological role in aquatic ecosystems. This taxonomic group was only represented by a single marine species, *Brachionus plicatilis*. Two developmental stages of *B. plicatilis* were used in exposure studies, neonates (Gambardella et al. 2018; Manfra et al. 2017) and nauplii (Beiras et al. 2018), both exposed via water. All studies investigated the effect of microplastic spheres, either composed of PS (Beiras et al. 2018; Gambardella et al. 2018) or PE (Manfra et al. 2017). In terms of size, two studies looked at particles <0.05 μm (Gambardella et al. 2018; Manfra et al. 2017), one study looked at particles 0.05–0.099 μm (Manfra et al. 2017), and one study looked at 1–9 μm sized particles (Beiras et al. 2018). Two studies described the surface of the particles, Gambardella et al. (2018) used plain PS spheres, and Manfra et al. (2017) looked at both COOH and $NH_2$ coated PS spheres.

All publications looked at individual-level effects, specifically mortality. No studies assessed subcellular or population-level effects and only one study considered ecosystem-level effects, specifically alterations in swimming speed (Gambardella et al. 2018). Neonates exposed to PS-$NH_2$ spheres (0.001–50 mg/L) exhibited significant mortality only when concentrations exceeded 10 mg/L (Manfra et al. 2017). On the other hand, PS-COOH spheres did not induce any effect at the same concentrations (Manfra et al. 2017). In another study, nauplii exposed to PE spheres were only significantly affected after 48 hrs of exposure, when concentrations exceeded 1 mg/L (Beiras et al. 2018). Finally, PS spheres (<0.05 μm) only affected the swimming speed of neonates after 48-hr exposure (0.001–10 mg/L) (Gambardella et al. 2018).

#### 7.3.2.5 Arthropoda

Arthropoda is the largest group of the animal kingdom, which includes invertebrate organisms that have an exoskeleton, a segmented body and jointed appendages. Arthropods are widely represented in every environmental compartment and include crustaceans, insects, isopods and amphipods, among others. Most of the studies conducted with Arthropoda (39 of 57) were in the freshwater environment, followed by 16 studies in the marine environment, 3 studies in terrestrial and only 1 in the brackish environment. Twenty-nine Arthropoda species from 5 classes, Branchiopoda, Entognatha, Hexanauplia, Insecta and Malacostraca, were studied: *Acartia tonsa*, *Amphibalanus amphitrite*, *Artemia franciscana*, *Asellus aquaticus*, *Calanus finmarchicus*, *Calanus helgolandicus*, *Carcinus maenas*, *Centropages typicus*, *Ceriodaphnia dubia*, *Chironomus tepperi*, *Corophium volutator*, *Daphnia galeata*, *Daphnia magna*, *Daphnia pulex*, *Echinogammarus marinus*, *Eriocheir sinensis*, *Folsomia candida*, *Gammarus fossarum*, *Gammarus pulex*, *Hyalella azteca*, *Idotea emarginata*, *Lobella sokamensis*, *Nephrops norvegicus*, *Palaemonetes pugio*, *Parvocalanus crassirostris*, *Platorchestia smithi*, *Porcellio scaber*, *Talitrus saltator* and *Tigriopus fulvus*. Fifteen of the species were Malacostraca, while there was only one study on Insecta (*Chironomus tepperi*; Ziajahromi et al. 2018). *Daphnia magna* was by far the most used species (n = 29 publications), followed by *Artemia franciscana* (n = 4 publications). Overall, 14 species were from the marine environment, 11 from freshwater, 3 terrestrial and 1 from brackish water.

Most of the Arthropoda species were filter/suspension feeders (6 species in 35 studies). Nine studies used eight detritivores species, seven studies included seven grazer species, and four studies used four scavenger species. Deposit feeders (two species), filter feeders (one species) and grazer and detritivores (one species) were represented by two publications each. Only one publication studied a predator species, *Eriocheir sinensis*. Most studies were carried out on adults (27 studies) and neonates (23 studies), while juveniles (7 studies), nauplii (6 studies), larvae (2 studies) and 1-week-old organisms (1 study) were less studied. Five publications studied the whole cycle of *D. magna* and *D. pulex*. Filter/suspension feeders and predators were exposed via water (37 studies). On the other hand, detritivores were exposed via water (three studies), sediment (two studies), soil (two studies) and food (two studies). Grazers were also exposed via water (five studies), sediment and food, and deposit feeders were exposed via water and sediment. Lastly, scavenger organisms were only exposed via food (four studies).

Fourteen polymer types were studied using Arthropoda, in a total of 57 publications. PS was the most studied polymer, followed by PE (31 and 14 studies, respectively). PET was represented by five publications, while PA, PMMA and PP had four each. Proprietary polymer and PVC had three and two studies, respectively. All the other particle types (ABS, nylon, PC, PHB, POM and SAN) were represented by one study each. Most of the studies used spheres (30 and 11 using PS and PE, respectively), while the remaining particle shapes had less than 5 studies each. Regarding size, PS particles between 1–9 µm, 0.1–0.99 µm and 0.05–0.099 were used in 13, 12 and 10 publications, respectively. Seven studies used PE particles between 20 and 49 µm. The remaining size classes were used in five or less studies. ABS, PC, PHB, POM and SAN were only studied within the size range 20 to 49 µm. Regarding particle surface, PS-COOH was the most studied with seven publications, followed by PS plain and PS-$NH_2$ with six studies each, all particles within the nano-scale. Particles with other surface modifications were used in five or less publications each.

Effects at the individual level (51 studies, corresponding to 89% of studies) were the most commonly determined in arthropods, followed by effects at the population (18 studies, 32% of studies) and subcellular, ecosystem and organ levels (11, 7 and 5 studies, corresponding to 19%, 12% and 9% of studies). When comparing the different levels of biological organization, the percentage of reported effects was comparable to those reporting no effects. Gene expression was the most common endpoint determined within the subcellular level (Bergami et al. 2017; Fadare et al. 2019; Gambardella et al. 2017; Heindler et al. 2017; Imhof et al. 2017; Lin et al. 2019b; Liu et al. 2018, 2019; Tang et al. 2019; Yu et al. 2018; Zhang et al. 2019), followed by enzymatic activity and neurotoxicity (Gambardella et al. 2017; Lin et al. 2019b; Yu et al. 2018) as well as oxidative stress (Lin et al. 2019b; Yu et al. 2018; Zhang et al. 2019). Energy reserves (Cole et al. 2019; Cui et al. 2017; Kokalj et al. 2018; Weber et al. 2018) and alterations in hepatosomatic index (Yu et al. 2018) were the endpoints targeted at the organ level. At the individual level, mortality (Au et al. 2015; Beiras et al. 2018; Bergami et al. 2016, 2017; Besseling et al. 2014; Bhargava et al. 2018; Blarer and Burkhardt-Holm 2016; Booth et al. 2016; Bosker et al. 2019; Bruck and Ford 2018; Canniff and Hoang 2018; Casado et al. 2013; Cole et al. 2015; Cui et al. 2017; Fadare et al. 2019; Gambardella et al. 2017; Gerdes et al. 2019; Gray and Weinstein 2017; Hämer et al. 2014; Horton et al. 2018; Imhof et al. 2017; Jemec et al. 2016; Kim et al. 2017; Kokalj et al. 2018; Lin et al. 2019b; Liu et al. 2018; Ma et al. 2016; Mattsson et al. 2017; Nasser and Lynch 2016; Ogonowski et al. 2016; Pacheco et al. 2018; Redondo-Hasselerharm et al. 2018; Rehse et al. 2016, 2018; Rist et al. 2017; Tang et al. 2019; Tosetto et al. 2016; Ugolini et al. 2013; Vicentini et al. 2019; Weber et al. 2018; Wu et al. 2019a; Yu et al. 2018; Zhang et al. 2019, p. 201; Ziajahromi et al. 2017) and growth (Au et al. 2015; Bergami et al. 2016; Besseling et al. 2014; Bruck and Ford 2018; Cole et al. 2019; Gerdes et al. 2019; Hämer et al. 2014; Imhof et al. 2017; Jemec et al. 2016; Kokalj et al. 2018; Liu et al. 2019; Ogonowski et al. 2016; Pacheco et al. 2018; Redondo-Hasselerharm et al. 2018; Rist et al. 2017; Jinghong Tang et al. 2019; Vicentini et al. 2019; Weber et al. 2018; Welden and Cowie 2016; Yu et al. 2018; Zhao et al. 2015; Zhu et al. 2018; Ziajahromi et al. 2017) were the most studied, alongside feeding behaviour (Blarer and Burkhardt-Holm 2016; Bruck and Ford

2018; Cole et al. 2013, 2019; Hämer et al. 2014; Kokalj et al. 2018; Ogonowski et al. 2016; Redondo-Hasselerharm et al. 2018; Rist et al. 2017; Straub et al. 2017; Watts et al. 2015; Weber et al. 2018; Welden and Cowie 2016; Zhu et al. 2018), development (Blarer and Burkhardt-Holm 2016; Ma et al. 2016; Straub et al. 2017), energy reserves (Watts et al. 2015; Welden and Cowie 2016), respiration rate (Cole et al. 2015) and gut microbial diversity (Zhu et al. 2018). Endpoints related to population level included alterations in reproductive output (Au et al. 2015; Besseling et al. 2014; Bosker et al. 2019; Canniff and Hoang 2018; Cole et al. 2015; Cui et al. 2017; de Felice et al. 2019; Heindler et al. 2017; Imhof et al. 2017; Liu et al. 2019; Ogonowski et al. 2016; Pacheco et al. 2018; Rist et al. 2017; Vicentini et al. 2019; Zhu et al. 2018; Ziajahromi et al. 2017, 2018), followed by larval development (Ziajahromi et al. 2018) and population size (Heindler et al. 2017). At the ecosystem level, only alterations in behaviour (e.g. swimming activity, phototactic response, distance and acceleration) were recorded upon exposure (Booth et al. 2016; Chae et al. 2018; de Felice et al. 2019; Frydkjær et al. 2017; Gambardella et al. 2017; Kim and An 2019; Lin et al. 2019b; Tosetto et al. 2016).

