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# What is known and unknown about the effects of plastic pollution: A meta-analysis and systematic review

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*Abstract.* As a consequence of the global ubiquity of plastic pollution, scientists, decision-makers, and the public often ask whether macroplastics (>5 mm) and microplastics (<5 mm) have a realized ecological threat. In 2016, we conducted a systematic review of the literature and made a call for further research testing hypotheses about ecological effects. In the subsequent years, the amount of relevant research has risen tremendously. Here, we reassess the literature to determine the current weight of evidence about the effects of plastic pollution across all levels of biological organization. Our data spans marine, freshwater, and terrestrial environments. We extracted data from 139 lab and field studies testing 577 independent effects across a variety of taxa and with various types, sizes, and shapes of plastic. Overall, 59% of the tested effects were detected. Of these, 58% were due to microplastics and 42% were due to macroplastics. Of the effects that were not detected, 94% were from microplastics and 6% were from macroplastics. We found evidence that whether or not an effect is detected, as well as the severity and direction of the effect, is driven by dose, particle shape, polymer type, and particle size. Based on our analyses, there is no doubt that macroplastics are causing ecological effects, however, the effects of microplastics are much more complex. We also assessed the environmental relevancy of experimental studies by comparing the doses used in each exposure to the concentrations and sizes of microplastics found in the environment. We determined that only 17% of the concentrations used in experimental studies have been found in nature, and that 80% of particle sizes used in experiments fall below the size range of the majority of environmental sampling. Based on our systematic review and meta-analysis, we make a call for future work that recognizes the complexity of microplastics and designs tests to better understand how different types, sizes, shapes, doses, and exposure durations affect wildlife. We also call for more ecologically and environmentally relevant studies, particularly in freshwater and terrestrial environments.

*Key words:* *ecological impact; effects; environmental relevance; marine debris; meta-analysis; microplastic; plastic debris; systematic review.*

## Introduction

Over the past decade, it has become apparent that plastic debris is a globally ubiquitous pollutant. Plastic is found in terrestrial, marine, and freshwater ecosystems around the world (Rochman 2018). The same properties that make plastic useful also lend to its potential for environmental harm: it is inexpensive to manufacture, durable, lightweight, and hydrophobic (Andrady 2015). Due to its low cost, plastic is mass produced and primarily used for single-use packaging, which is designed for immediate disposal (Jambeck et al. 2015). Despite the increasing global production and use of plastic products, waste management remains inadequate or nonexistent in many locations. As a consequence, plastic is ending up in the natural environment at the astounding rate of 31.9 million metric tons per year (Jambeck et al. 2015). Once it reaches the environment, its durability allows it to persist for hundreds to thousands of years (Barnes et al. 2009) and its light weight allows it to be transported long distances via wind and air currents (Barnes 2002). Finally, because of its hydrophobicity, plastics sorb contaminants from the surrounding environment (Ogata et al. 2009), altering the fate of chemicals across environmental matrices, including wildlife.

In the environment, macroplastics (plastic >5 mm) are exposed to sun and/or wave action that cause them to break up into smaller and smaller pieces, called microplastics (plastic <5 mm) (Andrady 2015). Over time, macro- and microplastics have accumulated in terrestrial and aquatic environments. Plastic debris has been reported in agricultural fields (Rillig et al. 2017), soil (Hurley and Nizzetto 2018), roadways (Kole et al.

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2017), storm water runoff from urban centers (Mason et al. 2016), freshwater lakes and streams (Dris et al. 2015), freshwater and marine shorelines (Browne et al. 2011), the open ocean (Cózar et al. 2014), coral reefs (Lamb et al. 2018), deep sea sediments (Woodall et al. 2014), submarine canyons (e.g., Pham et al. 2014), and Arctic sea ice (Obbard et al. 2014).

Terrestrial and aquatic wildlife are in a constant state of exposure to plastic, as it can be found in virtually every habitat on Earth. In the aquatic environment, organisms at every level of the food chain have been reported to ingest or interact with plastic. This includes primary producers such as algae (Carson et al. 2013), primary consumers such as zooplankton (Desforges et al. 2015), and higher-order consumers such as fish (Mcneish et al. 2018), turtles (Duncan et al. 2018), sea birds (Reynolds and Ryan 2018), seals (Donohue et al. 2019), and whales (Nelms et al. 2019). In the terrestrial environment, there are noticeably fewer primary research articles that report similar interactions. However, due to the pervasiveness of plastic pollution in the terrestrial environment, interactions with plastic pollution by wildlife are likely quite common. For example, microplastic ingestion has been reported in terrestrial birds (Zhao et al. 2016), and bees have been reported to incorporate anthropogenic debris into their nests (MacIvor and Moore 2013). Appropriately, concerns regarding the effects of plastic on aquatic and terrestrial wildlife are often mentioned in scientific literature, policy documents, and in the media.

Scientists are beginning to understand that plastics are a complex pollutant (Paul-Pont et al. 2018) and can cause a variety of sub-lethal and lethal effects (Rochman et al. 2016). Interacting with macro- and microplastics exposes an animal not only to the physical plastic, but also to a complex suite of chemicals with which the plastic is associated (Alimba and Faggio 2019). Plastic debris can be physically harmful to an animal via entanglement or ingestion, causing lacerations, suffocation, and/or starvation, all of which may lead to death (Wright et al. 2013). Ingestion of small microplastics (<100 μm) may also be physically harmful if the particles translocate across the cell membrane into the circulatory, lymphatic, respiratory, and/or other biological systems (Brennecke et al. 2015; M. Browne et al. 2008). Additionally, plastics can be chemically harmful due to the complex suite of chemicals with which they are associated. This "chemical cocktail" consists of the residual monomers that make up the plastic polymer, the additives that are added during manufacturing, and the contaminants that sorb from the surrounding environment (Rochman 2015). Many of the chemicals associated with plastics are listed by the U.S. EPA as priority pollutants because they are persistent, bioaccumulative, and/or toxic (U.S. EPA 2014). Furthermore, recent laboratory studies have shown lethal and sublethal effects in organisms exposed to plastic with sorbed environmental contaminants (Browne et al. 2013, Lithner et al. 2012, Rochman et al. 2014).

Due to the potential threats of plastic pollution, the public, scientists, and decision-makers often ask whether there is a realized ecological threat from plastic debris in the environment. In 2016, Rochman et al. (2016) published a systematic review of the literature through 2013 and made a call for further research testing hypotheses about effects. In the subsequent years, the literature regarding plastic pollution has grown tremendously. There continue to be new observational studies that show fatal interactions with macroplastics in marine mammals, fish, birds, and reptiles (Alomar et al. 2017, Franco-Trecu et al. 2017, Reinert et al. 2017). For microplastics, however, a consensus has not yet been reached with regard to their toxicity. Although the body of literature demonstrating their effects is increasing, exposure to microplastics does not seem to result in a straightforward response. In laboratory studies, microplastics have been shown to cause a variety of biological effects, predominantly in crustaceans and molluscs. These include changes in gene expression (Paul-Pont et al. 2016), inflammation (von Moos et al. 2012), disruption of feeding behavior (Cole et al. 2015), decreases in growth (Au et al. 2015), decreases in reproductive success (Au et al. 2015, Sussarellu et al. 2016), changes in larval development (Nobre et al. 2015), reduced filtration and respiration rates (Paul-Pont et al. 2016), and decreased survival (Au et al. 2015, Cui et al. 2017). However, there are also studies that test for an effect but do not detect one (Hämer et al. 2014, Batel et al. 2016, Espinosa et al. 2018). Additionally, the effects of microplastics seem to be exacerbated when organisms are exposed to plastic with sorbed contaminants (Rochman et al. 2013, Martínez-Gómez et al. 2017). The discrepancy in whether or not microplastics cause an effect may be a consequence of treating microplastics as one contaminant rather than a class of contaminants with differences in polymer type, shape, size, and chemical cocktail (Foley et al. 2018, Paul-Pont et al. 2018, Rochman et al. 2019). Whether or not an effect is detected is likely dependent on these factors in addition to others, including the species used for testing, the dose used during the exposure, and the duration of the exposure.

