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Blueprint for the ideal microplastic effect study: Critical issues of current experimental approaches and envisioning a path forward

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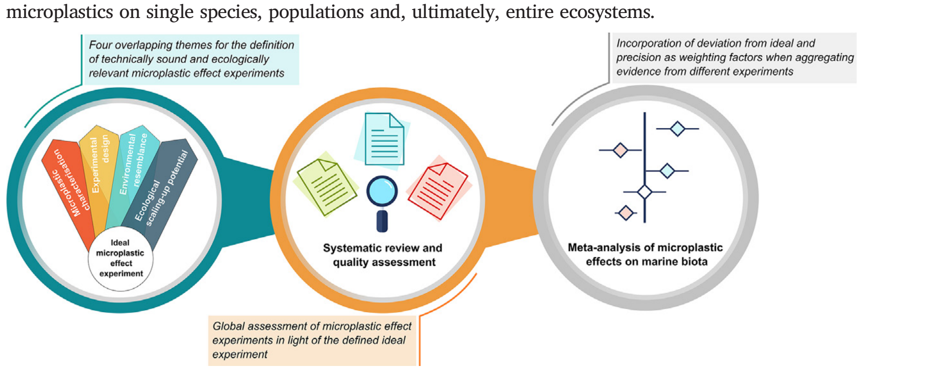
HIGHLIGHTS

- Effects of microplastic on marine biota reflect the quality of experimental research.
- The quality of published experiments can be quantified from an "ideal" experiment.
- Previously published experiments have significantly deviated from "ideal".
- Implementation of proposed criteria can improve future microplastic experiments.

GRAPHICAL ABSTRACT

Conceptual blueprint for an 'ideal' microplastic effect study and associated meta-analysis to provide a better basis for the design of ecologically relevant and technically sound experiments with which to understand the effects of microplastics on single species, populations and, ultimately, entire ecosystems.



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ABSTRACT

This article presents a novel conceptual blueprint for an 'ideal', i.e., ecologically relevant, microplastic effect study. The blueprint considers how microplastics should be characterized and applied in laboratory experiments, and how biological responses should be measured to assure unbiased data that reliably reflect the effects of microplastics on aquatic biota. This 'ideal' experiment, although practically unachievable, serves as a backdrop to improve specific aspects of experimental research on microplastic effects. In addition, a systematic and quantitative literature review identified and quantified departures of published experiments from the proposed 'ideal' design. These departures are related mainly to the experimental design of microplastic effect studies failing to mimic natural environments, and experiments with limited potential to be scaled-up to ecosystem level. To produce a valid and generalizable assessment of the effect of microplastics on biota, a quantitative meta-analysis was performed that incorporated the departure of studies from the 'ideal' experiment (a measure of experimental quality) and inverse variance (a measure of the study precision) as weighting coefficients. Greater weights were assigned to experiments with higher quality and/or with lower variance in the response variables. This double-weighting captures jointly the technical quality, ecological relevance and precision of estimates provided in each study. The blueprint and associated meta-analysis provide an

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improved baseline for the design of ecologically relevant and technically sound experiments to understand the effects of microplastics on single species, populations and, ultimately, entire ecosystems.

1. Introduction

Since the 1940s, plastics have revolutionised society to the extent that most people now use plastic products daily (Geyer et al., 2017). The global annual production of plastics has increased rapidly from about 2 million tonnes in 1950 to 348 million tonnes in 2017 (PEW and SYSTEMIQ, 2020). The recent global COVID-19 pandemic alone has added 8 million tonnes of pandemic-associated plastic waste (Canning-Clode et al., 2020; Peng et al., 2021). Annual plastic waste production could attain 155–265 million tonnes by 2060 and about one-tenth of which would eventually enter the oceans (Lebreton and Andrady, 2019; Borrelle et al., 2020). Once released into the marine environment, light, mechanical abrasion, waves, temperature fluctuations, and biodegradation reduce plastics into smaller particles (GESAMP, 2015). These small-sized plastic particles, so-called microplastics (1–1000 μm, Hartmann et al., 2019), have become ubiquitous contaminants in the marine environment (Andrady, 2017; Gestoso et al., 2019).

Direct contact with and/or the ingestion of microplastics can adversely affect the metabolic activity of organisms, their feeding and growth rates, reproductive output and larval development as well as their behaviour (Galloway et al., 2017; Toussaint et al., 2019; Petersen and Hubbart, 2021; Khalid et al., 2021). Although not all modes of effects exerted by microparticles on organisms have been identified, evidence indicates that microplastics can injure epithelia, clog both feeding appendages and respiratory organs, block digestive tracts, and accumulate in tissues and organs, which may ultimately be fatal (Wright et al., 2013). Moreover, by absorbing other chemicals and host pathogens, microplastics can serve as vectors for additional stressors (Brennecke et al., 2016; Naik et al., 2019; Bowley et al., 2021). These individual-level effects are expected to extend to population demography, community structure, the nature and intensity of biological interactions, energy and matter transfer in ecosystems (Galloway et al., 2017; Khalid et al., 2021).