From the terrestrial species included in the ecotoxicological assessments reviewed, effects on feeding behaviour, growth, gut microbial diversity and reproduction were seen for *F. candida* in response to PVC (1000 mg/kg soil, size range 80–250 μm) (Zhu et al. 2018). These effects were attributed to changes in soil structure due to the presence of microplastics that led to alterations in feeding behaviour and capacity to find high-quality food, thus influencing nutrient absorption (Zhu et al. 2018). Similar findings were found for *L. sokamensis* exposed to PE (1000 mg/kg soil, size range 20–49 μm) and PS (4, 8 and 1000 mg/kg soil, size ranges 0.1–0.99, 20–49 and 200–500 μm) (Kim and An 2019). In this study, springtails showed altered behaviour in response to microplastic movement into soil bio-pores, at lower concentrations and size ranges than those reported for *F. candida* (4 and 8 mg/kg soil for PS 0.1–0.99 μm compared to 1000 mg/kg soil PVC 80–250 μm). Both studies highlight that the behaviour of plastic particles in soil does not only affect the behaviour of soil-dwelling organisms and lead to high adverse effects (e.g. impaired growth and reproduction), but their presence can also have wider implications for effective management of soils (Kim and An 2019; Zhu et al. 2018).

Several biological endpoints have been determined in freshwater arthropods in response to both nano- and microplastics, with toxicity being dependent on polymer type (e.g. Au et al. 2015), particle size (e.g. de Felice et al. 2019), surface chemistry (e.g. Lin et al. 2019b) and time of exposure (e.g. Liu et al. 2019). As mentioned previously, the crustacean *Daphnia* sp. was the most used organism to assess the ecotoxicological effects of plastic particles via water exposure, for which acute and chronic toxicity has been reported for different particles. Adverse effects including mortality (LOEC 0.005 mg/L, PS spheres 10–19 μm (P. Zhang et al. 2019)), abnormal development (adults LOEC 0.1 mg/L and offsprings LOEC 5 mg/L, PS spheres 0.05–0.099 μm (Liu et al. 2019 and Cui et al. 2017, respectively)), swimming behaviour (LOEC 1 mg/L for PE fragments 10–19 μm, PS spheres 0.1–0.99 μm and PS-$NH_2$ 0.05–0.099 μm (Frydkjær et al. 2017; Lin et al. 2019b)) and reproductive output (LOEC 0.02 mg/L, proprietary polymer 1–9 μm (Pacheco et al. 2018)) were the most commonly described. In terms of sediment exposure, the effects of PE at environmentally relevant concentration (500 particles/kg sediment, size range 1–49 μm) were evaluated using the chironomid *C. tepperi* (Ziajahromi et al. 2018) after 5 and 10 days of exposure. The authors reported that exposure to PE negatively affected the survival, growth (i.e. body length and head capsule) and emergence of chironomids, with the observed effects being strongly dependent on particle size.

Ecotoxicological studies of marine arthropods showed that smaller sized plastic particles had a stronger impact, with surface chemistry playing a significant role for the effects seen. This is the case of *A. franciscana* exposed to PS nanoplastics with different surface alterations, for which the lowest LOECs for different endpoints were recorded. Also, when comparing the long-term toxicity of PS-COOH and PS-$NH_2$ (size range 0.05–0.099 μm), Bergami et al. (2017) observed a concentration-dependent mortality in brine shrimp after 14 days, with the latter showing a higher impact ($EC_{50}$ = 0.83 mg/L). In addition, alteration in genes involved in moulting were also recorded at the lowest concentration tested of 0.01 mg/L, further suggesting that the disruption of larval moulting and energy metabolism may play a role in the toxicity of nanoplastics towards arthropods. In another study by Gambardella et al. (2017), short-term exposure of *A. franciscana* and *A. amphitrite* to PS nanoplastics (size range 0.1–0.99 μm) at low concentrations (0.001 to 10 mg/L) did not affect survival but impacted swimming behaviour, increased expression of catalase and inhibited acetylcholinesterase activity in exposed organisms. As only sublethal effects were observed, the authors highlight that behavioural responses seem to be more sensitive than mortality in plastic toxicity assessments, especially after short-term exposure.

Arthropoda was the most heterogeneous of the taxonomical groups assessed, including a wide range of species belonging to the terrestrial and aquatic compartments with different developmental stages and feeding strategies. Several effects covering different levels of biological organization were reported, with impacts on feeding behaviour, growth, development, reproduction and lifespan being highlighted as the most significant. These findings emphasize the need to perform long-term exposures covering whole cycle assessments to fully understand the magnitude and consequences of plastic particles to the aquatic environment. This is particularly important for species belonging to zooplankton, an important food source for secondary consumers, as these represent a possible route by which plastic particles could enter food chains and be transferred up the trophic levels. In addition, a significant impact on the lifespan of these organisms might have serious consequences in the balance of aquatic ecosystems (Botterell et al. 2018).



#### 7.3.2.6 Annelida

The Annelida group is composed of segmented worms, such as earthworms, lugworms and leeches. Annelids can be found in all types of habitat, and one of their most important ecological roles is reworking of soils and sediments. The terrestrial environment was represented by nine studies (covering three species) and the marine environment by seven studies (also covering three species). The marine environment was represented by three species belonging to the Polychaeta class: *Arenicola marina* (five studies), *Hediste diversicolor* (one study) and *Perinereis aibuhitensis* (one study). The terrestrial environment was represented by three species of the Clitellata class: *Eisenia fetida* (five studies), *Lumbricus terrestris* (three studies) and *Eisenia andrei* (one study). All but one of the studies (where life stage was not specified) used adult organisms. In the terrestrial environment, soil was spiked with microplastics in eight out of nine studies, the remaining study using spiked food (leaf litter). However, both dermal and trophic exposure can be expected from these two exposure scenarios, due to constant burrowing and feeding activity of the earthworms. For the aquatic environment, spiked sediment was also the main exposure scenario (six out of seven studies), with only one study using spiked water.

The most studied polymer type was PE (nine studies, Besseling et al. 2017; Huerta Lwanga et al. 2016; Judy et al. 2019; Prendergast-Miller et al. 2019; Rillig et al. 2017; Rodríguez-Seijo et al. 2017; Rodríguez-Seijo et al. 2018a, b; Wang et al. 2019a), followed by PS (five studies, Besseling et al. 2013; Cao et al. 2017; Leung and Chan 2018; Van Cauwenberghe et al. 2015; Wang et al. 2019a), PVC (four studies, Browne et al. 2013; Gomiero et al. 2018; Judy et al. 2019; Wright et al. 2013) and PET (one study, Judy et al. 2019). The morphology of the particles was not always provided by the authors, but when it was the case, spheres and fragments were the most common shapes, each covered by six studies. Interestingly in one study, characterization by scanning electron microscopy revealed that particles sold as spheres were in fact flakes (Cao et al. 2017). Overall, particles ranging from below 1 μm to 5 mm were studied, with most studies focusing on particles above 100 μm (12 out of 16 studies). When particles were prepared in the laboratory, the lowest and largest particle sizes were not always provided (e.g. Huerta Lwanga et al. 2016). None of the 16 studies on Annelida reported any surface characterization or functionalization.

The individual level was assessed in all 16 studies on annelids, followed by subcellular (9 studies), ecosystem (6 studies) and population (3 studies) levels. Only one study covered effects at the cellular and organ level. At the individual level, mortality and growth were the most studied endpoints (both covered by 10 studies), although being the least affected endpoints across species, environmental compartments, polymer types and sizes. Mortality was never observed, except in one study with PS flakes at environmentally irrelevant concentrations (5 and 20 g/kg soil dry weight). Growth was rarely affected, and only at environmentally irrelevant concentrations for pristine plastic particles (from 10 g/kg PS flakes and from 4 g/kg PE spheres).

The lowest concentrations inducing effects at the subcellular level were observed for exposure to PE fragments (size classes 200–500 and > 500 μm), which increased protein, lipid and polysaccharide contents in earthworms at 62 mg/kg, decreased catalase activity at 125 mg/kg and increased lipid peroxidation at 250 mg/kg (Rodríguez-Seijo et al. 2017, 2018a). PS fragments of similar size (200–500 μm) were found to increase peroxidase activity in earthworms at 10 g/kg (the lowest concentration tested by Wang et al. 2019a). In marine annelids, PVC fragments

(100–199 μm) induced inflammation at 5 g/kg (the lowest concentration tested by Wright et al. (2013)).

At the ecosystem level, negative results were most frequently reported, e.g. no avoidance of PE fibres (40 × 400 μm) at up to 10 g/kg (Prendergast-Miller et al. 2019) and PE, PET and PVC fragments (>500 μm) at 5 g/kg (Judy et al. 2019) by earthworms and no effect of PE spheres (particle size distribution ranging from <50 μm to >100 μm) at up to 12 g/kg on burrow formation by earthworms (Huerta Lwanga et al. 2016). The only effects seen were on the feeding activity of marine annelids, where PVC fragments (100–199 μm) at 10 and 50 g/kg increased the feeding activity of *Arenicola marina* (Wright et al. 2013).

Overall, the data on the ecotoxicological effects of plastic particles on Annelida is very limited but seem to suggest a moderate to low risk to these organisms. One of the reasons could be linked to the ecological traits of annelids, adapted to continuously ingest vast amounts of non-nutritious particles, through their burrowing and feeding activities. It should also be noted that the absence of avoidance behaviour and detrimental effects on annelids make them efficient vectors of plastic particles not only to their predators but also to the whole ecological compartment, due to their intense bioturbation activity.



#### 7.3.2.7 Mollusca

The Mollusca group includes several ecologically and commercially important filter feeders (e.g. mussels and clams) that due to their habitat and feeding behaviour are likely to encounter plastic particles of varying sizes. Most of the studies for Mollusca focused on marine species (29 studies, 13 species), followed by freshwater (6 studies, 4 species) and terrestrial species (a single study, 1 species). The 17 species belonged to 2 classes, Bivalvia and Gastropoda: *Abra nitida*, *Achatina fulica*, *Corbicula fluminea*, *Crassostrea gigas*, *Dreissena polymorpha*, *Ennucula tenuis*, *Meretrix meretrix*, *Mytilus edulis*, *Mytilus galloprovincialis*, *Mytilus* sp., *Ostrea edulis*, *Perna perna*, *Perna viridis*, *Pinctada margaritifera*, *Potamopyrgus antipodarum*, *Scrobicularia plana* and *Sphaerium corneum*. The most commonly studied species was the mussel *M. galloprovincialis* (in 11 studies). Most of the species used were filter feeding (13 species in 33 studies), followed by grazer species (2 species in 2 studies), while only 1 study used deposit feeders (2 species). Most studies were carried out on adults (28 studies), with 7 studies using larvae, 4 studies embryos, 2 studies gametes and 1 study juveniles. Filter-feeding organisms were exposed mainly via water (28 studies) and 1 via water plus muddy sediment. For these organisms, two studies used exposure via food and two studies via sediment. The deposit feeders were exposed via sediment, while the grazers via food and soil.