Here, our primary aim was to update the analysis of Rochman et al. (2016), and determine the state of evidence regarding the effects of macro- and microplastics to freshwater, marine, and terrestrial organisms across all levels of biological organization. In this paper, we also aim to examine any underlying trends between whether or not an effect was detected and the direction and severity of the effect, in relation to the particle size, dose, exposure duration, and type of plastic used in the experiment. Further, we assess the environmental relevancy of laboratory experiments that investigate effects by comparing the dose and size of microplastics used in the exposures with the concentrations and sizes of microplastics found in nature.

## Methods

### *Literature review*

We systematically reviewed the literature for papers that tested or observed a biological response to an organism or group of organisms from plastic debris (both macro- and microplastic). We searched the literature using Web of Knowledge (all databases) for the key word terms: "marine debris," "plastic debris," and "microplastic" from the year 1898 through 26 November 2017. Our search resulted in a collection of literature spanning the fields of oceanography, limnology, conservation and marine biology, toxicology, and ecology. All peer-reviewed studies discussing impacts relevant to plastic debris in marine, freshwater, or terrestrial ecosystems were included in our analysis. This differs from Rochman et al. (2016) only in that we did not include studies relevant to other fields (e.g., studies about nanoplastic materials from the medical literature).

### *Data extraction and quality assessment*

All publications were checked by two authors to make sure they were relevant to our objectives based on the title and abstract. We then each reviewed all manuscripts for relevance to effects (see DataS1:All Effect Studies for a full list of references used in this study). All papers included were primary literature from peer-reviewed scientific journals, i.e., no reviews were included. For each study, we examined the tested and/or observed effects of plastics across 14 levels of biological organization: subatomic particle, atom, small molecule, macromolecule, molecular assemblage, organelle, cell, tissue, organ, organ system, organism, population, assemblage, and ecosystem. For each study, we recorded information regarding the size of the debris (and whether it was macroplastic >5 mm or microplastic <5 mm), the ecosystem studied (i.e., marine, freshwater, terrestrial), the taxonomic group, the organism studied, the characterization of the plastic (polymer type, chemicals added, shape, color, size), the effect tested, the level of biological organization of the tested effect, the type of study (i.e., observation in nature, field experiment, laboratory experiment), the experimental design (i.e., use of controls, dose, length of exposure, environmental relevance of exposures) and whether or not the effect was detected. An effect was detected when it was an observation in nature or when it was statistically significant in an experiment. Each experimental study was also assessed to determine whether it included information relevant to an $LC_{50}$ (dose required to kill one-half of the population). All synthesized data were double-checked by two authors to confirm the data that was extracted was correct before including it in the analysis. Any discrepancy was discussed among co-authors to reach an agreement. Fig. 1 summarizes the data collection and extraction process.

### *Synthesis of data*

We synthesized experimental and observational studies that tested impacts of plastic debris across several levels of biological organization in increasing order of ecological relevance using an established framework for pollutants (Adams et al. 1989). We classified effects that were most ecologically relevant as effects to the (1) organism (an individual organism's growth, behavior or death was a direct result of debris), (2) population (population size changed as a result of debris), (3) assemblage (there was a change in the structure or composition of assemblages as a direct result of debris), and (4) ecosystem (there was a change in ecosystem structure or function as a direct result of debris).

To visualize the weight of evidence related to the effects of plastic pollution, all tested or observed effects were plotted on a matrix with the level of biological organization at which the effect was observed as a function of the size of the debris, ranging from 1 nm (e.g., nanoplastics) to 1 km (e.g., fishing net; Fig. 2). In studies that tested or observed effects at multiple levels of biological organization and debris size, each effect was accounted for individually. All tested effects that were detected are depicted in Fig. 2A and all effects that were tested but not detected are depicted in Fig. 2B. See DataS1: All Effect Studies for a list of studies included in Fig. 2.

To better understand the underlying trends in whether or not an effect was detected, the results from laboratory experiments using microplastics (without the addition of chemicals) were organized in dot plots by plastic type, plastic shape, taxa, level of biological organization, particle size, and exposure duration (Fig. 3; see DataS1: Scatterplot Data for a list of studies used). For these plots, we only included studies that reported their doses in particles per volume of water to facilitate comparison between studies with different plastic types and sizes. Thus, we excluded studies that reported dose as mass per volume of water, particles per kilogram sediment, and mass per kilogram sediment.

### *Meta-analysis*

All laboratory studies that tested the effects of microplastics were reassessed to determine if they contained sufficient information to be included in a meta-analysis to compare effect sizes between studies. These papers were categorized by taxonomic group and by the biological endpoints assessed in each study. To minimize bias, an endpoint was excluded from the meta-analysis if it was tested for in fewer than three papers (e.g., in crustaceans, only one study investigated the effects of microplastics on swimming speed; therefore, this endpoint was excluded from further analysis). From each relevant paper, we extracted the mean of the treatment group, the mean of the control group, the standard deviation (or standard error, or confidence interval), and the

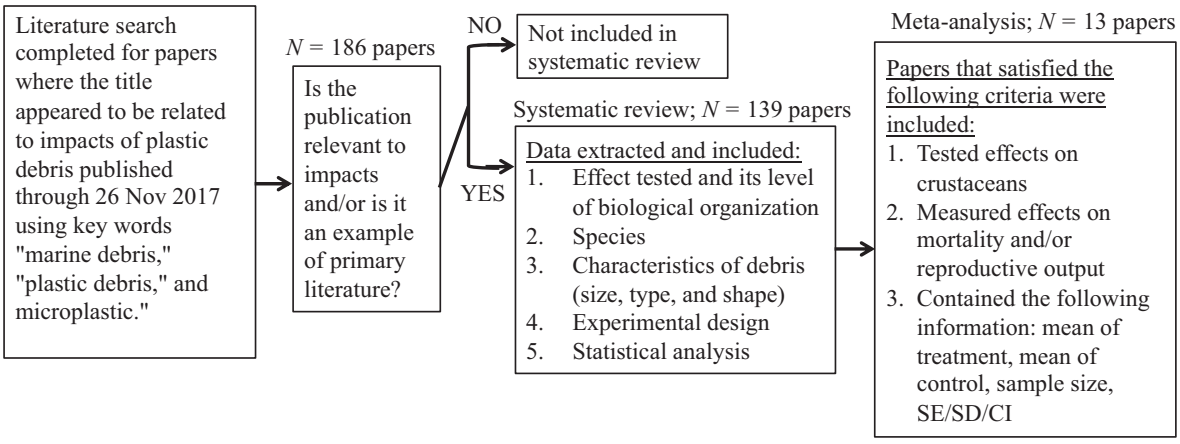


FIG. 1. A schematic representation of our literature search and decision-making tree for extraction of data for the systematic review and meta-analysis.

