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Microplastics and Nanoplastics (2022) 2:20
https://doi.org/10.1186/s43591-022-00040-4

Microplastics and Nanoplastics

RESEARCH Open Access

Characterizing microplastic hazards: which concentration metrics and particle characteristics are most informative for understanding toxicity in aquatic organisms?

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Abstract

There is definitive evidence that microplastics, defined as plastic particles less than 5 mm in size, are ubiquitous in the environment and can cause harm to aquatic organisms. These findings have prompted legislators and environmental regulators to seek out strategies for managing risk. However, microplastics are also an incredibly diverse contaminant suite, comprising a complex mixture of physical and chemical characteristics (e.g., sizes, morphologies, polymer types, chemical additives, sorbed chemicals, and impurities), making it challenging to identify which particle characteristics might influence the associated hazards to aquatic life. In addition, there is a lack of consensus on how microplastic concentrations should be reported. This not only makes it difficult to compare concentrations across studies, but it also begs the question as to which concentration metric may be most informative for hazard characterization. Thus, an international panel of experts was convened to identify 1) which concentration metrics (e.g., mass or count per unit of volume or mass) are most informative for the development of health-based thresholds and risk assessment and 2) which microplastic characteristics best inform toxicological concerns. Based on existing knowledge, it is recommended that microplastic concentrations in toxicity tests are calculated from both mass and count at minimum, though ideally researchers should report additional metrics, such as volume and surface area, which may be more informative for specific toxicity mechanisms. Regarding particle characteristics, there is sufficient evidence to conclude that particle size is a critical determinant of toxicological outcomes, particularly for the mechanisms of food dilution and tissue translocation.

Keywords: Microplastic, Nanoplastic, Aquatic organisms, Toxicity, Particle size, Food dilution, Tissue translocation

Introduction

By weight, macroplastics make up the greatest percentage of plastic floating in our oceans; however, by count, microplastics are by far the most numerous [1, 2]. Here, we define microplastics in accordance with the California State Water Resources Control Board as any plastic particle between 1 nm and 5 mm in size [3]. As a contaminant suite, microplastics incorporate a large diversity of plastic types, morphologies, and sizes alongside added chemicals and impurities [4, 5]. Microplastics include small particles that were produced as such (e.g., microbeads, pre-production plastic pellets) and those that are degraded pieces of larger plastic products (e.g., tire dust, microfibers; [5]. This diverse contaminant suite is no longer considered just a marine pollutant, and it is now recognized that microplastics are found in all

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ecosystems—including freshwater, terrestrial, and atmospheric [6, 7].

The ubiquity and broad size range of microplastics make them bioaccessible to hundreds of species across food webs [8]. In aquatic organisms, ingested or inhaled small microplastics (< 100 μm) may translocate across cell membranes into the circulatory, lymphatic, respiratory, and/or other physiological systems [9, 10]. Microplastics have been shown to cause a variety of sub-lethal and lethal effects, potentially harming individual organisms, populations, and communities [11].

Adverse biological effects evidenced in the literature have led to concerns regarding the health of ecosystems exposed to microplastics [12], prompting governments and institutions including those in the United Kingdom, European Union, and Canada to release reports aimed to address plastic pollution [13–15]. In the United States, the state of California passed Senate Bill 1263, mandating the development of a Statewide Microplastics Strategy [16]. Key components of the strategy include a research plan and framework to develop risk assessments in coastal habitats. Although such frameworks exist for many environmental contaminants, they cannot be directly translated to microplastics due to the contaminant suite's complexity and diversity [5].

Microplastic toxicity is multivariate, driven by many biological factors and particle features (e.g., size, morphology, density, volume, chemical additives, polymer type) [17]. Moreover, many risk assessment frameworks report effect-based thresholds as a mass concentration (e.g., dissolved chemicals), or particle metrics such as particle count, surface area, or aspect ratio (e.g., airborne particulates, silica, asbestos, (engineered) nanomaterials) [18]. For a diverse group of materials such as microplastic, the accuracy of concentration metrics is surrounded with uncertainty. Because microplastics are insoluble particles by definition, toxicity may be related to mass, count, or other metric(s) such as volume or surface area which have been recognized in other fields of particulate research but only recently for microplastics [19, 20]. In the literature, microplastic toxicity data is reported most often in mass or count, with no standard metric yet defined. The current recommendation is to report both metrics [21] and to provide detailed descriptions of key particle characteristics (e.g., morphology, density) so that conversions may be made with confidence [22]. At the same time, environmental data is generally reported in count concentrations for microplastics. A framework is needed that incorporates the mechanism(s) of toxicity (e.g., physical, chemical) and is comparable to environmental monitoring data.

Here, we aim to inform the California Statewide Microplastics Strategy by using the existing scientific literature to identify 1) which concentration metrics are most informative for risk assessment and the development of health-based thresholds and 2) which microplastic characteristics (e.g., size, morphology, polymer type) are of greatest toxicological concern. We also briefly discuss critical gaps in knowledge regarding microplastic toxicity and how our recommendations may be incorporated into existing research for microplastics.

Methods

Exploration of existing toxicity data

Literature was explored using the Toxicity of Microplastics Explorer (ToMEx), a repository for microplastics toxicity data. The database houses over 160 microplastic ecotoxicity studies and captures data pertaining to the microplastic particles tested (e.g., size, shape, polymer type), test organisms (e.g., organism types, species, age), experimental design (e.g., sample size, exposure duration), biological effects (e.g., effect type, dose–response), and quality criteria (e.g., controls, validation of particle characteristics). The database is also accompanied by a web-based application which allows users to intuitively search the database, create custom graphics, and analyze data. For more details see [23]. Our analyses used data as it is, without quality screening. However, we assume that any noise in the data caused by this is random and thus implicitly included in comparisons and statistical significance tests.

To provide recommendations for which concentration metrics and particle characteristics to include in threshold development and risk assessment, study participants were instructed to use the ToMEx database as well as their own expertise, knowledge, and statistical tools to explore existing microplastics toxicity data. Exploratory data analysis allows for effect phenomenon detection through inductive reasoning, which has advantages over the more commonly practiced hypothetico-deductive data analysis, namely the greater likelihood for scientific replication (i.e., less susceptible to "fishing", "data snooping," and "p-hacking"), as well as maximizing the value of the data [24].

In addition to exploratory data analysis, a priori deductive hypothesis testing was used. For example, participants were asked to create graphics using ToMEx to determine microplastic concentrations where effects were reported in the literature. Data were visualized where concentrations were expressed as mass, count, volume, and surface area per volume of water to explore which concentration metrics might be most informative for understanding toxicity. The potential influences of different particle characteristics were investigated by selecting comparable subsets of data, keeping as many variables constant as possible, while varying the characteristic of interest. For instance, to explore the influence of particle size (i.e.,

length), data may be filtered to only include chronic exposures in Cladocera using polystyrene (PS) spheres and then grouped by particle size. During these exercises, it was also noted when specific data types were limited or absent (Fig. 1). Finally, it is important to note that the exploratory analyses described here are by no means exhaustive. Further assessments using this dataset are encouraged.