Just as the literature addressing the biological impacts of microplastic debris has increased exponentially over the past decade (Cunningham, 2019), so have concerns about methodological weaknesses of microplastic effect studies and, in turn, the reliability of the research (Burns and Boxall, 2018; de Ruijter et al., 2020). Microplastic concentrations in experiments are typically orders of magnitude greater than the concentrations currently observed in aquatic environments, and the types of microplastics used in experiments are atypical to those in the natural environment. Moreover, most effect studies fail to assess the bioavailable particle concentrations in order to verify whether the targeted concentrations have been reached and thereby fail to quantify correctly their independent effect. Furthermore, effect studies are usually much shorter than the life span of their test organisms and typically focus on only a single life stage.

Until recently, little effort had been made to systematically and thoroughly evaluate these experimental weaknesses. de Ruijter et al. (2020) was the first study to critically review — from a quality-assurance perspective — the relevant methodology of published studies. The quality assurance-based criteria and the scoring system defined by de Ruijter et al. (2020) to evaluate microplastic effect studies represent excellent building blocks for defining a standardised protocol to perform technically sound experiments. However, the quality of microplastic experiments also depends on how well experimental settings mimic environmental conditions, and to what degree the experimental results can be extrapolated to the ecosystem level. This extrapolation is essential for helping ecosystem management to sustain ecosystem integrity (Galloway et al., 2017).

In this context, this article presents the concept of an 'ideal' microplastic effect study that encompasses both technical soundness and ecological relevance. Although conducting this definitive 'ideal' experiment is currently unattainable, the concept serves as a theoretical reference with two concrete applications. First, it serves as a benchmark for future experimental research. Depending on the nature of a forthcoming study, it is possible to choose recommended tactics that improve experimental quality and enhance its ecological relevance. Second, it is possible to measure the departure or 'distance' of an existing experiment to the 'ideal' experiment, thereby quantifying the experimental quality of an existing study. By combining experimental quality with the precision of a set of studies, it is possible to perform a meta-analysis (Gurevitch et al., 2018) of the effects of microplastics on marine biota in which experimental quality and design are incorporated in the outcomes. Traditional meta-analysis typically considers quality as a binary function, i.e. either to include or exclude a study, or as a nominal variable (depending on overall quality and scientific rigor) in which poorer quality studies are omitted from subsequent analysis (e.g. Mikolajewicz and Komarova, 2019). Evidence from those studies that passed the quality criteria is then weighted by giving studies with larger sample sizes and lower experimental errors greater weight (Hartung et al., 2008; Gurevitch et al., 2018). This approach obviously carries the risk of over-weighting study precision at the expense of experimental quality, thereby undermining the results of the meta-analysis.

2. Material and methods

2.1. The ideal microplastic experiment

The 'ideal' microplastic effect study can be defined by carefully considering how a microplastic pollution scenario can be mimicked in a laboratory setting and how long experiments need to run before they begin to detect a relevant effect. These theoretical considerations can then be compared with the experimental parameters and the ecological relevance of the methodology used in published studies. This comparison would allow the assessment of the shortcomings of previous experiments and their reproducibility, from which recommendations for future experiments can be derived. The recent accumulation of literature on the potential biological effects of microplastics and the availability of tools for automated searches and data extraction provide an unprecedented opportunity to systematically screen studies and draw conclusions at a global scale (see, e.g. Lebreton and Andrady, 2019; Toussaint et al., 2019; de Ruijter et al., 2020).

The 'ideal' experiment needs to encompass four broad and non-mutually exclusive themes: (1) microplastic characterization, (2) experimental design, (3) environmental resemblance, and (4) ecological scaling-up potential (see Supplement 1 Table S1). The definition and relevance of these four themes are as follows:

(1) ***Microplastic characterization***: expands on de Ruijter et al.'s (2020) and describes the microplastic particle characteristics that determine interaction between microplastics and organisms and the environment (Hartmann et al., 2019; de Ruijter et al., 2020). Polymer type, shape, colour, and size can affect the perception, ingestion, and retention of microplastic particles by organisms.

(2) ***Experimental design***: Under this theme de Ruijter et al. (2020) focused on the quality control of microplastics in the experimental medium, i.e., chemical purity, potential contamination and quantifying exposure concentrations as well as statistical replicability and description of the potential ecological mechanisms underlying ecological impacts. Here the theme is expanded to include indicators of procedural controls, i.e. assessing the effects of natural microparticles under the same experimental conditions and the type, quantity and frequency of food supplied to test organisms.

(3) ***Environmental resemblance***: These criteria quantify how closely experimental conditions resemble those in the field These criteria include a) identifying the nature (i.e. size, shape, polymer type, colour),

composition and concentration of microplastics in the habitat from which the test organisms were collected to select the most appropriate particles or the most realistic mix of particles for the experiment, b) assessing the temporal variability in the composition and the concentration of microplastic particles in the habitat, c) exposing the experimental microplastic particles to field conditions to allow natural ageing and the establishment of a biofilm on the particles prior to the experiment, d) providing appropriate experimental space for the test organisms, e) accounting for potential interactions between microplastics and other environmental stressors in pelagic and benthic habitats and f) assessing the impact of microplastics on multiple species.

