Studying microplastics: lessons from evaluated literature on animal model organisms and experimental approaches

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ABSTRACT

Although we are witnesses of an increase in the number of studies examining the exposure/effects of microplastics (MPs) on different organisms, there are many unknowns. This review aims to: (i) analyze current studies devoted to investigating the exposure/effects of MPs on animals; (ii) provide some basic knowledge about different model organisms and experimental approaches used in studying MPs; and to (iii) convey directions for future studies. We have summarized data from 500 studies published from January 2011 to May 2020, about different aspects of model organisms (taxonomic group of organisms, type of ecosystem they inhabit, life-stage, sex, tissue and/or organ) and experimental design (laboratory/field, ingestion/bioaccumulation/effect). We also discuss and try to encourage investigation of some less studied organisms (terrestrial and freshwater species, among groups including Annelida, Nematoda, Echinodermata, Cnidaria, Rotifera, birds, amphibians, reptiles), and aspects of MP pollution (long-term field studies, comparative studies examining life stages, sexes, laboratory and field work). We hope that the information presented in this review will serve as a good starting point and will provide useful guidelines for researchers during the process of deciding on the model organism and study designs for investigating MPs.

Keywords: environment; taxonomic group; laboratory; field research; intraspecific differences

1. Introduction

The manufacture and use of plastics have progressively increased over the past 50 years. Because of characteristics such as lightweight, durability, low-cost, low thermal conductivity, plastic has found its place in everyday life and is found in virtually all ecosystems. The plastics industry provides direct employment to more than 1.6 million people in Europe. In 2018, global plastics production reached almost 360 million tons. In Europe, the production of plastics reached nearly 62 million tons (Plastic Europe, 2019). This enormous expansion of the plastic industry confers significantly increased convenience but simultaneously it has also created a huge quantity of plastic trash and raised numerous environmental concerns. It is estimated that by the end of this century, there will be 2.5×10^7 to 1.3×10^8 tons of microplastics (MPs) debris on the ocean's surface (Everaert et al., 2018).

The concept of microplastics was introduced for the first time in 2004 (Thompson et al., 2004). Generally, small plastic debris are classified into microplastics (100 nm-5 mm) and nanoplastics (<100 nm) (Ng et al., 2018). A new phrase, "mesoplastics", has been introduced to make a distinction between relatively small plastic fragments that are visible to the naked eye and fragments that are only microscopically visible (Andrady, 2011). The smallest fragments, nanoplastics, are regarded as potentially more hazardous than larger microplastic parts since they can more easily penetrate organelles and cell membranes, carry various toxic chemicals, thus raising toxicity (Browne et al., 2013). Based on their source, MPs can be categorized as primary or secondary (Yao et al., 2020). Primary MPs are tiny fragments aimed for industrial and domestic use and include various plastic fragments, fibers, pellets, seeds and spheres used in agriculture, cosmetic and pharmaceutical industries, products produced during the ship-breaking process and materials applied in air-blasting technology (Fendall and Sewell, 2009; Guzzetti et al., 2018). Secondary MPs are the outcome of long-term, extended physical, chemical or biological breakdown of the larger plastic parts (Guzzetti et al., 2018). These small parts are difficult to collect and remove from the environment. Microplastic parts are easily dispersed in atmospheric, aquatic and terrestrial surroundings, and represent a considerable potential environmental risk due to their difficult decomposition in nature.

MP debris is not localized in one specified habitat (air, water or the ground) and migrates between different environments. The land is considered as one of the principal sources of MPs, however, the ocean is an aggregate space for a large number of MPs (Gong and Xie, 2020). The freshwater system serves as a link between terrestrial and marine MP polluted habitats. MPs enter the ocean primarily through water flows. Larger pieces of plastic are not difficult to eliminate via various technological processes, but the elimination of MPs is demanding and not feasible due to its worldwide distribution in all ecosystems and difficulties to detect them (Gong and Xie, 2020).

A substantial quantity of these products arrives in the environment and accumulates in terrestrial and aquatic ecosystems worldwide. These plastic parts decompose very slowly over time and remain in the living world for a long period after the lifespan of the plastic products. Consequently, MPs have been discovered in a broad spectrum of living organisms (Chang et al., 2019). The dimensions of MPs are mostly alike to that of zooplankton, and the outcome could be direct ingestion by aquatic animals (Fernández et al., 2020). Many ecotoxicological investigations have revealed that as the result of the ingestion of MPs, some organisms can undergo reproductive problems, oxidative stress, organ injuries, a general weakening and eventual death (Lu et al., 2019; Wang et al., 2019a; Burgos-Aceves et al., 2020). Therefore, the

European Parliament in 2019 offered a proposal for the regulation of MP pollution in sewage sludge and treated water (Sol et al., 2020).

Recently it was shown that MPs can be dangerous for the living world as the result of not only direct ingestion but also indirect intake by trophic transfer (Nelms et al., 2018). The harmful effects of MPs on living systems are not only limited to the aquatic ecosystem. Microplastics interrelate with terrestrial animals, invertebrates, and some plant-pollinators (de Souza Machado et al., 2018). According to Horton et al. (2017), MP pollution on land could be 4-23-times greater than in the aquatic environment, especially in what is referred to as agricultural soil. Small particles can arrive in the ground by different physical, biological, and anthropogenic processes (Rillig et al., 2017).

There are suggestions that MPs can be associated with other hazardous materials, which can further modify the adverse impact on the environment (Bakir et al., 2014; O’Donovan et al., 2018). However, the potential of MPs to behave as reservoirs in the environment for different chemicals and their ability for long-distance transmission and increased bioavailability for animals can significantly depend on the environmental conditions and properties of both the MP and the adsorbed chemical (Rodrigues et al., 2019; Ma et al., 2020; Santos-Echeandía et al., 2020). This issue remains to be clarified (Rodrigues et al., 2019).

Over the years, the number of articles studying MPs has been quite variable. Zhang et al. (2020) performed a comprehensive study of 2501 papers from 1986 to 2019. Before 2010, only 10 articles per year were published, but since 2011, the number of investigations related to this area has started to grow. In 2016 the number of published research papers was more than 200, and from 2017 there were 300 publications. According to the authors, 60.86% of all the included investigations were released during the last two years (1295 studies on microplastics) (Zhang et al., 2020). Of all analyzed papers, 8.01% were review articles, which points to the great interest in investigations and analytical studies of MP pollution (Zhang et al., 2020).

Although there has been an increase in research dealing with different effects of MPs on various organisms, many questions remain unanswered. We hope that the present study will facilitate the assessment of the MP contamination risk as it will summarize data from investigations published thus far, which have examined the quantifiable link between various animal groups (model organisms) and the plastics they have absorbed. This review aims to (i) present the current state of MP research in relation to their exposure/effects on biota; (ii) to contribute to the existing knowledge of different model-organisms and experimental methodologies used in MPs studies, and to (iii) identify promising fields for future research.