To visualize potential interactions between particle size, exposure concentration (and metrics), and toxicity, a series of three-term logistic regression binomial models were generated using the glm() function native to R (version 4.1.2) and the ggplot package. For these analyses, only organismal-level toxicity data from ToMEx were utilized to control differences in responses between different levels of biological organization, and toxic effect response data were binarized such that effect concentrations which are not statistically significant from controls were assigned a value of "0" and effect concentrations in which statistically significant effects relative to control were observed were assigned a value of "1."

Results and discussion

Concentration metrics to inform toxicity, threshold development, and risk assessment

Microplastic toxicity studies most often reported concentrations either as mass or number of particles per volume of water. Recently, a greater number of studies have begun to report concentrations as both mass and counts (Fig. 2). This has been repeatedly recommended to provide maximum utility when comparing concentrations to other toxicity studies or environmental concentrations, which are typically reported as particle count per volume or surface area of water [21, 25, 26].

Reporting concentrations in both mass and count will increase comparability amongst toxicity and occurrence studies, but specific concentration metrics may be more informative or appropriate in certain toxicological scenarios. For instance, concentrations expressed as particle count may be more informative when studying endpoints such as changes in swimming speed or locomotion due to entanglement where the absolute number of particles encountered is of primary importance [27], and concentrations in mass may best inform effects dependent on the total amount of material present, such as those driven by polymer type or chemical additives [28, 29]. Alternatively, using an inappropriate concentration metric may hide or amplify some patterns in toxicity, causing adverse effects to be under- or overestimated. Thus, it is important to carefully consider which concentration metrics may be most appropriate for different exposure scenarios and mechanisms of toxicity.

It is important to consider that the standard reporting of microplastic concentrations as either count or mass

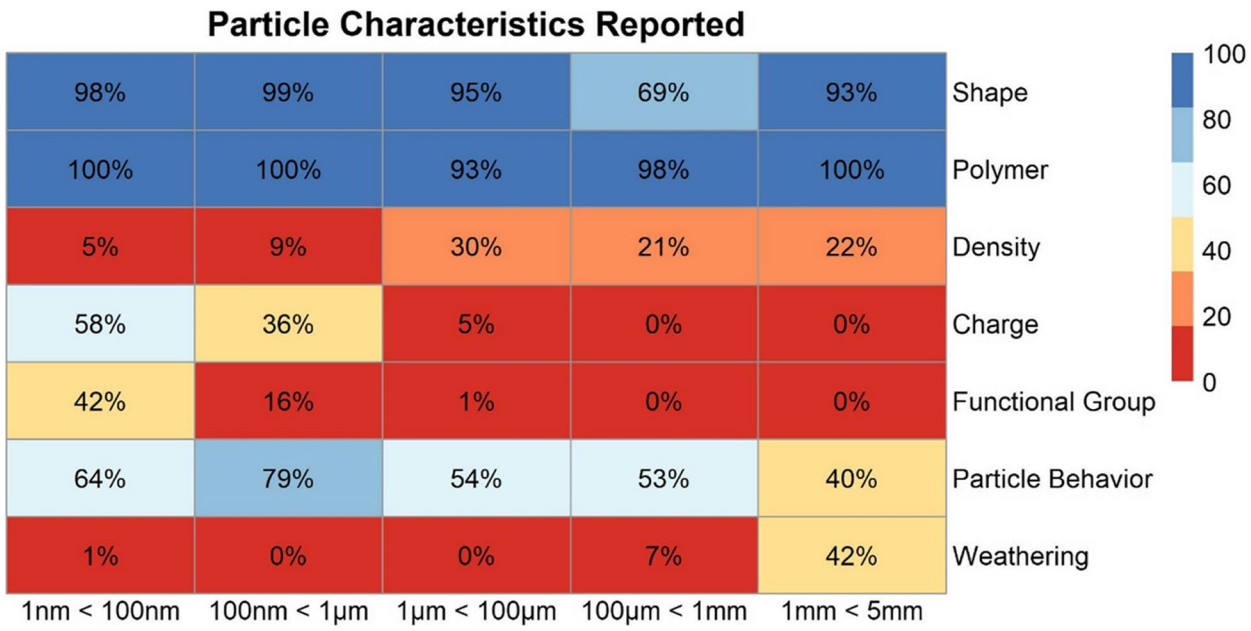

Fig. 1 The percentage of measurements in the Toxicity of Microplastics Explorer database reporting different particle characteristics. Descriptions of particle characteristic categories are as follows: Shape, physical morphology (e.g., sphere, fragment, fiber); Polymer, material type (e.g., polystyrene); Density, material density; Charge, electrostatic properties/ zeta potential (e.g., positive, negative); Functional Group, chemical surface modifications (e.g., carboxylation); Particle Behavior, quantitative or qualitative descriptions of how particles behave in media (e.g., sedimentation, clumping, dispersed); Weathering, descriptions particle biofouling prior to exposures

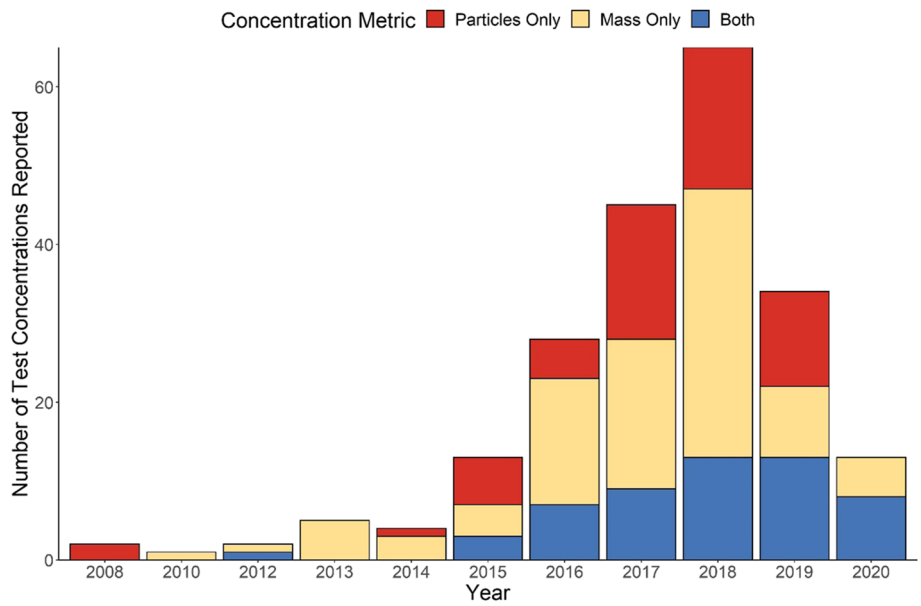

Fig. 2 Frequency of reported concentration metrics by study as mass, count (labeled as particles only), or both in toxicological studies for aqueous exposures included in the Toxicity of Microplastics Explorer database

based per unit volume is partially the result of existing methods for measuring microplastics. Specifically, microplastics are most often quantified in environmental samples by manually counting particles via microscopy [30] whereas many soluble chemical contaminants are expressed in mass. Though concentrations expressed in mass or count have proven useful and informative thus far, it is critical that alternative concentration metrics beyond mass and count are reported in future microplastic toxicity studies as they may be more meaningful for the toxicological mechanism at hand. For instance, [31] argue that the total volume of ingested plastic is the most relevant dose metric when looking at effects driven by food dilution where the amount of space taken up in the gut by microplastics (i.e., the volume) is of primary importance. On the other hand, increases in oxidative stress and inflammation are likely driven by surface area as previous nanotoxicity studies have documented strong correlations between particle surface area and reactive oxygen species generation as well as the expression of inflammatory markers in vitro [32, 33].