(4) ***Ecological scaling-up potential:*** This measures the degree to which the experimentally documented biological impacts of microplastics can be extrapolated to the ecosystem level. The extrapolation potential evaluates how well experimental conditions reproduce ecosystem structure and interactions between abiotic environment and the biota. In this sense, aspects such as the diversity of species and stressors included in an experiment, the assessment of impacts on different life stages, or the choice of response variables with a clear connection to relevant functions performed by species in the ecosystem define the scaling-up potential of experimental results.

2.2. Systematic literature review

Following the systematic quantitative literature review approach of Pickering and Byrne (2014), ISI Web of Science (WoS) and Scopus were used to identify experimental studies investigating the effects of microplastics on marine biota. An advanced search in WoS was applied selecting all databases for all available years (i.e. 1945–2020). The following search string was used: ALL = ((microplastic*) AND (marine OR ocean* OR sea*) AND (effect* OR impact* OR influence* OR consequence*)). A similar search strategy was used in Scopus: TITLE-ABS-KEY (microplastic*) AND TITLE-ABS-KEY (marine OR ocean* OR sea*) AND TITLE-ABS-KEY (effect* OR impact* OR influence* OR consequence*) AND (LIMIT-TO (LANGUAGE, "English")). These searches resulted in 2431 unique publications for subsequent scanning.

These publications were screened to include studies that (i) were carried out in marine waters, including open ocean, coastal and brackish ecosystems; (ii) provided original data; (iii) experimentally studied the effects of microplastics on biota (studies reporting no effects and studies on nanoplastics were excluded); and (iv) compared an affected state (microplastics present) to a control state (microplastics absent) either spatially or temporally.

The inclusion assessment was performed in three successive steps. The first stage was an evaluation of the titles to exclude non-relevant studies. The second stage entailed an assessment of abstracts. Several team members independently assessed a subset of the abstracts (n = 50), and a multi-rater Kappa statistic was calculated on the basis of the assessments (Fleiss, 1971). The value of the resulting Kappa statistic was far >0.6, suggesting that the reviewers were consistent in their evaluation, thereby confirming the appropriateness of the inclusion criteria (Koricheva et al., 2013). The third stage was an evaluation of the full texts of the remaining studies (n = 425).

All relevant information and quantitative statistics of all measurements were extracted for studies fulfilling the inclusion criteria. A subset of studies (n = 10) was evaluated independently by three of the study's authors to eliminate potential ambiguities in the assessment. First, each experiment was evaluated with respect to all previously defined quality criteria (Supplement 1 Table S1): binary criteria were scored as the availability or unavailability of a particular information; ordinal criteria were scored into ranked categories (e.g. single species effects, 2 to 3 species, community or ecosystem-level effects); continuous criteria were obtained by reported values (e.g. ratio of body size of the test organism to the volume of the experimental container). Second, arithmetic means with measurement units, standard errors (or standard deviations) and sample sizes of the control and the affected state(s) were extracted directly from tables and the text of the articles. When values were reported graphically, ImageJ software (Schneider et al., 2012) was used to extract these mean and error values. The extracted data were catalogued in a systematic database (Supplement 2 Research data). All experimental parameters that help to evaluate the technical appropriateness and ecological relevance of a study (Supplement 1 Table S1) were listed in columns in the database, in which each row represented an observation, thereby adhering to the tidy data principles suggested by Wickham (2014). The database also listed variables related to the background of the study (time, location, region), the study description (study focus, microplastic type and shape, studied organisms, developmental stage, other stressors involved in the experimental design), and the effect of microplastics on the biota (means of the collected response variables in the affected and in the control states and their errors).

2.3. Statistical approach

The database was used to perform two meta-analyses: the first to quantify experimental quality by calculating the departure or 'distance' between each study and the 'ideal' experiment and the second to quantify the precision of individual estimates of effect size given in each study (Jackson et al., 2017; Riley et al., 2018). The first meta-analysis was used to evaluate experimental quality with respect to the four evaluation themes and a set of geographic, experimental and ecological aspects. The two meta-analyses were merged to indicate the current state of knowledge of the effects of microplastics on marine biota.

Each experimental quality criterion was normalised to a unit interval scale. This normalisation was calculated by dividing each criterion by a threshold value that corresponds to an optimal quality level (considering infrastructural, methodological and analysis limitations of state-of-the-art facilities and tools) and by setting all quality criteria that exceeded this level to one (Supplement 2 Research data). Binary quality criteria simply indicated whether a study included particular aspects (e.g. the characterization of different features of microplastic particles, the inclusion and characterization of reference particles or a comparison of experimental microplastic particles and those present on site). These criteria were assigned one when included and zero when excluded.

To assess the concordance of each experimental quality criterion within the four evaluation themes, the RMSE (root mean square error; a measure of the differences between the observed values and the model predictions) of a linear model was calculated for each criterion with all other criteria within the same theme as predictors. To achieve a fair comparison of the RMSE values, all criteria were standardised to unit variance (i.e. by dividing all values by the standard deviation). Assigning weights based on the RMSE scores that are raised to a positive or negative power allows the up-weighting or down-weighting of non-concordant quality criteria (e.g. Abramson, 2021). Weighted means of the respective quality criteria within each theme were calculated to assign a quality value of a broad evaluation theme for each experiment. The average of these four themes represents the total experimental quality.