2. Material and methods

For this review article, we searched for research papers written in English that are available in the ScienceDirect database from January 2011 to May 3, 2020, using the following term "microplastic effects" (search covered both nano- and microplastic effects). ScienceDirect was chosen as the database that indexes the greatest number of articles on MPs according to Zhang et al. (2020). A total of 2,928 records on MPs were identified, from which 500 met the criteria of this review paper and were further analyzed. We selected articles that included one or more criteria: the ingestion, bioaccumulation and/or effects of MPs on animals. From each paper (the Abstract, Materials and Methods and Results sections) we analyzed and extracted the following factors: (i) the groups of organisms studied (invertebrates/vertebrates) and taxonomic groups (phylum, subphylum and class depending on species); (ii) the environment (marine, freshwater or terrestrial); (iii) the life stage (larvae, juvenile, adult, as well as not specified); (iv) sex; (v) type of study (laboratory, acute or chronic; field, short or long term) or comparative (ontogenetic stages and sexes, localities, seasons, field vs laboratory); (vi) the studied tissue and organ; and (vii) ingestion, bioaccumulation and effects (fitness, behavioral, cytohistological, morphological, neurotoxicological, immunological, genotoxic and physiological). Information from one article may include several criteria from one category, for example, in some studies both invertebrate and vertebrate species were investigated, different ontogenetic stages and often more than one parameter, therefore the number of studies presented in each category may differ from the total number of publications. Some species, such as birds and reptiles that are terrestrial but feed exclusively in the aquatic environment were classified in both environments. For laboratory examinations, acute and chronic studies were determined according to the data provided by the authors of original papers, or if the information was not provided, it was determined depending on the investigated species and current literature (for vertebrates, acute studies that lasted up to 14 days were examined, while chronic studies included those that lasted more than 14 days). Short-term field studies included any type of sample collection (punctual and/or repeated) for three months or less. Long-term studies referred to a research period longer than three months. For the tissue/organ factor we merged the results for the stomach and intestine in the gastrointestinal tract, also if a part of an organ was examined, we considered it as a whole organ.

3. Results and Discussion

3.1. Model organisms for studying microplastics in both laboratory and field studies

3.1.1. Invertebrates vs vertebrates

Our results showed that a slightly higher percentage of studies examined the exposure/effects of MPs on invertebrates than on vertebrate organisms (Fig. 1). Invertebrates are considered as potentially good model organisms for studying various types of pollution. In the case of MP pollution, their potential depends on different species’ characteristics (the ability to tolerate environmental stress, its ecological niche, feeding type, behavioral plasticity and life history strategies), and also on the characteristics of the MPs (their type, size and concentration) (Haegerbaeumer et al., 2019). Detailed analyses among invertebrates revealed that Mollusca, with 106 studies, and Arthropoda, with 100 studies, were the most studied taxonomic groups (Fig. 1). In Mollusca, Bivalvia species (95 studies) were predominantly used, while for Arthropoda different species of Crustacea- 84 studies (Branchiopoda and Malacostraca) were examined. The main characteristics that render Bivalvia and Crustacea susceptible to MPs and interesting for scientific research are as follows: filtration type of feeding, omnipresence, their role in the trophic system (most of them are primary consumers), and their significant share in human nutrition (Ribeiro et al., 2017; Garrido Gamarro et al., 2020). These two groups (Molluscs- 18% and Crustacean- 9%) were also marked as the most studied among invertebrates according to a review of 220 MP-related articles published between 2010-2019 by Ajith et al. (2020). Li et al. (2019) highlighted mussels as the target species for monitoring MP pollution. Mussels efficiently accumulate MPs and display various MP-induced changes (Fernández and Albentosa, 2019a, 2019b). On the other hand, crustaceans were one of the most studied groups of aquatic organisms in both marine and freshwater ecosystems, followed by molluscs, in a review article covering the ecotoxicological effects of MPs from 2010-2017 (de Sá et al., 2018). The findings obtained from our review and two review papers that considered different invertebrate groups, as examined by Ajith et al. (2020) and de Sá et al. (2018), are generally in agreement about the two most examined groups, however, the use of different databases, the period of published studies, keywords and the number of examined studies could result in disagreement about the most predominantly studied group.

Among the Mollusca and Arthropoda, we found that some groups, such as the Cephalopoda and Insecta (the largest group among invertebrates), were overlooked. Even though the use of cephalopods in research is strictly regulated, their wide use in human gastronomy as well as their economic importance can be one of the reasons to examine their ability to accumulate MPs and the effects that MPs can elicit (Oliveira et al., 2020). The absence of a larger number of studies on insects, mostly terrestrial organisms, is in agreement with the absence of studies of MPs in terrestrial ecosystems in general (see Section *3.1.2.*). Other groups of invertebrates, such as Annelida, Nematoda, Echinodermata, Cnidaria and Rotifera, were less investigated (Fig. 1). The lack of adequate information about the effects of MPs on these groups, together with difficulties in extrapolating the results obtained from laboratory research on ecosystems, prevented us from obtaining precise information for the impact of MPs on the environment. Even though, species from these groups may not have high economical and nutritional values, they play significant ecological roles in food webs (Smith et al., 2018).

Invertebrate species offer many advantages in comparison to vertebrate models, such as a small size body, inexpensive maintenance, ease of breeding, a short lifecycle, high fecundity, year-round spawning, fewer legal restrictions (Matozzo et al., 2016; Burgos-Aceves and Faggio, 2017; Stara et al., 2020). However, the level of bioaccumulation, the mode of exposure to MPs and the effects that vertebrates experience can to some extent differ from invertebrates (Lillicrap et al., 2016). Vertebrates are more complex, long-lived and positioned higher in the food chain, which makes them even more exposed to MP toxicity (due to longer exposure to MPs) (Bhagat et al., 2020). Our results revealed similar interest for studies of invertebrates and vertebrates, however, a greater variety of studied groups has been reported for invertebrates. In vertebrates, most of the studies were conducted on different fish species (182 studies), while other groups were much less studied (mammals 25, birds 10, amphibians 6 and reptiles 5) (Fig. 1). The results of other review articles confirmed fish as most investigated organisms as regards MP pollution (de Sá et al., 2018; Ajith et al., 2020). Fish species tend to be exposed to MPs (through the gills and by water and food ingestion), that produce adverse effects on various biological processes (Garrido Gamarro et al., 2020; Wang et al., 2020). The paucity of data on other vertebrate groups attracted our attention, as the results obtained on fish models in most cases cannot be properly extrapolated to other groups of vertebrates, especially endotherms and terrestrial species. For other ectotherms (amphibians), results showed that larvae of anuran species accumulated MPs that further altered their biological functions (laboratory examinations conducted by Hu et al., 2016; da Costa Araújo and Malafaia, 2020; da Costa Araújo et al., 2020a, 2020b; Boyero et al., 2020, as well as the field study of Karaoğlu and Gül, 2020). All used amphibian species have status according to the IUCN category of Least Concern (LC). In our previous review on the ecotoxicological effects of MPs, we highlighted amphibians as promising model organisms for MP research because of their biphasic life (aquatic and terrestrial) and a complex life cycle (eggs, tadpoles, juveniles, subadults and adults) (Prokić et al., 2019a). Studies on reptiles were conducted in the field on turtles and included only the examination of the ingestion potential, while the information on bioaccumulation and other effects was missing (Colferai et al., 2017; Pham et al., 2017; Caron et al., 2018; Rizzi et al., 2019; Digka et al., 2020 – all investigated species have IUCN status as Vulnerable (VU) or Endangered (EN)). Reptiles, especially long-lived species such as turtles and crocodiles can be used as an efficient model organism to determine how harmful MPs are in a long-term (multiyear) exposure period. For endothermic organisms, we established that in birds as for reptiles, only MP ingestion was evaluated from natural populations on species that are mostly considered as of Least Concern, and only two species as Near Threatened (NT) (Holland et al., 2016; Terepocki et al., 2017; Drever et al., 2018; Provencher et al., 2018; Verlis et al., 2018; Lavers et al., 2019; Masiá et al., 2019; Rossi et al., 2019; Carlin et al., 2020; Le Guen et al., 2020). In birds, the presence of both aquatic and terrestrial ecosystems (where they are often top predators or are near the top of the food chain) make them susceptible to the biomagnification process of MPs, and consequently to the negative effects of MPs. For mammalian species, we noted studies that used standard laboratory model organisms, rats and mice (in 9 studies) in attempts to examine the possible negative consequences of MPs on humans, and on aquatic mammals such as seals and dolphins, which showed the ingestion of MPs under environmental conditions (16 studies in which most of the examined species were considered as LC, with one having the status of NT, two were VU and three were EB). The lower number of studies conducted on some species of higher vertebrates that were limited mostly to ingestion/exposure can be due to ethical reasons and/or legal restrictions. However, the solution for this could be the use of animal laboratory model species or in field studies species that are not endangered (with least concern, according to the IUCN), while in cases of endangered species, information about the effects of MPs should be obtained by opportunistic sampling and non-invasive methods (by taking blood samples, feces, dead animals, or for some species, parts that can be regenerated (lizard tail), including skin biopsy). A new and efficient non-invasive method, scat-based molecular techniques (metabarcoding), allows the quantification of ingested MPs for the purpose of investigating dietary exposure to MPs in predators (Nelms et al., 2019). Pirsaheb et al. (2020) suggested investigation of gastrointestinal microbiota alternation as a suitable and non-invasive approach in the initial evaluation of exposure to MPs. The development of new non-invasive methods especially in determining the possible effects of MPs should be one of the priorities in studying the effects of MPs, even though it would be very challenging to use them under field conditions due to effects of other factors.

