Micro- and nanoplastic effects on the reproduction of *Daphnia* spp. - a meta-analysis

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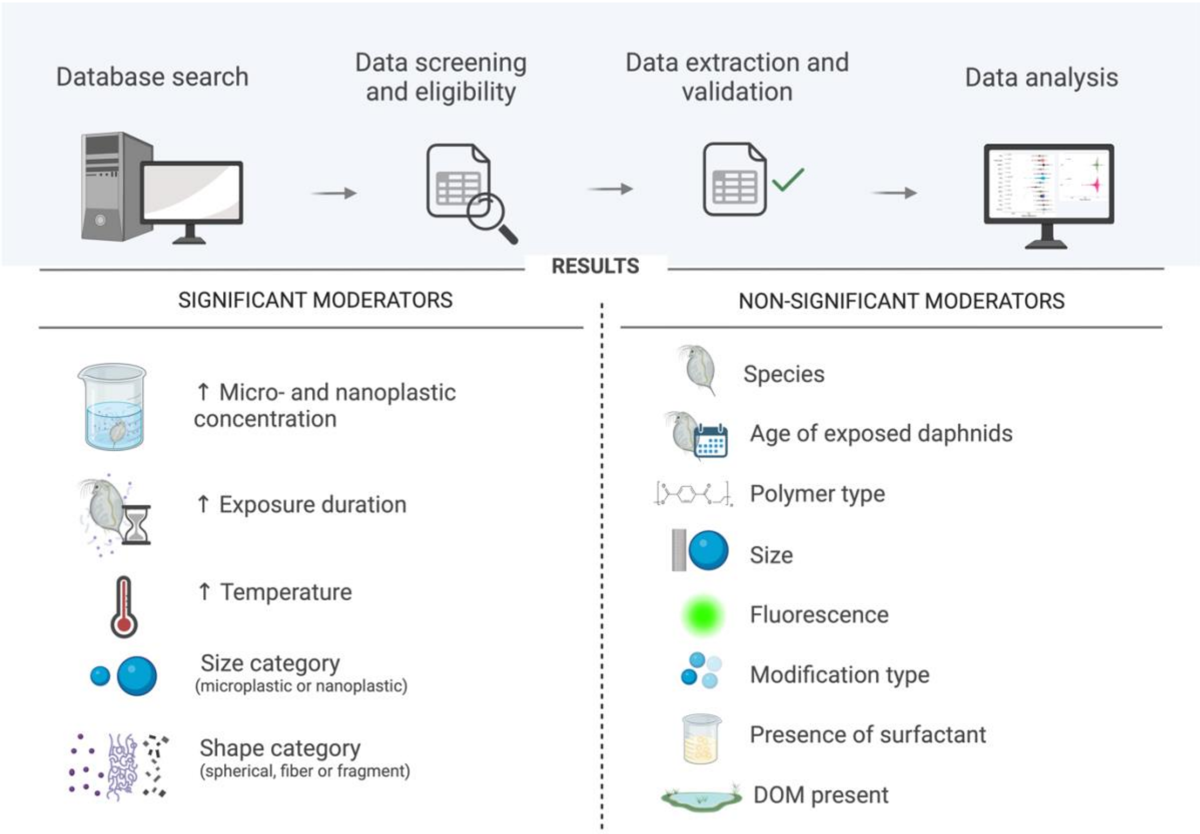
1 Abstract

Several traits of micro- and nanoplastic particles (MNPs), including among others, polymer type, size, and shape, have been shown to influence MNP toxicity. The direction and size of these moderating effects are however often unclear and generalizations from single studies are difficult to establish. Meta-analyses, which quantitatively aggregate data on a specific topic, can be used to increase generalizability of results and derive more accurate and precise effect size estimates by combining measurements from published studies. We conducted a meta-analysis to investigate the effects of MNP exposure on the reproductive output of water fleas of the genus *Daphnia* by aggregating 369 data points from 64 published studies. We show that *Daphnia* individuals exposed to MNP produce on average 13.6 less neonates, which represents a reduction of 20.8% compared to the particle free controls (control mean = 65.37 neonates). This effect is moderated by the particles' concentration and shape category, exposure duration, experimental temperature, and size category with microplastic particles eliciting a stronger negative effect than nanoplastic particles. Species, age of the test organisms, polymer type, size (as continous), fluorescence, modification type, presence of surfactant and DOM present did not influence effect sizes significantly. Based on the high residual heterogeneity in the data, we suggest that additional factors likely influence observed effects and discuss how a better characterization of particles could improve our understanding of the drivers of MNP toxicity.

Keywords: ecotoxicology, nano-plastics, microplastics, offspring, water flea, neonates

Graphical Abstract



2 Introduction

Despite significant research efforts, there are still gaps in our knowledge about the effects of micro- and nanoplastic particles (MNPs) on organisms (Brehm et al., 2023; Thompson et al., 2024). While some studies suggest potential toxic effects (Guzzetti et al., 2018; Pannetier et al., 2020), others report no adverse effects of MNPs (Malinich et al., 2018; Weber et al., 2021). Furthermore, reported effect sizes vary substantially among experiments (Brehm et al., 2023; Foley et al., 2018; Salomon et al., 2024). This inconsistency may be due to the fact that each MNP tested has a unique set of traits, and experimental setups are often not standardized across different studies (Brehm et al., 2023; Thompson et al., 2024).