Formal comparisons of the experimental quality were calculated in different subgroups (e.g. between different study region, microplastic types and shapes, studied organisms, developmental stage, other stressors involved in the experimental design). These comparisons can establish if the studies in the meta-analysis constitute a single population (i.e. no difference in experimental quality) or if study background, design, organism (among others) affect experimental quality (Borenstein and Higgins, 2013). This subgroup analysis was performed using linear mixed models with study ID as the random effect. Models were fit using the R package lme4 (Bates et al., 2015). In addition, the R package emmeans (Lenth, 2021) was used for multiple pairwise comparisons with Tukey adjustment to test for significant differences between means.

The second meta-analysis used Hedges' g as a measure of effect size (e.g. Borenstein et al., 2009) to estimate the impact of microplastics. Because some studies comprised several experiments and experiments typically provided several estimates of effect size, the data were hierarchical structured.

This multilevel nature of the data was taken into account by using a proper random effects structure in the model, i.e. studies and experiments within a study represented levels of the random effects. Each estimate of effect size in the analysis was weighted with a combination of its inverse variance (i.e. precision assessment; Hartung et al., 2008) and experimental quality. Consequently, studies with larger sample sizes and lower variance were assigned more weight, while smaller and less precise studies were assigned less weight. Likewise, studies with high experimental quality received more weight; studies with low experimental quality received less weight. This dual weighting accounts for both the precision and experimental quality in estimates of the effects of microplastics on the biota. The meta-analysis was carried out using the R package metafor (Viechtbauer, 2010).

3. Results

3.1. Synthesis of the experimental approaches in microplastic effect research

In total 425 publications fulfilled the assessment criteria of the systematic quantitative literature review and generated 7063 unique experimental results on the effects of microplastics on marine biota. Most experimental research on the effects of microplastics to date has been conducted in the temperate northern Atlantic and temperate northern Pacific (Supplement 3 Fig. S1). In contrast, less experimental research has been done in the tropics and almost none in polar regions (Supplement 3 Fig. S1).

Globally, polyethylene and polystyrene were the two most commonly used types of microplastics in experiments, 38% and 34%, respectively (Supplement 3 Fig. S2). Most studies were limited to a single polymer type rather than the polymer mixtures existing in natural marine waters. Moreover, most effect studies used non-weathered spherical microplastics (Supplement 3 Fig. S3).

The reviewed microplastic effect studies focused primarily on fish and bivalves (both 27%) with some studies that aimed at copepods, corals and sea urchins (5–8%); research on other groups of organisms was much more limited (Supplement 3 Fig. S4). Most experiments were performed on adult specimens (57%), considerably fewer on juveniles (22%). Similarly, only a few experiments involved several developmental stages or even multiple life cycles (Supplement 3 Fig. S5). The studies addressed a wide variety of potential microplastic effects, including metabolism, feeding, growth and reproduction (Supplement 3 Fig. S6); however, most experiments were short-term. In addition, most experiments examined only the effects of microplastics on the study organism, while few studies included other stressors (Supplement 3 Fig. S7).

3.2. Experimental quality: distance of microplastic effect studies from the 'ideal' experiment

In terms of quality, previous experiments characterized microplastic particles well, but often failed to include good practices of experimental design. Furthermore, experimental conditions tended to differ greatly from natural conditions (Fig. 1). Overall, experimental quality varied little across biogeographic realms, although the few studies conducted in the Arctic all exhibited good quality (Supplement 3 Fig. S8).

Despite the abundance of studies focused on bivalves and fish (Supplement 3 Fig. S9), most failed to exhibit good quality according to the assessment criteria, primarily because experimental containers were small (often <1 l) relative to the size of the test species and because studies were considerably shorter than the life span of the species. Moreover, most experiments lacked reference particles, and did not consider the true microplastic concentrations in the habitats in which the test organisms were collected. Furthermore, effects were assessed mainly on the level of single individuals of the same species, and the response variables chosen often lacked association with the functions performed by the species in the ecosystem and/or its ecological fitness. The feeding mode of the test organisms did not affect the overall quality of microplastics effect studies. However, due to the smaller size and shorter life span of pelagic test organisms, the experimental quality of their studies was greater than the quality of benthic species (Supplement 3 Fig. S10). Importantly, total experimental quality is a function of the combined qualities of all four themes. Therefore, studies exhibiting a high quality in one theme did not necessarily attain an overall high-quality score (Supplement 3 Fig. S11).