For Mollusca, 36 studies looked at the effects of 9 different polymers, with PS being the most studied polymeric material (total 20 studies). Overall, 12 studies used PE and 4 studies used PVC and PET. There were two studies for PLA and two for proprietary polymer, while all the other polymers (PA, PC and PP) only had one study each. Most of the studies were performed with PS spheres (n = 14), followed by PE and PS fragments (eight and three studies, respectively). Two studies used PET fibres and spheres of proprietary polymer, while the remaining morphologies only had one study each. Regarding size, the highest number of studies (12 in total) used PS particles between 1 and 9 μm. Studies with PE particles used size ranges of 20–49 μm and 50–99 μm with five studies each, along with PS particles with sizes 0.1–0.99 μm, 20–49 μm and 20–49 μm. All the other particle size distributions had less than five studies each. Only studies using PS particles reported particle surface information, for which four studies used PS-$NH_2$, three studies used plain and COOH and one used PS with sulphate groups, where all particles were within the nano-scale. Most of the reviewed studies only reported effects for particles above 1 μm, with only a small number showing impacts with particles within nano-range, more specifically PS and PE. This is the reflection of the size-dependent threshold commonly associated with the particle-selection feeding behaviour characteristic of most of the species included in this taxonomical group (Van Cauwenberghe and Janssen 2014; Wegner et al. 2012).

In terms of levels of biological organization, effects at the subcellular (23 studies, with 18 reporting effects) and individual level (22 studies, with 12 reporting effects) were the most studied. There was only one study at an ecosystem level (reporting effects) but 11 analysing effects at the population level (7 with observed effects). Overall, 11 studies analysed effects on organs (with 6 reporting effects) and 7 in cells (6 reporting effects). The most studied endpoint was related to impacts in feeding behaviour (15 studies), with 9 reporting significant effects related to filtration and ingestion rate, absorption and assimilation efficiency (Capolupo et al. 2018; Cole and Galloway 2015; Gardon et al. 2018; Green 2016; Guilhermino et al. 2018; Oliveira et al. 2018; Revel et al. 2019; Rist et al. 2016, 2019; Rochman et al. 2017; Santana et al. 2018; Song et al. 2019; Sussarellu et al. 2016; Wegner et al. 2012; Woods et al. 2018). Endpoints related to oxidative stress were the second most common endpoint, with 14 studies, 8 of which showing impacts on lipid peroxidation, formation of reactive oxygen species and total oxyradical scavenging capacity (Avio et al. 2015; Brandts et al. 2018b; Gonçalves et al. 2019; González-Fernández et al. 2018; Guilhermino et al. 2018; Magni et al. 2018; Oliveira et al. 2018; Paul-Pont et al. 2016; Revel et al. 2019; Ribeiro et al. 2017; Santana et al. 2018; Song et al. 2019; Sussarellu et al. 2016; von Moos et al. 2012). In combination with oxidative stress, alteration in enzymatic activity was also one of the main endpoints determined in molluscs (reported in 12 studies), with 10 studies showing alterations to antioxidant enzymes (Avio et al. 2015; Brandts et al. 2018b; Franzellitti et al. 2019; Gonçalves et al. 2019; Guilhermino et al. 2018; Magni et al. 2018; Oliveira et al. 2018; Paul-Pont et al. 2016; Pittura et al. 2018; Revel et al. 2019; Ribeiro et al. 2017; Song et al. 2019). Alterations in gene expression were also a common endpoint in most of the reviewed studies (12 studies), with 10 reporting up- and downregulation of genes involved in different metabolic pathways as detoxification, immunity, apoptosis, energy reserves, etc. (Avio et al. 2015; Balbi et al. 2017; Brandts et al. 2018a; Capolupo et al. 2018; Détrée and Gallardo-Escárate 2017, 2018; Franzellitti et al. 2019; Paul-Pont et al. 2016; Pittura et al. 2018; Revel et al. 2019; Rochman et al. 2017; Sussarellu et al. 2016). Histopathological alterations were also included in some of these studies to understand the effects of particle ingestion in different organs (total nine studies), with five studies reporting alterations in the gills and digestive glands of exposed organisms (Bråte et al. 2018; Gardon et al. 2018; Gonçalves et al. 2019; Guilhermino et al. 2018; Paul-Pont et al. 2016; Revel et al. 2019; Rochman et al. 2017; Song et al. 2019; von Moos et al. 2012). Five out of eight studies reported significant genotoxicity of the plastic particles used, expressed as DNA damage or micronuclei formation (Avio et al. 2015; Brandts et al. 2018a; Bråte et al. 2018; Magni et al. 2018; Pittura et al. 2018; Revel et al. 2019; Ribeiro et al. 2017; Santana et al. 2018). Seven studies also analysed the neurotoxicity of particles, with six reporting significant alterations in acetylcholinesterase activity (Avio et al. 2015; Brandts et al. 2018a; Guilhermino et al. 2018; Magni et al. 2018; Oliveira et al. 2018; Pittura et al. 2018; Ribeiro et al. 2017). Several endpoints related to population effects were determined in molluscs, most of which related to fecundity (six studies, Gardon et al. 2018; González-Fernández et al. 2018; Imhof and Laforsch 2016; Luan et al. 2019; Sussarellu et al. 2016; Tallec et al. 2018), offspring viability (one study, Capolupo et al. 2018), larval development (seven studies, Balbi et al. 2017; Beiras et al. 2018; Cole and Galloway 2015; Luan et al. 2019; Rist et al. 2019; Sussarellu et al. 2016; Tallec et al. 2018) and juvenile development (one study, Imhof and Laforsch 2016). Of these endpoints, only those related to fecundity (e.g. fertilization yield, gamete quality hatching rate, etc.) and larval development showed a significant effect. General health endpoints including growth (eight studies, Détrée and Gallardo-Escárate 2018; Gardon et al. 2018; Green 2016; Imhof and Laforsch 2016; Redondo-Hasselerharm et al. 2018; Rist et al. 2019; Santana et al. 2018; Song et al. 2019), energy reserves (five studies, Avio et al. 2015; Bour et al. 2018; Brandts et al. 2018a; Pittura et al. 2018; von Moos et al. 2012), condition index (six studies, Bour et al. 2018; Revel et al. 2019; Ribeiro et al. 2017; Santana et al. 2018; Sussarellu et al. 2016; von Moos et al. 2012), respiration rate (three studies, Gardon et al. 2018; Green 2016; Rist et al. 2016) and scope for growth (one study, Gardon et al. 2018) were also included in several studies; however, these were the less sensitive endpoints, where only one to two studies reported a significant effect.

Of the four freshwater species used in the studies reviewed, significant impacts were only recorded for *D. polymorpha* exposed to PS (1–9 μm, LOEC 50000 particles/L) (Magni et al. 2018) and *C. fluminea* following exposure to a proprietary polymer (1–9 μm, LOEC 0.13 mg/L) (Guilhermino et al. 2018; Oliveira et al. 2018), as well as PET, PE, PVC and PS fragments (Rochman et al. 2017). In the study by Rochman et al. (2017), *C. fluminea* was exposed to environmental concentrations and sizes of PET, PE, PVC and PS fragments (sizes range 50 to >500 μm) for 28 days, after which histopathological alterations were recorded (LOEC 2.8 mg/L). The authors highlight that the effects observed in exposed clams were specific to the polymer type used.

Several ecotoxicological effects across the different levels of biological organization were recorded for marine molluscs. Interestingly, mortality was one of the least sensitive endpoints in organisms exposed either via sediment or water, even at very high concentrations. Only Rist et al. (2016) reported substantial mortality in *P. viridis* exposed to PVC after 91 days of exposure (size range 1–49 µm, 2160 mg/L); however, no significant statistical differences were found compared to the control condition. Mussels belonging to the genus *Mytilus* were the most used marine species used in the reviewed studies, for which a wide range of biological endpoints were determined. The biological endpoints for which significant effects were recorded included byssus production and immunity deficiency (LOEC 0.025 mg/L, PE fragments >500 µm) (Green et al. 2019), mortality, concentration and phagocytic activity of circulation haemocytes, histopathological alterations, ROS production and lipid peroxidation (LOEC 0.032 mg/L, PS spheres 1–9 µm) (Paul-Pont et al. 2016), antioxidant enzymatic activity and genotoxicity (LOECs of 0.000008 mg/L and 0.01 mg/L, respectively, mixture PE and PP fragments, 200–500 µm) (Revel et al. 2019), feeding behaviour (LOEC 3000 particles/L, PET fibres 200 to >500 µm) (Woods et al. 2018), alterations in gene and protein expression, growth (LOEC 0.03 mg/L, PE and PLA fragments 1 to 50 µm) (Détrée and Gallardo-Escárate 2018), larval malformations (LOEC 0.00042 mg/L, PS spheres, 1–9 µm) (Rist et al. 2019), lysosomal membrane stability (LOEC 1500 mg/L, PE and PS fragments size range from <0.05 to 99 µm) (Avio et al. 2015) and neurotoxicity (LOEC 0.05 mg/L, PS spheres 0.1–0.99 µm) (Brandts et al. 2018b).

The gastropod *A. fulica* was the only terrestrial species in the ecotoxicological studies reviewed, for which effects were recorded following 28 days of exposure to PET fibres (length 1260 µm, diameter 76 µm) at concentrations ranging from 14 to 710 mg/kg sediment (Song et al. 2019). The authors reported alterations in feeding behaviour (LOEC 14 mg/kg sediment) upon exposure that resulted in histopathological alterations in the gastrointestinal tract (LOEC 140 mg/kg sediment) and oxidative stress in the liver (LOEC 710 mg/L).

Mollusca was the taxonomical group for which a wider range of biological endpoints were determined. Overall, the reviewed data highlighted that acute and chronic toxicity of plastic particles in molluscs seem to be dependent not only on particle characteristics such as polymer type (Avio et al. 2015; Rochman et al. 2017), concentration range (Gardon et al. 2018; Rochman et al. 2017), particle size (Tallec et al. 2018) and surface chemistry (Cole and Galloway 2015; Luan et al. 2019) but also on organism-specific traits such as developmental stage (Balbi et al. 2017; Rist et al. 2019) and tissue analysed (Brandts et al. 2018b; Revel et al. 2019; Ribeiro et al. 2017). Furthermore, the reviewed findings further emphasize the need to conduct studies with freshwater and terrestrial species, especially when considering their higher risk of exposure to plastic particles. It is also worth mentioning that this taxonomical group includes many filter-feeding species with a high tendency for particle retention, thus representing a possible source of transfer across higher trophic levels and potentially to humans.

