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Environmentally Accurate Microplastic Levels and Their Absence from Exposure Studies

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Synopsis Microplastics (synthetic polymers; <5 mm) are ubiquitous, in the environment and in the news. The associated effects of microplastics on flora and fauna are currently only established through laboratory-based exposure trials; however, such studies have come under scrutiny for employing excessive concentrations with little environmental relevance. This critical review is intended to summarize key issues and approaches for those who are considering the need for local microplastics research, both in terms of environmental pollution and the impacts on aquatic species. A meta-analysis of results from published experimental ($n = 128$) and environmental ($n = 180$) studies allowed us to compare the reported impacts from experiments that expose organisms to microplastics, and the concentrations of environmental microplastics found in the wild. The results of this meta-analysis highlight three issues that should be modified in future work (1) use of extreme dosages, (2) incompatible and incomparable units, and (3) the problem of establishing truly informative experimental controls. We found that 5% of exposure trials examined did not use any control treatment, and 82% use dramatically elevated dosages without reference to environmental concentrations. Early studies in this field may have been motivated to produce unequivocal impacts on organisms, rather than creating a robust, environmentally relevant framework. Some of the reported impacts suggest worrying possibilities, which can now inspire more granular experiments. The existing literature on the extent of plastic pollution also has limited utility for accurately synthesizing broader trends, as has been raised in previous reviews; environmental extraction studies use many different units, among which only 76% (139/180) could be plausibly converted for comparison. Future research should adopt the units of microparticles/kg (of sediment) or mp/L (of fluid) to improve comparability. Now that the global presence of microplastic pollution is well established, with more than a decade of research, new studies should focus on comparative aspects rather than the presence of microplastics. Robustly designed, controlled, hypothesis-driven experiments based on environmentally relevant concentrations are needed now to understand our future in the new plastic world.

Introduction

The unprecedented production of synthetic polymers (plastic) since the 1940s has improved the lives of billions of people, while simultaneously creating one of the most pressing environmental concerns the world faces today—the plastic pollution crisis. Due to global population and consumer pressure, plastic production has increased exponentially since the mid-20th century to become an industry worth billions to the worldwide economy (Cole et al. 2011). Consequently, the manufacturing and subsequent waste of plastic items is one of the leading factors that scientists have used to propose a new transition in Earth's history, the Anthropocene (Zalasiewicz et al. 2016). It is believed that a distinct layer of plastic, among other factors, integrated in Earth's sedimentary record will separate this contemporary geological epoch from the Holocene (Waters et al. 2016; Geyer et al. 2017). Roughly 335 million tons of plastic are produced globally every year, and of the 60 million tons deriving from within the European Union alone, 70% are wasted without recycling (PlasticsEurope 2018). Studies of potential impacts in aquatic systems have focused mainly on the marine environment, with estimates as a high as 10% of all global plastic production entering marine systems annually (Mattsson et al. 2017), and between 60% and 80% of all litter in the marine environment

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deriving from plastic (Derraik 2002). Marine plastic pollution mostly originates from land-based waste sources (80%; Landon-Lane 2018) with the remainder as lost equipment from shipping traffic and fishing vessels. In recent years, studies of marine and freshwater plastic pollution have changed direction, with efforts shifting away from large-scale macroplastic waste to the focus now mostly on micro-sized plastic particles and their impact on aquatic species.

The distribution of microplastic particles was first described in the coastal waters of southern New England (Carpenter et al. 1972), a finding which also first demonstrated the ingestion of microplastics by fish species. Another study carried out in subsequent years demonstrated the distribution of microplastic particles in the Northwest Atlantic using surface water trawls (Colton et al. 1974). These studies in the 1970s set the stage for what is now a global research topic, but microplastic pollution had little attention over the next 30 years and the term microplastics was not coined until 2004 (Thompson et al. 2004).

Research surrounding the persistence and impacts of microscopic plastic particles (synthetic polymers <5 mm; Thompson et al. 2004) in aquatic environments has increased exponentially over the past decade (increasing 200-fold; Fig. 1). It is well known that microplastics are now globally distributed and are prominent even in some of the most remote areas of earth, including the Arctic (Lusher et al. 2015) and the deep sea (Bergmann et al. 2017; Jamieson et al. 2019). Generally, microplastic pollution is divided into two groups: primary and secondary microplastics. Primary microplastics are industry-manufactured beads used in oil and gas exploration or industrial abrasives (Sharma and Chatterjee 2017), and also cosmetic products such as facial scrubs and toothpaste (Napper et al. 2015). Secondary microplastics are the result of fragmented macroplastic items (>5 mm) degraded by UV and wave exposure to below the 5 mm threshold (Gall and Thompson et al. 2015). Secondary microplastics are the most common form of microplastic pollution, as the input of macroplastics in aquatic environments continues to grow exponentially every year (Strand et al. 2013). Microplastic fibers are released during domestic washing machine cycles, potentially over 1900 fibers from a single polyester item of clothing (Cesa et al. 2017), and fibers are also introduced into marine and freshwater environments through the breakdown of fishing equipment such as ropes, nets, and traps (Chen et al. 2018). As a result, microplastic fibers, a secondary microplastic, are responsible for >90% of all microplastic pollution in aquatic systems (Lehtiniemi et al. 2018).