MNP toxicity has been shown to be influenced by several MNP traits (Lambert et al., 2017) including *(i) physical* (e.g., polymer type, size, shape, zeta potential and other surface properties; Gray & Weinstein, 2017; Pochelon et al., 2021; Saavedra et al., 2019; Schwarzer et al., 2022), *(ii) chemical* (e.g., presence of surfactants, additives or other plastic-associated chemicals; Boháčková & Cajthaml, 2024; Schrank et al., 2019) and *(iii) biological* (e.g., existence and form of an eco-corona or biofilm; Ramsperger et al., 2020; Salomon et al., 2024) factors. Moreover, characteristics connected to the biology of the organisms like the age of exposed individuals or characteristics connected to the experimental setup such as temperature (Chang et al., 2022; Klasios et al., 2024), MNP concentration (Fekete-Kertész et al., 2018), and exposure duration can influence experimental outcomes further (Pikuda et al., 2023).

While acute effects of MNP on survival are studied best among all endpoints (see Microplastics Toxicity Explorer (ToMEx), Thornton Hampton et al., 2022; Brehm et al. 2023), sublethal effects such as behavioral changes and alterations in life span, body size, growth, and reproduction are getting increasingly into the focus of scientific research and regulatory risk assessment (Barascou et al., 2021). Over longer exposure durations, sublethal effects can have substantial impacts on population dynamics by reducing the individuals' fitness (Santadino et al., 2014; Barata et al., 2008; Bellehumeur et al., 2016; Connell, 1995) and the overall health of ecosystems (Mayer-Pinto et al., 2020). Within the same time frame, sublethal effects often occur at lower concentrations than mortality (Horie et al., 2017; Wolf & Segner, 2023). Therefore, considering sublethal effects is essential for understanding the long-term impact of potentially toxic substances on populations and ecosystems. Among them, alterations in reproduction is of particular relevance (OECD, 2012, 2016) but its effect size is rarely reported.

Daphnia, a genus of filter-feeding crustaceans, is widely used in ecotoxicity studies, including both studies on survival and on sublethal effects (Ebert, 2022; OECD, 2012). *Daphnia* spp. have a comparably short generation time, they reproduce through parthenogenesis (i.e., producing genetically identical clones), and play a key role in lentic aquatic food webs by linking primary production with higher trophic levels (Baird et al., 1989; Ten Berge, 1978). These characteristics make *Daphnia* an efficient model organism which is used widely in ecotoxicological research and within the regulatory context (OECD, 2004, 2012). *Daphnia* spp. is one of the main organism groups used in the ecotoxicity assessment of pharmaceuticals (Tkaczyk et al., 2021), toxic metals (Kim et al., 2015), and also in MNP studies (Thornton Hampton et al., 2022). However, inconsistencies in the effects of MNPs on *Daphnia* reproduction are often observed, likely because material properties and experimental conditions have been rarely considered (Besseling et al., 2014; Khosrovyan & Kahru, 2022).

Meta-analyses summarize evidence on a particular topic by combining measurements or statistical results of multiple previous studies (Grewal et al., 2018; Spector & Thompson, 1991). They are broadly used in medical research (Wang et al., 2021) and increasingly also in ecotoxicology (e.g., Huo et al., 2022; Vilas–Boas et al., 2020; Yang & Nowack, 2020). By pooling the results of multiple studies, meta-analyses enable a more precise estimation of the true effect size (Ellis, 2010) and allow for a comprehensive assessment of how additional factors moderate these effects (Dekkers, 2018).

A first attempt to investigate generalized effects of MNPs on *Daphnia* reproduction by means of a meta-analysis was published in 2024 (Funke et al. 2024). In this first study, Funke et al. (2024) modelled the number of offspring by concentration classes. However, the heterogeneity in the models remained high, which indicates that grouping the data according to exposure concentration classes alone does not sufficiently account for the variance in the data. In contrast, it is likely that other factors including MNP traits and additional experimental characteristics might influence the true effect sizes. Additionally, the literature on MNP effects on *Daphnia* reproduction has increased considerably since the period covered by Funke et al. (2024) as the last publication search was in April 2022. A re-evaluation based on an extended dataset which takes into account further experimental parameters and MNP traits as predictors could thus improve our understanding of MNP effects on *Daphnia* spp. reproduction.

We performed a meta-analysis to obtain a most precise and accurate estimate of the effect size that MNPs impose on *Daphnia* spp. reproduction. In our analysis, we aimed to identify MNP traits and experimental conditions particularly associated with observed offspring number variations. To this end, we extracted 369 data points from 64 published papers, focusing on both experimental factors (e.g., species, age of individuals, exposure duration, and temperature) and different MNP traits (e.g., polymer type, size category and shape category).

3 Materials and methods

3.1 Search Strategy

We performed a literature search to identify studies investigating the effects of MNPs on the reproductive output of water fleas of the genus *Daphnia.* After search string optimization, the final search was performed on March 5th, 2024, using the Web of Science (WoS) database with the search string *"(("microplastic*" OR "micro plastic*" OR "micro-plastic*" OR "nanoplastic*" OR "nano plastic*" OR "nano-plastic*") AND ("Daphnia*") AND ("reproduction" OR "offspring*"))"*. Additionally, we added references listed in Brehm et al. (2023) and Funke et al. (2024). After removing duplicates, we screened the titles and abstracts based on the following inclusion criteria: (1) experimental research (i.e., excluding review articles, proceeding papers and book chapters); (2) measurements of reproductive output of *Daphnia* spp. under MNP exposure, with explicit reference to relevant terms (e.g. offspring, neonates, reproduction). The full text of studies that passed these initial criteria were screened based on two more criteria: (3) inclusion of a control treatment without MNP exposure (particle-free control), while maintaining all other experimental conditions; (4) no co-exposure with additional chemical stressors (e.g., toxic substances). Studies that did not meet all criteria were excluded and the reason(s) for exclusion were noted. Studies passing all four criteria were included for data extraction.