Ensuring that microplastic concentrations are comparable to other toxicity studies and environmental occurrence data, while also considering the exposure route and toxicity mechanism(s) at play, may seem to be an impossible challenge for researchers. However, concentration metrics may be expressed in a variety of ways if microplastic particles are well-characterized [34]. For instance, exhaustive characterization of particle size distributions is particularly helpful because these metrics can allow concentrations to be converted between mass, count, and volume if the plastic density is also reported [19, 31, 35]. If detailed data sets are available for the habitat or matrix of interest, probabilistic distributions can be used to convert concentrations not only from mass to count and vice versa, but concentrations can also be aligned to any particle characteristic of the user's choosing for which data are available [19, 31, 35].

Based on our analyses, we recommend (at minimum) that researchers publish microplastic concentrations from toxicity studies as both mass and counts. Alternative concentration metrics such as volume, specific surface area (i.e., surface area divided by mass), or surface area should be reported in future toxicity studies, particularly when they are hypothesized to be of primary importance to the toxicological mechanism(s) of interest. The use of alternative concentration metrics will be greatly facilitated by extensive particle characterization.

Microplastic characteristics of toxicological concern

The diversity of microplastics in both physical and chemical composition calls for the identification and prioritization of particle characteristics regarding their potential toxicity. Previous studies have hypothesized about which characteristics may be most important for driving specific toxicological mechanisms, and some studies have sought to describe the relative toxicities of varying particle characteristics (e.g., [28–, 36, 37, 38, 39]). Of the multitude of characteristics that may comprise microplastics, particle size, morphology, and polymer type are the most

well-studied (Fig. 1). On the other hand, other particle characteristics such as surface area and particle charge were rarely reported or explored as potential toxicological drivers. Therefore, microplastic polymer type, morphology, and size were the focus here.

Results from previous studies suggest that polymer type may influence toxicity. For example, polyvinyl chloride (PVC), polylactic acid (PLA), and polyurethane (PUR) fragments < 59 µm caused differential effects on reproductive output in *Daphnia magna* where PVC > PLA > PUR [29]. However, these types of comparative studies are rare, making it difficult to determine if specific polymer types are more toxic than others. Furthermore, the literature continues to be dominated by a handful of polymer types such as PS (51% of studies in the ToMEx database) and polyethylene (35% of studies in the ToMEx database). Though these metrics only represent data within the ToMEx database, previous literature reviews have also reported similar findings [25]. The importance of polymer type in influencing toxicity is uncertain, with hypothesized significant differences based on monomer toxicities [40] and statistically significant differences based on ecotoxicological effect studies [41]. At the same time, a recent meta-analysis of 17 in vitro mammalian toxicity studies suggests that polymer type is not a driving factor for toxicity [42]. Given these conflicting conclusions, the lack of studies comparing polymer toxicities, and the lack of diversity of particle types used in toxicity experiments, it remains uncertain whether polymer type is a key driver of microplastic toxicity in aquatic organisms.

There are more data regarding the potential influence of particle morphology on toxicity in comparison to polymer type. Results from previous laboratory effect studies and meta-analyses suggest that complex morphologies such as fibers or fragments may be more harmful than spheres to both aquatic organisms and mammalian cell lines [27, 36, 42, 43]. For instance, [43] exposed copepods, *Calanus finmarchicus*, to nylon fibers or granules of similar size and found that exposure to fibers significantly decreased algal ingestion rates whereas exposure to granules did not. Additionally, [42] found a statistically significant relationship between particle shape irregularity and thresholds at which cell death occurs across several cell lines. In the ToMEx database, effects were detected at lower particle count concentrations for fibers, followed by fragments and spheres (Fig. 3). Similarly, Bucci et al., found that fibers triggered effects in 62% of cases whereas fragments and spheres caused significant effects in 21 and 49% of cases, respectively [11]. Though these findings may suggest that morphology is a toxicological driver for microplastics, the data remains limited due to the overuse of spheres and biases toward different test concentrations for different morphologies (e.g., test concentrations were typically much lower for fibers, Fig. 3). More studies are needed to definitively confirm these patterns in toxicity, particularly those that have been demonstrated for non-plastic particles [44]

In contrast to polymer type and morphology, size-dependent patterns in toxicity are evident in the literature. Initial exploration of the ToMEx database revealed patterns in toxicity when data were binned by size, particularly when concentrations were expressed as counts (Fig. 4) – demonstrating the high variability in count-based toxicity thresholds and potential pitfalls when data are reported in size bins. Here, significant effects were observed for larger particles at lower count concentrations whereas effects for smaller particles were observed at higher concentrations. However, this may be the result of concentration selection biases where larger particles are more frequently tested at lower concentrations and smaller particles are more frequently tested at higher concentrations. Similar trends are observed when comparing species sensitivity distributions (SSDs) binned by arbitrary size bins (Fig. 5). Specifically, extreme variability between size bins is observed when exposure concentrations are reported as particle count per volume of exposure media – resulting in ~ 10 order of magnitude differences between SSDs between the smallest and largest size bins (i.e., 1–100 nm and 100 – 1,000 µm), however when mass per volume of exposure media is used as an exposure metric, SSDs range by just 2 orders of magnitude between size bins.

Similar patterns were observed when data were analyzed using a binomial logistic model to further investigate the relationship between the aqueous concentration of microplastics at which statistically significant effects occur relative to controls and the particle size (i.e., length) (Fig. 6). Here, regardless of whether concentrations are expressed in counts, mass, volume, or surface area, the inflection point where smaller particles become more toxic than larger particles as the concentration increases is readily apparent. Interestingly, both particle size and the interaction term between particle size and exposure concentration were statistically significant (p-value < 0.001) in general linear models for all log10-transformed exposure concentration metrics (i.e., mass, particle count, volume, surface area). Akaike's Information Criteria (AIC) was lowest for the general linear model which considered mass as the dose predictor (AIC = 4180), followed by volume (AIC = 4202), then surface area (AIC = 4208), and finally particle count (AIC = 4228), However, exposure concentration itself was only a statistically significant predictor of toxicity for surface area (p-value = 0.0012) and volume (p-value = 0.038), suggesting that volume and surface