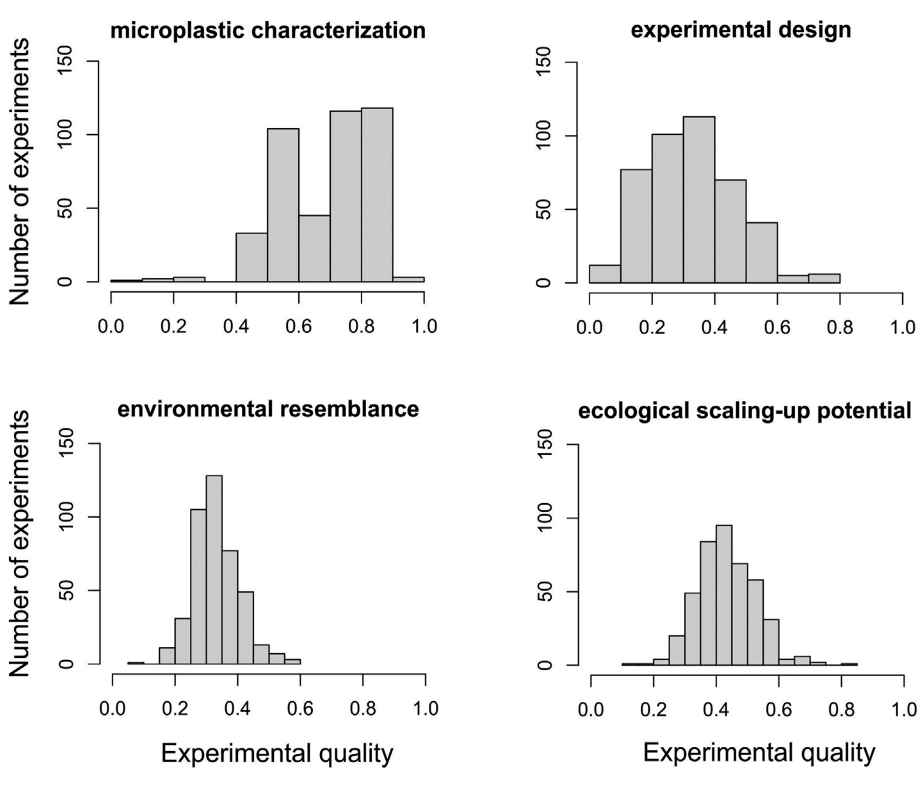

Fig. 1. Experimental quality ('distance') assessment of microplastic effect studies across the four evaluation themes. Zero denotes the lowest possible quality, and one represents the highest possible quality.

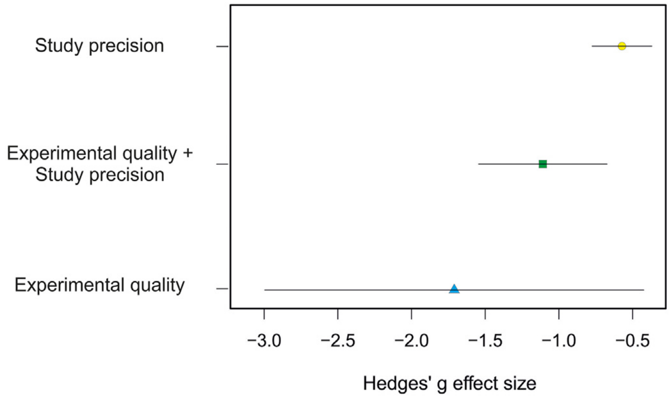

Fig. 2. Global effects of microplastics on marine biota (mean ± 95 % CI) as a function of the weighting of studies by study precision and experimental quality. This analysis used three different scenarios: weighting based only on study precision; equal shares for experimental quality and precision; and weighting based only on study quality. Hedges' g was used as a measure of effect size.

3.3. Meta-analyses combining study precision and study quality

The overall effect of microplastics on biota, as determined by the dual weighting factors of study precision and experimental quality, was negative (Fig. 2). Interestingly, the overall effect was only slightly negative when based solely on study precision. However, the effect was strongly negative when the meta-analysis was based only on experimental quality, but showed great variability. The overall effect when weighted for study precision and experimental quality was between the two individual meta-analyses.

The direction and size of the effects of microplastics on marine biota varied across biogeographic realms and taxonomic groups. Differential weighting resulted in negative effects of varying magnitude in Temperate Northern Atlantic and Temperate Northern Pacific. In the other biogeographic regions, the overall effects were mostly negligible. Among taxonomic groups, jellyfish and corals exhibited the greatest negative effects, while lesser negative effects were observed for microalgae and barnacles. Other groups were only marginally affected. Benthic filter feeders exhibited the greatest statistically significant negative effect. Interestingly, the effects of microplastics varied little among different life stages (Fig. 3). Importantly, this comparison involved multiple species as few studies tested the effects of microplastics on the different life stages of the same species.

4. Discussion

4.1. The 'ideal' microplastic experiment: recommendations and a strategy to improve experimental quality

This study presents a novel conceptual blueprint for conducting an 'ideal' microplastic effect study based on four broad themes (1) microplastic characterization, (2) experimental design, (3) environmental resemblance and (4) ecological scaling-up potential (Supplement 1 Table S1). In addition to forming the basis for evaluating the quality of published experiments, the 'ideal' experiment also serves to establish a set of recommendations to improve the quality of future studies. These recommendations are listed according to their level of difficulty and likewise pertain to the same (non-exclusive) themes (Fig. 4, for more detailed guidelines along broad themes see Supplement 4). The ranking of difficulty is not universal, but rather depends on the experiment, i.e. it is a function of the organism and the specific objectives of the experiment. The position of the themes in the ranking also provides insights into the general degree of difficulty associated with each theme.