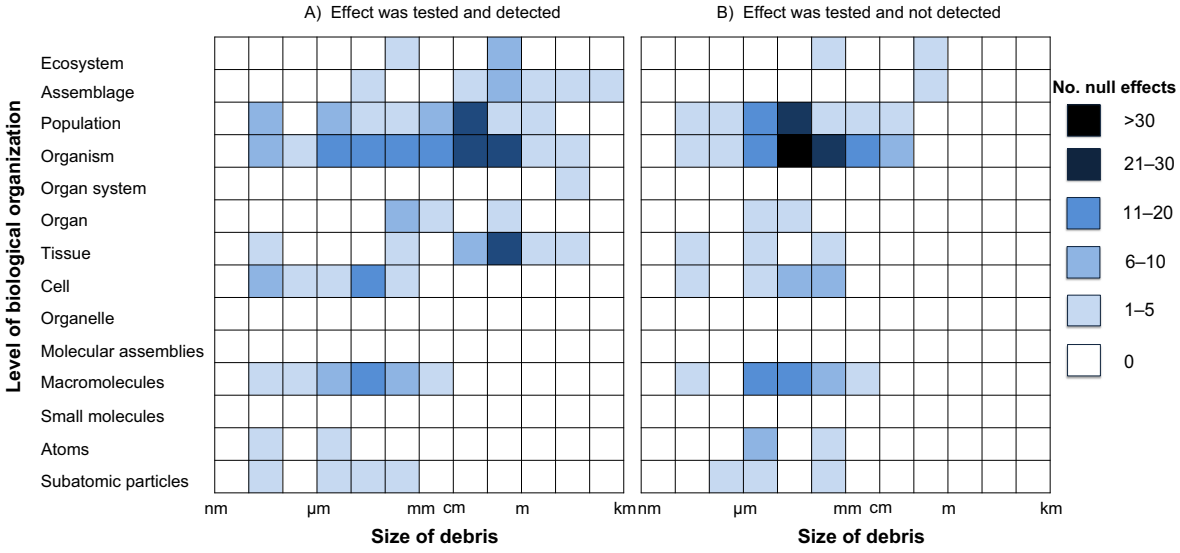


FIG. 2. (A) Detected and (B) non-detected impacts of plastic debris. Rows in each matrix represent different levels of biological organization. Columns represent order-of-magnitude sizes of debris from smallest (left) to largest (right). Shading in the individual cells of the matrix represents the number of impacts of debris in peer-reviewed literature identified during our literature search. All impacts described at multiple size ranges and levels of biological organization are represented, such that there are more impacts than there are papers.

sample size. If a study reported standard error or a 95% confidence interval, the value was converted to standard error using $SD = SE * \sqrt{n}$ or $SD = CI * \sqrt{n}/1.96$, respectively (Foley et al. 2018). If a paper lacked the required information, the authors were contacted to obtain raw data.

Ultimately, crustaceans were the only taxonomic group with sufficient data for this analysis, and only for two endpoints: mortality and reproductive output (DataS1: Effect Size Data). Thus, effect sizes were calculated for these endpoints. For each individual record, Hedges' $g$ (Hedges and Olkin 1985) was calculated by multiplying Cohen's $d$ by a correction factor to account for small sample sizes (Borenstein et al. 2009)

$$g = \frac{\text{Mean}_T - \text{Mean}_C}{\sqrt{\frac{(n_T-1)*\text{SD}_T^2+(n_C-1)*\text{SD}_C^2}{n_T+n_C-2}}} * \left(1 - \frac{3}{4*(n_T+n_C-9)}\right).$$

here, $T$ and $C$ represent treatment and control, $n$ represents the sample size, and SD represents the standard deviation. We used R statistical software (R Core Team 2018) to plot the effect sizes against three explanatory variables: dose, particle size, and duration of exposure (Fig. 4).

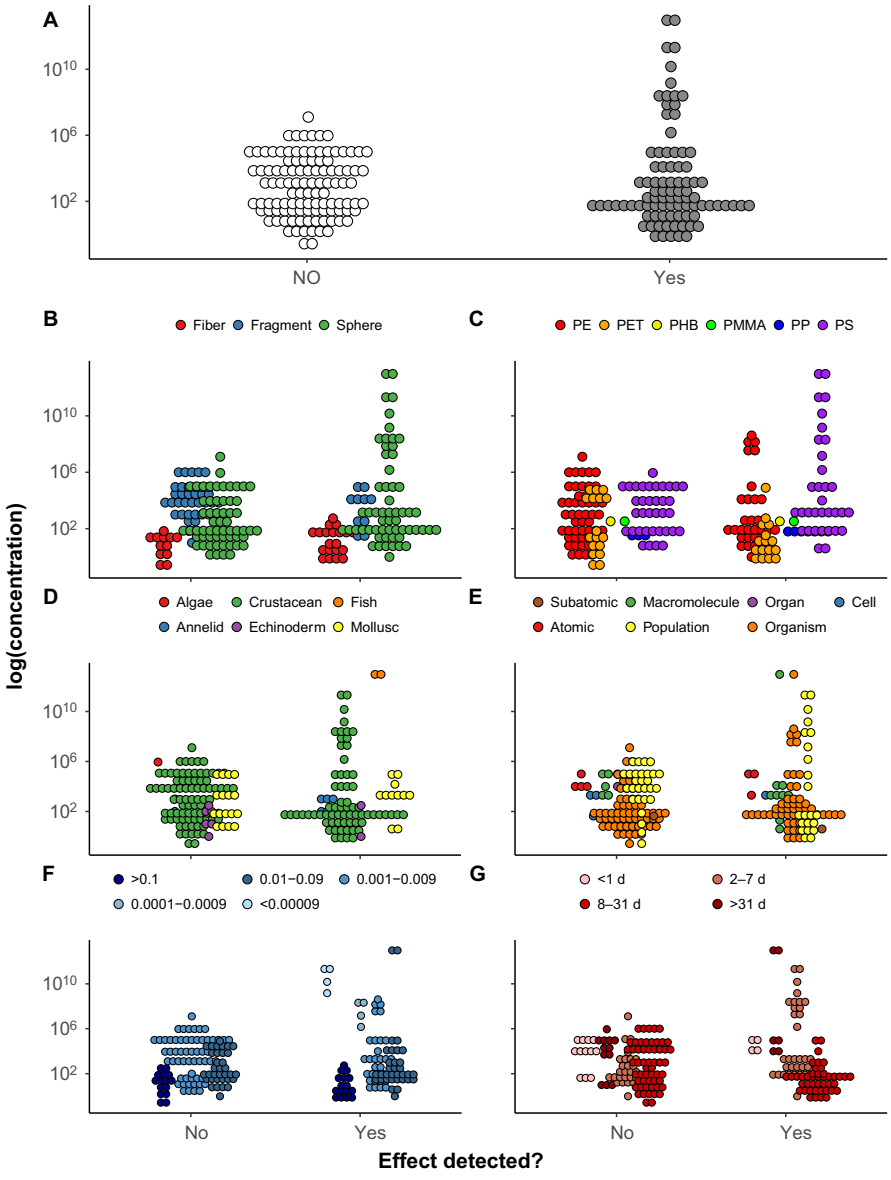


FIG. 3. Dot plots where each point depicts one tested effect. (A) The concentration (no. particles/mL) used to test the effect is on the *y*-axis and whether or not the effect was detected is on the *x*-axis. In each plot, the same data is organized by (B) plastic shape, (C) plastic type, (D) taxa, (E) duration of exposure, (F) particle size, and (G) exposure duration.

In some instances, we were unable to calculate Hedges' *g* using the above formula (Jemec et al. 2016, Rehse et al. 2016, Ziajahromi et al. 2017). This occurred when there was no variation among the replicates in both the control and treatment groups, making both standard deviations equal to zero. This will cause the denominator of the Cohen's *d* equation to be zero, resulting in an undefined value. One situation where this can occur is when all individuals in both the treatment group and the control group survive for the duration of the exposure. In this case, because there is no difference in mortality between the two groups, Hedges' *g* was recorded as zero (Rehse et al. 2016). Another situation that results in an undefined value for Cohen's *d* is when there is consistent mortality across all replicates in both the control and treatment groups (e.g., 0% mortality in all control replicates and 100% mortality in all treatment replicates). In Ziajahromi et al. (2017), this occurred in six instances, when there was 0% mortality in all control replicates and either 10%, 40%, or 100% mortality in all treatment replicates. Although these are meaningful results, Hedges' *g* cannot be calculated and therefore the effect size was not included in the analysis. However, because these points reflect instances where a large effect