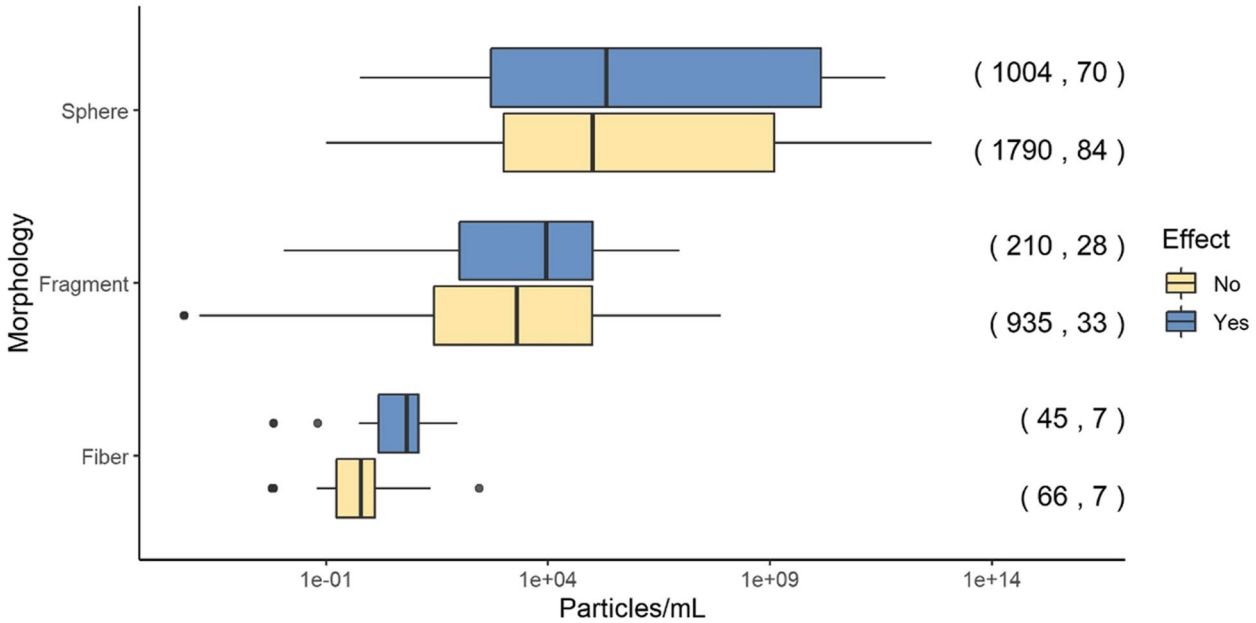

Fig. 3 Representative box and whisker plot generated from the Toxicity of Microplastics Explorer database displaying the microplastic concentrations (count) where statistically significant effects were detected ("Yes") or not detected ("No") as reported by the original studies for different morphologies within the organism groups "Crustacea", "Fish", and "Mollusca" for the specific endpoint categories "Growth", "Body Condition", "Development", "Reproduction", and "Mortality". The center line represents the median. Upper and lower hinges correspond to the first and third quartiles. Outliers (i.e., values greater than 1.5 multiplied by the interquartile range) are represented as individual data points. Text labels represent the total number of datapoints and studies, respectively. Underlying data may be found in the Supplementary Information

area may be more accurate predictors of toxicity than mass or particle count. It is important to note that surface area and volume exposure concentrations were estimated based on reported characteristics (particle shape, size, polymer density, count and/or mass – see [20] for methods), so comparisons with these indirectly derived values (i.e., volume or surface area) would have greater uncertainty than comparisons between true values.

Size has long been recognized as an important factor for particle toxicity. For instance, air quality standards are based on distinct size classes of particulate matter, acknowledging that small particles, particularly those less than 2.5 µm are more likely to travel deep into the respiratory tract and cause health problems [45]. Particle size is also known to be a critical determinant of agglomeration and toxicity for engineered nanomaterials [46]. Based on this previous knowledge, the influence of particle size has also been explored for microplastics and similar findings have been reported. For example, [47] exposed the marine copepod *Paracyclopina nana* to PS spheres of different sizes (0.05, 0.5 and 6 µm) and found that only smaller particles (0.05 and 0.5 µm) negatively affected growth and fecundity. Similarly, Yang et al. [48] exposed larval Goldfish (*Carassius auratus*) to either 70 nm or 5 µm PS spheres and found that nanosized particles entered fish muscle tissue and caused greater negative effects on larval movement compared to larvae exposed to 5 µm PS spheres. Long fibers may be more likely to entangle small organisms than shorter ones [27]. Chemicals may also leach more easily from smaller particles [49], though many laboratory studies use "additive-free" particles.

It is well known that particle size also affects the ingestibility of microplastics based on the development stage and mouth size opening of the organism [31, 50], as well as the ability for particles to translocate into tissue [51] and cross the blood–brain barrier [52]. In addition to ingestability, toxic effect endpoints differ due to size-based differences in uptake and distribution of particles. Distinct biases in the sizes administered to organisms based on group are readily apparent (Fig. 7) – a phenomenon which is likely due to researchers choosing particle sizes based on the relative size of the organism and their ability to ingest them. This bias in the literature may skew effect concentration data for a given species or organism group if using particle count as the exposure metric, and to a lesser extent, mass-based exposures. No obvious visual trend is observed when comparing effect endpoint measured (e.g., fitness, immune response, neurological, etc.) in laboratory effect studies as a function of particle size (Fig. 7), though there is an apparent bias toward using particles within the $1 < 100$ µm size