3.1.2. Environments – marine, freshwater and terrestrial

Microplastic particles are detected across all environments (water, soil, and air), suggesting that all organisms are or can be exposed to MPs (Phuong et al., 2016). However, analyses of data collected for this review paper showed that the most studied organisms were from marine environments (61.4%), as compared to freshwater environments (26.1%), and that terrestrial organisms were less studied (11.5%). Studying the problem of MPs began with research conducted on marine ecosystems (Huntley et al., 1983). Through the years (from 2011 to May 2020), the number of studies on marine organisms was on a constant rise (Table S1). Marine ecosystems are considered as final sinks for MPs (Alimba and Faggio, 2019), and a wealth of studies on a variety of species (more than 300 different species in 320 studies in this review) have demonstrated that MPs can affect marine biota. Most examined marine organisms were mussel species from the genus *Mytilus* (*M. galloprovincialis* and *M. edulis*) at 12.9% (Table S1).

On the other hand, the effects on freshwater and terrestrial animals are still considered as one of the gaps in investigating MP pollution (O'Connor et al., 2019; Strungaru et al., 2019). Studies on freshwater organisms according to our results started to gain increased attention only from 2018 when we noted 28 studies, and 40 during 2019, and are on constant increase, and up to 50 until May 2020 when we completed data collection. This increased interest in the examination of freshwater organisms was the result of improved knowledge and understanding of the concentration and behavior of MPs in freshwater environments (Ajith et al., 2020). Invertebrates such as *Daphnia* (*D. magna* and *D. pulex*) species were used as model organisms in

70% of all analyzed studies, while among freshwater vertebrates, nearly half of the studies (49.1%) were conducted on zebrafish (*Danio rerio*) (Table S1), highlighting the potential of both species for monitoring the presence of MPs in freshwater ecosystems. The use of zebrafish as a model organism for studying micro- and nano-pollution was suggested in the review by Bhagat et al. (2020). However, the damage of MPs produced on freshwater ecosystems remains poorly understood (Akdogan and Guven, 2019).

Considering that nearly 80% of all MPs in marine and coastal environments are produced, used and often disposed of in terrestrial environments, very little is known about the possible threats of MPs on terrestrial ecosystems (Ng et al., 2018). Our results also evidenced this, as only 65 of 500 analyzed studies dealt with the exposure/effects of MPs on terrestrial organisms. From all studies that involved terrestrial animals, almost all were performed on soil invertebrates (on Nematoda 15 studies were registered, mainly on *Caenorhabditis elegans*, and species that belong to the Annelida phylum with 12 studies, both groups are suggested by the OECD as a good model organism for soil treatment experiments). Transfer of MPs through the terrestrial food chain, and the ecological impact of MPs on soil involves effects that can be observed on various levels (Huerta Lwanga et al., 2016, 2017). Studies on vertebrates are scarce. We observed some vertebrate species that belong to both terrestrial and aquatic environments, such as birds and reptiles. However, because they feed primarily in aquatic environments, authors of analyzed papers these species usually considered in context of MP pollution of aquatic environments. Research on mice and rats can provide possible mechanisms of MP action on terrestrial vertebrates but in all 9 reported studies, they were used as model organisms to examine the potential effects of exposure to MPs (through water and air) on human health. We did not find any study that was conducted on vertebrate organisms that inhabit exclusively terrestrial ecosystems and they were not standard laboratory model organisms. There are some suggestions that terrestrial species may be exposed to levels of plastic pollution capable of shifting the baselines of physiological and ecosystem processes (de Souza Machado et al., 2018). Carlin et al. (2020) evidenced that *Buteo lineatus* (red-shouldered hawk) bird species that consume terrestrial prey had a greater number of MPs in the digestive tract in comparison to fish-feeding osprey (*Pandion haliaetus*).

3.2. Intraspecific characteristics

Intraspecific characteristics (life stage, sex, population size) of model organisms can represent one of the important factors during experimental design. However, they have often been overlooked, even though the obtained results and the whole study can significantly depend on them. Kögel et al. (2020) marked the developmental stage and sex as two of nine factors that can determine the effects of MPs. Their review study included one study on the differences between sexes in fish, and four on the development phases (2 on Crustacean, 1 on Gastropod and 1 on fish species). To our knowledge, there is no comprehensive review study covering the effects of intraspecific characteristics of examined organisms on MP pollution to a greater extent. Therefore, this section was devoted to summarizing the information about life stages and sexes of model organisms used in studying MPs.

3.2.1. Life stages (larval, juvenile and adult)

During their life, organisms undergo changes that depend on the complexity of the examined species. For most of the organisms we could clearly define juvenile (immature) and adult (mature) stages, and for some groups of animals also the larval stage. Our result revealed that the most examined life stage in MP studies was the adult stage with 46% (vertebrates 42%, invertebrates 49%), followed by 18% of studies on larvae (vertebrates 15.3%, invertebrates

24.4%), while 16% of studies were conducted on juvenile/subadults (vertebrates 20.7%, invertebrates 11.1%), and almost 20% of studies examined undefined life stages (vertebrates 22%, invertebrates 18.5%). The comparison between vertebrates and invertebrates revealed a similar distribution of studied life stages. Ten studies included all life stages, and all were performed on invertebrate species because of the short life cycle. However, it would be interesting to gain data on some vertebrate models; we suggest the use of *Xenopus leavis* as it is an aquatic anuran species with a complex life cycle and is an established experimental model. For some of the studies that did not provide information about the life stage of the organism, the authors provided some basic biometric parameters (body length and size) that could be used as an indicator of the life stage. During ontogenetic stages, organisms may differ in the rate of MP bioaccumulation and their response to MP contamination (Alomar et al, 2017; Steer et al., 2017; Bernardini et al., 2018; Eltemsah and Bøhn, 2019; Luan et al., 2019; Pannetier et al., 2020). For example, body growth that organisms experience during life is one of the obvious factors that can affect the accumulation rate of MPs. This could mean that a larger body size observed in adults or older individuals requires a greater amount of food intake and consequently MP bioaccumulation. Significant positive correlations between body size and ingested MPs were reported in freshwater fish (Horton et al., 2018), and for plastic particles in a long-term study on seabirds (Spear et al., 1995); in contrast, lower intake or increased elimination capacity in larger individuals were reported for loggerhead turtles (Rizzi et al., 2019). Ontogenetic changes in some species are also followed by a change in food source or even the entire lifestyle as is the case in amphibians (Prokić et al., 2018). During early development and/or specific states such as reproduction, metamorphosis and hibernation, individuals tend to respond in a different manner to external stressors (Gavrić et al., 2017; Prokić et al., 2017, 2019b).