Secondary microplastics continue to proliferate in freshwater (Shruti et al. 2019), marine (Zhang et al. 2019), and terrestrial ecosystems (Hüffer et al. 2019) and their ubiquity is now well established; however, publications are still aiming toward quantifying and identifying microplastics in the environment with little scientific novelty or comparative context. Most studies employ methods such as hypersaline density separations to extract plastic from sediment (Thompson et al. 2004; Hidalgo-Ruz et al. 2012; Wang and Wang 2018) and subsequent identification of plastic polymers through Raman spectrometry (Wen et al. 2018) or Fourier Transform Infrared (FT-IR) spectrometry (Kunz et al. 2016; Naji et al. 2017; Li et al. 2018). Despite the same methods being used for most extraction studies, not all researchers quantify microplastic abundance with the same units. As a result, the abundance of microplastics in sediment or water among different environments is often not comparable due to the variety of units used (Burns and Boxall 2018). At present, we know that plastic is everywhere, but there is little data to really determine how much plastic, or the rate of accumulation. This lack of quantification protocol for environmental sampling has also caused difficulties when applying known concentrations of microplastics to exposure studies (Huvet et al. 2016), which are currently the only available medium to determine the impacts of microplastics on aquatic species.

Despite studies showing that microplastics are prominent in most ecosystems, are readily consumed by species (Garnier et al. 2019), and can transport persistent organic pollutants (POPs; Rodrigues et al. 2018), the general research area has come under scrutiny in recent years and has been labeled as a "bandwagon" topic. This has both advantages and disadvantages in terms of research and environmental advocacy. Recent media coverage has promoted plastic pollution as one of the greatest threats to the planet (Stafford and Jones 2019). Increased media attention, including documentaries such as BBC's *Blue Planet*, and a surge in microplastic research publications has helped to influence policy to reduce plastic waste around the world, including cosmetic microbead bans in Canada, UK, and USA. Microplastics working groups have also been established to push for cosmetic microbead bans in Australia (Lam et al. 2018). The majority of critical scrutiny has surrounded the use of microplastics in laboratory-based exposure trials, as there have been

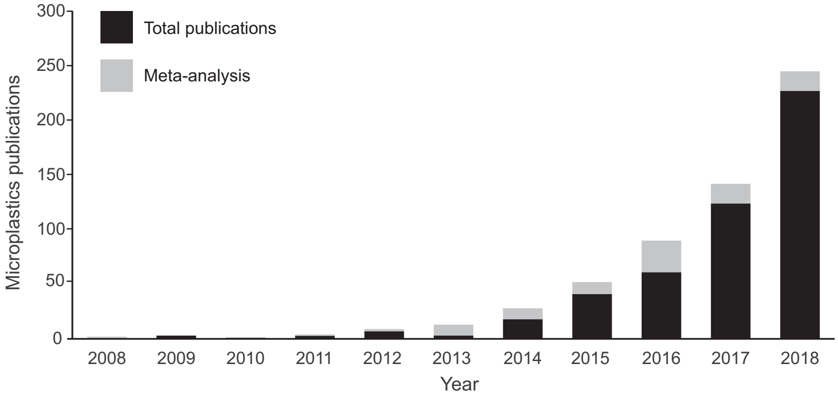

Fig. 1 The increase in microplastic publications over an 11-year period from 2008 to 2018, based on articles listed in ISI Web of Science with topic "microplastics" excluding journal subjects exclusively in materials science, engineering, physics or chemistry. The gray portion of each bar indicates the number of studies analyzed in detail for the meta-analysis herein.

an increasing number of studies using elevated microplastics dosages to determine their impacts on aquatic taxa (Huvet et al. 2016; Troost et al. 2018). Additionally, uniform virgin microplastic beads bought directly from industry manufacturers are commonly used in exposure experiments with aquatic taxa (Jemec et al. 2016; Rehse et al. 2016). As microplastic beads will become biofouled in aquatic systems, the abundance of pristine virgin microbeads in the natural environment is limited (Lehtiniemi et al. 2018). The "debate" about global climate change has demonstrated clearly how important it is to vigilantly maintain high standards of research and experimental design, especially in areas subject to heightened public interest.