#### 7.3.2.8 Echinodermata

Echinoderms are exclusively marine invertebrate species that have a widespread distribution throughout the ocean. These organisms inhabit a diverse array of cold water and tropical ecosystems including habitats from coastal, intertidal zones to offshore, as well as deep water areas. Common echinoderms include sea cucumbers, starfish and sea urchins. Four microplastic ecotoxicology studies were reviewed for echinoderms representing the marine environment. Sea urchin species were used in all studies: *Paracentrotus lividus* was used in three studies (Beiras et al. 2018; Della Torre et al. 2014; Messinetti et al. 2018), while *Tripneustes gratilla* was used in one study (Kaposi et al. 2014). Early life stages of sea urchins were used for all studies (larvae/embryo (Beiras et al. 2018; Della Torre et al. 2014; Messinetti et al. 2018)). All studies with echinoderms were performed via water exposure. Reviewed studies used PS (two studies) and PE (two studies) microparticles. Experimental studies on echinoderms varied with PS with two different surface charges being used at the 40–50 nm size range and 10 µm PS spherical microparticles. PE of similar size ranges similar as natural food of zooplankton organisms (1–500 µm) were also used, as well commercial PE ranging from 10 to 45 µm.

The individual level was studied in all four studies and one study included endpoints at the cellular level (Della Torre et al. 2014). The effects of carboxylated PS (PS-COOH) and amine PS (PS-$NH_2$) nanoplastics were used to evaluate embryotoxicity in *P. lividus*, specifically disposition, embryo development and gene expression. No embryotoxicity was observed for PS-COOH which formed microaggregates and was anionic up to 50 µg/mL. However, PS-$NH_2$, which was better dispersed and cationic, caused developmental defects ($EC_{50}$ 3.85 µg/mL 24 hours post fertilization and $EC_{50}$ 2.61 µg/mL 48 hours post fertilization). These findings suggest that surface charge and particle aggregation dynamics in seawater influence embryotoxicity. Collectively, the findings of Della Torre et al. (2014) highlight the importance of different aggregation states and surface properties of nanoplastics and how they lead to differences in uptake, exposure and disposition routes and overall impacts.

The effects of ingesting microplastics in larval *T. gratilla* were proportionally related to the concentration of PE microspheres and ingestion was reduced in the presence of biological fouling and phytoplankton food. An unrealistically high concentration of PE microspheres (300 spheres/mL) affected larval growth with no significant effect on survival observed. Conversely, at environmentally realistic concentrations, there was little effect observed on growth or survival (Kaposi et al. 2014).

The planktotrophic larvae of *P. lividus* were utilized to evaluate the effects of PS microbeads on juvenile development. *P. lividus* larvae were able to ingest microplastics, albeit at a lower rate, in comparison to the sessile filter-feeding ascidian (*Ciona robusta*) juveniles. No effect of PS microbeads, at any concentration (control vs. 0.125, 1.25, 12.5 and 25 µg/mL), was observed on larval survival, whereas growth was negatively affected, with shorter larvae observed in the 25 µg/mL treatment (Messinetti et al. 2018).

#### 7.3.2.9 Chordata: Fish

Marine and freshwater environments are evenly represented in fish studies, with 19 and 20 studies, respectively. Overall, 18 different species were used in fish studies (*Acanthochromis polyacanthus*, *Acanthurus triostegus*, *Bathygobius krefftii*, *Carassius carassius*, *Clarias gariepinus*, *Cyprinodon variegatus*, *Danio rerio*, *Dicentrarchus labrax*, *Lates calcarifer*, *Oncorhynchus mykiss*, *Oreochromis niloticus*, *Oryzias latipes*, *Oryzias melastigma*, *Pimephales promelas*, *Pomatoschistus microps*, *Sparus aurata*, *Symphysodon aequifasciatus*). The most commonly studied species is the zebrafish *D. rerio* (12 studies, corresponding to 26% of studies). The European seabass (*D. labrax*) and the common goby (*P. microps*) are the most commonly studied marine species (six studies, 13% of studies each). Most studies were carried out on embryo/larvae (11 studies, 28% of studies) or juvenile (16 studies, 41% of studies) fish, while studies on adult fish only represent 18% of the studies (7 studies). Six studies did not report the developmental stage of the test species.

Fish exposure to microplastics was performed either directly via water (27 studies, 69% of studies) or via the trophic route (13 studies, 33% of studies). For the later, two main methods are found in the literature. The first method consists in exposing living prey to microplastics then feeding them to fish (Cedervall et al. 2012; Mattsson et al. 2015, 2017; Skjolding et al. 2017; Tosetto et al. 2017). The second method consists in spiking artificial food with known concentrations of microplastics and feed it to fish (Ašmonaitė et al. 2018a, b; Caruso et al. 2018; Granby et al. 2018; Jovanović et al. 2018; Mak et al. 2019; Mazurais et al. 2015; Rochman et al. 2013). While the first method is more representative of trophic interactions in the environment, microplastic ingestion by living prey is not a controlled parameter, and spiking artificial food therefore offers better control of exposure concentrations. The numbers of studies reporting adverse effects, as well those reporting an absence of effect, are similar for marine and freshwater environments and for the different exposure routes. This suggests that these parameters are not likely to influence the occurrence of effects in fish following exposure to microplastics.

More than 92% of studies conducted on fish species used PS (45% = 18 studies) or PE (47.5% =15 studies) microplastics. Commercially available (micro)spheres are the most represented particle morphology and are used in 56% of the studies (22 studies). Undetermined fragments are used in 46% of the studies (18 studies), and close to 13% of the studies (5 studies) did not disclose particle morphology. Four studies used microplastics produced by grinding larger plastic items (Caruso et al. 2018; Choi et al. 2018; Lei et al. 2018b). A broad range of particle sizes have been tested, with the vast majority of studies using microplastics comprised between 0.1 and 500 µm. Most studies investigating the effects of microplastics presenting different properties compared different particle sizes: 49% (19 studies) studied microplastics presenting different sizes, while only one and two studies compared microplastic morphology and polymer type, respectively.

In fish studies, the subcellular level is the most frequently studied level of biological organization (23 studies, 59% studies), followed by the individual, ecosystem, organ and population levels, respectively (16, 16, 13 and 8 studies, respectively, corresponding to 41%, 41%, 33% and 21% of studies). For each organization level, all the studied endpoints were listed and sorted as "impacted" or "not impacted" following exposure to microplastics. For most organization levels, the numbers of endpoints not impacted are very close to the numbers of impacted endpoints. At cellular and subcellular levels, oxidative stress is the main endpoint studied (Ašmonaitė et al. 2018a; Chen et al. 2017; Choi et al. 2018; Ding et al. 2018; Ferreira et al. 2016; Karami et al. 2017; LeMoine et al. 2018; Luís et al. 2015; Mak et al. 2019; Oliveira et al. 2013; Rochman et al. 2013; Wang et al. 2019c), as well as lipid peroxidation (Barboza et al. 2018; Ding et al. 2018; Ferreira et al. 2016; Fonte et al. 2016; Oliveira et al. 2013; Wen et al. 2018a), immune and/or inflammatory responses (Brandts et al. 2018a; Choi et al. 2018; Granby et al. 2018; Mazurais et al. 2015), neurotoxicity (Barboza et al. 2018; Ding et al. 2018; Ferreira et al. 2016; Fonte et al. 2016; Luís et al. 2015; Oliveira et al. 2013; Rainieri et al. 2018), energy production (Barboza et al. 2018; Oliveira et al. 2013; Wen et al. 2018a), endocrine disruption (Wang et al. 2019c) and gut tight junctions proteins, as well as active transport through gut (Ašmonaitė et al. 2018b). At the organ level, most studies focus on histological changes (Ašmonaitė et al. 2018b; Choi et al. 2018; Jovanović et al. 2018; Karami et al. 2016, 2017; Lei et al. 2018b; Mak et al. 2019; Rainieri et al. 2018; Rochman et al. 2013; Wang et al. 2019c), but other endpoints were also studied, such as intestine permeability (Ašmonaitė et al. 2018b; Jovanović et al. 2018), blood and plasma chemistry and metabolite concentrations (Jovanović et al. 2018; Mattsson et al. 2015, 2017), brain weight and water content (Mattsson et al. 2015, 2017), liver glycogen (Karami et al. 2016; Rochman et al. 2013), lipid metabolism (Cedervall et al. 2012) and gut microbiota (Caruso et al. 2018; Jin et al. 2018). Endpoints studied at the population level comprise fish fecundity (e.g. number of eggs laid and hatching rate) (Cong et al. 2019; LeMoine et al. 2018; Wang et al. 2019c), embryo survival and development (Batel et al. 2018; Pitt et al. 2018) and larval survival, development and behaviour (Chen et al. 2017; Choi et al. 2018; Malinich et al. 2018). Endpoints at the ecosystem levels relate to behaviour and include feeding behaviour (e.g. feeding time, foraging, predatory performance), environment exploration and fish locomotion (Cedervall et al. 2012; Choi et al. 2018; Critchell and Hoogenboom 2018; de Sá et al. 2015; Ferreira et al. 2016; Fonte et al. 2016; Guven et al. 2018; Jacob et al. 2019; Luís et al. 2015; Mak et al. 2019; Malinich et al. 2018; Mattsson et al. 2017; Pitt et al. 2018; Skjolding et al. 2017; Tosetto et al. 2017; Wen et al. 2018a). Contrary to the above-described levels of biological organization, for which the numbers of impacted and non-impacted endpoints are similar, at the individual level more studies report an absence of effects (11 studies) than the observation of adverse effects (3 studies) following microplastic exposure. Mortality was reported for medaka larvae exposed to PS sphere (10 μm, 100,000 part./L) for 14 days (Cong et al. 2019) and for juvenile goby exposed to PE spheres (1–5 μm, 184 μg/L) for 4 days (Fonte et al. 2016), and weight loss was observed in crucian carp exposed to PS nano-spheres via trophic chain for 42 days (Cedervall et al. 2012). Other studies investigating fish mortality, growth or body condition reported an absence of effect (Critchell and Hoogenboom 2018;

Ding et al. 2018; Granby et al. 2018; Jovanović et al. 2018; Karami et al. 2017; Lei et al. 2018b; LeMoine et al. 2018; Mazurais et al. 2015; Oliveira et al. 2013; Wen et al. 2018a, b;), and in one case reported mortality only at the highest concentration test (PMMA nano-spheres, 20 mg/L) (Brandts et al. 2018a).