Differences between the life stages and the effects of exposure to MPs can be mainly the result of differences in feeding ecology and strategy. After a careful review of studies that were performed at more than one life stage, we considered only those in which it was clearly stated which stage was more sensitive to the effects of MPs. A total of 16 papers met the above criteria. Comparisons between the two stages were presented in 14 studies, while only 2 papers compared all life stages within the same species. The uptake of MPs (mainly by ingestion) was examined in 11 studies (7 field and 4 laboratory); in 2 studies there were no significant differences between juveniles and adults, 3 adults ingested more MPs than juveniles, 5 juveniles ingested more MPs than adults and in one study, larvae ingested more MPs than adults. A higher tendency of MP ingestion at younger life stages than in adults can be explained by the higher demands for energy necessary for development and growth that increase the pressure on individuals at earlier stages to search for and consume greater amounts of food, together with a lower experience with differ preys and MPs that can lead to increased ingestion of MPs. For example, juvenile *Galeus melastomus* and *Prionace glauca* sharks were found to ingest significantly higher quantities of MPs than adults (Alomar and Deudero, 2017; Bernardini et al., 2018). Terepocki et al. (2017) investigated the ingestion of MPs by seabirds and showed that 85% of juveniles and only 41.7% of adults contained plastic. Starting from higher ingestion of MPs at earlier stages and taking into account that they are not fully developed (e.g. xenobiotic decontamination enzymes in the liver/hepatopancreas), it could be expected that earlier stages (larvae, juvenile) would not be able to cope with MP pollution as well as adults. MPs can block the gastrointestinal tract and cause an insufficient supply of nutrients and energy to organisms, affecting somatic growth and/or metamorphosis (Messinetti et al., 2018); altered metabolic rates and MPs induce ROS production that can damage biomolecules (Choi et al., 2020), while sensitive (undeveloped) immunity together with MPs lower the immune response of individuals at earlier stages, making them more susceptible to infections (Burgos-Aceves et al., 2020); the ability of accumulated MPs to alter behavior can affect the hunting behavior or the ability of prey to detect and avoid predators (Yin et al., 2019). The effects of MPs were examined in 5 laboratory studies (from a total of 16 studies). In all investigations of effects, differences between stages were detected. The higher sensitivity of the earlier stages than of later stages was noted in studies; in 2 studies, the larvae were more sensitive than juveniles, in 2 studies the larvae were more sensitive than the adults, and in one study, juveniles were more sensitive than adults. For example, Eltemsah and Bøhn (2019) reported that juveniles of *Daphnia magna* exhibit a higher sensitivity to MPs than adults. Environmental MPs induced higher toxicity effects in larvae than in juveniles of Japanese medaka fish (Pannetier et al., 2020). Based on the analyzed data, it could be concluded that the earlier stages were more prone to the effects of exposure to MPs. The importance of such studies is reflected in the fact that the impact of MPs that was manifested at earlier life stages could cause negative consequences at the latter adult stage, and also modify the population dynamics (Steer et al., 2017; Luan et al., 2019). Future comparative studies should address the impact of MPs with life-stage-related aspects to a greater extent.

One of the dangers that exposure to MPs can have is not only the ability to bioaccumulate and alter biological processes in animals and humans, but also to exert long-term influence on offspring and induce chronic diseases in adulthood. Two studies provided shreds of evidence of possible maternal (generational) transfer of micro and nanoplastic to offspring (Pitt et al., 2018; Luo et al., 2019). Assessment of F1 embryos and larvae of zebrafish individuals revealed accumulated nanoplastic particles in the yolk sac, gastrointestinal tract, liver and pancreas of maternally and co-parentally exposed F1 embryos/larvae. The transferred nanoplastic did not lead to major physiological disturbances in offspring under laboratory conditions, however, a more detailed examination of other physiological endpoints is warranted in order to establish the risk of exposure to nanoplastics (Pitt et al., 2018). In another report, the effect of MPs on offspring, a multigenerational effect, showed that exposure of pregnant mice to MPs induced metabolic disorders in offspring, providing basic insight into the potential relationship between MPs and health risk even in the next generation (Luo et al., 2019). The exact mechanisms and all effects of MP transfer through generations remain to be investigated.

3.2.2. Sex differences

One of the intraspecific aspects that are generally neglected in studies on MPs are sex differences and their possible effects on the responses of different species to pollution with MPs. In this study, we observed that only 30 studies determined the sex of the investigated model organisms (12 papers with segregated sexes, in only 11 males were used, and in only 7 females were used). This implies that most of the authors that investigated MPs did not take into account possible sex differences, even though meta-analyses conducted on some parameters (physiological and immunological) between sexes, revealed significant sex-specific responses for most vertebrate species (Costantini, 2018; Kelly et al., 2018). Use of sex pooled data (when statistical analyses confirmed sex differences in the examined trait), or the use of just one sex can possibly lead to an inaccurate picture of species' response to MPs. The pooled average phenotype, which is a by-product of the mean response of both male and female phenotypes, does not exist in nature and is not realistic (Bennett, 1987; Cripps et al., 2014; Ellis et al., 2017). Overall, this can create a false basis for conclusions on how biological systems work under exposure to MPs. In defense of some authors, the investigation of male/female differences can in some species have its limitations. Sexual dimorphism exists in many adult organisms but for some invertebrates (e.g. Bivalve), morphological distinction can be unreliable (Yusa, 2007), and also there are issues with hermaphroditic species.

The initial assumptions for possible differences between sexes in MP ingestion and subsequent responses can be due to differences in characteristics, such as body size and/or behavior. Sex together with a larger body needs to consume more food because of increased energy demands, which increases the potential for ingesting MPs. Differences in behavior, the example of species with sex-trophic segregation niches (a behavior observed in some fish, birds and mammalian species) (Catry et al., 2005; Scharnweber et al., 2011; Kernaléguen et al., 2015), or with a more sedentary behavior of one sex (characteristic of some lizard species) (Olsson et al., 2012), could lead to unequal exposure to MPs. A study conducted on king penguins showed that different behaviors of three groups of individuals, incubating, chick-rearing and non-breeding birds, affected MP ingestion (Le Guen et al., 2020). The data regarding possible differences between the sexes in terms of MP exposure and effects were investigated only in a few studies (11 identified papers). The ingestion of MPs in different invertebrate and vertebrate species was examined in 9 studies (8 field and 1 laboratory study). No gender-dependent differences in MP ingestion were reported in sharks (Alomar and Deudero, 2017; Bernardini et al., 2018), Norway lobster (Murray and Cowie, 2011) and common periwinkle (Doyle et al., 2019) in field observations, as well as copepods in a laboratory study (Vroom et al., 2017). However, 4 studies on wild fish and crustacean populations revealed that females contained higher levels of MPs than males, probably due to gender-dependent differences in body size, energy requirements, gastrointestinal tract structure and/or feeding behavior (Welden and Cowie, 2016; Bordbar et al., 2018; McGoran et al., 2018; Su et al., 2019). Based on the analyzed studies, it could be concluded that females have a higher tendency to ingest MPs in the field than males, but significantly more research is needed to confirm this. The higher the number of MPs ingested by females of some species, may produce greater adverse health effects in comparison to males. However, in response to MPs, differences in physiological and immunological functions between sexes need to be taken into consideration. As for data dealing with the distinction between genders regarding the direct effects of MPs, only 2 laboratory studies were conducted (Wang et al., 2019a; Park et al., 2020). Both studies showed some sex differences in response to MPs (pathological changes of tissues and gene expressions), however, it is difficult to draw any general conclusions from such a small number of studies. Given the possibility of sex-dependent differences in the exposure/effects of MPs, it is important to provide information on the sex of the animals in laboratory studies and to determine the ratio between genders in the field samples to avoid premature conclusions. Unfortunately, the lack of sufficient information in this area limits our knowledge about sex-specific responses to this rising threat. Greater efforts are needed to weigh up the influence of sex throughout an organism's life cycle and its contribution to the variability of species-level responses to MPs.