This critical review was conceived in order to provide a baseline for prospective students and researchers interested in pursuing environmental microplastics as a research topic. We aim to highlight research gaps, the best possible methods for future studies, and the problems arising from microplastic research. By conducting a meta-analysis of recent literature, this review aims to highlight the extent of elevated microplastic dosages used in exposure studies on aquatic species. We also explore which aquatic species are at the highest or lowest risk to microplastics exposure using environmentally-relevant concentrations taken from recent exposure trials. Moreover, this review highlights the variability in environmental microplastic concentrations and the subsequent units of measurement used within extraction studies. Our analysis demonstrates the need for future experiments to build a robust, environmentally relevant framework that can be used to predict future impacts of microplastics on marine and freshwater systems.

Meta-analysis

A total of 101 peer-reviewed journal articles on environmental microplastics, published on or before 1 February 2019, were collated to form the data used in this meta-analysis. The set of published articles were taken from keyword and cross-referenced searches using multiple academic search engines (ISI Web of Science, Science Direct, Google Scholar) and also from Supplementary Material provided in a previous exhaustive review by Burns and Boxall (2018). We did not assign a lower time bound, but the earliest paper recovered by our approach was published in 2008. Our publication search focused solely on environmental microplastic research and was limited to relevant topics, excluding an increasing number of engineering or manufacturing publications from the wider microplastics area. Among the available papers we selected those with clear outcomes relevant to two specific areas: (1) environmental extraction from environmental samples of aquatic sediments or (2) experimental exposure of aquatic animals to simulated microplastic pollution under controlled conditions; these papers form the basis of our meta-analysis. This more detailed analysis excludes research on ingestion (i.e., extraction of plastics from animal carcasses or feces), interaction of species with environmental plastic debris, dispersion of plastic litter, and extraction of environmental microplastics from water samples (Fig. 1). Each of the selected articles was examined and separated into one or multiple individual experimental trials, and these experiments or results ("studies") were treated as the major unit in our analysis. (For example, a paper comparing animal behavior in an experimental treatment with plastic exposure and a treatment with no plastic, would represent two "studies.")

We identified 180 studies on the extraction of microplastic from environmental sediment samples (Supplementary Table S1). The metadata extracted from each study were as follows: the microplastic concentration found, the units used to quantify microplastic concentrations, the sediment type (freshwater, estuarine, mangrove, beach, coastal, off-shore, deep sea), the geographic location, and the size classification of the microplastics found. To draw comparisons between the microplastic concentrations found at each ecosystem, unit conversions were used where possible to convert values into microplastic particles (mp) per kilogram of sediment (mp/kg). Studies using mp/m^2 (or variations thereof) were not used within the comparison between ecosystems.

We identified a further 128 microplastic exposure studies on marine and freshwater species (Supplementary Table 2). The impact of microplastic exposure was classified as either high (mortality, decreased reproductive output, organ damage) or low (transient behavioral changes such as increased respiration, reduced feeding, and reduced energy). The dosages of microplastics used in the study were classified as either high ($>$100 mp/L water; $>$100 mp/kg sediment) or low ($\leq$100 mp/L water; $\leq$100 mp/kg sediment). The cut off between the two categories was determined using the highest recorded environmental surface water concentrations as a threshold (Burns and Boxall 2018). This threshold of 100 mp/L is intended to represent the highest values of microplastics reported at a given time. Microplastic concentrations in surface waters will vary over time due to wind and currents (Lusher et al. 2015); however, long-term and spatial variability data are unavailable and would be difficult to represent in laboratory experiments. Environmental sediment microplastic values were not used as a threshold in this meta-analysis as most benthic species are exposed to suspended microplastics in laboratory conditions (Green et al. 2019). The metadata identified from each study were as follows: microplastics dosage, the dosage units used, whether the species was pelagic or benthic, the life stage of the species (adult/larvae), the species group (benthic/pelagic), the species name, the impact of microplastics on the species, the impact classification (high/low), the dosage classification (high/low), and the location of the study. This analysis included only zoological impacts, and studies that included the impact of microplastics on microalgae species were omitted.

Results and discussion

Our meta-analysis highlights three critical problems that undermine much of the existing knowledge base and that must be considered by current and future experimental research on microplastics impacts: (1) use of extreme dosages relative to recorded environmental concentrations, (2) incompatible and incomparable units, and (3) an issue of how to establish informative control conditions for experimental results.