3.2 Data Extraction and Preparation

For each MNP treatment and the particle-free controls, we extracted the reported reproductive output (mean and standard deviation (SD), standard error (SE) or confidence interval (CI)) and noted the exact endpoint that was measured (e.g. neonates per adult or neonates per brood). In addition, we extracted the following parameters of the experimental setup, if reported: the number of replicates (i.e. number of test vessels), the number of individuals per replicate (i.e. number of adults per test vessel) at the start of exposure, species name, age of the test individuals at the start of exposure (in days), exposure duration (from start of exposure to reproduction measurement in days), presence of surfactant (yes or no), whether the exposure media contained dissolved organic matter (DOM) or particles used were incubated in e.g., natural freshwater or media supplemented with DOM, resulting in the formation of an eco-corona or biofilm), and experimental temperature. As MNP characteristics, we noted polymer type, mean size (and standard deviation if provided), fluorescence (yes or no), modification type (either one of aluminium oxide, aminated, BP-3, carboxylated, DiNP, incubation of MNP in DOM containing media prior to exposure, or UV-weathered), and shape category (one of either spherical particle, fiber or fragment). When details were not reported, but both the manufacturer and lot number were provided, we checked for additional information on the manufacturer's website. Recycled LDPE and virgin LDPE were grouped as LDPE. Polymer types with three or less data points were aggregated in the polymer type group "others". We included polymer categories for "Thermoset" and "Tire wear" (TW) for cases where authors only reported these polymer categories without providing more detailed information.

We extracted the concentration of MNPs used in each treatment in mg/L and particles/ml when available. If necessary, concentrations reported in particles/ml were transformed to mg/L according to Thornton Hampton et al. (2022) based on particle dimensions and polymer densities. For experiments that reported reproduction over time, offspring for the time point closest to 21 days was noted when possible. Otherwise, we extracted the last cumulative time point. Reproduction rates for all other time points were disregarded. When reproductive output was provided as daily average, we multiplied the number of neonates per exposure duration. In case both neonates per brood and the number of broods were provided, we multiplied the numbers to obtain an estimate for the total number of neonates, which was then divided by the number of adults in the replicate. If only the number of neonates per brood was provided and the number of broods was not specified, we assumed five broods for a 21-day experiment. For all these cases, we calculated the overall SD by taking the square root of the sum of the squared SDs of single broods or daily measurements ($SD = \sqrt{(SD_1^2) + (SD_2^2) + \cdots + (SD_N^2)}$). When only the SE was reported, the SD was calculated by multiplying the SE with the square root of the number of replicates ($SD = SE \times \sqrt{N}$). If only the number of eggs (inside the breeding cavity) was provided, we considered that as the reproductive output. For transgenerational experiments, we only considered the reproductive output from adults that were directly exposed to MNPs (i.e., not the recovery generation). If the measure of uncertainty (SD, SE or CI) was not explicitly stated, we assumed it to be SD. Whenever feasible, we directly extracted the reproductive output from the tables, main text or supplemental material. If measurement means were not reported directly, but raw data were provided, means and SDs were calculated from the raw data. When experimental temperature was not reported specifically, but breeding temperature was mentioned, we assumed them to be the same. A total of 4 data points from 1 study did not mention the age of individuals at the start of the exposure. We assumed an age similar to the median age across all data points in the dataset. If data were presented only in figures, we used the R package *metaDigitise* (version 1.0.1; Pick et al., 2018) to obtain mean values and SD from the plots. If extraction was not possible in any instance, we tried to contact the authors via email to request the raw data.

3.3 Data Analysis

As a measure of effect size, we calculated the *mean difference* (MD) using the *escalc* function from the *metafor* package (version 4.2; Viechtbauer, 2010). Our primary model was a multivariate random-effects meta-regression model without intercept using the *rma.mv* function. The full model included all potential moderators: species name, age of individuals (in days), exposure duration (in days), temperature (in °C), concentration (in mg/L), polymer type, mean particle size (µm), shape category, whether the particles were microplastics (> 100 nm) or nanoplastics (≤ 100 nm; size category), fluorescence (yes or no), modification type (if applicable), presence of surfactant (yes or no), and whether DOM was present in the media or particles were pre-conditioned in DOM containing media prior to exposure (yes or no). In addition, we added random effects for samples (sample_ID) nested within studies (pub_ID) to allow the model to account for heterogeneity between studies and between samples within the same study. To test the significance of each moderator individually, reduced models were fitted by excluding one moderator at a time from the full model. The *anova* function was finally used to compare each reduced model with the full model via *likelihood ratio tests*. Results were illustrated with regression plots for the continuous and forest plots for categorical variables using the *orchaRd* package (version 2.0; Nakagawa et al., 2023). For these illustrations, meta-regression models were fitted including only one predictor at a time. All statistical analyses and data visualizations were performed in R (version 4.4.1; R Core Team, 2024).