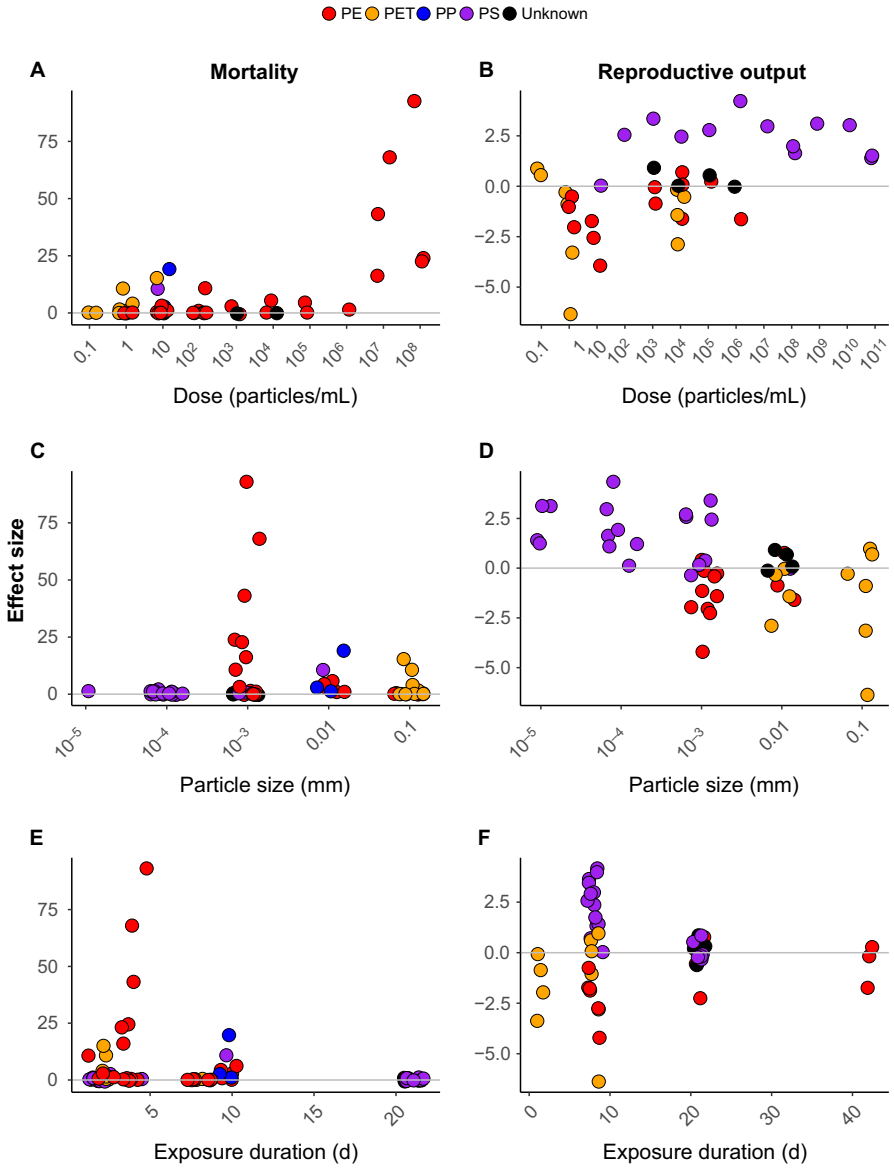


FIG. 4. Effect size figures comparing three explanatory variables (A and B, dose; C and D, particle size; E and F, exposure duration) with the mortality (A, C, E) and reproductive output (B, D, F) of crustaceans exposed to microplastics. Each point represents the size of the effect of one treatment compared to the control (i.e., each laboratory experiment will have multiple treatments and, consequently, multiple points on a graph). Points are further categorized by polymer type, represented by color.

was detected, we can assume these values to be relatively large, and discuss where this data fits in with the rest of the studies to help elucidate any patterns.

### *Environmental relevancy of laboratory experiments*

To assess the environmental relevancy of laboratory experiments, studies that tested the effects of microplastics were compared to studies reporting concentrations of microplastics found in nature. Using Web of Knowledge (all databases), a second search was conducted with the keyword "microplastic" to find all studies that report concentrations in nature. First, the titles of all search results from the year 1898 to the end of 2018 were assessed to isolate the appropriate studies. The abstract and methods section were then read to confirm that each paper was relevant. Papers were excluded from the analysis if they reported values as a concentration per unit area (e.g., particles/km$^2$) or as a concentration per mass of sediment or soil (e.g., particles/kg dry mass), as they cannot be compared to laboratory studies that report doses in particles of plastic per unit volume. This led us to only include studies that reported concentrations of microplastics in surface water, subsurface water, or from

the water column, to compare them to the experimental studies from our systematic review. Papers that were not found in the Web of Knowledge database, but were known to the authors and met the criteria, were also included. Using the 106 papers that met this criteria, we extracted the following information: the average concentration of microplastics per volume, the maximum concentration of microplastics per volume, the mesh size used to take the sample or the mesh size used during processing (for grab samples), whether the sample was taken in marine or freshwater, and from what matrix the sample was taken (surface water, subsurface water, water column). See DataS1: Concentrations in Nature Data for a list of all papers included in this analysis.

To assess whether laboratory experiments are dosing with environmentally relevant concentrations of microplastics, we created a stacked histogram to visualize whether or not there is overlap in the concentrations found in nature and those used in exposures. To facilitate this comparison, the concentrations in all studies were converted to particles/mL. In studies reporting concentrations found in nature, the maximum reported concentration was used in the analysis. In cases where different sampling techniques were used (e.g., studies comparing grab samples and Manta trawls), the highest concentration from each technique was included. In experimental studies that dosed with multiple concentrations, each dose was included in the analysis. The concentrations were then categorized into bins by order of magnitude and plotted as a stacked histogram. See DataS1: Doses in Lab for a list of all studies included in this analysis.

To assess whether laboratory experiments are dosing with environmentally relevant sizes of microplastics, we compared the average size of microplastics used in laboratory exposures with the lowest mesh size used to collect samples from the environment. When studies used a Manta or similar type of trawl to collect their samples, the size of this mesh was used in the analysis. When studies collected grab samples, the size of the filter used to process the sample was included in the analysis. In laboratory studies, the mean particle size was used. Finally, the particle and mesh sizes were categorized by order of magnitude and plotted as a stacked histogram. See DataS1: Experiment Particle Size for a list of all studies included in this analysis.

## Results

### *Overall weight of evidence*

Our initial literature search resulted in 186 studies. Based on a closer read of the abstract and manuscript, 139 of these studies were relevant for our systematic review. Data were extracted from each paper and synthesized to describe the weight of evidence behind the effects of plastics in the environment (process shown in Fig. 1). Across 139 studies, we found 577 effects that were tested or observed at several levels of biological organization (Fig. 2), a variety of taxa, from marine, freshwater, and terrestrial ecosystems, and with various plastic types, sizes, and shapes (Fig. 3).

In our analysis, 59% (341) of tested or observed effects were detected while 41% (236) were not. Of the effects that were detected, 42% (142) were due to macroplastics and 58% (199) were due to microplastics. Of the effects that were not detected, 6% (14) were due to macroplastics and 94% (222) were due to microplastics (see Table 1 for more details).

The majority of tested effects (77%, or 443 effects), were investigated at the organism or sub-organism levels of biological organization. Of the 205 effects tested at sub-organismal levels (36% of all effects), 61% (126) were detected while 39% (79) were not. Of the 238 effects tested at the organism level (41% of all effects), 56% (134) were detected while 44% (104) were not. Of the 134 effects tested at the population, assemblage, and ecosystem levels (23% of all effects), 60% (81) were detected while 40% (53) were not. For a more detailed breakdown of the effects tested at each levels of biological organization, see Table 1 and Fig. 2.

Finally, the vast majority of effects (76%, or 441 effects) were relevant to marine ecosystems. Only 21% (122) were relevant to freshwater ecosystems, while 3% (14) were relevant to terrestrial ecosystems. For a further breakdown of the effects relevant to each ecosystem and whether or not they were detected, see Appendix S1: Figs. S1, S2, and S3.

### *Field observations and experiments*

Our literature search found 69 observational or manipulative field studies investigating the effects of macro- and microplastics. Of these, 67 were related to macroplastics and only two were observational studies with microplastics. Of the two field experiments using microplastics, one study found a significant relationship between microplastics and the population size of *Halobates sericeus,* a marine insect (Goldstein et al. 2012). The other study found a significant correlation between plastic ingestion and enzyme activity, but not with lipid peroxidation (Alomar et al. 2017).