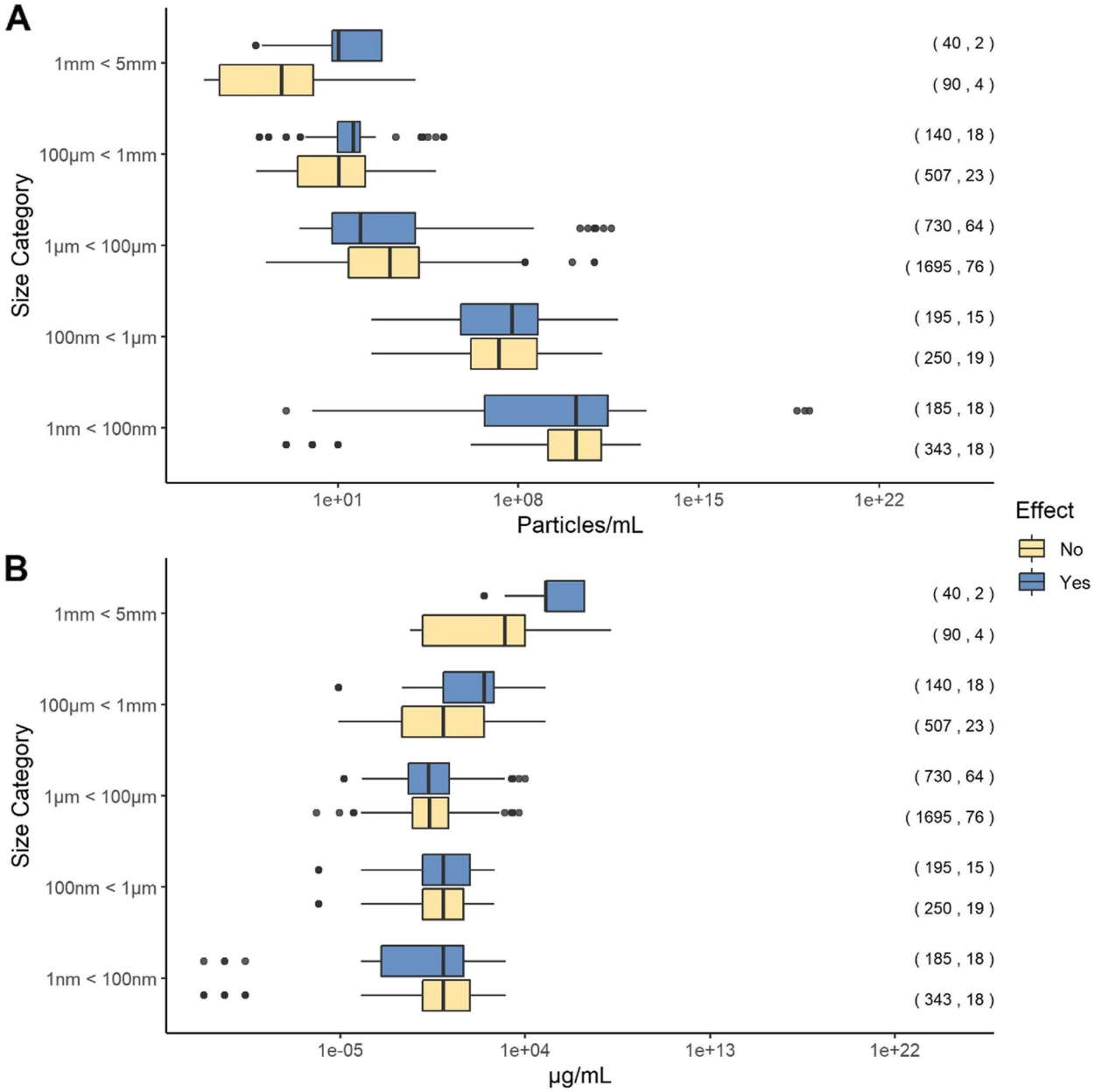

Fig. 4 Representative box and whisker plot generated from the Toxicity of Microplastics Explorer database displaying the microplastic concentrations where statistically significant effects were detected ("Yes") or not detected ("No") as reported by the original studies for different size categories expressed as count (**A**) and mass (**B**). Both panels are represented by the same underlying data. The center line represents the median. Upper and lower hinges correspond to the first and third quartiles. Outliers (i.e., values greater than 1.5 multiplied by the interquartile range) are represented as individual data points. Text labels represent the total number of datapoints and studies, respectively. Underlying data may be found in the Supplementary Information

category. The lack of obvious relationships could potentially be due to the non-specific toxic targets of microplastics (i.e., food dilution and oxidative stress cause general toxicity).

For most microplastic characteristics, more data are needed to determine how they influence toxicity in aquatic organisms. Preliminary data suggest that polymer type and morphology are likely important for microplastic toxicity, but more studies using well-characterized and diverse particle types as well as environmentally realistic mixtures of microplastics are needed [34]. Other particle characteristics (e.g., weathering, charge, biofouling,

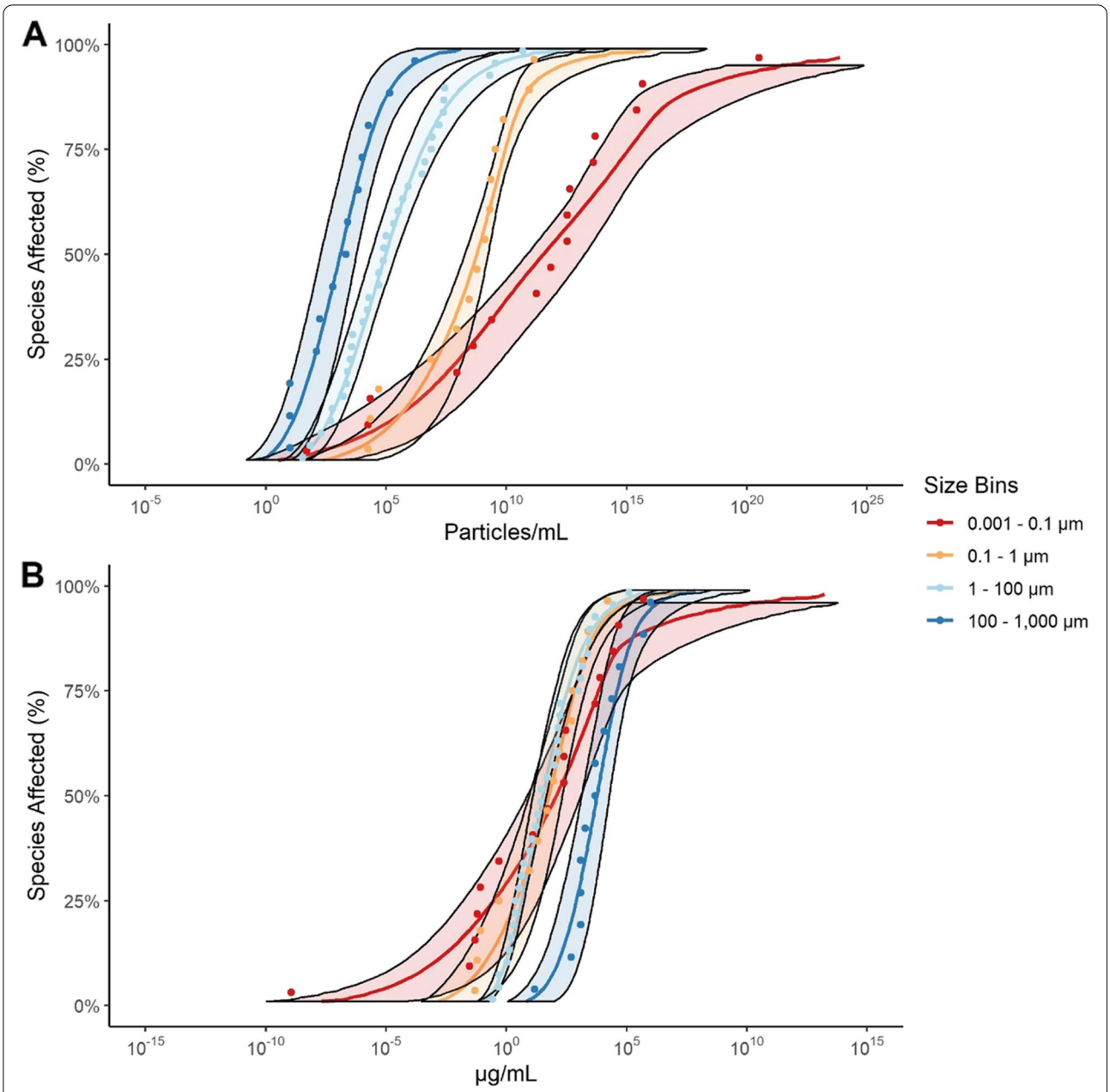

Fig. 5 Species sensitivity distributions generated from the Toxicity of Microplastics Explorer database for microplastics binned by size reported as particle count per volume of exposure medium (**A**) or mass per volume of exposure medium (**B**). Each point represents the average no-observed effect concentration for a given species. Smoothed lines represent modeled distributions, with ribbons representing the 95% confidence interval

etc.) are also likely to influence toxicological outcomes [28, 53, 54], but these characteristics are rarely investigated in toxicity studies (Fig. 1). Thus, researchers are also encouraged to describe and report these characteristics in future studies and consider their possible influences on toxicity. Despite the lack of data for the majority of microplastic particle types and characteristics, there is substantial evidence that particle size is a critical factor for microplastic toxicity in aquatic organisms. Therefore, risk frameworks today can focus on size (i.e., particle length) as a driver of toxicity.