4 Results

4.1 Literature search and data extraction

We identified a total of 122 publications (see PRISMA diagram; Fig.1) through the Web of Science (WoS) database search, 84 publications from Brehm et al. (2023), and 32 from Frunke et al. (2024). After removing duplicates and triplicates (n = 42), we screened the titles and abstracts of 172 individual publications. Following this screening, 96 papers were excluded as they did not meet the inclusion criteria. We thoroughly assessed the full text of a total of 76 papers for eligibility and the possibility to extract all necessary data, with 12 further studies being excluded (see Fig.1 for reason for exclusion and supplementary online material for the full literature list including all screening results). Ultimately, our meta-analysis resulted in the successful extraction of 369 data points from 64 publications. All extracted data can be found in the supplementary online material.

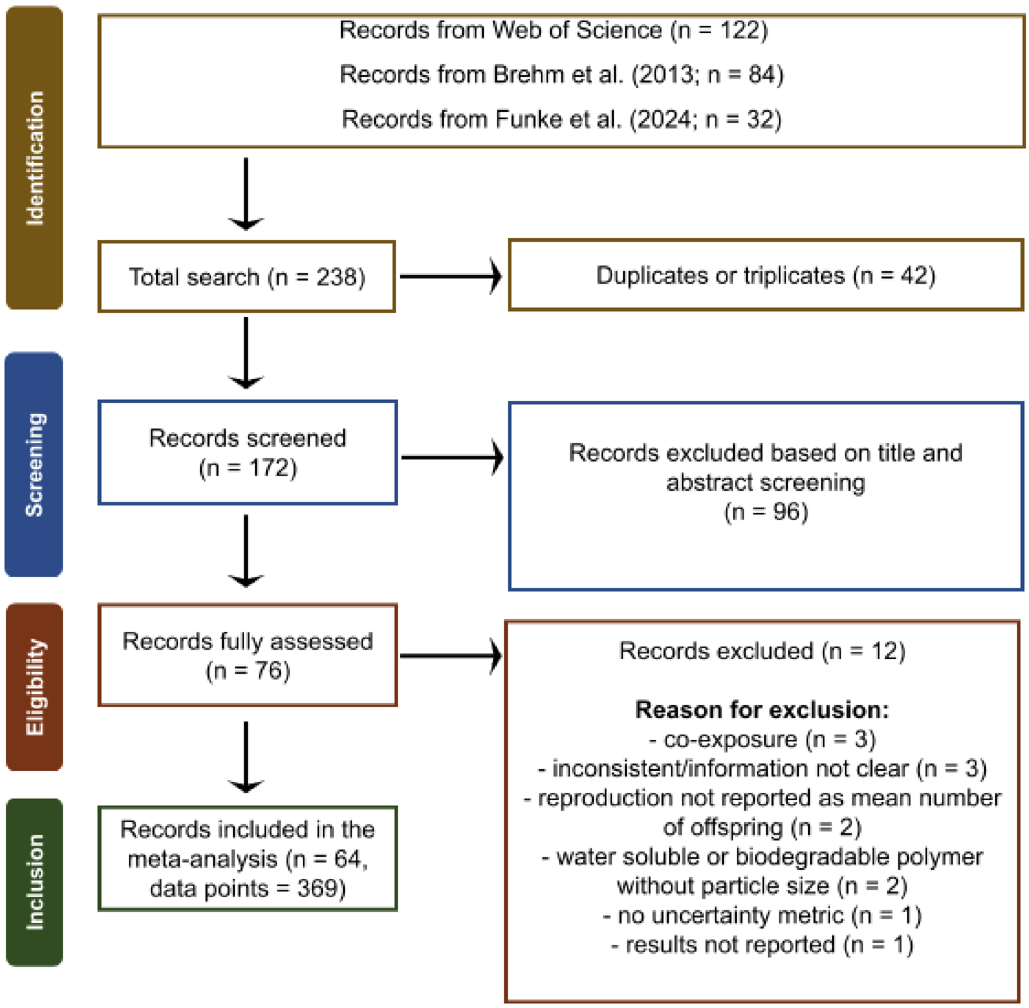


Fig. 1. Adapted PRISMA Flow Diagram illustrating the systematic process used to include eligible studies in our meta-analysis. WoS: Web of Science. MNP: Micro- and nanoplastic particles.

4.2 Meta-analysis of *Daphnia* spp. reproduction

Averaged over all studies, the number of neonates produced per *Daphnia* in the particle-free control groups was 65.37 (mixed random effects meta-regression model: MD = 65.37; SE = 2.85; 95% CI = [59.77, 70.96]; z = 22.90; $p < 0.001$). When exposed to MNPs, the number of neonates decreased by 20.8% (MD = -13.60; SE = 2.77; 95% CI = [-19.03, -8.17]; z = -4.90; $p < 0.001$). However, this effect estimate is likely not derived from a common true effect (residual heterogeneity of the model: Q (df = 368) = 29979.05; $p < 0.001$).

Collectively, all the moderators together explained a significant amount of variability in effect sizes (full mixed meta-regression model [test of moderators: QM (df = 35) = 103.55; $p < 0.001$; residual heterogeneity of the model QE (df = 333) = 20215.55; $p < 0.001$). Adverse effects of MNP on reproduction increased with increasing particle concentration (full vs. reduced mixed meta-regression model: likelihood ratio (LR) = 29.98; $p < 0.001$; concentration range = 7.19e-08 to 500 mg/L, median = 1.25 mg/L, mean = 18.01 mg/L; Fig. 2), increasing exposure duration (LR = 12.56; $p < 0.001$; range of exposure durations = 6 to 39, median = 21, mean = 20.29; Fig. 3A) and increasing temperature (LR = 37.60; $p < 0.001$; range of temperature = 15 to 30, median = 20, mean = 20.85; Fig. 3B). Effects were further moderated by size category (LR = 4.35; $p = 0.03$) and shape category (LR = 6.17; $p = 0.04$). Species (LR = 1.44; $p = 0.69$), size (LR = 3.25; $p = 0.07$; Fig. S1), age of individuals at the start of exposure (LR = 0.05 $p = 0.81$; range of age = 0 to 18, median = 0, mean = 0.7; Fig. S2), polymer type (LR = 12.44; $p = 0.41$), fluorescence (LR = 0.90; $p = 0.34$; Fig. S3), modification type (LR = 2.21; $p = 0.94$; Fig. S4), presence of surfactant (LR = 2.57; $p = 0.10$; Fig. S5), and DOM present (LR = 0.07; $p = 0.78$) were not significant.