Of the 67 macroplastic field studies, 58 studies were observational and nine were manipulative experiments. From the 58 observational field studies with macroplastics, 83 effects were detected. These effects were predominantly related to entanglement, ingestion, or smothering. The organisms that were observed in these studies included 23 species of marine mammals (including pinnipeds, manatees, whales, and dolphins; 36 effects), four species of sea turtles (20 effects), 11 species of birds (16 effects), four species of fish (four effects), one species of algae (one effect), and many species of invertebrates (six effects). For detailed information on what species were studied refer to DataS1: All Effect Studies. Of these detected effects, 40% were at

Table 1. Breakdown of detected and non-detected impacts from micro- and macro-debris at each level of biological organization.

| Level | Effect detected ($n$ = 341) | | Effect not detected ($n$ = 236) | |
|---|---|---|---|---|
| | Micro (<5 mm) | Macro (>5 mm) | Micro (<5 mm) | Macro (>5 mm) |
| Fraction (%) | 58 | 42 | 94 | 6 |
| Total | 199 | 142 | 222 | 14 |
| Sub-organismal level | | | | |
| Subatomic (e.g., oxidative stress) | 8 | 0 | 6 | 0 |
| Atomic (e.g., greater concentrations of intracellular Ca) | 3 | 0 | 7 | 0 |
| Small molecules (e.g., toxic metabolites) | 0 | 0 | 0 | 0 |
| Macromolecules (e.g., protein, DNA damage) | 36 | 0 | 38 | 0 |
| Molecular assemblies (e.g., formation of protein chains) | 0 | 0 | 0 | 0 |
| Organelles (e.g., more micronuclei) | 0 | 0 | 0 | 0 |
| Cells (e.g., necrosis, less viable cells) | 29 | 0 | 17 | 0 |
| Tissues (e.g., inflammation, laceration) | 3 | 32 | 6 | 0 |
| Organs (e.g., change in size, lesions) | 10 | 4 | 5 | 0 |
| Organ system (e.g., poorly functioning digestive system) | 0 | 1 | 0 | 0 |
| Organismal level | | | | |
| Organism (e.g., reduced growth, death to an individual) | 75 | 59 | 96 | 8 |
| Ecological level | | | | |
| Populations (e.g., increase or decrease in size of population) | 28 | 24 | 44 | 1 |
| Assemblages (e.g., change in abundance or diversity of biota) | 4 | 14 | 0 | 3 |
| Ecosystem (e.g., change in ecosystem function) | 3 | 8 | 3 | 2 |

sub-organismal levels (34 effects), 50% at the organism level (42 effects), and 10% at the levels of population (four effects) and assemblage (four effects).

From the nine manipulative field experiments with macroplastics, we found 39 instances where an effect was tested, all of which were in the marine environment. The organisms tested in these studies included invertebrates, sea turtles, fish, and a plant. Of the 39 tested effects, 77% were detected (30 effects). Of these, 3% were at sub-organismal levels (one effect), 23% were at the organismal level (seven effects), and 73% were at the levels of population (seven effects), assemblage (seven effects, and ecosystem (eight effects). Of the nine effects that were not detected, 44% were at the organismal level (four effects) and 66% were at the levels of population (one effect), assemblage (two effects), and ecosystem (two effects).

### *Laboratory experiments*

Our literature search resulted in 69 studies that tested the effects of plastic with manipulative laboratory experiments. Of these studies, 66 tested with microplastics and three with macroplastics (DataS1:Scatterplot Data). For the three lab studies that used macroplastics, most were testing effects related to the leachates associated with the plastic rather than the plastic itself. The organisms tested in these studies included aquatic invertebrates and fish. From the three studies, we extracted 24 tested effects, 23 of which were detected. Of the 23 detected effects, 35% were at the organismal level (eight effects) and 65% were at the population level (15 effects). The one effect that was not detected was at the organism level.

From the 66 lab experiments using microplastics, we extracted 421 instances where an effect was tested. Of the tested effects, 47% (199) were detected while 53% (222) were not (for a more detailed breakdown see Table 1; Fig. 2). Of the 66 lab experiments, 43 tested on marine organisms, 20 on freshwater organisms, and three on terrestrial organisms. Experiments ranged in duration from 30 minutes to eight months. Overall, these 66 studies included 33 short-term tests (six were <24 h, four were 24 h, eight were 48 h, two were 72 h, nine were 96 h, and four were 120 h) and 42 tests that were one week or longer (11 were one to two weeks, 12 were two to four weeks, 15 were one to two months, and four were longer than two months). Various organisms were used in these lab studies, including crustaceans (24 studies; including zooplankton), molluscs (14 studies), fish (11 studies), annelids (seven studies), algae (six studies), echinoderms (three studies), fungi (one study), and plants (one study). Similarly, diverse polymer types have been used. The majority of the experiments exposed organisms to polyethylene (28 studies) and polystyrene (39 studies), while six studies used PVC, four used PET, three used polypropylene, two used polycarbonate, and one used PHG, PLA, PMMA, cellulose acetate, acrylic, and polyamide. Two studies used microplastics with unknown polymer types. Finally, various shapes were used in these experiments: 44 studies used spheres, 22 used fragments, eight used fibers, and two used pellets.

Of the 66 laboratory studies, 20 were used for further comparison to explore the patterns behind whether or not an effect was detected. Categorization of the data by plastic shape (Fig. 3B), plastic type (Fig. 3C), taxa used

(Fig. 3D), level of organization targeted (Fig. 3E), dose (Fig. 3F), and length of exposure (Fig. 3G) provides more insight into the underlying trends in the data. From these studies, we extracted 197 tested effects, 55% of which were detected (109) and 45% of which were not (88). The doses used in these experiments ranged from 0.27 to $2.91 \times 10^{11}$ particles/mL. In terms of plastic shape, 16% (32) of tested effects used microfibers, 24% (47) used fragments, and 60% (118) used spheres. An effect was detected in 62% (20) of cases using microfibers, 21% (10) using fragments, and 49% (58) using spheres. In effects tested with spheres, those that dosed with $>1.46 \times 10^7$ particles/mL always detected an effect (Fig. 3A). In terms of plastic type, 41% (81) of tested effects used PE, 35% (69) used PS, 17% (33) used PET, 5% (10) used PP, 1% (2) used PHB, and 1% (2) used PMMA. An effect was detected in 37% of cases using PE (30 of 69), 48% using PS (33 of 69), 48% using PET (16 of 33), 70% using PP (7 of 10), 50% using PHB (1 of 2), and 50% using PMMA (1 of 2). In terms of the taxa tested, 76.1% (150) used crustaceans, 14.4% (28) used molluscs, 4% (8) used echinoderms, 4% (8) used annelids, 1% (2) used fish, and 0.5% (1) used algae (Fig. 3D). An effect was detected in 45% of cases using crustaceans (67), 39% of cases using molluscs (11), 25% of cases using echinoderms (2), 75% of cases using annelids (6), 100% of cases using fish, and none of the cases using algae. In terms of the level of organization targeted, 15% (30) targeted sub-organism levels (i.e., sub-atom, atom, macromolecule, cell, organ), 60% (117) targeted individual levels, and 25% (50) targeted population levels (Fig. 3E). An effect was detected in 47% of cases at the sub-organism level (14), 33% of cases at the individual level (52), and 44% of cases at the population level (22). Particle sizes used in these experiments ranged from 0.00005 to 0.28 mm with a mean of 0.05 mm (Fig. 3F). Of the 197 tested effects, 16% (32) used particles larger than 0.1 mm, 34% (67) used particles between 0.09 mm and 0.01 mm, 46% (90) used particles between 0.009 mm and 0.001 mm, and 4% (8) used particles smaller than 0.0009 mm. When particles smaller than 0.0025 mm were used in testing, an effect was detected in 70% of cases. Length of exposure ranged from 3 h to 95 d (Fig. 3G). Of the 197 tested effects, 40% (78) were from experiments that lasted less than one week, 51% (101) were from experiments that lasted one to four weeks, and 9% (18) were from experiments that lasted over a month. In the experiments that lasted up to one week, 42% (33) of effects were detected and 58% (45) were not. In the experiments that lasted one to four weeks, 49% (49) of effects were detected and 51% (52) were not. Of the experiments that lasted over a month, 67% of effects (12) were detected and 33% (6) were not.