The majority of experimental exposure studies used highly elevated microplastic dosages when trying to assess the impacts of plastic on aquatic taxa (82%; 105/128), substantially above typical levels found in most ecosystems. Certain studies have been designed to determine a tipping point for ecotoxicological effects in species using higher values than what is found in the environment (Redondo-Hasselerharm et al. 2018), but others report comparative control/treatment effects. Only 23 studies (in a smaller number of published papers) tested the biological impacts from experimental dosages at environmentally relevant levels. The studies we considered to use "high" dosages occupied a range of multiple orders of magnitude, to extreme levels with questionable relevance. For context, consider that the smallest objects visible to the naked eye are around 0.1 mm. There is a physical limit of around 1.4 million particles per mL (the maximum theoretical number of spherical particles of diameter 0.1 mm that could be packed in a 1 mL volume), or 1.4 × 10^9 mp/L. We consider 100 mp/L to be "high" relative to environmental levels; multiple published studies have used dosage concentrations of 10^6 or 10^7 mp/L, and one reported concentrations of 10^6 mp/mL (10^9 mp/L), which is physically implausible, given that an experimental organism also takes up some volume in an aquarium (Supplementary Table S2).

There is a related and substantial issue around units, and equivalencies, in comparing microplastics studies. The lack of consistent units impedes real comparisons among extraction studies, which provide the baseline to determine what qualifies as environmentally relevant concentrations of microplastic pollution (Burns and Boxall 2018). We made every effort to convert units among experimental exposure studies, but some uncertainty around comparability requires that our visualization of exposure and effects is schematic and approximate (Fig. 2). Typical classification of microplastic particles includes any items <5 mm in length (Thompson et al. 2004); so this already encompasses plastic

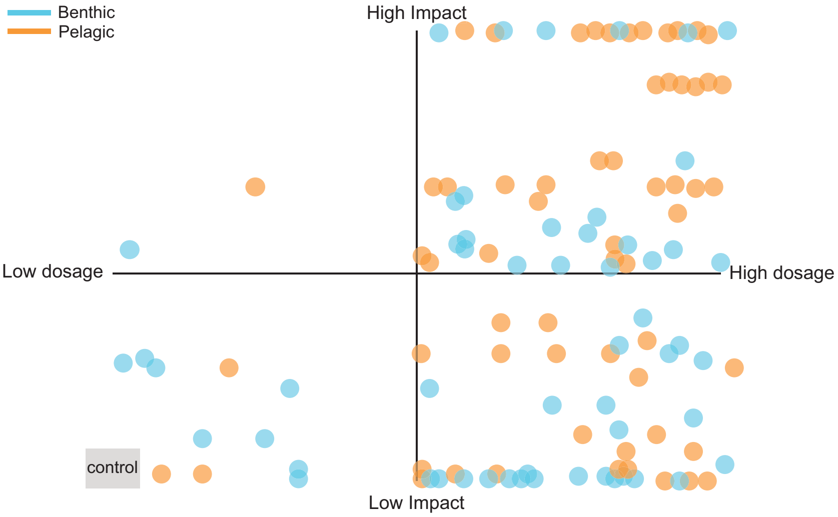

Fig. 2 Schematic plot of the relationship of experimental exposure of aquatic taxa to microplastic pollution and the impacts reported in 181 (53 controls) studies. The distinction between high and low dosages is 100 microplastic particles per kg of sediment or per L of fluid, or the equivalent concentration in other units. High impact responses are permanent (including mortality), low impact responses are transient metabolic anomalies. There is no apparent correlation between dosage and impact in either benthic (blue) or pelagic (orange) taxa nor overall.

objects over several orders of magnitude in length and volume. Unified measure of mp/L of water or mp/kg of sediment does not guarantee comparability in the concentration or the properties of plastic pollution.

Different sizes and chemical compositions of plastics would necessarily have different impacts. Nonetheless, the vast majority of experimental work published to date on impacts of microplastics has included control treatments. A small number of papers did not use any control treatment ($n = 2$); these studies instead presented comparisons of multiple plastic exposures (either comparing among multiple species and plastic dosages, or differing colors of plastics) with no measurement of animal responses in the absence of plastic. Generally, the control treatments in most studies compare replicates which contain increasing plastic dosages to replicates with no plastic (Lee et al. 2013); however, other studies compared a number of different pollutant treatments (surfactants or bioplastic), which were all controlled individually (Green et al. 2016; Kokalj et al. 2018). Since environmental plastic is so ubiquitous, it is arguably difficult to have a "no plastic" group, but it is essential for any experimental design to include a control without exposure to the added treatment. The next challenge is to determine what in particular about plastic is inducing a biological response? Many plastics are inert, but nonetheless provide vectors for POPs, visual interference, and a range of other potentially relevant effects. As far as we are aware, no attempt has been made to control for the effects of plastic particles by, for example, exposing animals to a non-plastic, space-filling pollutant such as additional organic particulates.