### 7.3.3 *Species Sensitivity Distributions*

Species sensitivity distributions (SSDs) are a common approach used in environmental protection, risk assessment and management practices to describe interspecies sensitivity and estimate community-level risks for a specific stressor. An SSD is derived by fitting a selected statistical model, in this case a lognormal distribution, to available ecotoxicity effect data for species from different taxonomical groups, after which predictions of the % of species affected can be calculated (Posthuma et al. 2019). The SSD captures the interspecies variability, which can then be used to derive key risk assessment components, such as the concentration at which 5% of the species in an ecosystem can be affected. This key regulatory parameter is commonly known as the "hazardous concentration for 5% of the species" or $HC_5$ and is normally used to derive environmental quality criteria standards (Besseling et al. 2019; Burns and Boxall 2018 and references therein). Even though this approach is commonly used to assess the risk of other environmental chemicals, only recently it has been applied to both microplastic and nanoplastic data (Adam et al. 2019; Besseling et al. 2019; Burns and Boxall 2018; Everaert et al. 2018; VKM 2019).

With the ecotoxicological data collected from the reviewed publications, three SSDs for microplastic were investigated for water, sediment/soil and food exposure routes, after which the $HC_5$ corresponding to concentrations expressed in mass and particle number when available were estimated (Fig. 7.6). However, the lack of ecotoxicological data for species covering the different environmental compartments limited the applicability of SSDs in this case, thus decreasing the overall success of the hazard assessment of microplastics and nanoplastics. SSDs are as robust as the quality of their ecotoxicological data, and usually at least 12 different species are considered a minimum for fitting an SSD (Posthuma et al. 2019). Accordingly, even though a total of 107 species covering key taxonomical groups were comprehensibly assessed in the 175 publications reviewed, only 12–58 were used to build the SSDs. This represents a subset of the total data, depending on the availability of data for the exposure matrix (water or sediment/soil) and the exposure quantification (mass or particles).

As the total microplastic toxicity data on freshwater and marine environments is still limited, information collected on marine, freshwater and terrestrial species were combined according to exposure route (water, sediment/soil and food) to increase the number of feeding strategies and trophic levels included in the SSDs, thus increasing statistical power. No distinction was made between particle characteristics due to insufficient data within a certain particle size and polymer type. In

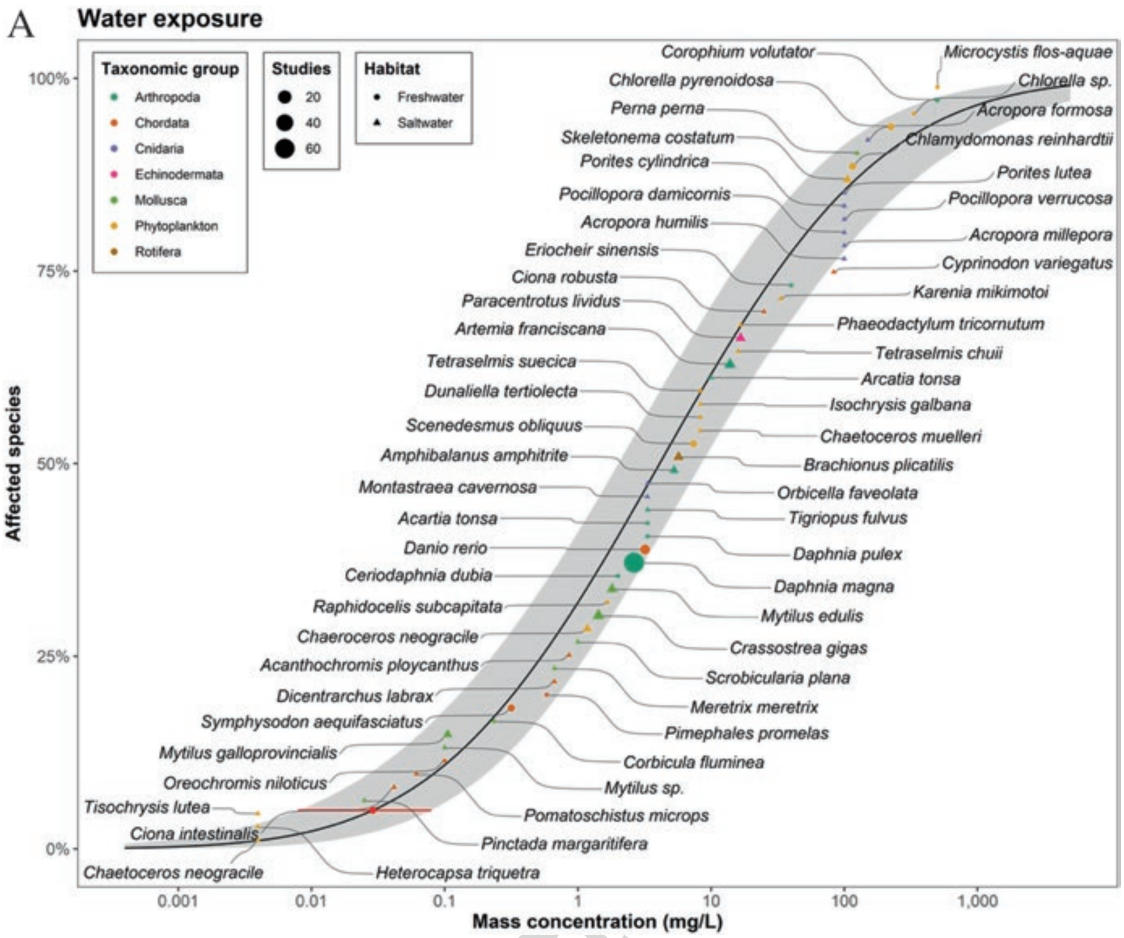


**Fig. 7.6** Species sensitivity distributions (SSDs) for (**a**) species exposed via the water phase with data divided by particle concentration expressed as mass (mg/L) ($n = 58$); (**b**) species exposed via the water phase with data divided by particle concentration expressed as particle number (*million* particles/L) ($n = 31$); and (**c**) species exposed via the sediment and soil phase with data shown only for particle concentration as mass number (mg/kg) ($n = 12$). The average SSDs are plotted as solid black lines, and the 95% credible interval as grey ribbon. The $HC_5$ (concentration at which 5% of the species are affected) is represented as a red point in combination with the 95% credible intervals. Taxonomic groups are represented in different colours, with the different habitats divided by shape and where size reflects the number of studies included



addition, only data pertaining to individual and population levels were considered (e.g. mortality, growth, reproduction), for which both NOECs and $EC_{50}$/$LC_{50}$ values were used.

The poor standardization in terms of reporting of experimental conditions was another factor influencing the construction of SSDs. For example, the lack of information on exposure concentrations expressed in mass and particle number further limited the usable data sets. Dose metrics were standardized to either mass- or particle-based concentrations. When it was not possible to perform this conversion, the studies were excluded from the SSD fitting. Most of the excluded studies were for exposure via food (e.g. fish), leaving insufficient data available to construct SSDs, as only 6 and 3 data points were available (for mass concentration and

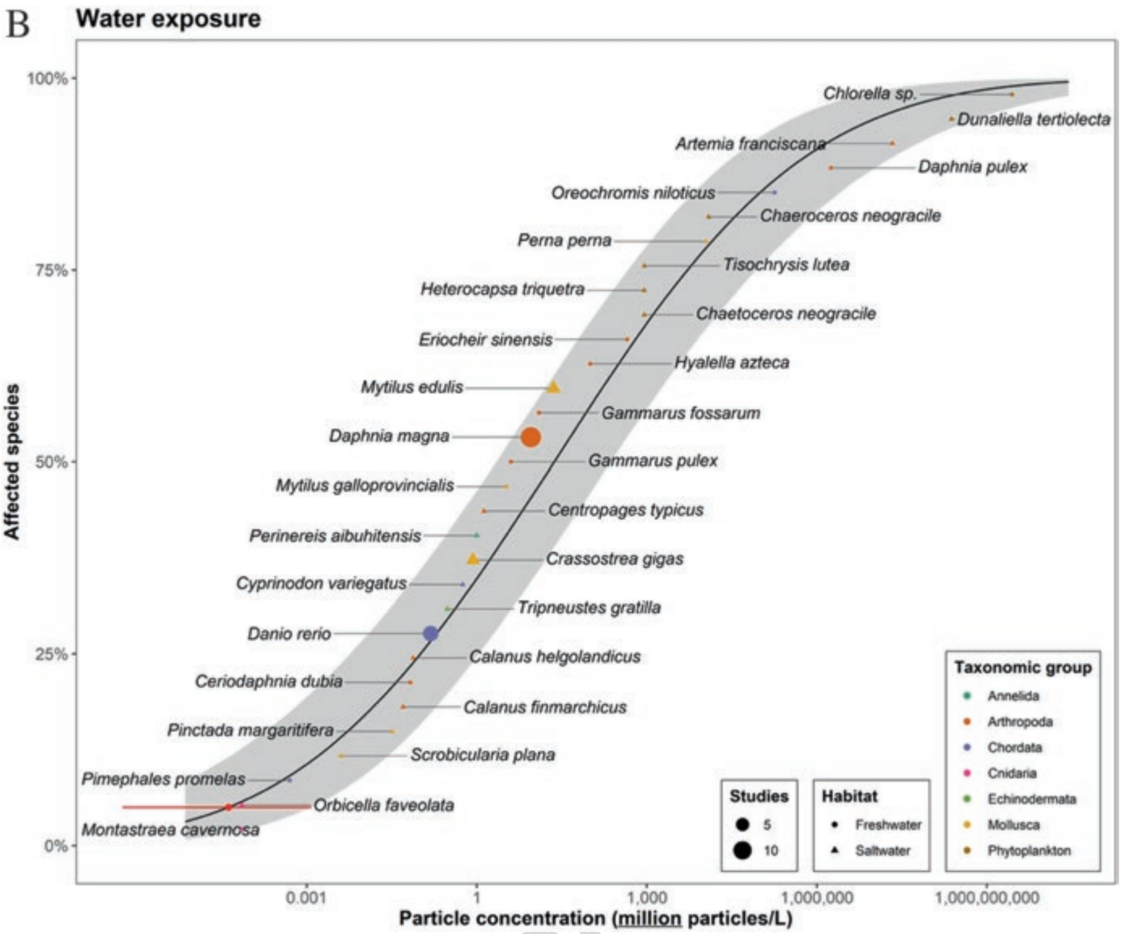


**Fig. 7.6** (continued)



particle concentration, respectively). Overall, tentative SSDs reflecting the combined variability of species sensitivity, plastic properties and effect mechanisms were only constructed for water exposure as a function of particle dosage (both mass and number) and sediment/soil exposures as a function of particle dosage (mass only). Due to insufficient data, the particle-based sediment exposure route and the entire dietary exposure route were excluded from the SSD analyses. The SSD for mass-based water exposure was fitted to data from 101 studies, covering 58 species across 7 taxonomic groups and 2 habitats. Its particle-based counterpart was fitted to data from 39 studies, covering 31 species across 7 taxonomic groups and 2 habitats. For the mass-based sediment exposure route, the SSD was fitted to data from 17 studies, covering 12 species across 4 taxonomic groups and 3 habitats; note that in terms of species coverage, this is considered a minimum acceptable coverage.