Without doubt, the simplest recommendation to fulfil is to report complete information on an experiment. Although this recommendation would seem self-evident, some publications fail even to provide basic information, e.g. failing to define the error bars shown (standard deviation, standard error, confidence intervals).

Although researchers tend to describe the application of microplastics in their experiments in an understandable and reproducible manner, the pervasive application of non-weathered particles of only a single polymer type and shape is a serious departure from the pollution scenario prevalent in marine habitats. While it would be relatively easy to improve the experimental quality by administering mixtures of microplastic shapes and polymers, it would be more challenging to artificially age microplastic or collect microplastic particles from the environment for the use in experiments. Likewise, administering microplastics at realistic concentrations and using the appropriate polymer types demands extensive a priori on site measurement and analysis. To date, little empirical data are available reflecting current microplastic concentrations, particularly for particle sizes < 100 μm (Conkle et al., 2018; Lindeque et al., 2020). As a result, much of the research on the effects of microplastics on marine biota cannot be related to any specific environmental pollution scenario. Furthermore, little is known about the variability of microplastic concentrations in space and time (Phuong et al., 2017; Setälä et al., 2018; Wong et al., 2020), largely due to uneven sampling efforts and the lack of standards in collecting and processing samples. As a result, levels of microplastic pollution in the environment are commonly either under- or overestimated (Shahul Hamid et al., 2018).

Recommendations with respect to experimental design and environmental resemblance exhibit much in common (Fig. 4). Feeding test organisms with natural rather than artificial food is relatively easy in most cases. However, food and microplastics need to be administered together and at natural intervals throughout the course of the experiment. However, many published studies provide only a single dose of both at the start of the experiment. In addition, adding natural microparticles such as clay or diatoms as a procedural control would further improve the experimental quality. Increased monitoring, e.g., measuring microplastic consumption and microplastic bioavailability during the experiment and in the natural environment of the study organism, is another recommendation to improve experimental design and environmental resemblance.

Improvements that enhance the ecological scaling-up potential of a study are by far the most difficult to achieve. Moving from simplicity to complexity vexes many experimenters, and clearly sophisticated modelling will be obligatory in future microplastic effect studies. This will arise as future microplastic research needs to consider a) the spatial variability in microplastic abundances (e.g. Gorman et al., 2020), b) non-lethal effects on biota across different life-stages of given test organisms and functional roles (Galloway et al., 2017), c) interactions with other environmental stressors (Segner et al., 2014), and d) a multi-species context as interactions between species can determine their interplay with microplastics and the resulting biological effects (Segner et al., 2014). As a result, the potential effects of microplastic exposure on biological interactions and on the community level need to receive more attention in future research. In summary, this is a call for complexity in experimental approaches that seek to elucidate the biological effects of microplastics. Presumably, it will become inefficient to perform a plethora of simple experiments by creating new combinations of, for instance, single species and single polymer types or microplastic shapes. Years or decades may never successfully reflect the influence of microplastics on natural populations, communities or ecosystems. Complex multifactorial experiments that address biological systems at the community level are a more promising approach, because they would allow for interactions between species during the exposure phase, while simultaneously enabling experimenters to assess relevant interactions between microplastics and abiotic or biotic stressors. The latter may be pivotal for capturing the possible effects of the synthetic microparticles, which may not emerge before a given test organism is faced with a second stressor. Such approaches, however, require adequate laboratory or mesocosm facilities with an infrastructure equipped to apply several abiotic stressors such as warming, desalination and oxygen deficiency along with exposure to microplastics. This requirement