The distribution of the results in our meta-analysis, looking for a correlation between microplastic dosage and resulting impacts, show no clear patterns (Fig. 2). Previous reviews on microplastic exposure studies reported similar findings (Foley et al. 2018; Hermsen et al. 2018). The 128 microplastic exposure studies included 44 different aquatic species, evenly divided between benthic (52%) and pelagic (48%) species (Fig. 3A). Both low and high impacts are widely associated with elevated microplastic dosages across all studies; however, variation in the particle size/composition/shape, and the exposure time may explain the variation. The present literature provides an unfortunately somewhat limited foundation to predict the future impacts of microplastic pollution, and we will discuss some gaps as well as emerging trends below.

Extraction studies

Among the 180 microplastic extraction studies, four separate types of units were used in published estimates of microplastic contamination from marine and freshwater benthic sediments (Fig. 4A). The majority report concentration by sediment weight, which is the recommended best practise (Lots et al. 2017). A smaller number of studies used other volume

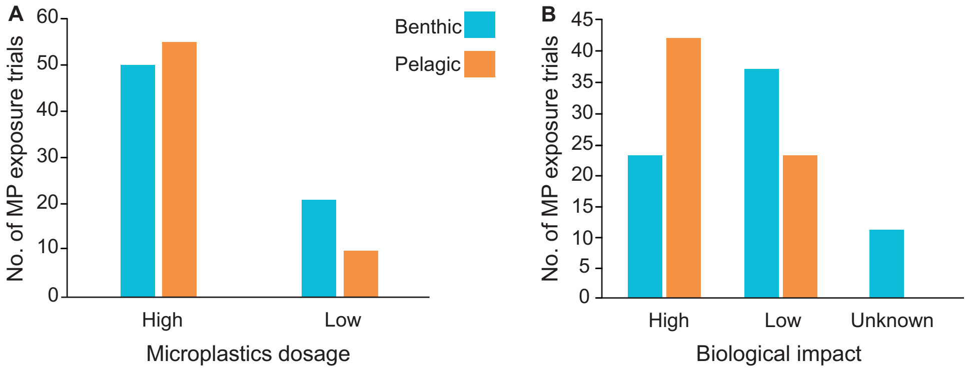

Fig. 3 Meta-analysis of microplastic exposure studies, showing (**A**) the number of studies on benthic and pelagic species that utilized a high or low microplastic dosage and (**B**) the number of benthic and pelagic species that reported unknown, low, or high impacts from microplastic exposure. High impact responses are permanent (including mortality), low impact responses are transient metabolic anomalies, and unknown impacts are cases in which the uptake of microplastics was reported with no post examination of effects.

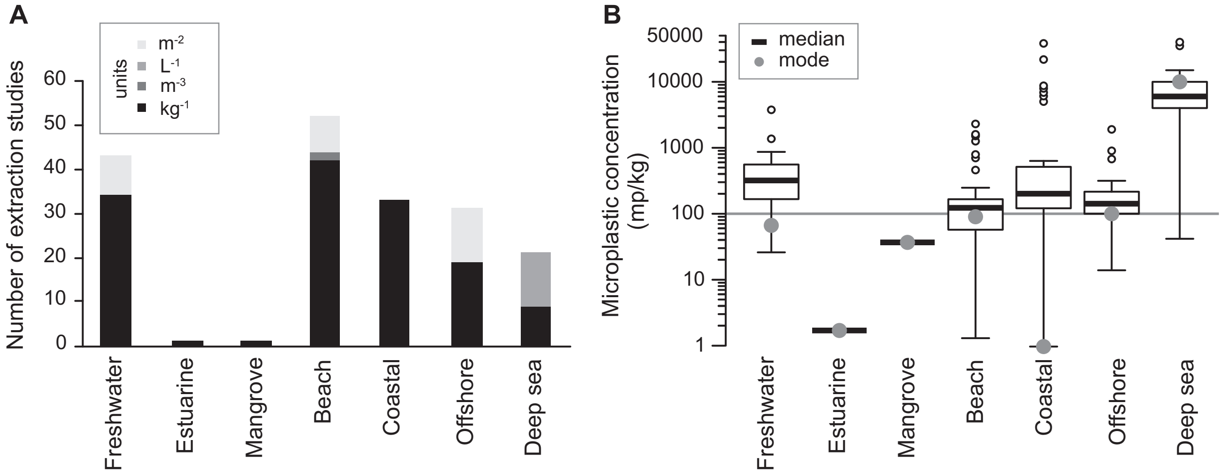

Fig. 4 Meta-analysis of microplastics extraction studies, showing (**A**) the number of studies and units utilized in published extraction studies for different aquatic habitats and (**B**) trends in the concentrations (reported in or converted to microplastic particles per kg of sediment), showing median and inner quartiles of the concentrations (box and whisker plots) and the mode of the frequency distribution of concentrations (gray circles).