### *Informing lethal concentrations*

Of the 139 studies we evaluated in our systematic review that investigated the effects of microplastics, only six measured one or more $LC_{50}$ value (Table 2). Reported $LC_{50}$ values ranged from 71.43 to 1 million particles/mL and from 0.04 to 65 mg/L. All studies reporting $LC_{50}$ values were conducted using species of zooplankton. $LC_{50}$ values have been calculated using various plastic types, shapes, and sizes for *Daphnia magna*, *Hyalella azteca*, and *Ceriodaphnia dubia*. All tests were conducted with either PE, PS, or PP, and for sizes of microplastics ranging from 200 nm to 75 μm.

### *Meta-analysis*

Of the 66 lab experiments using microplastics, only 13 studies, all on crustaceans, were relevant for our meta-analysis. In these studies, mortality and reproductive output were the only response variables that were measured consistently.

Mortality was investigated in 10 of the 13 studies. This yielded 73 instances where an effect size was calculated. Of these, 57% detected an increase in mortality in the treatment groups, 40% detected no change in mortality, and 3% detected a decrease in mortality (Fig. 4A, C, E). Seven of the initial 13 studies reported dose as a number of particles per unit volume and were therefore used to explore mortality with dose as the explanatory variable (Fig. 4A). Doses ranged from 0.27 particles/mL to $4.06 \times 10^8$ particles/mL, with a median of 90 particles/mL. While the majority of studies detected an increase in mortality, effect size tends to increase as the dose increases to extreme concentrations. For particle size, there seems to be no clear directional trend with mortality (Fig. 4C). Particle sizes ranged from 0.000052 mm to 0.7 mm with a median of 0.0025 mm. Very small particles (on the order of 0.00001 mm) and relatively large particles (on the order of 0.01 and 0.1 mm) produce larger effect sizes than the particles of an intermediate size (0.001 mm). Finally, exposure duration has no clear directional trend with mortality (Fig. 4E). However, these experiments ranged from 2 to 21 d, with a median of 4 d, and only 3 of the 10 studies lasted longer than 10 d.

Reproductive output was investigated in 7 of the 13 experimental studies. This yielded 46 instances where an effect size was calculated. Of these, 54% detected an increase in reproductive output in the treatment groups, 43% detected a decrease, and 2% detected no change (Fig. 4B, D, F). Six of the initial 13 studies reported dose as a number of particles per unit volume and were used to explore reproductive output with dose as the explanatory variable (Fig. 4B). Doses ranged from 0.27 particles/mL to $1.46 \times 10^{10}$ particles/mL, with a median of 1,000 particles/mL. The trend suggests that reproductive output increases with dose; however, this is driven largely by one study that exposed organisms to PS (Jeong et al. 2017). Particle size ranged from 0.00005 mm to 0.28 mm, with a median of 0.003 mm (Fig. 4D). The trend suggests that reproductive output decreases with particle size. Finally, exposure duration ranged from 2 to

TABLE 2. $LC_{50s}$ that have been calculated in studies.

| Study | Organism | Effect | Plastic type | Size | Length of exposure | Shape | $LC_{50}$ concentration |
|---|---|---|---|---|---|---|---|
| Ogonowski et al. (2016) | *Daphnia magna* | death | unknown (from cospheric) | 4 μm | 14 d | sphere | 1 mol/L particles/mL |
| Au et al. (2015) | *Hyalella azteca* | death | PP | 20–74 μm | 10 d | fiber | 46,400 particles/mL |
| Au et al. (2015) | *Hyalella azteca* | death | PE | 10–27 μm | 10 d | fragment | 71.43 particle/mL |
| Frydkejar et al., 2017 | *Daphnia magna* | immobilization | PE | 10–75 μm | 48 h | fragment | 65 mg/L |
| Ziajahromi et al. (2017) | *Ceriodaphnia dubia* | death | PE | 1–4 μm | 48 h | sphere | 2.2 mg/L |
| Ziajahromi et al. (2017) | *Ceriodaphnia dubia* | death | PE | 1–4 μm | 48 h | fiber | 1.5 mg/L |
| Rehse et al. (2016) | *Daphnia magna* | immobilization | PE | 1 μm | 96 h | sphere | 57.42 mg/L |
| Kim et al. (2017) | *Daphnia magna* | immobilization | PS | 200 nm | 48 h | sphere | 0.04 mg/L |

42 d, with a median of 8 d (Fig. 4F). There is no directional trend between length of exposure and reproductive output.

### *Environmental relevancy of laboratory experiments*

Twenty laboratory experiments and 106 environmental studies were compared for environmental relevancy on the basis of concentration in particles per unit volume (Fig. 5A; see DataS1: Concentrations in Nature Data for the environmental studies and DataS1: Doses in Lab for the experimental studies). Because lab studies often use a series of dose concentrations in their experiments, our analysis consisted of 89 experimental doses. The doses ranged from 0.27 particles/mL to $9.30 \times 10^{12}$ particles/mL, with a median of 333.33 particles/mL. From the 106 studies that reported concentrations of microplastics in nature, we obtained 137 concentrations for our analysis. These concentrations ranged from $2.80 \times 10^{-8}$ particles/mL to 1.77 particles/mL, with a median of 0.00014 particles/mL. When comparing the doses used in laboratory experiments with the concentrations of microplastics found in the environment, we determined that 17% (15 doses) were environmentally relevant.

Forty-four laboratory studies and 102 environmental studies were analyzed for environmental relevancy on the basis of particle size (Fig. 5B; see DataS1: Concentrations in Nature Data for the environmental studies and DataS1: Lab Experiment Particle Size for the experimental studies). Many lab studies used multiple particle sizes in their experiments, so our analysis consisted of 71 particle sizes. Particles used in laboratory studies ranged from 0.02 μm to 1 mm, with a median of 10 μm. From 102 environmental studies, we obtained 133 mesh sizes for our analysis. Mesh size ranged from 0.45 to 505 μm, with a median of 200 μm. When comparing the particle sizes used in laboratory experiments with the mesh sizes used for environmental sampling, we determined that 80% of the particle sizes used in laboratory studies (61 particle sizes) were relevant to what is being sampled for in nature.

## DISCUSSION

Studies investigating the effects of plastic pollution use various types, sizes, and shapes of plastics, test on various taxonomic groups, and target all levels of biological organization. These studies include laboratory experiments, observational field studies, and manipulative field studies. The majority of laboratory experiments investigate the effects of microplastics and have detected effects across all levels of biological organization, from sub-organismal to ecologically relevant levels. The majority of field studies (both observational and manipulative) investigate the effects of macroplastics and have detected effects predominantly at individual, population and assemblage levels of organization. Finally, the great majority of studies (lab and field) are relevant to marine ecosystems (76%), while relatively little has been done regarding freshwater and terrestrial.

In this study, we found evidence that plastic pollution of all shapes and sizes can affect organisms across all levels of biological organization: roughly two-thirds (60%) of tested or observed effects from lab and field studies have been detected. There is no doubt that plastic pollution can have an impact on wildlife, but the results for macroplastics seem to be more straightforward than for microplastics. The goals of our current systematic review were to provide an update on the weight of evidence regarding the effects of plastic pollution and conduct a systematic review and meta-analysis to explore the complexity of current trends to inform future research.