3. 3. Experimental approaches

3.3.1. Laboratory and field studies – overall ratio and ratio by groups

Most studies concerning the impact of MPs on animals have been performed in the laboratory. The number of publications we considered from January 2011 to May 2020 revealed that 60.16% of reviewed articles were laboratory-based studies, while 39.84% were field investigations (Fig. 2). However, a significantly different ratio between laboratory and field research within different animal groups was observed.

The reported trend on the overall study sample was primarily the result of research on invertebrate species performed under laboratory-controlled conditions in comparison to studies in the field (Fig. 2). Laboratory studies were more numerous in all groups of invertebrates compared to field studies (Fig. 2). For example, when considering the most investigated group of invertebrates – Arthropods, studies in the laboratory (81 articles) are about 4 times more numerous than environmental-based studies (21 articles). In the least investigated groups of invertebrates, only a few (Annelida, Cnidaria, Echinodermata) or no studies (Nematoda and Rotifera) on the impact of MPs in the field were found in 500 papers that we analyzed. Invertebrates were recognized as excellent animal models for laboratory investigations of the effects of MPs (Maes et al., 2020; Ribeiro et al., 2017), but in order to obtain a complete picture of the impact of MPs, it is necessary to expand future research to studies in natural conditions. Another reason why the number of field studies on invertebrates should be increased is the information that many invertebrate species served as appropriate indicators to assess the negative effects of MPs in the environment (Thushari et al., 2017; Nel et al., 2018; Qu et al., 2018).

There is around a 1:1 numerical ratio between laboratory and field studies in vertebrates (Fig. 2). This result is primarily due to numerous studies on specific fish species. It is important to point out that no study on the impact of microplastics on birds and reptiles in laboratory conditions was found in the reviewed literature. This may be related to restrictions for the use of different vertebrates in laboratory research. A lack of studies on field-collected frog species was also observed (only one study) (Karaoğlu and Gül, 2020). Filling these gaps in science using ethically approved approaches would provide valuable data on the effects of microplastics on these less investigated but highly endangered vertebrate groups.

3.3.2. Laboratory vs. field studies – lack of comparative-corroborating studies

Regardless of the type of study, it is important to point out that both laboratory and field research on the impact of MPs on animals have their advantages and disadvantages. Laboratory-controlled studies report experimental exposure and/or their effects that are crucial to examine the mechanisms of microplastic toxicity (Espinosa et al., 2017; Liu et al., 2018). However, there is the question of whether they can reflect the actual influence of MPs present in the natural environment. Laboratory studies usually do not have a sufficiently representative approach due to the limitation of the used form (in the context of shape, size, type, particle mixture, age-modification), and the concentrations of MPs that are elevated but not environmentally relevant (Phuong et al., 2016; Ribeiro et al., 2017). Therefore, more effort is needed to include natural-like MPs in laboratory investigations (Vroom et al., 2017; Qu et al., 2018). Furthermore, laboratory studies rarely take into account the possible modification of MP toxicity caused by other factors (biotic and abiotic) present in the environment, which makes it difficult to assess the actual toxicity (Aljaibachi et al., 2020; Maes et al., 2020). Although an environmental-like complex system cannot be duplicated in the laboratory, future studies should be aimed at achieving the most representative conditions (Qu et al., 2018).

Field studies mainly report on environmental exposure through different types of uptake, primarily ingestion. Studies that have addressed the toxicological effects potentially obtained after exposure to MPs in wild conditions are rare (Barboza et al., 2020; Carreras-Colom et al., 2020; Li et al., 2020). However, we should be careful in making conclusions on organisms for natural populations as they are also exposed to multiple factors that can influence their responses to MPs (Barboza et al., 2020; Carreras-Colom et al., 2020). Previous data indicated that organisms in the environment face more emergent situations than in laboratory-controlled conditions. As carriers or vectors of different types of contaminants (including heavy metals and organic xenobiotics), MPs act as multiple stressors in natural populations (Vethaak and Leslie, 2016; Wang et al., 2016; Fossi et al., 2017). The main problem of environmental studies is that co-exposure to the variety of the factors present in the environment may lead to underestimation or overestimation of the level of actual MP toxicity (Ferreira et al., 2019). Comparing organisms from the same habitat that contain MPs with those without MPs is a promising approach aimed at defining appropriate biomarkers of pollution with MPs (Barboza et al., 2020). Also, comparative studies on animals from sites where MPs are the predominant pollutant with those from a reference site can contribute to that goal (Li et al., 2020). Even in these cases, the authors indicated that the impact of compounds other than MPs cannot be excluded. To recognize other contaminants that might contribute to MP uptake/toxicity, it is crucial to perform comprehensive multi-year and multi-factor monitoring studies that will enable adequate modeling of the overall effects of MPs. However, this scientific challenge has yet to be filled.

A major challenge in examining the effects of MPs is conducting comparative-corroborating laboratory and field investigations (or at least mesocosm systems) with parallel exposure. We observed that a very few studies (only 2 from the total number of articles identified) dealt with this issue. Qu et al. (2018) investigated microplastic pollution in two mussel species and reported significant differences in results between laboratory and field observations. Even though the concentrations of MPs in water was higher in the laboratory than in the environment, the abundance of MPs by weight in mussels from the field was higher than in laboratory-exposed animals. It should not be overlooked that the abundance of MPs in mussels depends highly on the methods used. Differences in the proportions of the different shapes of MPs in mussels between laboratory and field samples were also observed. The authors concluded that more effort is needed to simulate natural conditions in the laboratory. Aljaibachi et al. (2020) used laboratory vs. seminatural conditions (laboratory-field linking mesocosm) and revealed that laboratory-based studies of the effects of MPs on *Daphnia magna* fitness parameters did not predict the responses in the environment. To perform comparative laboratory/field studies, it is necessary to meet several conditions. Field studies should include the measurement of contaminants other than MPs, while laboratory-controlled experiments should be more complex and aimed at clarifying the links between the uptake/effects of MPs and environmental variables. Parallels between laboratory experiments and field investigations can reveal shortcomings in both types of studies. Not only increasing the number, but also improving comparative laboratory-field research should be the primary scientific goal that will enable the assessment and understanding of the real-life impact of microplastics. Enhanced comparative laboratory-mesocosm-field studies will be an interesting setup for future analyses and perhaps the only solution for clarification of the actual effects of MPs.