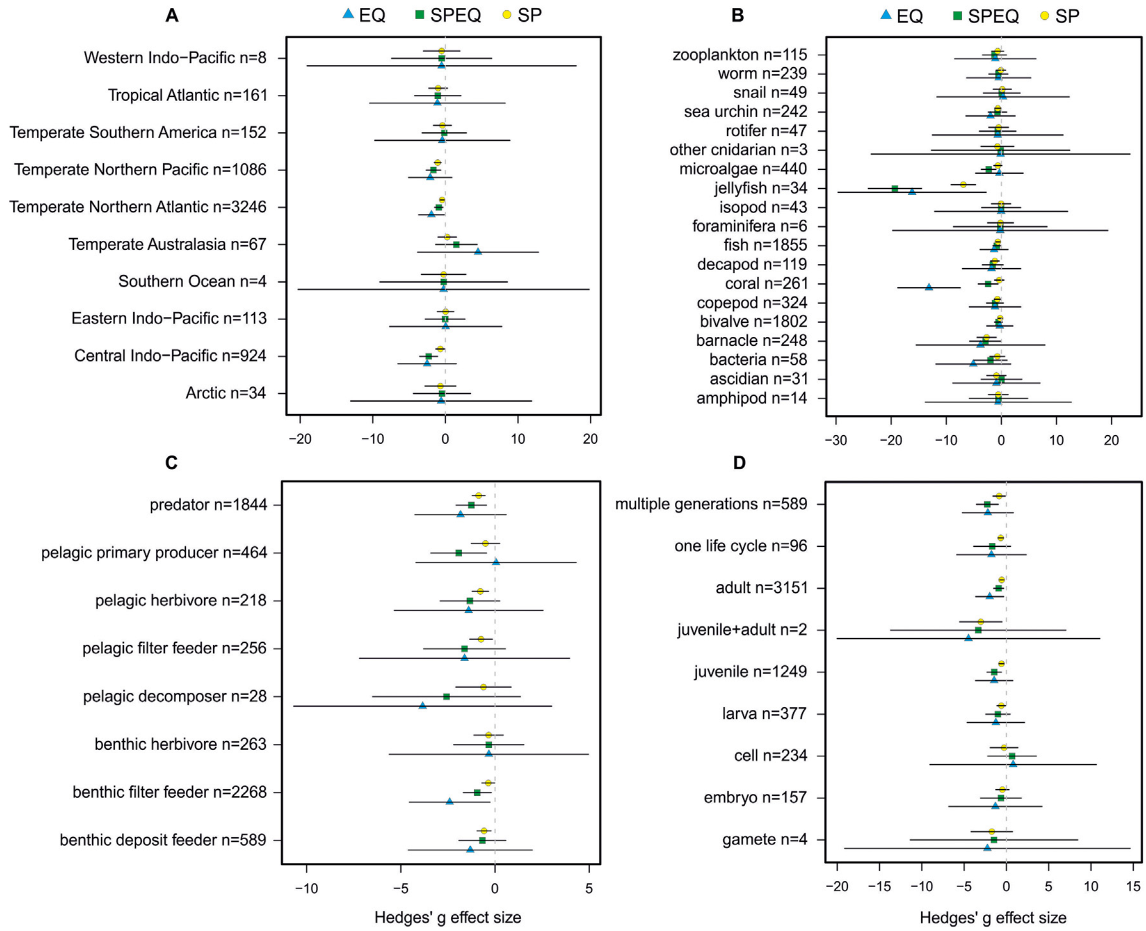

Fig. 3. Effects of microplastics on the biota (mean ± 95 % CI) across biogeographic realms (A), taxonomic groups (B), feeding modes (C) and life cycle stages (D) weighted with study precision (SP), experimental quality (EQ) or both (SPEQ).

would restrict research on the biological effects of microplastics to particular research institutions and would limit the research done at institutes that lack this infrastructure.

The simplicity-complexity dilemma in experimental research is faced by a wide range of environmental/ecological disciplines, e.g. microplastics in terrestrial ecosystems (Baho et al., 2021) and chemical pollutants in aquatic ecosystems (Schuijt et al., 2021). Consensus is emerging that a piecemeal collection of different combinations of simple experiments is ill-suited to developing an understanding of how pollutants affect ecosystems, calling for both more sophisticated 'realistic' experiments and experiments that focus on multiple stressors (Baho et al., 2021; Schuijt et al., 2021; Simmons et al., 2021). Aside from the effects on biota of microplastics themselves, microplastics can also serve as substrates for other pollutants, such as heavy metals (Liu et al., 2021) and hydrophobic organic contaminants (Kwon et al., 2017), and act as of source of chemicals leached from within particles (Baho et al., 2021). Consequently, even the distinction between the effects of microplastics and those of chemical pollutions is difficult to justify.

4.2. Published microplastic effect studies: meta-analysis including quantitative experimental quality

The main objective of meta-analyses is to perform a statistical analysis summarizing a set of conceptually similar scientific studies (Gurevitch et al., 2018). Meta-analysis typically weights individual results based on their respective precision, specifically the inverse variance (Hartung et al., 2008; Gurevitch et al., 2018). However, experimental quality is often neglected in meta-analyses (Mikolajewicz and Komarova, 2019). Nevertheless, experimental quality is particularly relevant in microplastic impact research and continuous improvements in the experimental design and the used technology had already enhanced the quality of more recent experiments.

In this study, a meta-analysis of the effects of microplastics on marine biota was performed with an additional weighting factor based on the quantified departure or 'distance' of each experiment from the proposed 'ideal' experiment. This quality weighting adds the aspects of experimental design and ecological relevance to the precision weighting of traditional meta-analysis.

The impact of adding experimental quality as a weighting factor becomes clear when compared to traditional meta-analysis (Fig. 2). Traditional meta-analysis suggests that microplastics have little impact on marine biota. However, when weighted by experimental quality, meta-analysis suggests that microplastics have a strong impact, although with substantial uncertainty and large differences between subgroups (Fig. 3). Therefore, studies with more rigorous experimental designs and/or better resemblance to the natural environment more often report adverse impacts on test organisms than those with a lower experimental quality. The microplastic effect studies assessed in the meta-analyses inevitably differed greatly in experimental quality, which explains why the meta-analysis based only on experimental quality resulted in a wide range of effect sizes. Nevertheless, this range can be reduced in the future when researchers follow the recommendations to improve experimental quality in microplastic effect studies.