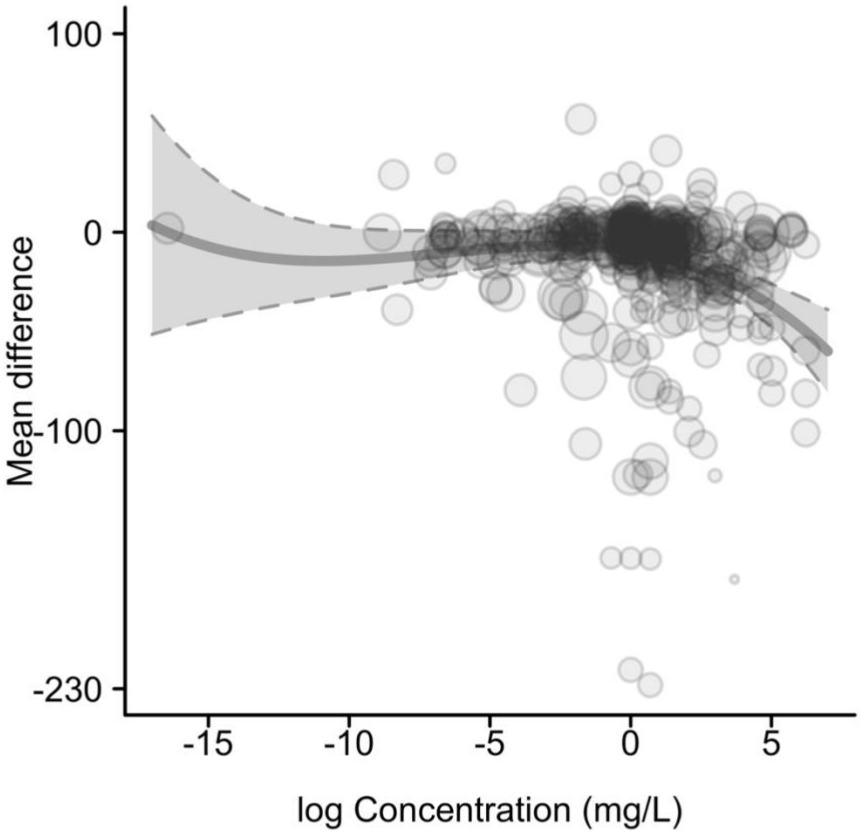


Fig. 2. Effect of MNP concentration in mg/L on the reproductive output of *Daphnia* spp. The size of the data points is proportional to the inverse standard error. The meta-regression line is the solid line, with the 95% confidence interval shown as the shaded area.

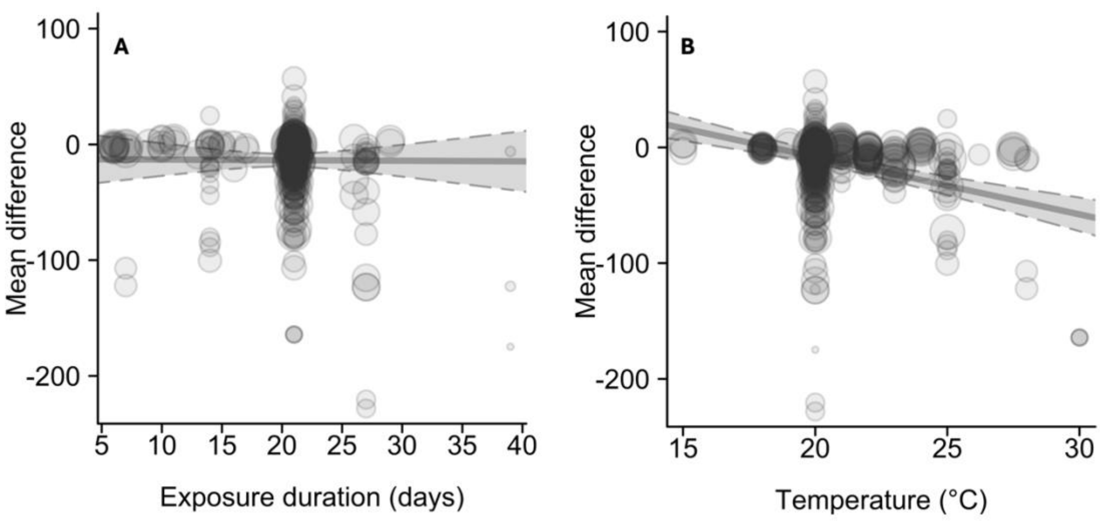


Fig. 3. Effect of experimental conditions on MNP effects on the reproductive output of *Daphnia* spp. A: exposure duration (in days); B: temperature (in degrees Celsius (°C)). The size of the data points is proportional to the inverse standard error. The meta-regression line is the solid line, with the 95% confidence interval shown as the shaded area.