3.3.3. Field studies – spatial comparison

Locality-dependent differences of microplastic-related parameters on field-collected animals were reported in 71 reviewed articles (out of a total of 202 studies in the field). The abundance of microplastics in nature revealed high spatial variations that depend on the natural and anthropogenic influences connected to the proximity of urban and industrial areas (Rodrigues et al., 2018; Yao et al., 2019). Thushari et al. (2017) noted that the accumulation rates of MPs in different field-collected marine invertebrate species varied depending on the location, and correlated with the degrees of anthropogenic input. Qu et al. (2018) also showed that the abundance of MPs in mussels depended on their concentration in the water. Similar results on MP ingestion were obtained for different fish species (McGoran et al., 2018). Zitouni et al. (2020) reported that MP-induced cellular toxicity in the gastrointestinal tract of marine fish was locality-dependent. Microplastic-related spatial differences in the responses of oxidative stress biomarkers were also observed in the skin of fin whales (Fossi et al., 2016). Considering the above, it is important that comparative environment-based studies include a wide range of localities for a more accurate estimate of the biological impact of MPs in the field.

3.3.4. Field studies – short-term vs. long-term – lack of temporal comparison

Short-term studies on the exposure/effects of MPs in the environment are more numerous in both vertebrates and invertebrates (58.59% and 63.01%, respectively), than long-term studies (41.41% and 36.99%, respectively). This trend was noted in all groups of invertebrates, while in vertebrates it was more pronounced in fish as the most studied vertebrate group. The constant overload of the environment with MPs as highly persistent compounds indicates that long-term exposure is almost the only one present in nature (Beer et al., 2018; Maes et al., 2020). Wild organisms are exposed to MPs not only throughout their lifespan but in succeeding generations. However, the possibility of conducting long-term field studies depends on several factors: they are much more time and resource demanding than short-term studies; during certain periods of the year it is difficult to collect samples from the field due to various external and unpredictable factors; study performance is highly dependent on species developmental characteristics. Despite the number of long-term studies and their aim to present the most realistic influence of MPs on natural populations, there is a seasonal aspect that was often overlooked and therefore will be briefly discussed.

Previous examinations indicated that MP abundance in nature displayed high temporal variations that depended on a large number of factors, such as seasonal and other natural conditions as well as anthropogenic influences (Rodrigues et al., 2018; Yao et al., 2019). The feeding behavior/activity of aquatic organisms was also dependent on seasonality (Thushari et al., 2017; Beer et al., 2018). All these seasonally conditioned factors must be considered when drawing conclusions about the levels of MP ingestion/bioaccumulation in different organisms during long-term monitoring of environmental conditions (Ferreira et al., 2019).

After analyzing all field studies, in only 12 papers samples from different seasons were compared and potential seasonal influences on the investigated parameters affected by MPs were assessed. All studies were performed on aquatic organisms in which the level of MP ingestion/accumulation were primarily examined. Only 3 studies covered the whole year (Bordbar et al., 2018; Ferreira et al., 2018; Ferreira et al., 2019), with sampling performed on a monthly basis, while 9 studies compared 2 or 3 seasons (with one or more months per season) (Devriese et al., 2015; Welden and Cowie, 2016; Beer et al., 2018; Catarino et al. 2018; McGoran et al., 2018; Nel et al., 2018; Phuong et al., 2018; Carreras-Colom et al., 2020; Zheng et al., 2020). Significant differences among animal samples between seasons were reported in 11 articles. Given the small number of studies (including lack of data for all seasons), because of differences between climatic zones, as well as the large number of factors influencing the level of MP uptake, it was difficult to draw general conclusions. However, based on the analyzed papers, two main season-dependent factors that lead to differences in the level of MP contamination in animals over the year were defined, including the rate of feeding activity and the input of MPs into the ecosystem (e.g. fishing activities, freshwater runoff). It was shown that fish caught in the summer ingested more plastic particles compared to the winter (McGoran et al., 2018) and spring (Beer et al., 2018), which may be explained by their higher feeding activity in the summer. Devriese et al. (2015) observed seasonal effects on MP contamination in brown shrimp *Crangon crangon* (higher uptake in October compared to March) as the consequence of seasonal fluctuations of food uptake as well as different patterns of MP levels in the water. In areas with dry and rainy seasons, aquatic animals were more exposed to MPs during the rainy season due to the higher input of MPs (Ferreira et al., 2018, 2019; Zheng et al., 2020). The situation is further complicated in field research concerning various biomarkers of different effects, from subcellular to organismal. Numerous time-varying factors other than only MPs could trigger a response of different non-specific biomarkers and/or even altered microplastic toxicity (Alomar et al., 2017). For example, the basic physicochemical properties of the environment were shown to influence a variety of biomarkers (Gavrić et al., 2015). Therefore, if long-term studies on the effects of MPs do not consider the seasonal aspect, conclusions will not be drawn correctly.

Taking into account the above, future field studies on the impact of MPs on animals should be directed not only towards increasing the number of long-term investigations but also their improvement in terms of monitoring temporal changes.

3.3.5. Laboratory studies – acute vs. chronic exposure

Over the past decade, a large number of laboratory-based studies of the effects of both acute and chronic exposure have been conducted in order to investigate the impact of MPs on various animal groups and to clarify the mechanisms of MP toxicity (Espinosa et al., 2017; Liu et al., 2018; Park et al., 2020). When all reviewed laboratory-based investigations in our study were considered, we observed higher percentages of acute (58.10%) in comparison to chronic (41.90%) studies. For vertebrate and invertebrate models, 61.54% and 56.64% were acute studies, respectively, and 38.46% and 43.36 % were chronic studies, respectively.

Acute studies are faster and easier to perform and represent a starting point in determining the potential toxicity of MPs, therefore this can be one of the reasons why there is a greater number of acute studies in comparison to chronic. Even though acute exposure of animals to MPs is useful for explaining some of the mechanisms of toxicity, there are significant differences in the assessment of MP toxicity between acute and chronic laboratory studies, (acute studies usually consider many fold higher concentrations than detected in the environment). Ribeiro et al. (2017) evaluated the effects of MPs in tissues of the clam *Scrobicularia plana* in laboratory conditions for 14 days and showed that genotoxicity increased with time. Time-dependent toxic effects were also obtained on fish larvae chronically exposed to environmental MPs (Pannetier et al., 2020). Maes et al. (2020) pointed out significant differences in the impact of MPs between acute/subchronic and long-term chronic studies.

Despite a higher number of acute studies, chronic studies on both vertebrate and invertebrate models should be favored because of the more faithful simulation of environmental conditions and possible equivalence with environment-based investigations. Previous sections of this paper also indicated that all types of studies (including laboratory, acute and chronic) should proceed in the direction of the examination of less-studied animal groups and biological parameters/biomarkers.

3.4. Ingestion and tissue bioaccumulation of MPs

Once MPs are introduced into the environment, they begin to interact with biota, primarily the consequence of ingestion by a variety of organisms that cannot distinguish between food and plastic. After uptake, MPs can be translocated from the intestine to other tissues and organs (Browne at al., 2008). Therefore, the ingestion and bioaccumulation of MPs provides a useful and necessary starting point in the investigation of harmful outcomes of exposure to MPs. Microplastic uptake, retention and translocation to tissues are not only related to particle characteristics (type, size, density and shape), but it might also vary with the taxon, feeding mode and the habitat of the exposed organisms (Browne et al., 2008; Jabeen et al., 2018).