Although meta-analysis consolidates the findings of a group of conceptually similar studies, it is rare for the group to comprise identical studies. Nonetheless, meta-analysis offers the possibility to analyse the effect of differences among the studies. Subgroup and meta-regression analyses are useful to test hypotheses about the influence of the experimental context

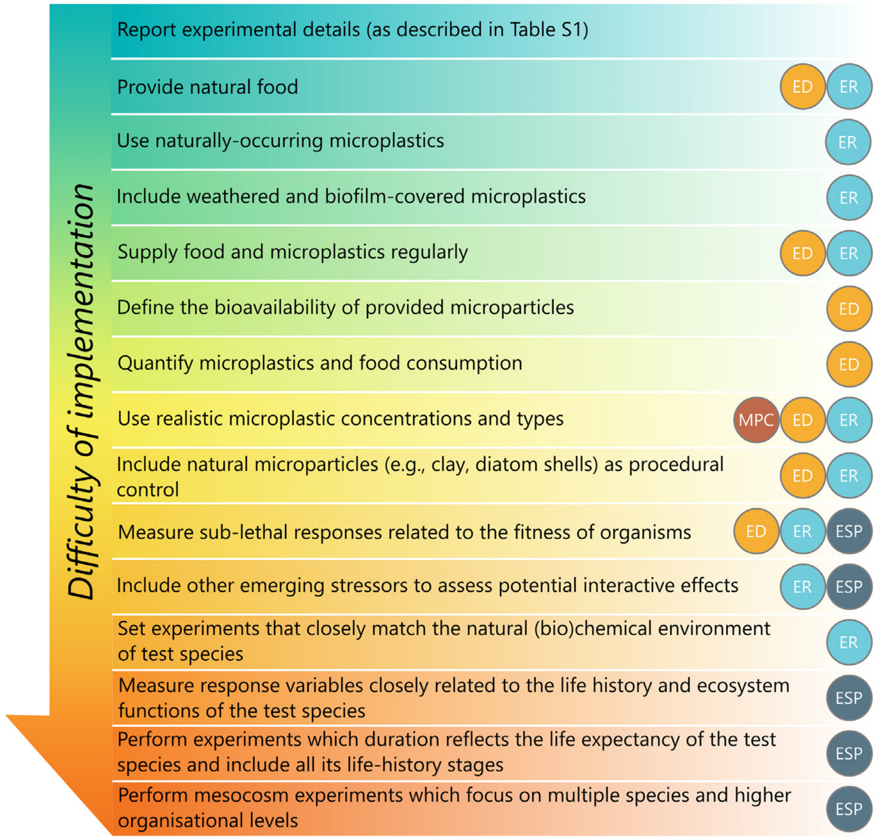

Fig. 4. Recommendations to improve the quality of future experiments listed by degree of difficulty.

and/or of the environmental conditions on the strength and direction of microplastic effects (Borenstein et al., 2009). Even when effects are pooled from different studies that assessed a diverse range of response variables, the negative effects of microplastics systematically increased with increasing concentrations of microplastics, while the effects notably declined shortly after the cessation of microplastics exposure (Supplement 3 Fig. S12, Supplement 4 Fig. S1). However, subgroup and meta-regression analyses may be biased if subgroup levels are represented by too few individual studies and/or exhibit poor experimental quality. For example, a lack of data — based on only two bivalve species (deposit and filter-feeder) — led to the overly positive (though insignificant) effects of microplastics on biota in Australia. These species are quite tolerant to environmental stress, experiments were short-term and the design included moderate microplastic concentrations and reference particles. Consequentially, meta-analysis – while a powerful analytical tool – must be tempered with discernment.

5. Conclusions

This paper elucidated the methodological variability upon which our current understanding of the effects of microplastics on marine biota is built. An array of experimental parameters of previous effect studies was compared to ecologically relevant scenarios. This analysis resulted in a set of criteria that define an 'ideal' microplastic effect study reliably evaluating the effects of microplastics on marine biota. Quantitative measures of the departure or 'distance' of previously published studies from this 'ideal' experiment led to the means to incorporate experimental quality into a meta-analysis of the effect of microplastics on marine biota. The results of this meta-analysis suggest that microplastics commonly exhibit greater detrimental effects in experiments with greater quality. In addition, a set of recommendations — based on the criteria to establish the 'ideal' experiment — was formulated to improve experimental quality. Although performing the 'ideal' is clearly unachievable, the recommendations serve as guidelines to perform what is feasible and to strive for future technological developments for what is lacking. Finally, recognition of the role of experimental quality is crucial and results of any microplastic experiment can be properly evaluated only in light of its experimental quality.

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Credit authorship contribution statement

Developed the study concept and performed the analysis: JK, FRB, ML, AK. Collected the data: JK, ML, FRB, HJ, PADG, AB, CVC, TH, JJ, GP, RSK, HOK, LL, SL, SKMG, AK. Wrote the manuscript: JK, FRB, HJ, RSK. All authors discussed the results and edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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