measurements that we converted to approximate per kg values (mp/mL or mp/m^3) in calculating trends in concentrations, but a large fraction of data (16% of studies) from several different habitats reported concentrations per sediment surface area, which has no clear equivalency to the other units. Within each habitat and overall, the frequency of microplastics concentrations follows a strongly skewed distribution (Fig. 4B). The overall mode of the dataset of comparable concentrations was 100 mp/kg, and all individual habitats except for deep sea also had modal concentrations of 100 or below, confirming our selection of that threshold for elevated concentrations. The median concentration among all studies was 192 mp/kg, meaning half of studies recovered that level or lower, and median values within all habitats except the deep sea are between 100 and 320 mp/kg or lower.

The frequency distribution of microplastic concentration is highly asymmetrical, with a long ‘tail’ of individual studies reporting elevated environmental concentrations (Fig. 4B). There are a large number of studies with very high local levels of plastic, with the highest reported levels of pollution up to ∼40,000 mp/kg in coastal (Manalu et al. 2017) and deep-sea extraction studies (Woodall et al. 2014). Those two studies employed FT-IR spectrometry to confirm the presence of polymers within the sediment; however, Manalu et al. (2017) only identified the polymer types of macroplastic particles, and therefore the reported microplastic concentrations may be lower than expected. Although an increasing number of freshwater studies have been carried out in recent years (Castañeda et al. 2014; Peng et al. 2018; Wang et al. 2019), freshwater systems are still underrepresented in comparison to marine systems, and we analyzed only one study from mangroves (Nor and Obbard 2014) or estuaries (Clunies-Ross et al. 2016), with 75% of the data focused on marine sediments. This highlights a significant research gap, as freshwater rivers and lakes are known to act as a vector for microplastic transportation to marine systems (Rochman 2018) and 80% of all marine plastic litter is land-based (Landon-Lane 2018). Microplastic concentrations in freshwater systems may be as high as marine systems (Eerkes-Medrano et al. 2015), and therefore future efforts should concentrate more on the impact of microplastics on freshwater species.

Exposure studies—benthic species

Microplastics accumulate in sediment, and benthic animals may be less able to physically escape such contaminants. Although the majority of published exposure studies use exaggerated contamination levels, actually most studies reported transient or low impact responses (Fig. 3B).

Most exposure trials using benthic species employed elevated microplastic dosages above the 100 mp/L threshold (50/67); despite this, high impacts were only reported in 23 of those trials (Fig. 3). The benthic species that seemed most at risk from elevated microplastic dosages were amphipods such as *Hyalella azteca*, which displayed decreased growth when exposed to microspheres (Blarer and Burkhardt-Holm 2016). Conversely, lower dosages of microplastic fibers (22.5–90/mL) showed to have a more detrimental effect on *Gammarus fossarum* than elevated dosages of microplastic particles (10–100,000 mp/mL; Au et al. 2015). This suggests that *G. fossarum* is at a high risk to microplastic exposure as fibers are known to be the most abundant microplastic type in aquatic systems (Lehtiniemi et al. 2018). Effects in the next most common species that experienced "high" impacts in high dosages, were cell damage and reduced byssal thread production in the blue mussel *Mytilus edulis* (Green et al. 2019). This reduction in byssal threads and consequent decreased potential for biogenic reef formation in *M. edulis* may reduce biodiversity due to the decreased habitat complexity (Koivisto 2011). Further studies using elevated dosages demonstrated tissue necrosis in coral reef species (*Acropora humilis*, *Pocillopora verrucosa*; Reichert et al. 2018), mortality in decapod crustaceans (*Eriocheir sinensis*; Yu et al. 2018), and reduced reproductive output in bivalves (*Crassostrea gigas*; Sussarellu et al. 2016). The lowest microplastic dosage used throughout the studies representing high impact/high dosage experiments was 4000 mp/L (Reichert et al. 2018, on coral species), and the highest was 10^8 mp/L (Au et al. 2015, on an amphipod); despite the extreme difference between those dosages, both studies recorded mortality of the study species. Yet the relatively small number of papers precludes any clear determination whether this is a species sensitivity. A further eight studies that used elevated microplastic dosages were aimed at understanding the uptake of microplastics by aquatic species as opposed to the associated impacts and therefore no impacts were recorded (Watts et al. 2014; Setälä et al. 2016). Such studies are needed to determine a tipping point for microplastic effects on species, and therefore can justify the use of high dosages.