### *Current weight of evidence*

In 2016, Rochman et al. (2016) reviewed the weight of evidence regarding what we knew about the ecological impacts of plastic pollution. At that time, there were

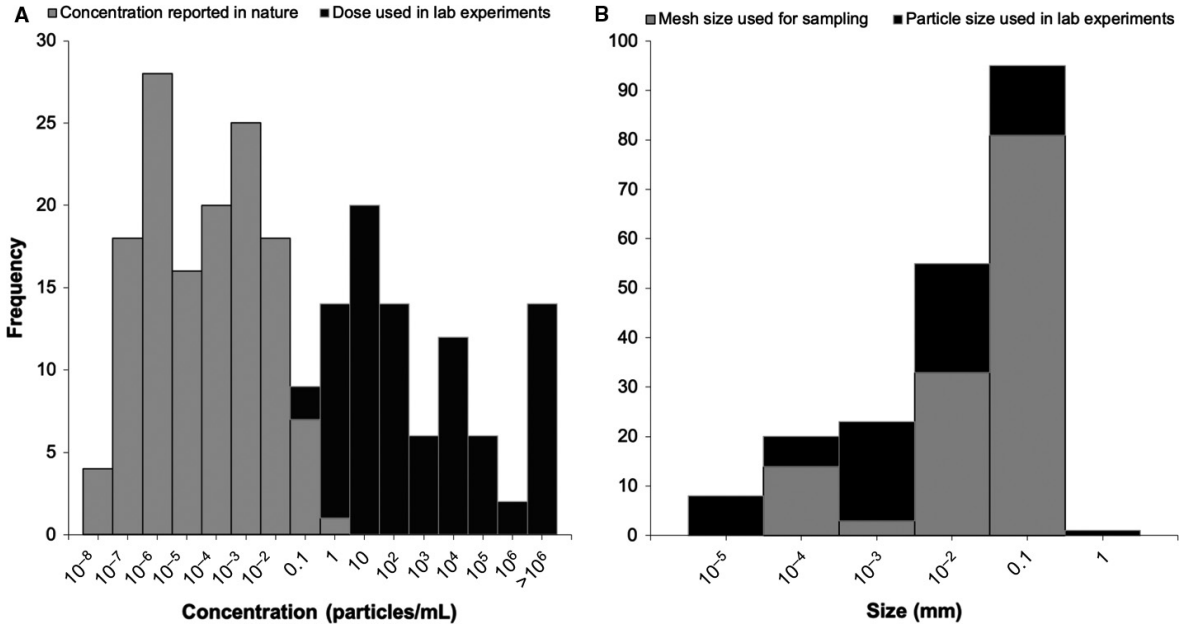


FIG. 5. (A) Concentrations of microplastics found in nature (gray) and doses of microplastics used in laboratory experiments (black). Only 17% of the doses analyzed were found to be environmentally realistic. (B) Particle sizes of microplastics used in laboratory experiments (black) and mesh size used in environmental sample collection (gray). Environmental samples are being collected with mesh sizes that make collection of very small particles (<1 μm) impossible, yet these sizes continue to be used in lab experiments.

plenty of studies about the effects of macroplastics, but a lack of studies investigating microplastics. As such, the authors made a call for further research, particularly for studies asking questions about the effects of microplastics. Here, we revisit the literature and determine the weight of evidence for the effects of plastic pollution through most of 2017.

Since the 2016 paper, there has been a dramatic increase in the number of studies testing hypotheses related to the effects of macroplastics and microplastics in the environment. In the first paper, Rochman et al. (2016) extracted 245 tested or observed effects of plastic pollution, many of which had been pulled from fields unrelated to plastics. Since then, this number has risen to 577 tested or observed effects (without having to pull data from unrelated fields). Further, the number of detected effects has risen from 70 to 142 for macroplastics, and from 175 to 199 for microplastics. Additionally, more microplastics studies are now targeting effects at ecologically relevant levels of organization. In the 2016 review, only four effects had been tested at organismal and ecological levels of organization for microplastics. Today, we quantified 253 effects tested for microplastics at ecologically relevant levels of organization. It is also noteworthy that in the 2016 review, there was only one instance where a tested effect was not detected (Browne et al. 2008). Here, we report 222 tested effects that were not detected for microplastics and 14 for macroplastics.

In 2016, Rochman et al. concluded that macroplastics can negatively affect organisms, and made a call for further research investigating the effects of microplastics. Today, there is overwhelming evidence of the detrimental effects of macroplastics to individuals, as well as compelling evidence for effects to populations, communities, and ecosystems. As such, the current weight of evidence is enough to motivate an immediate conservation response for affected populations, species, and assemblages (see the DataS1: All Effect Studies for details regarding which species are affected). For microplastics, however, the evidence surrounding effects is much more variable and seems to be context dependent. In 2016, Rochman et al. were unable to dig deeper into the data simply because there was not enough. In this study, we were able to synthesize and analyze the literature to better tease apart the complexity related to the effects of microplastics.

### *A deeper dive into studies testing the effects of microplastics*

While many laboratory studies have detected effects from microplastics, almost an equal number of studies have not. The discrepancy in these results is likely due to the diversity of experimental designs being employed to test for effects. Experimental studies have used a range of exposure durations, taxonomic groups, polymer types, shapes, sizes, and concentrations. When we explore the patterns underlying these studies, we found that an effect was more likely to be detected at higher concentrations of microplastics used (Fig. 3A). We looked further into

this trend by comparing effect sizes from all lab studies that tested the effects of microplastics to crustaceans. When mortality was investigated as an endpoint, larger effects were seen as the dose increased, especially for extreme, environmentally unrealistic concentrations (on the orders of 10 and 100 million particles/mL; Fig. 4A). Some experimental studies that use multiple concentrations have detected dose-dependent relationships, such as for mortality in *H. azteca* (Au et al. 2015), fecundity in *P. nana* (Jeong et al. 2017), growth in various benthic macroinvertebrates exposed to PS fragments (Redondo-Hasselerharm et al. 2018), and algae ingestion in *C. typicus* (Cole et al. 2011). Although dose-response studies provide useful information on the toxicity of different types of microplastics, they often lack environmental relevancy due to their use of extremely high doses. For reproductive output, the trend with dose is more complicated (Fig. 4B). Reproductive output appears to increase with extremely high doses, decrease with relatively low doses, and both increase and decrease at intermediate doses. The only obvious trend here is with polymer type, which will be discussed below. Finally, when we take into consideration the instances where it was not possible to calculate an effect size, this pattern is not reinforced as what would be calculated as very large effect sizes fall at lower concentrations, on the orders of 1, 10, and 100 particles/mL (Appendix S1: Fig. S4a).

Plastic shape also seems to be an important factor in whether or not an effect is detected. In studies that tested for an effect with fibers, the majority detected an effect. Studies that tested for an effect using fragments, however, were less likely to detect one (Fig. 3B). Some experimental studies that compare effects by plastic shape have reached a similar conclusion where fibers are more toxic than fragments in *H. azteca* (Au et al. 2015), fragments and spheres in shrimp (Gray and Weinstein 2017), and spheres in *D. magna* (Ziajahromi et al. 2017). This finding is important as microfibers are the most common type of microplastic found in nature (Barrows et al. 2018) and in a recent study, 70% of the microplastics found to be ingested by zooplankton were microfibers (Sun et al. 2017). In our analysis, we found that although studies that test with spheres have detected an effect more frequently than no effect, there are also far more studies that test with spheres at extremely high concentrations. At lower concentrations, there are slightly more studies testing with spheres that do not detect an effect (Fig. 3B).

Size is also a contributing factor in whether or not an effect is detected. In our analysis, we see that an effect is more likely to be detected with smaller particles while, for larger particles, there seems to be more variability in whether or not an effect is detected (Fig. 3F). For the smaller particles, this trend may be due to their increased bioavailability (Jeong et al. 2017) or due to their potential to translocate across the cell membrane (Browne et al. 2008). It may also be a result of the particles blocking the passage of food through the digestive tract or causing pseudo-satiation, which stops the organism from feeding (Anbumani and Kakkar 2018). We looked further into this trend by comparing effect sizes from all lab studies that tested the effects of microplastics to crustaceans. Larger particles generally seem to cause an increase in mortality, although the effect is larger at intermediate particle sizes (Fig. 4C). However, one study in particular (Rehse et al. 2016), which used an extremely high dose of microplastics of an intermediate size, is driving the trend with very large effect sizes at an intermediate particle size. When that study is removed from the analysis, mortality is simply seen to increase with particle size (although this also reverses the trend previously seen with mortality and dose; Appendix S1: Fig. S5). Finally, when we take into consideration the instances where it was not possible to calculate an effect size, what would be calculated as very large effect sizes would fall at intermediate (0.001 mm) and relatively large (0.1 mm) particle sizes (Appendix S1: Fig. S4b). Because our analysis includes only studies that test effects in crustaceans, it is likely that the larger particles are more lethal because of their size relative to the organism. It is possible that the larger particles can lacerate their digestive tract or cause the organism to starve (Anbumani and Kakkar 2018), while the smaller particles may be excreted (Cole et al. 2011).