The separately constructed SSDs for organisms exposed via water and sediment/soil (expressed in mass and particle number) are shown in Fig. 7.6. Of the studies where concentrations were expressed by particle mass, microalgae species were the most and least sensitive species to exposure via the water phase (Fig. 7.6a). The most sensitive species was the marine microalgae *C. neogracile* (PS-$NH_2$ spheres, <1 µm), (González-Fernández et al. 2019), while the most sensitive freshwater species was the clam *C. fluminea* (proprietary polymer, 1–9 µm) (Oliveira et al. 2018).

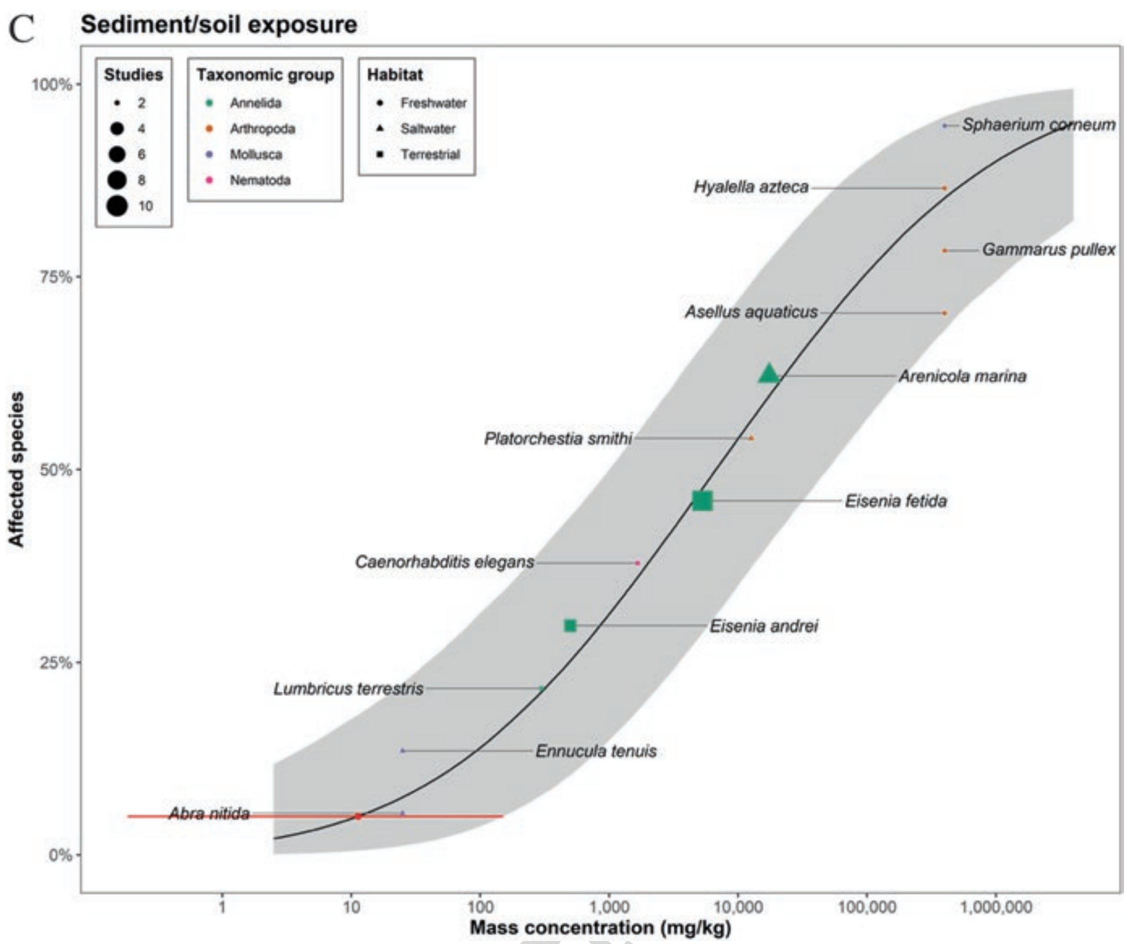


**Fig. 7.6** (continued)

The least sensitive freshwater species was *M. flos-aquae* (PVC and PP, 100–199 μm) (Wu et al. 2019b), while the cnidarian *A. formosa* was the least sensitive marine species (PE fragments, size range 50 to 500 μm (Syakti et al. 2019). The derived $HC_5$ for this SSD was 28.9 μg/L (95% CI 7.94–79.1 μg/L). For the water exposure SSD built with data expressed in terms of particle number (Fig. 7.6b), the cnidarians *M. cavernosa* and *O. faveolata* were the most sensitive species (PE beads, >50 μm (Hankins et al. 2018)), while the least sensitive was the freshwater microalgae *Chlorella* sp. (Thiagarajan et al. 2019). The derived $HC_5$ for this SSD was 41.6 particles/L (95% CI 0.58–1176 particles/L). For exposures either via sediment or soil (Fig. 7.6c), the SSDs obtained for particle concentration in mass showed that the most sensitive species were the marine clams *A. nitida* and *E. tenuis* (PE fragments >1 μm) (Bour et al. 2018), followed by the terrestrial annelid *L. terrestris* (PE spheres <1 to >500 μm) (Huerta Lwanga et al. 2016). The least sensitive species were the freshwater snail *S. corneum* (PS fragments >20 μm (Redondo-Hasselerharm et al. 2018)) and the freshwater arthropod *H. azteca* (PE and PS fragments 10–500 μm) (Au et al. 2015; Redondo-Hasselerharm et al. 2018). The derived $HC_5$ for this SSD was 11.3 mg/kg (95% CI 0.18–151 mg/kg). As mentioned above, construction of an SSD for particle-based sediment exposure was not possible due to lack of sufficient data.

The mass-based water exposure $HC_5$ value (28.9 μg/L) obtained in the present review is higher than that previously reported for microplastics (0.08–5.4 μg/L) (Table 7.2). The main reason for this difference is the inclusion of a higher number of species covering multiple taxonomical groups. On the other hand, the particle number-based $HC_5$ value was 41.6 particles/L, which is within the range provided by the VKM (2019) assessment. Even though this estimate included a larger data set (31 species) than other assessments, the number of studies that provide particle concentrations in number is still quite limited. No other $HC_5$ values expressed in mg/kg exist in literature for comparison.

Even though the SSDs presented here are more robust as they are based on larger data sets and add to the existing SSDs in literature, several knowledge gaps still need to be addressed to reduce uncertainties and improve the robustness and relevance of the obtained results (Besseling et al. 2019; Burns and Boxall 2018). For this reason, ecotoxicity testing of relevant particle sizes, shapes and polymer types,

**Table 7.2** – $HC_5$ values obtained from species sensitivity distribution analysis collected from literature

| $HC_5$ (μg/L) | $HC_5$ (particles/L) | $HC_5$ (mg/kg) | Notes | References |
|---|---|---|---|---|
| 28.9 (7.94–79.1) | 41.6 (0.58–1176) | 11.3[a] (0.18–151) | Freshwater and marine species exposed to micro- and nanoplastics via water and sediment/soil | Present review |
| 0.14 (0.04–0.64) | 71.6 (3.45–1991) | – | Freshwater and marine species exposed to micro- and nanoplastics | VKM (2019) |
| 0.08 (0.04–0.11) | 740 (610–1300) | – | Freshwater species exposed to microplastics. 25–75 percentile was used instead of confidence interval | Adam et al. (2019) |
| 5.4 (0.93–31 mg/L) | $5.97 \times 10^{10}$ ($1.6 \times 10^{10}$–$22 \times 10^{10}$) | – | Marine and freshwater species exposed to nanoplastics | Besseling et al. (2019) |
| 1.67 (0.086–32.6) | 1015 (101–10,223) | – | Marine and freshwater species exposed to microplastics | |
| – | 64,000 | – | Marine and freshwater species exposed to microplastics (10 to 5000 mm) | Burns and Boxall (2018) |
| – | 33.3 (0.36–13,943) | – | Marine species exposed to microplastics | Everaert et al. (2018) |
| – | 3214 (3.3900–84,261) | – | Marine species exposed via water and sediment to microplastics | Van Cauwenberghe (2016) |

[a]Note that the $HC_5$ value for mass-based sediment exposure is derived from a minimum of necessary data and needs to be interpreted with caution

standardized testing, improved reporting of experimental designs, methods and results, as well as a higher focus on freshwater and terrestrial compartments, need to be prioritized in order to enable a sound risk assessment of plastic particles in the environment.

### *7.3.4 Direct and Indirect Effects at the Ecosystem/Community Level*

Cascading effects through different levels of biological organization is a central paradigm of ecotoxicology: contaminant-induced subcellular changes, such as enzymatic activity or gene expression, can impact higher levels of organization and affect organism's performance (e.g. locomotion, feeding, reproduction). These alterations might impact an entire population and could ultimately have consequences at the ecosystem level. With that said, directly linking effects at the lowest levels of biological organization to impacts on ecosystems is extremely challenging for any environmental contaminant (Galloway et al. 2017). The data currently available on nano- and microplastic ecotoxicity does not allow firm conclusions to be drawn about such links. However, certain endpoints observed at the individual level are indicators of potential indirect effects on other species and/or on the functioning of ecosystems. Such endpoints are therefore categorized as endpoints relevant at the ecosystem level. For example, behavioural changes at the individual level can affect prey-predator interactions (Fonte et al. 2016; Wen et al. 2018a) and impact entire trophic webs, or impaired burrowing activity of dwelling organisms can alter bioturbation and soil/sediment oxygenation (Green et al. 2016). Changes in microbial activity can also result in altered essential ecosystem processes, such as nutrient cycling (e.g. nitrogen and carbon cycles) (Green et al. 2017).