Among the studies demonstrating low impacts under high dosages on benthic species, half (10/17) reported no impacts from plastic exposure. Both amphipod species, *Echinogammarus marinus* and *Gammarus pulex* displayed no impacts when exposed to 100,000 mp/L (Bruck and Ford 2018) and 4,000,000 mp/L (Weber et al. 2018), respectively. No impacts were also observed in *Mytilus galloprovincialis* (Gonçalves et al. 2019) , the oyster *Ostrea edulis* (Green 2016), the coral *Porites lutea* (Reichert et al. 2018), the isopod *Idotea emarginata* (Hämer et al. 2014), nor the crab *Eriocheir sinensis* (Yu et al. 2018). The lugworm (*Arenicola marina*) was exposed to concentrations of up to 100 g of microplastic per liter of sediment, but only showed reduced feeding throughout the duration of the experiment (Besseling et al. 2013). Lugworms also displayed increased respiration when exposed to microplastics that totaled 5% of the total sediment weight (Browne et al. 2013). Despite being exposed to high microplastics concentrations, lugworms have displayed low impacts in response to these exposures in multiple separate investigations (Besseling et al. 2013; Browne et al. 2013; Wright et al. 2013; Green et al. 2016). It is therefore likely that *A. marina* is relatively robust to microplastic exposure in the wild.

Further studies on benthic species displayed low impacts whilst using low dosages, the minority of which (6/17) reported no associated impacts. The shore crab (*Carcinus maenas*) also showed low or no impacts when exposed to more environmentally relevant microplastic dosages (Watts et al. 2015), and therefore may also be at relatively low risk. The Norway lobster (*Nephrops norvegicus*) was the only species within this analysis with effects we classified as high impact (reduced growth) when exposed to low and environmentally relevant microplastic dosages (Welden and Cowie 2016b). The demonstrated sensitivity of *N. norvegicus* to environmentally relevant microplastic dosages could have major implications for European fishing industries; *N. norvegicus* is the most valuable fishery resource in the UK with a value of over £110 M per annum (Ungfors et al. 2013; Becker et al. 2018). Reduced body mass could also impact upon key legislation surrounding minimum landing sizes (MLS) that are vital to maintain fishery sustainability in the NE Atlantic (Catchpole et al. 2006). Some of these results do have substantial economic importance and would be used in future fisheries management and risk assessment, underscoring the importance of assessing effects at environmentally relevant concentrations.

Exposure studies—pelagic species

The majority of exposure studies using pelagic species employed elevated microplastic dosages and reported high impacts (55/61 studies; Fig. 3B). Low or no impacts were found in 23% of exposure trials under exert high dosages. The water flea *Daphnia magna* was the most commonly used pelagic species in exposure trials, with studies showing that high dosages of microplastic exposure caused reduced feeding (Ogonowski et al. 2016), reduced reproductive output (Besseling et al. 2014), immobilization (Rehse et al. 2016), or mortality (Jemec et al. 2016). By contrast, a separate independent experiment with 'high' exposure levels found no impact on *D. magna* (Kokalj et al. 2018). Two of those studies used the same experimental concentration of microplastics by weight (12.5 mg/L), but one used nano-sized plastic particles (Rehse et al. 2016). Size and shape of particles are known to alter the impacts on aquatic species (Ziajahromi et al. 2017), and smaller particles have a proportionately larger surface area and different effects on species (Liu et al. 2019).

Fish species are also commonly used in high dosage exposure trials. Organ damage was found in *Danio rerio* (Lu et al. 2016), *Carassius auratus* (Jabeen et al. 2018), *Sebastes schlegelii* (Yin et al. 2018), and *Clarias gariepinus* (Karami et al. 2017). The majority of studies on fish applied elevated dosages (22/28); however, transient or no impacts were reported in four studies using *D. rerio* (Chen et al. 2017; Lu et al. 2016), *Sparus aurata* (Jovanović et al. 2018) and *Pomatoschistus microps* (Oliveira et al. 2013) . We found that fish species are rarely subjected to environmentally relevant or low microplastic dosages and no studies reported high impacts on fish when exposed to these treatments; however, this is likely due to the lack of data. More studies using environmentally relevant dosages are needed as fish have the potential to transfer microplastics to higher trophic levels (Donohue et al. 2019).

Pelagic experimental subjects also include the larvae of benthic invertebrate species. Larvae of the urchin *Tripneustes gratilla* was unaffected by increasing microplastic exposure from dosages of 1000–100,000 mp/L (Kaposi et al. 2014); however, this was the only echinoderm found within recent literature so further studies are needed to assess this species group in more depth.

Low or environmentally relevant microplastic dosages were utilized in only 10% of all the pelagic exposure trials. The majority of pelagic studies showed high impacts (69%) and such studies have come under scrutiny in recent years (Huvet et al. 2016). Interestingly, one study demonstrated high impacts from environmentally relevant microplastic dosages. Digestive tract damage was found in the brine shrimp *Artemia parthenogenetica* when exposed to 100 mp/L (Wang et al. 2019). *Artemia* occupies harsh, hypersaline environments (Moscatello et al. 2002) yet is evidently sensitive to microplastic pollution. As brine shrimp are a valuable prey for many species (Varo et al. 2011), this type of effect should be highlighted as a concern when assessing microplastic impacts at an ecosystem level.