The trend between particle size and reproductive output is, again, more complicated. Reproductive output appears to increase for small particles but decrease for relatively large particles. A decrease in reproductive output may be expected, and in fact has been shown in a number of studies (Cole et al. 2015, Ogonowski et al. 2016). A decrease in reproductive output has been attributed to a reduction in feeding behavior reducing energy allocation for reproduction (Ogonowski et al. 2016). For the small particles, however, we would not expect reproduction to increase. It is possible that this trend may be caused by another factor: polymer type. In fact, the majority of the studies reporting an increase in reproductive output had exposed organisms to polystyrene (in 18 out of 21 instances, from three different studies). The studies reporting a decrease in reproductive output had exposed organisms to either polyethylene (11 out of 20 instances) or polyethylene terephthalate (eight out of 20 instances), with one instance of exposure to polystyrene causing a decrease in reproductive output. Thus, it is possible that, while polyethylene and polyethylene terephthalate may decrease reproductive output by causing stress to the organism, polystyrene may cause an increase in reproduction, as it is known to contain chemicals that affect the endocrine system (Lithner et al. 2011).

In general, we did not detect a pattern in whether or not an effect was detected based on polymer type (Fig. 3C; despite the trend in the direction of effect seen in Fig. 4B, D, F), taxa used (Fig. 3D), or the level of biological organization tested (Fig. 3E). For exposure duration, no trend was apparent in whether or not an

effect was detected (Fig. 3G), nor was a trend detected in effect sizes (Fig. 4E, F). However, very few studies lasted longer than 10 d, making it nearly impossible to generate a conclusion from this data. Overall, robust analysis has been made difficult at this time due to the wide variation in the types, shapes, and sizes of plastic used in experiments, the different taxa being tested on, the dose used during testing, and the duration of the exposures.

### *Environmental relevancy of the current body of literature*

Ideally, the motivation for new environmental policy should be stimulated by scientific evidence that is environmentally relevant (Rochman 2016). Providing environmentally relevant data regarding the effects of microplastics requires using realistic concentrations and sizes of plastics in nature. In our analysis, 17% of doses used in experimental studies were concentrations of microplastics that have been found in nature (Fig. 5A). The highest environmental concentration was 1.77 particles/mL (Dubaish and Liebezeit 2013), while the highest experimental dose was 9.3 trillion particles/mL (Mattsson et al. 2015). Although we recognize that microplastics are heterogeneously distributed throughout lakes and oceans, and that we have yet to accurately quantify contamination below 300 μm (Covernton et al. 2019), there is clearly a disconnect between the concentrations found in nature and those used in laboratory experiments. Interestingly, when looking only at the 35 instances where an environmentally relevant dose is used, 49% detected an effect while 51% do not. This same trend was seen when looking at all microplastic studies regardless of dose. Despite there being a benefit to exposing organisms to concentrations of microplastics higher than what is found in nature (e.g., calculating $LC_{50}$ values, future projections, determining the mechanism of an effect), more of an effort should be made to investigate effects at environmentally relevant doses, i.e., around 10 particles/mL.

Another metric by which we can measure environmental relevancy is particle size. In our analysis, the smallest mesh size used for environmental sampling was 0.45 μm (Barrows et al. 2017), while the smallest particle size used in a laboratory experiment was 0.02 μm (Bhattacharya et al. 2010). Despite only 9% of environmental studies using a mesh size that would capture particles smaller than 10 μm, 52% of laboratory studies dose with particles in that size range. A recent study suggests that environmental samples collected with mesh smaller than 100 μm in size capture concentrations of microplastics one to four orders of magnitude greater than samples collected with a standard 300 μm mesh (Covernton et al. 2019). Thus, using smaller mesh sizes during environmental sampling will give a better indication of how many particles are present in nature, and inform laboratory studies that wish to dose with small particle sizes. This is especially important as governments discuss the effects of nanoplastics and make hypotheses about greater effects based on their smaller size (Lusher et al. 2017), which may be irrelevant if we cannot accurately quantify small particles in nature. Our comparison was limited by the tendency for lab experiments dosing with extremely small particle sizes to report dose as a mass concentration. Moreover, very few field studies report concentrations of microplastics smaller than 10 μm in the environment. As a long-term solution to this issue, the field requires technological developments that will allow scientists to accurately count extremely small particles. Finally, it is worth noting that dose and size are not the only factors that should be considered when assessing environmental relevancy of laboratory experiments. Other factors that should be considered include polymer type (including mixtures of multiple polymer types), shape (fiber, fragment, sphere), and chemicals associated with the plastics (Rochman et al. 2019).

### *Data gaps and areas for future research*

Despite the increase in laboratory studies investigating the effects of microplastics, a consensus has not yet been reached regarding their impacts. This is likely a result of "microplastic" being generalized as a single contaminant, rather than a suite of contaminants that vary in polymer type, size, shape, color, and chemical cocktail. Future experimental work should be conducted strategically such that we can begin teasing apart these factors and determine whether and how they change the observed impacts of microplastics. For example, by conducting an experiment with multiple plastic types as unique treatments while holding every other factor constant, we can elucidate the potential impacts of different polymer types. This could be repeated with different sizes or shapes of microplastics, plastics with different chemical mixtures (additives from manufacturing and chemicals sorbed from the environment), and with different taxa. Additionally, performing experiments with more harmonized methods would provide data useful for conducting a more robust meta-analysis (e.g., reporting concentrations in particles per volume, investigating particular endpoints). As we gain a better understanding of the individual effects of different microplastic characteristics, it will be valuable to investigate complex, environmentally realistic mixtures of microplastic types, shapes, and sizes.

While laboratory studies that strategically test different aspects of microplastics are necessary, future experimental work should also begin asking questions about how microplastics affect ecosystems under more natural scenarios. For example, observational experiments can be conducted in the field, or manipulative experiments can be conducted within mesocosms that mimic an ecosystem (Green 2016) or in an experimental lake. Moreover, to forecast how present or future scenarios of plastic contamination may affect populations, assemblages, and other ecosystem dynamics, modelling exercises can be conducted as they have been for other

anthropogenic contaminants (Huang et al. 2015). Such experiments will allow us to extend our knowledge of how microplastics act in the lab to real-world scenarios.

Finally, in addition to a call for using more ecologically relevant scenarios and teasing apart the effects of different characteristics of microplastics, we still have a lot to learn about ecological effects outside of the marine environment. For freshwater and terrestrial environments, the work remains in its infancy. We suggest using lessons learned from the marine environment and applying it to new studies that specifically ask questions about the effects of micro- and macro- plastics to freshwater and terrestrial ecosystems across all levels of biological organization.

## Conclusion

In 2016, Rochman et al. concluded that evidence for the ecological effects of plastic pollution of all sizes was not necessarily lacking because plastics do not cause ecological effects, rather that scientists were not asking questions relevant to ecological processes. Today, we continue to see evidence for the ecological effects of macroplastics, and more research asking questions regarding the effects of all sizes of plastics across all levels of biological organization. However, the current evidence for microplastics remains split between detected and non-detected effects. Here, we make a call for future laboratory work to continue with a greater recognition of the complexity of microplastics as a diverse suite of contaminants, and to try to understand the underlying mechanisms causing the mixed evidence. We also make a call for continued observational and experimental work at diverse scales (e.g., from microcosm to mesocosm to the field) and across all levels of organization, that aims to truly understand the ecological effects of macro- and microplastics. In addition, we call for holistic work beyond the marine environment, recognizing that plastic pollution is a global issue across all ecosystems. We suggest that it is time to ask more contextual questions and use more strategic experiments to begin to tease apart the complex effects of plastics on wildlife and ecosystem processes globally.

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