Effect mechanisms influenced by size

Though our understanding is continuously evolving, relationships between microplastic size, exposure, and toxicity have been partially described. Current research

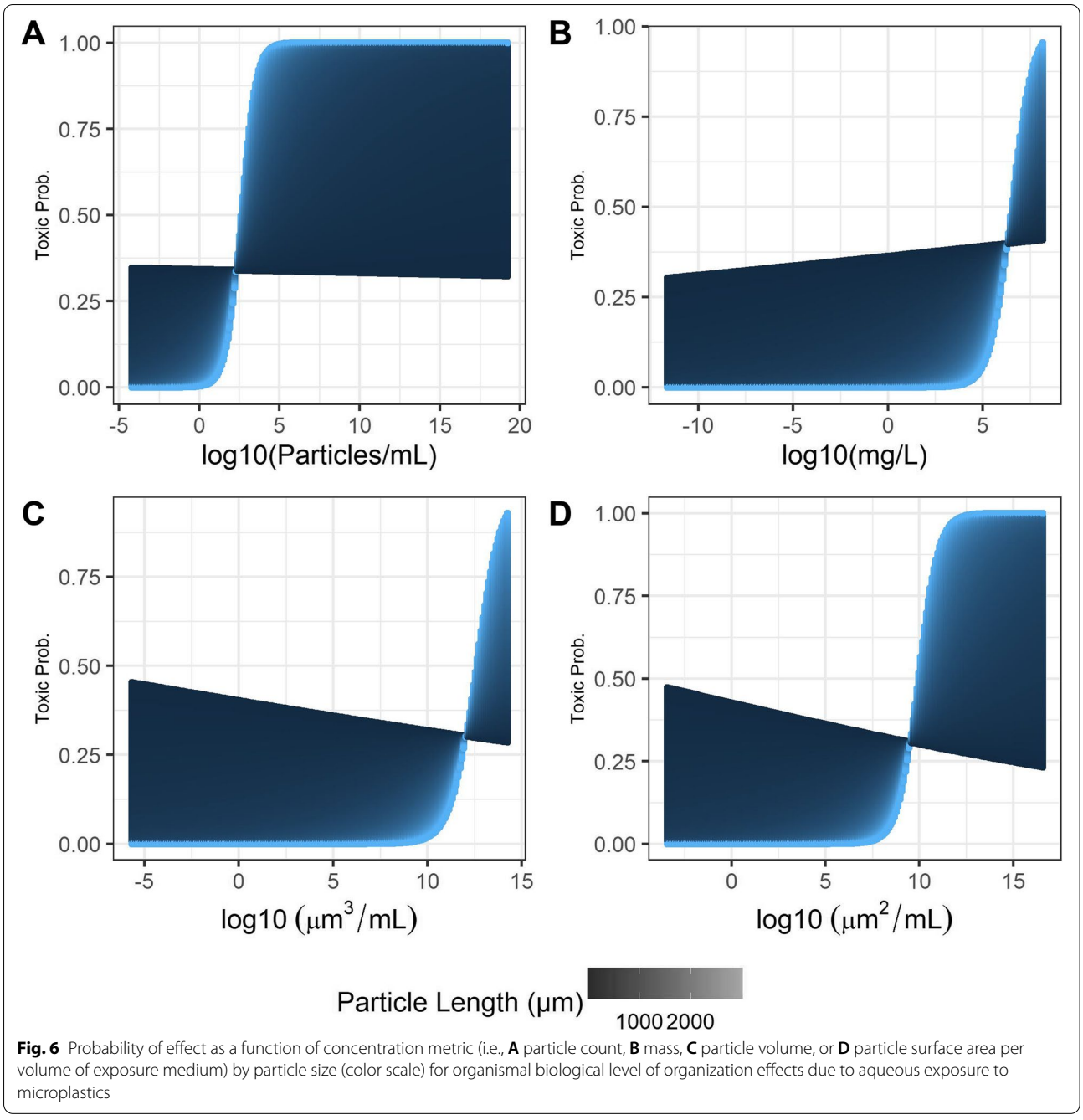

Fig. 6 Probability of effect as a function of concentration metric (i.e., **A** particle count, **B** mass, **C** particle volume, or **D** particle surface area per volume of exposure medium) by particle size (color scale) for organismal biological level of organization effects due to aqueous exposure to microplastics

collectively indicates that two toxicological mechanisms related to microplastics are heavily influenced by particle size. These are food dilution, which occurs due to reduced food intake or nutrient acquisition when macro or microplastics occupy space in an organism's gut [31] and tissue translocation-mediated toxicities such as inflammation and oxidative stress [51].

Food dilution

Food dilution takes place when macro or microplastics are ingested, either directly or via transfer from prey [21, 31]. Rather than contributing towards the overall nutritional value of an organism's diet, plastics (or any other substance with no nutritional value) occupy space in the stomach and digestive tract, and thus contribute to a sense of false satiation [21, 55, 56]. This results in a reduction in the amount of food ingested and possibly