A total of 253 (51%) studies reported the ingestion of MPs and/or their accumulation in the digestive tract, while bioaccumulation in other tissues was documented in 182 (28%) studies (for more information see Supplementary Table and Section 3.5). In 82 invertebrate-related, and in 114 vertebrate-related studies, the ingestion and bioaccumulation were examined without assessment of any other MP-associated effects at the subcellular, cellular, individual or population levels. The ingestion and bioaccumulation of MPs were reported across various groups of invertebrates (99 and 110 studies, respectively), and vertebrates (154 and 72 studies, respectively). Among invertebrates, MP uptake and bioaccumulation were the most studied in Mollusca (33 and 66 studies, respectively) and Arthropoda (42 and 25 studies, respectively). In the study that investigated the occurrence of MPs in a total of 38 invertebrates, including gastropods, bivalves and crabs collected on mudflats and sandy beaches in Hong Kong, MPs were found in 32 species with the highest abundance in gastropods (Xu et al., 2020). Even though there is a lack of data related to terrestrial species, it is expected that MPs also interact with soil invertebrates (Dioses-Salinas et al., 2020). Earthworms could be highly exposed to MPs as they burrow through and ingest soil (Prendergast-Miller et al., 2019). Fluorescence imaging pointed to the ingestion of polyethylene and polystyrene particles by *Eisenia fetida* (Wang et al., 2019b). The observed increase in burrowing time of *Hediste diversicolor* exposed to polystyrene may have relevant implications in the ecosystem considering the important ecological role of polychaetes in soil and sediment bioturbation (Silva et al., 2020). Except by direct swallowing, MPs could also be ingested and accumulated from prey to predator through trophic transfer. Moreover, in predators, trophic transfer is the dominant route of MP uptake rather than direct ingestion (Nelms et al., 2018). Zhang et al. (2019) showed MP transfer from marine crustaceans to several fish species and concluded that fish at a higher trophic level likely accumulated MPs, making them convenient MP indicator species. We observed that all examined vertebrate groups displayed a tendency to ingest MPs, however, except for studies on fish (121 studies), there were relatively few studies on other vertebrate groups (4 on amphibians, 5 on reptiles, 9 on birds and 15 on mammals). According to Carlin et al. (2020), 55% of investigated birds associated with freshwater habitats and 56% of seabirds had microplastics in their stomachs. Bioaccumulation was documented in fish (58 studies), amphibians (6 studies), birds (1 study) and mammals (6 studies), while for reptiles there was no data.

Based on our examination of scientific literature, it can be concluded that MP ingestion and accumulation in the digestive tract has been largely investigated, but little is known about MP translocation from the intestine to other organs. Due to their smaller size, invertebrates were usually examined in a whole-body distribution pattern (Rist et al., 2017; Nel et al., 2018; Patterson et al., 2019), and therefore bioaccumulation was reported slightly more often in relation to ingestion. In vertebrates, the number of studies that provide evidence of MP ingestion is twice as high as evidence of their accumulation. Investigations of tissue accumulation are important because they have implications for the physiological effects and other harmful consequences of the consumption of plastic debris. There is also growing concern regarding human health since it was confirmed that plastic debris present in the environment can be ingested and then retained in the tissues of many species widely used in the human diet (Barboza et al., 2018). However, limited information and a research gap in this field are noted. For example, although muscle is the most edible part of fish, only a few studies examined its content of MPs (Akhbarizadeh et al., 2018; Fang et al., 2019; Akoueson et al., 2020; Zitouni et al., 2020).

3.5. Report of tissues analyzed in studies with MPs

The presence of MPs in biological samples is usually estimated via digestion of tissues and following analysis of filtrates. It was shown that the KOH digestion technique is suitable for a range of tissues (Thiele et al., 2019). Relating to the accumulation and/or the effects of MPs, invertebrates are generally investigated in whole (whole bodies were analyzed in 82 studies and soft tissue of Bivalvia, Gastropoda and Crustacea in 53 studies). The gastrointestinal tract and gills as the main routes through which organisms can incorporate plastic debris (de Sá et al., 2018; Schirinzi et al., 2020), and they were analyzed in 73 and 23 studies, respectively. Other investigated tissues included the hepatopancreas/digestive glands/liver (26), hemolymph (20), muscle (11), mantle (6), gonads (5), kidneys (1), and the coelomic fluid and epidermis (1). Among the reports of MPs in vertebrate species, the gastrointestinal tract was the most commonly studied tissue (161 studies), followed by the liver (45 studies), the whole body (32 studies), the gills (30 studies), muscle (20 studies), blood (16 studies), brain (16 studies), kidneys (13 studies) and gonads (9 studies). There are relatively few studies of other tissues - skin (Espinosa et al., 2017, 2019; Fossi et al., 2016, 2017; Abbasi et al., 2018; Brandts et al., 2018; Feng et al., 2019), spleen (Karbalaei et al., 2019; Mancia et al., 2020; Park et al., 2020; Zhu et al., 2020), heart (Pitt et al., 2018; Karbalaei et al., 2019; Park et al., 2020), bladder (Oliveira et al., 2013; Yin et al., 2018; Batel et al., 2020), lungs (Park et al., 2020), nervous tissue (Borysov et al., 2020), the uterus (Park et al., 2020) and thymus (Park et al., 2020). Thus, there is a need to better understand the distribution and accumulation of MPs across different tissues in order to reveal their potentially adverse consequences. The widely accepted assumption is that MPs are mostly present in the gastrointestinal tract; indeed, because of its quick and easy dissection and the subsequent reduction in mass of the tissue to be analyzed, it is frequently used in MP studies, especially those related to larger animals (Bour et al., 2018). Investigations of stomachs and intestines are relevant for microplastic >0.5 mm in size, as these particles do not readily pass through the gut wall (Lusher et al., 2017). Feces were also used to ascertain not only the consumption of MPs but also their fate in organisms of both invertebrates (5 studies) and vertebrates (7 studies).

4. Conclusions

After reviewing 500 representative articles that examined the ingestion/accumulation and effects of MPs on animals, we provide some general future recommendations for studies which should be followed to fill the gaps in scientific knowledge relevant to this worldwide problem.

- To obtain a complete picture of the impact of MPs on the environment, more information about less investigated but not less important groups of Mollusca (e.g. Cephalopoda) and Arthropoda (e.g. Insecta), as well as almost all other invertebrate groups (Annelida, Nematoda, Echinodermata, Cnidaria, Rotifera) is necessary (respecting all regulatory restrictions). Considering the vertebrates, all main groups (amphibians, reptiles, birds, and mammals) except fish, are still insufficiently researched (probably due to ethical reasons and the more restricted research from a legal standpoint), especially in the context of the effects of MPs. A solution for this could be the development and usage of animal laboratory model species or in field studies the use of species that are not endangered or are considered as least concern according to IUCN, while in cases of endangered species, information about the exposure/effects of MPs should be obtained by opportunistic sampling and non-invasive methods (taking blood samples, feces, dead animals).
- Most studies on the impact of MPs are based on organisms associated with the aquatic environment. There is a need to expand the knowledge on freshwater and especially

terrestrial organisms. The lack of studies on terrestrial vertebrates that are not standard laboratory models prevents the acquisition of valuable information identifying the potential risk to human health.