Conclusions

Microplastic concentrations in the wild will increase, due to ongoing input compounded with the further fragmentation of larger plastic debris accumulating in the oceans and freshwater systems. Studies using elevated concentrations of microplastics may become more relevant in time; nonetheless, the use of extremely high concentrations (Au et al. 2015; Ogonowski et al. 2016; Watts et al. 2016; Yin et al. 2018) may have been useful to determine a potential tipping point for ecotoxicological effects on a particular species, but are not in line with current or future environmental values. Microplastics accumulate in the water column in closer proximity to ocean gyres (Brach et al. 2018) but transportation by wind and currents creates dynamic fluctuations in surface water concentrations (Lusher et al. 2015). The 100 mp/L threshold we used to separate nominally "high" and "low" or relevant dosage levels is not absolute or permanent; while the idea of separating "normal" from extreme exposures is important to interpreting experimental results, the value of that threshold must vary depending on the environment, and may increase over time. Experimental work that is useful to predict future environmental consequences should be planned in context of realistic present and future pollution levels.

The range of units used in both concentrations found in the wild, and the range of units used during exposure trials to assess potential impacts, can obscure the environmental relevance of a study. Replicating environmentally accurate dosages of microplastics in exposure trials means having to convert concentration units found in water and/or sediment, and then apply that to treatment dosages for experimentation. Although such conversions do exist (in the simplest cases, $mp/cm^3 = mp/mL$), certain environmental measurements cannot be converted (such as mp/m^2) and are subsequently disregarded (Burns and Boxall 2018). These conversions work for concentrations of microplastics in volumes of fluid; however, no such conversions exist to the most common sediment quantification units (mp/kg). As a result, we urge future microplastic researchers to adopt units of mp/L for fluid and mp/kg for sediment extraction and quantification. Microplastics are highly abundant in sediment (Thompson et al. 2004), but the recovery of microplastics within and among sites follows a skew distribution, with a few very highly concentrated patches and many samples of much lower concentrations. Conducting comparative and highly sampled studies will allow for a greater understanding of how microplastic concentrations vary on a small scale between neighboring environments and on a large scale among habitats and across latitudinal gradients.

Microplastic pollution is not uniform in composition, in size or in chemical or physical properties. This is a significant confounding issue in comparing extraction studies, over and above the issue of measurement units, and more in comparing experimental trials. The meta-analysis we assembled herein did not consider the type of plastics used in the studies analyzed; however, many authors are cognizant of and have compared impacts of different polymers (Kokalj et al. 2018). Although the use of virgin microplastics in exposure trials lacks environmental relevance, the use of the same polymer types/shapes/sizes can be isolated easily. The use of extracted microplastics from sediment or water in exposure trials would not help to determine the associated impacts of microplastics on aquatic species as polymer types/shapes and sizes would not be consistent across a large scale experiment.

The purpose of experimental controls is to limit extraneous variables. In microplastics research, presence of absence of particulate plastic is not necessarily sufficient to determine the cause of an observed biological response, and plastic is almost impossible to eliminate from any lab setting. Virgin microplastics are commonly used in exposure studies (Jemec et al. 2016; Rehse et al. 2016) but will have different impacts than biofouled microplastics and secondary microplastics encountered in the field. Efforts have been made to biofoul virgin microplastics with algae to improve the environmental relevance of exposure impacts on European oysters (*O. edulis*; Green 2016), lugworms (*A. marina*; Green et al. 2016), and blue mussels (*M. edulis*; Green et al. 2019). Plastic polymer composition, size, shape, surface area, color, surface texture, surface fouling, may all have separate, relevant, effects that differ among species.

Now that the global presence of microplastic pollution is well established, with more than a decade of research, new studies should focus on comparative aspects rather than the presence of microplastics. There is an opportunity now to establish extraction studies to build long term data sets to monitor rates of microplastic accumulation. We recommend that established microplastic concentrations should be used alongside quantities found within species native to a particular area, and therefore applied to exposure trials to achieve environmentally relevant outcomes. This would help to reduce the number of laboratory studies demonstrating adverse but potentially uninformative impacts of exaggerated dosages on particular species, and move the research area in a more environmentally relevant direction. Robustly designed, controlled, hypothesis-driven experiments based on environmentally relevant concentrations are needed now to understand our future in the new plastic world.

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Supplementary data

Supplementary data available at *ICB* online.

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