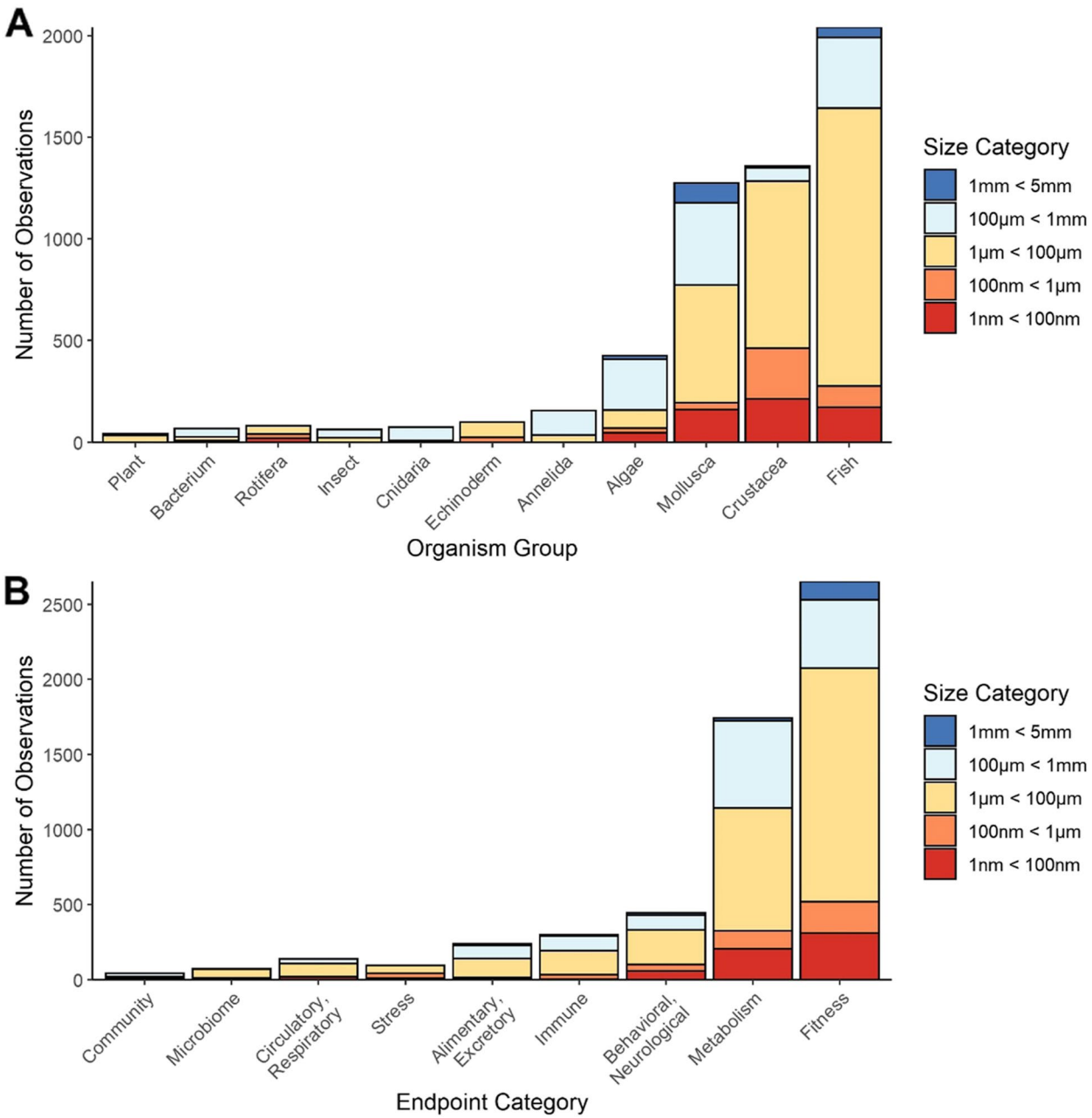

Fig. 7 Number of observations in Toxicity of Microplastics Explorer database demonstrating laboratory effect study biases of particle sizes as a function of **A** Organism Groups and **B** Endpoints assessed

a lower tendency to seek out prey. Plastic ingestion may also affect nutrient assimilation in more subtle ways such as through damage to the gut wall or changes to the microbiome [36, 39, 57] or cause effects on the cellular pathways by which macromolecules are processed or absorbed, reducing the overall energy budget [58–, 59, 60, 61]. Long term impacts connected to decreased energy reserves include altered swimming behavior, decreased growth, altered reproduction, lowered fecundity, altered respiration, and in limited cases, reduced survival. These impacts can be dependent on life stage, phylogenetic group, habitat type, abiotic factors such as temperature or salinity, as well as the morphology and polymer composition of the internalized microplastic [62–, 63, 64, 65, 66, 67, 68]. Biofilms on the surface of microplastics may partly ameliorate the food dilution

effect in some organisms [69], thus reinforcing the need to characterize particle characteristics in studies as well as use realistic particle exposures. Ultimately, these effects on physiological processes critical to survival in the wild could cumulatively reduce population size and even scale up to effects on entire communities, and the associated ecosystem services they provide [70, 71].

Investigations across taxa have determined food dilution to be a driver of negative impacts on aquatic organisms, and modeling efforts support these findings [31]. For example, an early and highly influential study on polychaete worms (*Arenicola marina*) found that exposure to virgin PVC in sediments had significantly reduced energy reserves (e.g., lipids) in comparison to controls [67],the authors posited that decreased reserves could ultimately have deleterious effects on body condition and growth, as well as time to maturity and reproduction. Subsequent research findings are in alignment with this earlier work, with a study in the Marine Jacopever (*Sebastes schlegelii*) observing not only reduced feeding activity following exposure to PS microplastics, but also altered shoaling behavior (an indication of stress), decreased exploration ability and hence fewer encounters with potential prey as a likely consequence of overall reduced energy reserves [68]. Similarly, in another fish species, the Amazonian Cichlid (*Symphysodon aequifasciatus*), regardless of the absence of effects on body size and survival, elevated microplastic ingestion altered the activity of critical digestive enzymes (e.g., trypsin, amylase) and impaired predatory performance [60]. These patterns hold true in bivalves, such as the pearl oyster *Pinctada margaritifera* [72], and in representative crustaceans—the decapod *Carcinus maenas* and cladoceran *Daphnia magna* [73, 74]. Collectively, evidence suggests that internalization of microplastics causes food dilution by two routes, 1) reducing the capacity of the animal to physically digest food and/or obtain nutrition either by altered digestive processes or internal damage and 2) by altering predation behaviors which leads to reduced intake of prey.

Particle size is a critical factor for food dilution regarding both ingestibility and magnitude of effect. For food dilution to occur, it must first be physically possible for an organism to ingest the particle(s) in question [31]. Ingestion is directly related to the size of the particle relative to the gape size of a given organism. For example, the mouth opening of a larval fathead minnow (*Pimephales promelas*) is estimated to be between 240–280 microns [75], suggesting that any microplastic particle less than this size range in at least one dimension may be orally ingested. Of course, other factors such as the environmental microplastic concentration, biofilm [76], and species-specific foraging behaviors [77, 78] will influence the likelihood of oral ingestion, but particle size is the key microplastic characteristic when determining if consumption is physically possible. Once ingestion has occurred, the cumulative volume taken up by microplastics is of primary interest [31]. This concept is supported by the results of the previously described general linearized model where volume was found to be a statistically significant predictor of toxicity (Fig. 5). However, most toxicological studies only report the presence or absence of microplastics in the gut, though total plastic volume in the gut of field-collected benthic invertebrates was just recently reported [79, 80].

Tissue translocation

Tissue translocation occurs when smaller microplastic particles (estimated to be < 83 μm; [20]) move from the digestive tract to other tissues. Particle translocation is a phenomenon not exclusive to microplastics and is hypothesized to occur when epithelial cells or immune cells associated with the gastrointestinal tract lining take up particles or, in some rare cases, pass between epithelial cells into other tissues [51, 81]. Though there have been multiple observations of translocated particles in fish [82–84], crustacea [77, 85], and mollusks [10, 86], translocation has been primarily documented in laboratory studies rather than the field, and the exact mechanisms of translocation in aquatic organisms are not fully understood.

Once particles translocate, they may cause adverse effects such as oxidative stress and inflammation [17]. Many pollutants and environmental stressors, including microplastics, induce the production of reactive oxygen species [87], which can harm cellular components and structures (e.g., membranes) and even underscore larger-scale physiological damage if unchecked (e.g., [88–90]. Similarly, excessive inflammation can be a precursor to other adverse effects, such as damage to DNA and cancer [91]. Previous studies have observed inflammatory and oxidative stress responses in aquatic organisms in tissues where microplastics have translocated. For instance, Chinese mitten crabs (*Eriocheir sinensis*) accumulated 0.5 μm PS spheres in their liver tissue after seven days of exposure. After 21 days of exposure, gene expression and enzyme analyses in the liver were indicative of alternations in both inflammation and oxidative stress responses [92]. Similarly, PS spheres (32–40 μm) were also found to accumulate in the liver tissue of grass carp (*Ctenopharyngodon idella*) where biochemical alterations in oxidative stress responses were also observed [93].