Species. In total, we extracted data for four *Daphnia* species (Fig. 4): *D. pulex* (32 data points; 6 studies), *D. magna* (331 data points; 56 studies), *D. galeata x longispina* (2 data points; 1 study), and *D. carinata* (4 data points; 2 studies). The meta-regression showed that for *D. pulex*, the reproductive output was on average reduced by 22.06% (control mean = 79.01; mixed meta-regression model: MD = -17.43; SE = 7.59; 95% CI = [-32.30, -2.55]; z = -2.29; p = 0.02) and 19.74% for *D. magna* (control mean = 62.76; mixed meta-regression model: MD = -12.39; SE = 2.84; 95% CI = [-17.97, -6.81]; z = -4.35; p < 0.001). *Daphnia carinata* also showed an overall decrease by 28.08% in reproductive output under MNP exposure (control mean = 189.42; mixed meta-regression model: MD = -53.19; SE = 17.58; 95% CI = [-87.66, -18.72]; z = -3.02; p = 0.002). The data for *D. galeata x longispina* were limited to only 2 data points from 1 study and, therefore, no summary statistics were calculated.

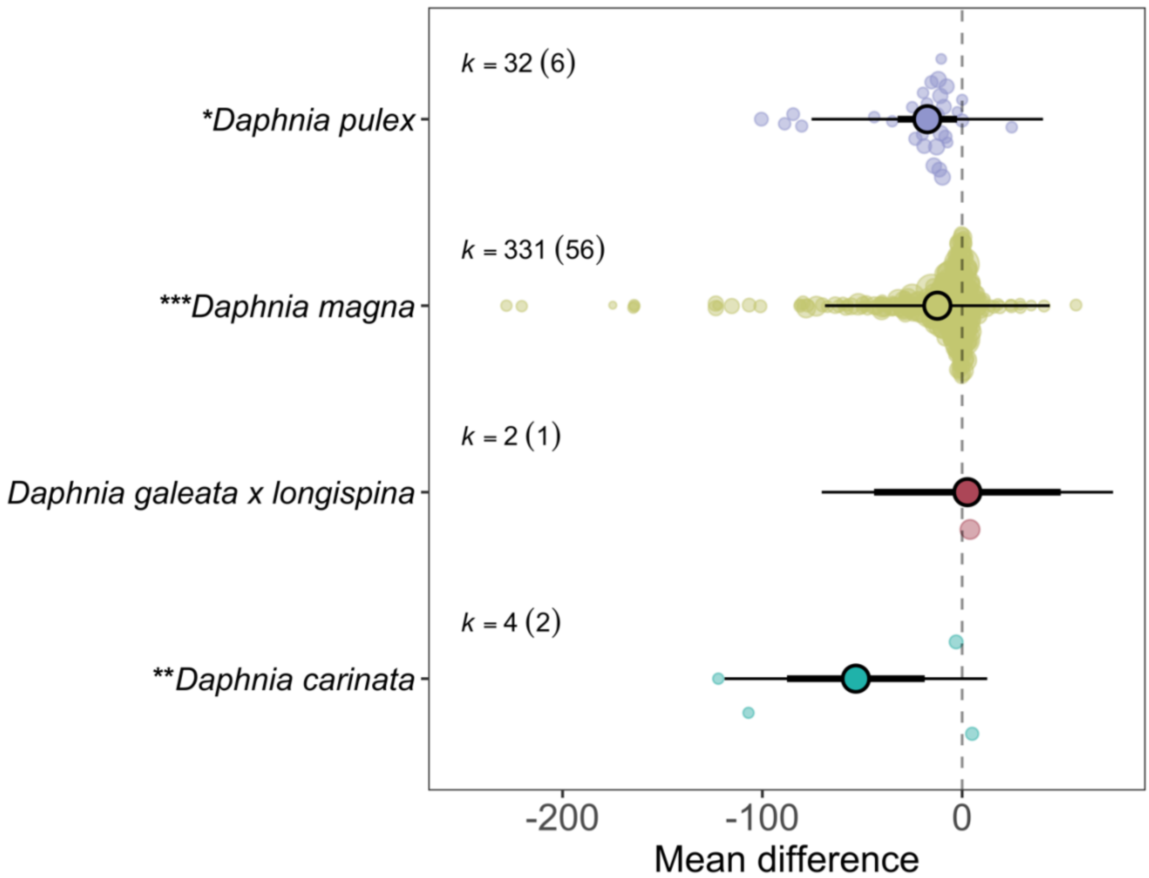


Fig. 4. Mean difference in reproductive output among individuals of four *Daphnia* species exposed to MNPs compared to particle-free controls. Narrow lines represent prediction intervals, black bold lines show 95% confidence intervals; ‘k’ indicates the number of data points, with the number in parentheses corresponding to the number of publications. The dashed line at zero represents the point where no effect is detected; *p < 0.05, **p < 0.01, ***p < 0.001.