- Future studies should include data about intraspecific characteristics of organisms as an important factor in assessing the impact of MPs. More comparative studies examining differences in the responses to MP exposure between life stages as well as different sexes are also required to obtain accurate conclusions on species' susceptibility. At present, results on a very restricted number of studies (16 for differences between life stages and 11 for sex differences) suggested that earlier life stages and females displayed a tendency to ingest more MPs and to be more sensitive to MP pollution.
- Increasing the number of comparative laboratory-field investigations with parallel exposure should be an important future scientific target that will enable the assessment and improve our understanding of the real-life impact of microplastics.
- Future long-term field studies should be more complex in their approach as regards the time dynamics of the investigated biological parameters and their dependence on the seasonal aspect of MP occurrence, as well as other environmental factors.
- MP-related monitoring should not be limited to the gastrointestinal tract but should also include a variety of other tissues and organs. One suggestion for the examination of the accumulation of MPs is the study of tissues of animals used for human nutrition (e.g. fish muscle) in view of their potential importance in human health.

By reviewing the representative literature dealing with the exposure/effects of MPs on animals, it can be concluded that the impact of MPs needs further comprehensive research. We hope that this review will help researchers to choose appropriate animal models and experimental approach in order to significantly expand knowledge about the risk that MPs pose to the living world.

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Conflict of interest

All authors declare no conflict of interest.

Credit author statement:

Marko D. Prokić: conceptualization, formal analysis, investigation, data curation, writing the original draft; **Branka R. Gavrilović**: investigation, writing the original draft; **Tijana B. Radovanović:** investigation, writing the original draft; **Jelena P. Gavrić:** investigation, writing the original draft; **Tamara G. Petrović**: investigation, writing, review and editing, visualization; **Svetlana G. Despotović:** investigation, writing, review and editing; **Caterina Faggio**: supervision, project administration, writing, review and editing.

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Figure 1. Invertebrates and vertebrates (given in percentages) and taxonomic groups (given as the number of studies) of model organisms used in studies of MPs.

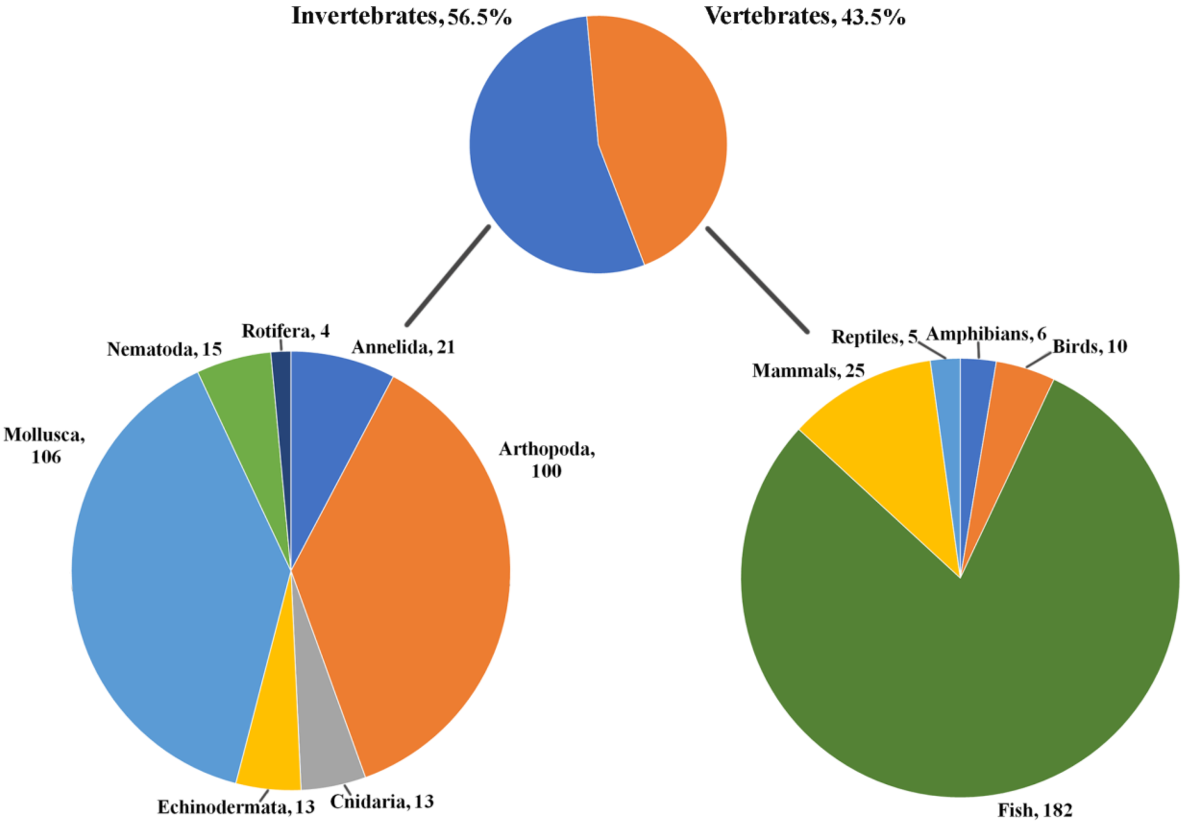
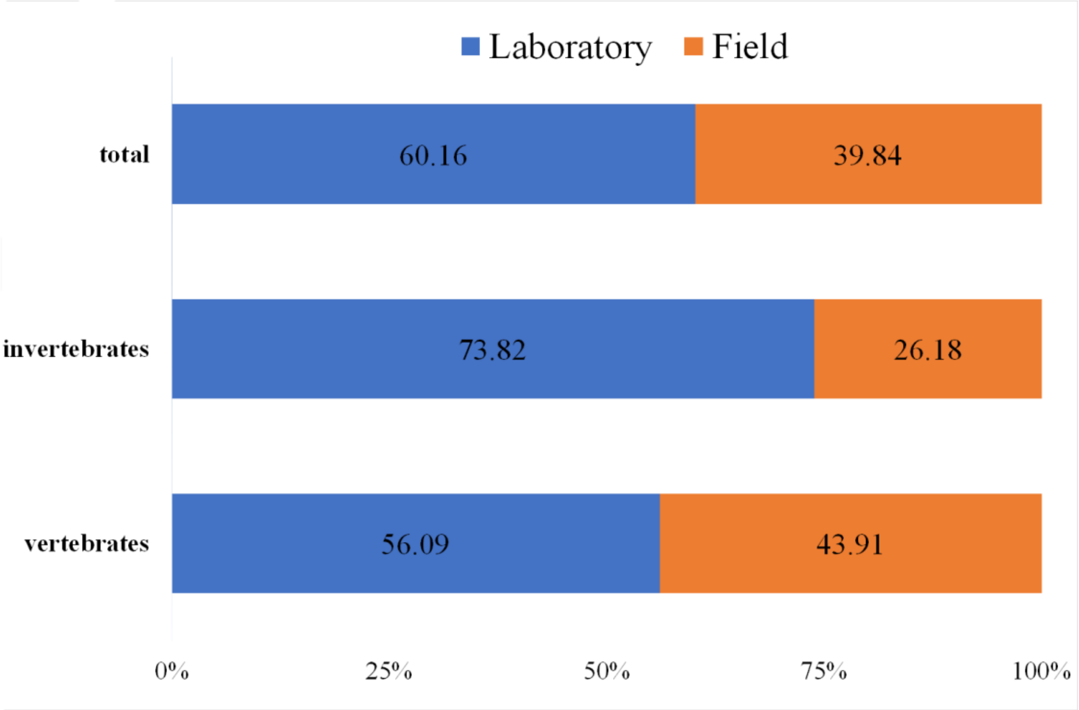


Figure 2. The ratio between field and laboratory investigations in total, for invertebrates and vertebrates in studies of MPs.



Graphical abstract