Among the studies reviewed in this chapter, endpoints relevant at the ecosystem level were most studied on three taxonomical groups: Annelida, Arthropoda and Chordata. The recorded endpoints were related to behaviour: feeding activity (Besseling et al. 2013, 2017; Browne et al. 2013; Cedervall et al. 2012; Green et al. 2016; Guven et al. 2018; Malinich et al. 2018; Mattsson et al. 2017; Wright et al. 2013), burial and burrow formation (Booth et al. 2016; Huerta Lwanga et al. 2016), cast production (Green et al. 2016; Prendergast-Miller et al. 2019), locomotion (Chae et al. 2018; Choi et al. 2018; Critchell and Hoogenboom 2018; de Felice et al. 2019; Frydkjær et al. 2017; Gambardella et al. 2017; Kim and An 2019; Lin et al. 2019b; Mattsson et al. 2017; Pitt et al. 2018; Skjolding et al. 2017; Tosetto et al. 2016, 2017; Ziajahromi et al. 2017), prey-predator interactions (de Sá et al. 2015; Ferreira et al. 2016; Fonte et al. 2016; Jacob et al. 2019; Luís et al. 2015; Mattsson et al. 2017; Wen et al. 2018a) and aggression (Critchell and Hoogenboom 2018). Studies focusing on such ecologically relevant endpoints are currently underrepresented (16% of the reviewed studies), although the available data shows that these endpoints can be impacted by plastic particles, especially locomotion (Cedervall et al. 2012; Choi et al. 2018; de Felice et al. 2019; Frydkjær et al. 2017; Kim and An 2019; Lin et al. 2019b; Mattsson et al. 2017), feeding activity (Besseling et al. 2013, 2017; Green et al. 2016; Guven et al. 2018; Mattsson et al. 2017; Wright et al. 2013) and prey-predator interactions (Fonte et al. 2016; Wen et al. 2018a).

Only a single study looked at the ecosystem-level effects on Cnidaria, more specifically on *P. damicornis* (Tang et al. 2018). The results obtained in this study suggest that acute exposure to PS particles can activate stress responses at the individual level, repressing detoxification and immune systems, which in turn can compromise the anti-stress capacity of exposed organisms. However, this study found a minimal impact in community shifts (symbiont density and chlorophyll content) in the short term. In a similar study, Reichert et al. (2018) suggested that species-specific effects might promote community shifts in coral reefs. For example, if growth, health and photosynthesis are affected, this might amplify the coral's susceptibility to other stressors such as increased seawater temperatures, contributing to shifts in coral reef assemblages. Like cnidarians, only one study considered the effects of nanoplastics at the ecosystem level in phytoplankton (González-Fernández et al. 2019). This study analysed the impact of PS-$NH_2$ (50 nm) on a diatom (*C. neogracile*), which led to changes of the concentration of associated bacterial communities. It is important to study effects following exposure to plastic particles in phytoplankton not only due to their susceptibility (as seen in the SSD) but also due to their importance in the ecosystem. As already stated, these organisms are at the base of the aquatic food web, and changes in their communities may disturb the productivity of an entire ecosystem (Prata et al. 2019). Moreover, particles may end up higher in the food web due to algae-particle interaction as the first step in the biomagnification (Nolte et al. 2017), as previously shown in other studies with suspension-feeding bivalves (Ward and Kach 2009). Finally, one study addressed the impacts of microplastics on the health and biological functioning of oysters (*O. edulis*) and on the structure of associated macrofaunal assemblages using an outdoor mesocosm experiment (Green 2016). The author found that exposure to high concentrations of microplastic resulted in alterations of assemblage structure, diversity, abundances and biomasses of several taxa in vegetated oyster habitats, whose cascade effects can lead to significant impacts in marine ecosystems.

Indirect, secondary effects are effects occurring on species not necessarily exposed to plastic particles but which are impacted by changes resulting from their direct exposure. In their mesocosm study, Green et al. (2016) exposed the lugworm *A. marina* to microplastics and observed a decrease in cast production, as well as decreased microbial biomass with increasing concentrations. One of the hypotheses discussed by the authors to explain the decreased microbial biomass was that reduced sand reworking by the worms would have resulted in less nutrients available in the sand to support primary productivity. No firm conclusion about indirect effects of microplastics could be drawn from this study, as microplastics could have directly affected microbial communities, but this scenario is one of the potential examples of indirect microplastic effects. In another recent study, reduced survival and reproduction were observed for the terrestrial invertebrate *Enchytraeus crypticus* following exposure to synthetic fibres (Selonen et al. 2020). However, fibre ingestion could not be confirmed, and the authors hypothesized that the observed effects could be due to changes in environmental conditions, such as microbial activity and physicochemical properties of the soil, resulting from microplastic exposure. In both cases, the authors (Green et al. 2016; Selonen et al. 2020) present indirect effects of microplastics as a hypothesis, but investigating microplastic indirect effects was not the main purpose of the study. Although highly ecologically relevant, studies on nano- and microplastic indirect effects are currently almost non-existent. Such studies are needed to help link effects at the organism level to impacts on the ecosystem level. Future studies should consider potential direct and indirect nano- and microplastic effects at the ecosystem level, to fill these major gaps in the field of plastic ecotoxicology.

### *7.3.5 Interaction of Plastic Particles with Chemicals*

The challenge of assessing the impact of plastic particles in the environment is further complicated by the presence of chemicals, which can potentially pose additional hazards towards organisms. These chemicals comprise polymerization catalysts and additives, which are incorporated during production to endow plastics with specific characteristics (e.g. flame retardants, plasticizers, antioxidants, UV stabilizers and pigments) (Gallo et al. 2018) and non-intentionally added substances (NIAS). Furthermore, chemicals already present in the environment (e.g. polycyclic aromatic hydrocarbons (PAHs) and metals) may also be incorporated/adsorbed by plastic surfaces depending on the polymer physico-chemical properties (e.g. Teuten et al. 2009).

Few studies have identified nano- and microplastics as vectors for other contaminants (Trojan horse effect), and even fewer have focused on the presence and leaching of chemical additives. Of the 175 references reviewed, 48 addressed these combined effects, with a focus on chemicals present in the environment, such as PAHs (e.g. benzo(a)pyrene (BaP), phenanthrene, fluoranthene, pyrene), polychlorinated biphenyls (PCBs), organophosphates (e.g. chlorpyrifos), metals (e.g. gold, mercury, cadmium, chromium and copper), metal nanomaterials (gold and titanium nanoparticles) and pharmaceuticals (roxithromycin, cefalexin, carbamazepine, florfenicol, doxycycline and procainamide). Only a small percentage of these studies (12.5%) focused on chemicals known to be used as plastic additives (e.g. benzophenone, polybrominated diphenyl ethers (PBDEs), perfluorooctane sulfonates (PFOs), bisphenol A (BPA), triclosan), surfactants (e.g. nonylphenol) as well as chemical leachates extracted from plastic particles. In addition, the combined effects of plastic particles with natural acidic organic polymers (e.g. palmitic acid, humic acid and fulvic acid) were also considered in some of the reviewed publications.

Most of these studies were conducted in arthropods (28%), followed by fish (20%), molluscs (17%), phytoplankton (15% studies), annelids (9%), echinoderms (2%), nematodes (2%) and rotifers (2%). No studies on the combined effects of plastic particles and other contaminants were reported for cnidarians. Of the 57 studies reviewed for arthropods, 15 addressed the interaction between plastic particles and chemicals. These chemicals included benzophenone (Beiras et al. 2018), fluoranthene (Bergami et al. 2016, 2017; Horton et al. 2018; Vicentini et al. 2019), humic acid (Fadare et al. 2019; Wu et al. 2019a), PCBs (Gerdes et al. 2019; Lin et al. 2019a; Watts et al. 2015), phenanthrene (Ma et al. 2016), gold (Pacheco et al. 2018), BPA (Rehse et al. 2018), PAHs (Tosetto et al. 2016), palmitic acid (Vicentini et al. 2019) and roxithromycin (Zhang et al. 2019). Several effects at the subcellular, individual and population levels were seen in arthropods upon exposure to nano- or microplastics combined with these chemicals. The most reported effects where impacts on reproduction, mortality, development and growth. Eleven studies conducted on fish used microplastics sorbed with chemicals. In seven of those, the tested microplastics were purposely spiked with chemicals, such as BaP (Batel et al. 2018); antibiotics (Fonte et al. 2016); heavy metals such as mercury (Barboza et al. 2018), cadmium (Lu et al. 2018) and chromium (Luís et al. 2015); gold nanoparticles (Ferreira et al. 2016); and a cocktail of environmental contaminants comprising PCBs, PBDEs, PFOs and metals (Granby et al. 2018). Additionally, in four studies, the tested microplastics were deployed in environmental matrices (i.e. harbour, sewage effluent, urban bay), and further analyses confirmed the presence of environmental contaminants, such as surfactants and PAHs (Ašmonaitė et al. 2018a, b; Rochman et al. 2013; Tosetto et al. 2017). Interestingly, for every level of biological organization covered in these fish studies, the presence of chemicals sorbed on microplastics does not change the occurrence of adverse effects, indicating that microplastic-associated chemicals would play a minor role in microplastic effects. Studies on combined effects of micro- and nanoplastics and chemical exposure using molluscs included pyrene (Avio et al. 2015), carbamazepine (Brandts et al. 2018b), florfenicol (Guilhermino et al. 2018), mercury (Oliveira et al. 2018), fluoranthene (Paul-Pont et al. 2016; Rist et al. 2016), BaP (Pittura et al. 2018) and PCBs (Rochman et al. 2017). Effects at the cellular and subcellular levels were often reported for this taxonomical group, followed by impacts at the organ and individual level. Additionally, in one of the studies reviewed, no effects were reported for *M. galloprovincialis* exposed to benzophenone (Beiras et al. 2018). In the eight studies reported for phytoplankton, adverse effects of micro- and nanoplastics in combination with other contaminants were reported for metal mixtures (Baudrimont et al. 2020), copper (Bellingeri et al. 2019), titanium nanoparticles (Thiagarajan et al. 2019), fulvic and humic acid (Liu et al. 2019), chlorpyrifos (Garrido et al. 2019), doxycycline and procainamide (Prata et al. 2018), triclosan (Zhu et al. 2019) as well as leachate mixtures (Luo et al. 2019). Overall, the documented effects in these studies included reduction in growth, oxidative stress, membrane stability and reduction in protein content and natural pigments. From the 16 studies conducted with annelids, five included co-exposure with contaminants, namely, PCBs (Besseling et al. 2013, 2017), chlorpyrifos (sprayed to the surface of PE spheres (Rodríguez-Seijo et al. 2018b)), BaP (Gomiero et al. 2018), nonylphenol, phenanthrene, triclosan and PBDE-47 (sorbed to microplastics (Browne et al. 2013)). Of the effects found in annelids, alterations in behaviour (i.e. reduced feeding) were most commonly reported associated with exposure to PCBs (Besseling et al. 2013, 2017). Reduction in growth was also observed at lower concentrations when plastic particles were sprayed with chlorpyrifos (Rodríguez-Seijo et al. 2018b) or co-exposed with PCBs (Besseling et al. 2013). Of the reviewed studies for Echinodermata, only Beiras et al. (2018) utilized microplastics spiked with benzophenone-3, an organic, hydrophobic chemical found in cosmetic products, using *P. lividus* as a test organism. Even though ingestion of virgin and BP-3 spiked PE microplastics was observed at 1 and 10 mg/L, no acute toxicity was observed above concentrations considered environmentally relevant (low treatment = 20 µg/L and high concentration treatment = 200 ng/L) (Beiras et al. 2018). When it comes to nematodes, in the study by Judy et al. (2019), microplastics were added to soil amended with municipal waste compost. The presence of trace metals was assessed in amended soils and in microplastics (PE, PET, PVC), and GC-MS analysis revealed the presence of phthalates in PVC, which could have accounted for the effects observed in exposed organisms. Only one study looked at combined effects of PE spheres and benzophenone using the rotifer *B. plicatilis*, for which no effects were reported (Beiras et al. 2018).