Polymer. A total of 19 polymer types were included in the meta-analysis (see Table S1). The number of studies per polymer type ranges from 1 to 34. Although polymer was overall not a significant moderator, we found differences among the polymer types (Fig. 5). The reproductive output of *Daphnia* spp. was overall significantly reduced when exposed to the four most tested polymers: Polystyrene (PS; 231 data points from 34 studies; mixed meta-regression model: MD = -11.31, SE = 3.61; 95% CI = [-18.39, -4.23]; z = -3.13; p = 0.001); Polyethylene (PE; 36 data points from 14 studies; MD = -17.91; SE = 6.16; 95% CI = [-30.00, -5.82]; z = -2.90; p = 0.003); Thermoset (24 data points from 5 studies; MD = -25.23; SE =

8.07; 95% CI = [-41.07, -9.40]; z = -3.12; p = 0.001); and TW (13 data points; 2 studies; MD = -25.30; SE = 12.85; 95% CI = [-50.50, -0.10]; z = -1.96; p = 0.04). Additionally, reproductive output was also reduced by polyethylene terephthalate exposure (PET; 4 data points; 2 studies; MD = -51.38; SE = 16.51; 95% CI = [-83.74, -19.01]; z = -3.11; p = 0.001). All other polymer types did not significantly change MNP effects on *Daphnia* reproduction (mixed meta-regression model: all p > 0.09).

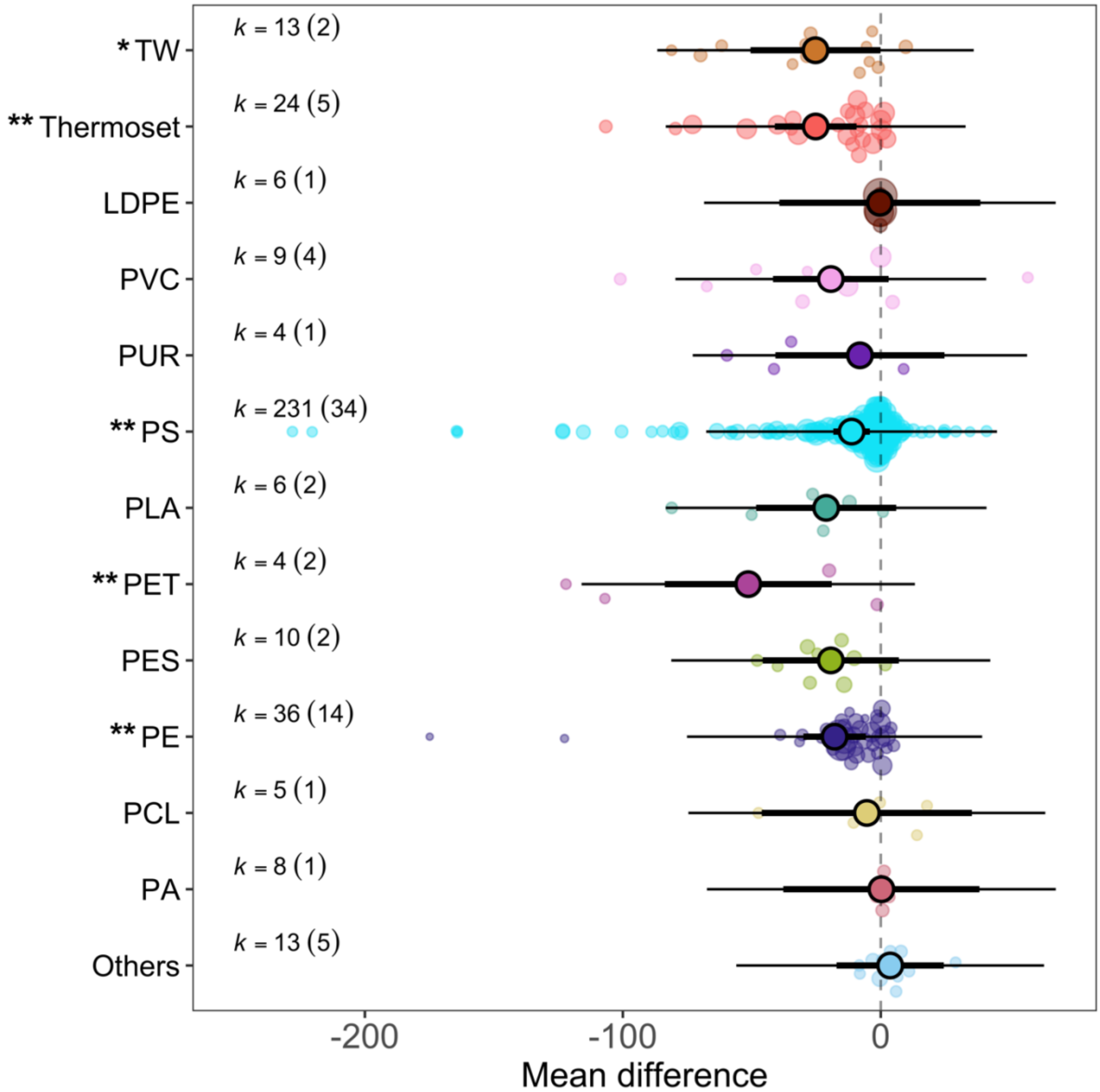


Fig. 5. Mean differences between the reproductive output of *Daphnia* individuals in response to MNP treatment and particle-free control dependent on polymer type. Narrow lines represent prediction intervals, and black bold lines show 95% confidence intervals; 'k' indicates the number of data points, with the number in parentheses

corresponding to the number of publications. Dashed line at zero represents the point where no effect is detected. Polymer types with three or less data points were aggregated as "others"; *p < 0.05, **p < 0.01, ***p < 0.001.