Tissue translocation is highly dependent on particle size [94]. Evidence thus far supports the hypothesis that smaller microplastics may be more likely to trigger

oxidative stress and related downstream damage (e.g., lipid peroxidation, altered membrane permeability), possibly because these particles have a greater tendency to translocate within an organism (e.g., [67, 82, 95–97]. Translocation of micro and nanoplastics is a phenomenon that remains poorly understood, but in aquatic organisms it appears more likely to occur with particles that are < 80 microns in diameter or length [20, 55, 98, 99], with the likelihood increasing as particles near the upper limit of the nanoscale range (< 1 μm). In these tissues, as well as in the gut, the likelihood of particles to interact with cells and tissues may also be related to their available surface area as has been previously demonstrated for nanoparticles. For example, a strong correlation between surface area and inflammation and markers of oxidative stress was observed in vitro following exposure to titanium dioxide or carbon black nanoparticles [32]. In addition, surface area was also found to be a significant predictor of toxicity here (Fig. 5). However, microplastic surface area is rarely reported in toxicity studies, if ever (Fig. 1), and the potential connection between increases in surface area and increases in oxidative stress has not yet been experimentally demonstrated in aquatic organisms.

Recommendations moving forward

While it is advised that researchers report microplastic concentrations as both counts and mass, it is especially emphasized that other concentration metrics (e.g., volume, surface area) are reported in future studies as they may be more informative for specific toxicological mechanisms. For instance, researchers interested in the ability of microplastics to cause food dilution should determine the total volume of plastic ingested by test organisms and assess biological responses associated with food dilution (e.g., nutrient assimilation, growth, etc.). Determination of particle volume requires extensive particle characterization, which will also facilitate the identification of particle characteristics driving toxicity.

If detailed data about particle characteristics are available for the habitat or matrix of interest, probabilistic distributions can be used to convert concentrations not only from mass to count and vice versa, but doses may also be aligned to a variety of particle characteristics [19, 31, 35]. Additionally, other methods will inevitably be modified and improved over time as technologies for detecting and characterizing microplastics and nanoplastics rapidly emerge. As they do, relationships between specific particle characteristics and how they relate to exposure and toxicity should be reevaluated. At a minimum, reporting toxicity dosing metrics as both mass and particle count allows flexibility as the field of microplastics develops and environmental managers seek to balance time, cost, and resource constraints in pursuit of high quality and informative monitoring and toxicity data upon which regulatory decisions may be made with high confidence.

Extensive particle characterization is not only key for the expression of various microplastic concentrations, but it is also essential for identifying other drivers of microplastic toxicity. We identify particle size (i.e., length) as an important toxicological determinant due to its close relationship with volume and surface area, but it is likely that other characteristics are important determinants of toxicity as well. For some characteristics, such as morphology and polymer type, more data are needed. Other characteristics are not described or reported in the literature. Thus, researchers should carefully consider which particle characteristics may drive toxicity while formulating study designs and hypotheses and strive to describe particles as extensively as possible. For more details regarding recommendations for particle characterization, see [34].

Here, we describe several key areas to advance microplastic toxicity research including reporting multiple concentration metrics, improving particle characterization, and identifying novel, sensitive responses to microplastics. Addressing these recommendations will not only better our understanding of microplastics toxicity, but it will also facilitate harmonization amongst microplastic toxicity studies and beyond. For instance, if toxicity studies report test concentrations as both mass and count per unit volume, concentrations are more likely to be directly comparable to other studies, increasing the utility of the data. More detailed reporting of test concentrations and particle characteristics will also facilitate direct comparisons to microplastic concentrations in environmental samples. Compatible data sets are essential for robust evaluations of ecological health, including risk assessment where data describing exposure (i.e., occurrence data) must be directly compared to hazard concentrations, which are derived from toxicity data. Thus, it is critical that microplastics toxicity research and methods for monitoring microplastics evolve in tandem to ensure that environmental concentrations can be appropriately contextualized regarding potential hazards and risks.

Conclusions

Within the last decade, researchers globally have demonstrated the ubiquity of microplastics in the environment as well as their potential to cause harm to aquatic organisms. These findings have motivated the passing of numerous legislative mandates aimed at increasing our understanding of microplastic exposure and devising environmental management strategies to address plastic pollution. To effectively manage

microplastics, clarity is needed regarding which concentration metrics should be used for future monitoring and toxicity initiatives. As emphasized above, we conclude that researchers exploring microplastic toxicity should at least express microplastic concentrations in both counts and mass and apply other metrics as they relate to specific toxicological mechanisms (e.g., total volume of particles and food dilution, surface area and oxidative stress). In addition, it is also important to identify which particle characteristics (e.g., size, morphology, polymer type) drive toxicity. Here, we conclude that particle size, critical to determining ingestibility, is the primary particle characteristic of biological concern. Larger ingestible particles have a greater volume (relative to an organism's size), take up more space in the gut, and may contribute to food dilution. On the other hand, smaller particles are more likely to translocate and cause oxidative stress and inflammation. Other particle characteristics likely also influence toxicity, but data are limited. Future research should be designed in a way to help us better understand which characteristics should be considered in risk assessment frameworks while ensuring harmonization across studies.

Abbreviations

PLA: Polylactic Acid; PS: Polystyrene; PUR: Polyurethane; PVC: Polyvinyl Chloride; ToMEx: Toxicity of Microplastics Explorer.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-022-00040-4.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Additional file 5.

Acknowledgements

The authors acknowledge all participants of the California Health Effects Workshop for their contributions towards conceptualizing these recommendations for the advancement of microplastics scientific research.

Peer review

In keeping with research integrity principles, all SCCWRP workshop articles co-authored by Dr. Steve Weisberg, Dr. Albert Koelmans, and other Microplastics and Nanoplastics editors were handled by independent editors of the journal.

Authors' contributions

L.M.T.H., S.M.B., S.C., M.C., L.H., A.A.K., and C.M.R. all assisted in the conceptualization and preparation of the manuscript. The author(s) read and approved the final manuscript.

Funding

Funding for this project was provided by the California State Water Resources Control Board.

Availability of data and materials

The Toxicity of Microplastics Explorer database and web application may be accessed at https://microplastics.sccwrp.org. The database is readily accessible via the web applications.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Competing interests

The authors L.M.T.H. and S.C. declare having no known competing financial interests or professional relationships that could have appeared to influence the work reported in this paper.
The following authors declare financial interests/professional relationships which may be considered as potential competing interests:
- S.M.B. reports a relationship with Ocean Science Trust that includes consulting or advisory and acknowledges funding from the National Science Foundation under grant agreement No. 1935028.
- M.C. acknowledges financial support from the MICRO-OPT project, Norwegian Retailers Fund (Handles Miljøfund, HMF).
- L.H. acknowledges fundings from Environment and Climate Change Canada.
- A.A.K. reports a relationship with World Health Organization, European Food Safety Authority, United Nations and SAPEA & European Commission Group of Chief Scientific Advisors that includes consulting or advisory, and financial support in the form of funding grants or travel reimbursement. AAK also acknowledges research funding from the European Chemical Industry Council, the Dutch Research Council, the Norwegian Research Council and Rijkswaterstaat Water Traffic and Living Environment. All research is carried out under the Dutch code of conduct for scientific integrity.
- C.M.R. reports a relationship with Ocean Conservancy as a Scientific Advisor.

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Received: 11 January 2022 Accepted: 13 July 2022
Published online: 06 August 2022

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