Overall, the studies reviewed on the joint toxicity of plastic particles and chemicals (either adsorbed to particles or additives) showed that their interaction can elicit a wide range of biological responses in exposed organisms. In addition, chemicals associated to plastic particles can also influence their bioavailability and potential transfer through food chains, possibly causing effects at the ecosystem level. Nonetheless, these findings need to be interpreted with caution as most of these studies differ in how they approach vectoral transfer kinetics and exposure mechanisms for chemicals under realistic natural conditions and thus overestimate the role of plastic particles as the delivery system of chemicals to organisms. The majority of these laboratory experiments use simplified exposure settings, in which clean organisms placed in clean media/sediment/soil are exposed to plastic particles pre-treated with chemicals. These controlled exposure settings create conditions that promote rapid dissolution of the chemicals from the plastic particles into the surrounding environmental compartment, which then become easily bioavailable to organisms through a more conventional exposure route (Diepens and Koelmans 2018; Booth and Sørensen 2020). Under more environmentally relevant exposure scenarios, currently available data suggests that chemicals accumulated in organisms are derived to a very small extent from ingested plastic particles, especially when compared to natural pathways of bioaccumulation as water, sediment and food (Koelmans et al. 2016; Besseling et al. 2017). For this reason, it is important to consider the relative importance of plastic particles as an exposure route for chemicals in the context of other uptake pathways that may be more relevant under realistic natural conditions (Lohmann 2017; Diepens and Koelmans 2018). To understand how plastic particles can act as vectors for other chemicals and what is the contribution that additives make to overall exposures, a thorough control of exposure mechanisms is therefore necessary. This will ensure that any observed biological effects are a consequence of exposure to the chemicals adsorbed and/or incorporated in the particles and not derived from their leaching, desorption and dissolution into environmental compartments (Booth and Sørensen 2020; Gallo et al. 2018;

Hermabessiere et al. 2017). In addition, there is a pressing need for studies addressing synergistic/antagonist effects following short- and long-term exposure to plastic particles in combination with contaminants of high concern, as well as studies on their cumulative effects in both terrestrial and aquatic species and potential biomagnification throughout food chains. For further information on the impacts of environmental contaminants and plastic additives in terrestrial and aquatic organisms, see reviews by Gallo et al. (2018) and Hermabessiere et al. (2017). For additional studies on the importance of exposure pathways for a range of chemicals present in plastic particles under natural conditions, the readers may refer to Koelmans et al. (2016), Lohmann (2017) and Diepens and Koelmans (2018).

## 7.4 Challenges and Future Directions

Exposure experiments focusing on the ecotoxicological effects of plastic particles in a wide range of organisms have increased exponentially over the past few years. A consensus from the reviewed literature is that plastic particles can impact organisms across successive levels of biological organization, covering effects from the subcellular level up to the ecosystem level (Galloway et al. 2017; VKM 2019). Nonetheless, our understanding on the mechanisms behind any toxic effects recorded is still minimal, partially due to a lack of attempt to link the physical and chemical properties of the particles being tested with the recorded toxic effects. Many of the reviewed studies relate to common chemical exposure endpoints rather than particle related endpoints, including how particles directly interact with the cellular environment and organisms, their uptake mechanisms, tissue distribution and subsequent impacts (e.g. tissue alterations due to inflammation or other physical impacts). Accordingly, understanding and distinguishing the potential physical and chemical effects of plastic particles across the whole spectrum of biological levels is needed to improve environmental risk assessment of plastic pollution, as a means to ensure a better protection and mitigation of its impacts in the different environmental compartments.

The comparability of existing ecotoxicological data is being hampered by numerous factors such as the use of wide array of experimental testing approaches, unrealistic environmental concentrations, lack of relevance in terms of particle characteristics (polymer type, shape or size), use of appropriate controls, incomplete/inadequate particle characterization (physico-chemical properties and chemical additives), variability in reporting units (e.g. in mass and/or particle number, % particles in food or sediment) and experimental conditions (e.g. exposure duration). Many of these limitations were found during the evaluation of data quality in the reviewed references, in which the use of appropriate controls, confirmation of exposure concentration and polymer type as well as presence of chemical leachates and particle size distributions were the most common issues. The ubiquitous nature of microplastic contamination, widespread geographical distribution, abundance and small size have also raised significant concerns regarding their interactive effects with chemicals, not only by increasing the bioavailability of contaminants in organisms but also by eliciting common toxic effects. This is especially true when considering the potential risk of chemical accumulation in higher trophic levels including humans, as well modifications in population structure and ecosystem dynamics (e.g. negative effects at lower trophic levels) that may potentially result in a reduced productivity of the whole ecosystem. However, the role of plastic particles as the delivery system of chemicals to organisms is currently overestimated and additional data is required to understand the relative importance of exposure to chemicals (either adsorbed or additives) from particles compared to other exposure pathways (e.g. water and natural diet).

This overview is consistent with the tendencies observed by other authors, calling into question the environmental relevance and proposed risks caused by nanoplastic and microplastic exposure (e.g. Burns and Boxall 2018; de Ruijter et al. 2020; Kögel et al. 2020; VKM 2019). To determine if these plastic particles are in fact posing significant risks to organisms, future work needs to focus on the development of reporting guidelines to improve the reproducibility and comparability of plastic-related research, as highlighted by Connors et al. (2017) and Cowger et al. (2020). Several research priorities are thus recommended to better understand the ecological risks of plastic particles in the terrestrial and aquatic environments:

1. **Standardization.** It is fundamental for ecotoxicological investigations to be comparable. A standardized approach from experimental design to reporting is required. To this end, quality assessments should be conducted throughout the whole duration of any laboratory studies (including concentrations and exposure conditions with quality assessment) to obtain reliable and comparable data.
2. **Environmental relevance.** Researchers should endeavour to conduct experiments which have relevance to current and future scenarios of plastic concentrations and characteristics in the different environmental compartments. These include partially degraded and irregularly shaped particles commonly found in the environment, with varying polymer types, sizes and surface properties. As fibres and fragments are prevalent in environmental samples, these should be prioritized in future studies.
3. **Particle vs. chemical effect.** The combination of particle and associated additives must be considered in ecotoxicological studies, such that it is possible to discriminate between effects derived from particles from those resulting from additive chemicals. Therefore, it is paramount that a thorough characterization of exposure materials is carried out, including the chemical profiles of organic and metal additives. To really understand whether plastic particles are relevant carriers for chemicals, environmentally realistic exposure settings also need to be taken into account when looking at particle-chemical interactions, more specifically leaching/desorption kinetics, chemical bioaccumulation from water/sediment/soil, natural diet and percentage of ingested particles.
4. **Ecosystem compartments.** As highlighted throughout this chapter, there is disproportion between the number of studies conducted on marine, freshwater and terrestrial biota. Moving forward, it is important to direct attention towards

freshwater and terrestrial ecosystems, as these are considered the main sources and transport pathways of plastic particles to the marine environment.

5. **Test species.** Species utilized for ecotoxicological testing are generally focused on model organisms used for standard ecotoxicological testing. This originates a significant knowledge gap on the effects of plastic particles in other species that have critical roles in ecosystem balance. Species considered at highest risk of exposure due to their feeding strategies and position in the water column need to be prioritized in terms of ecotoxicity testing, e.g. planktonic species not included in ISO and OECD guidelines. Species ecology and time spent in various environmental compartments are also important considerations for choice of test species, with particular emphasis on early developmental stages that have been shown to be highly susceptible to the impacts of plastic particles. Moreover, given that soil/sediment is considered the ultimate sink for plastic particles and other conventional contaminants, increased testing with suspension and deposit feeders is also warranted.
6. **Physiological perspective.** Currently there is a lack of mechanistic understanding of the effects of microplastics and nanoplastics on biota. Additional efforts are needed to understand the differences in physical and chemical behaviour of plastic particles compared to conventional contaminants. The direct and indirect interaction of nano- and microplastics within the cellular environment and organisms, uptake mechanisms (size dependency), tissue distribution and impacts must therefore be comprehensibly assessed and linked to the physical and chemical properties of the particles being used. Modifications in experimental design and proper characterization of the particles (e.g. presence of additives) can also assist to explain the underlying mechanisms responsible for the observed responses and help distinguish physical from chemical toxicological effects.
7. **Integrated and multi-level approaches.** Long-term experiments with multiple species (e.g. model ecosystems) are required to examine effects with higher ecological relevance. Therefore, small- and large-scale mesocosm experiments mimicking environmentally relevant scenarios and covering links from primary producers (e.g. microalgae) to top predators (e.g. fish) are encouraged.

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# Author Queries

Chapter No.: 7 0005156912

| Queries | Details Required | Author's Response |
|---|---|---|
| AU1 | Please check "Material and Methods section" for correctness as there is no mention of such section in the text. | |
| AU2 | Please check "18" for correctness. | |
| AU3 | Please provide page number in Kallenbach et al. 2021. | |
| AU4 | Please provide publisher location details for Prakash et al. (2020) and Rochman (2015). | |