Size category. The compiled studies included particles on the nanoscale (< 1µm; 70 data points; 15 studies) and microscale (≥ 1µm; 299 data points; 54 studies) with particle sizes ranging from 0.003 µm to 600 µm (median = 4.10 µm; mean = 22.59 µm). For two data points in one study, particle size was not reported. For these cases, the median of the reported sizes was assumed, and the missing data points were included as microplastic in the size category. While particles categorized as microplastic significantly reduced the number of offspring by 16.55 (Fig. 6; mixed meta-regression model: MD = -16.55; SE = 2.97; 95% CI = [-22.39, -10.71]; z = -5.55; p < 0.001), particles categorized as nanoplastic had no significant moderating effect (MD = -1.30; SE = 5.30; 95% CI = [-11.70, 9.09]; z = -0.24; p = 0.80).

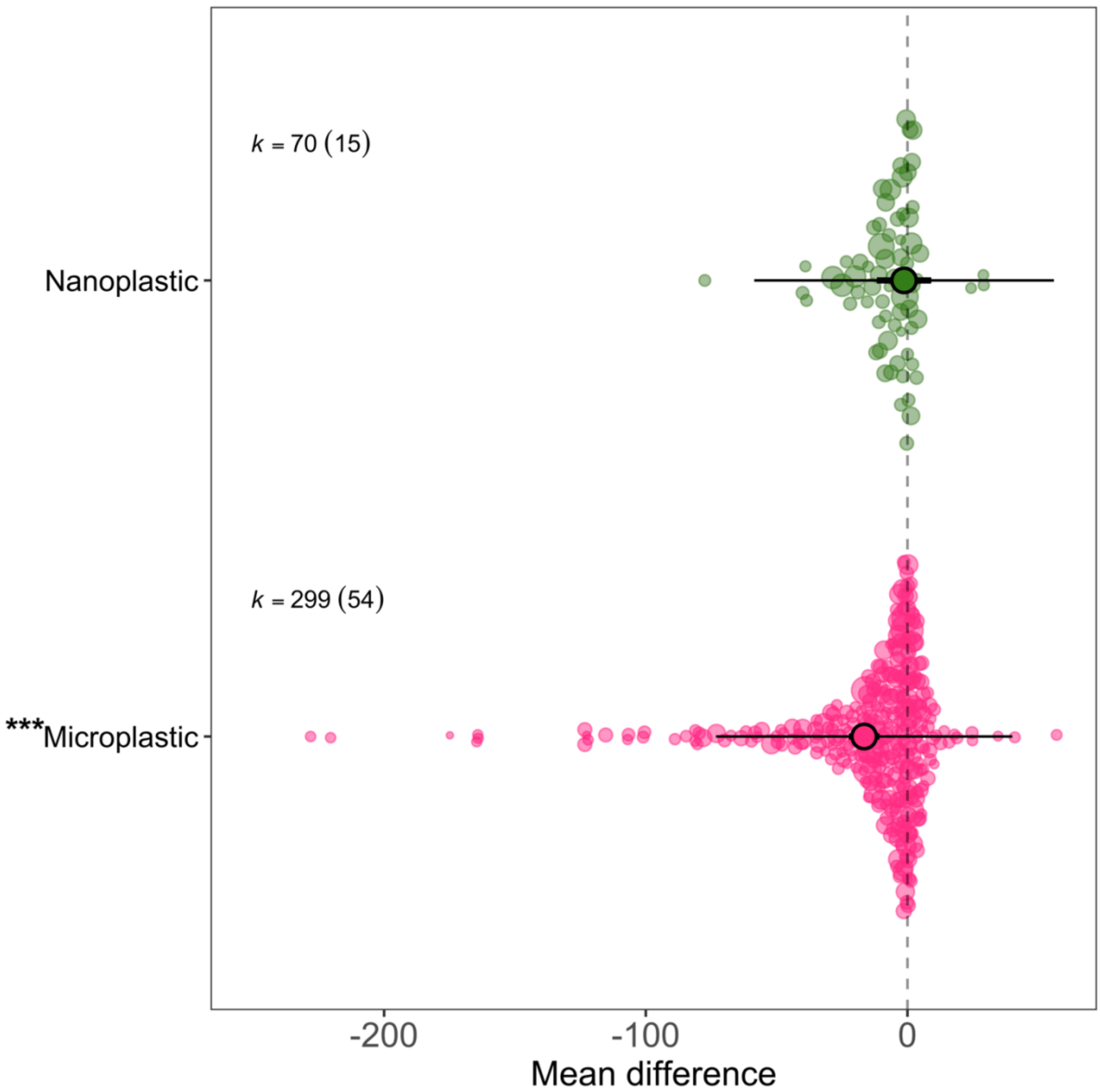


Fig. 6. Mean differences in reproductive output between exposure to particles categorized as nanoplastic or microplastic, and particle-free control. Narrow lines represent prediction intervals, and black bold lines show 95% confidence intervals; 'k' indicates the number of data points, with the number in parentheses corresponding to the number of publications. Dashed line at zero represents the point where no effect is detected; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Shape category. Our meta-analysis included MNPs of all three particle shape categories: Spherical particles (231 data points; 45 studies), fragments (124 data points; 22 studies) and fibers (14 data points; 4 studies). Fragments and spherical particles moderated MNP effects on reproductive output similarly (Fig. 7), with spherical particles reducing offspring numbers by 13.12 (mixed meta-regression model: MD = -13.12; SE = 3.19; 95% CI = [-19.38, -6.86]; z = -4.10; p < 0.001) and fragments by 13.23 neonates (MD = -13.23; SE = 4.21; 95% CI = [-21.48, -4.98]; z = -3.14; p = 0.001). Exposure to fibers had a stronger negative effect, reducing offspring on average by 23.82 neonates (MD = -23.82; SE = 9.30; 95% CI = [-42.06, -5.58]; z = -2.56; p = 0.01).

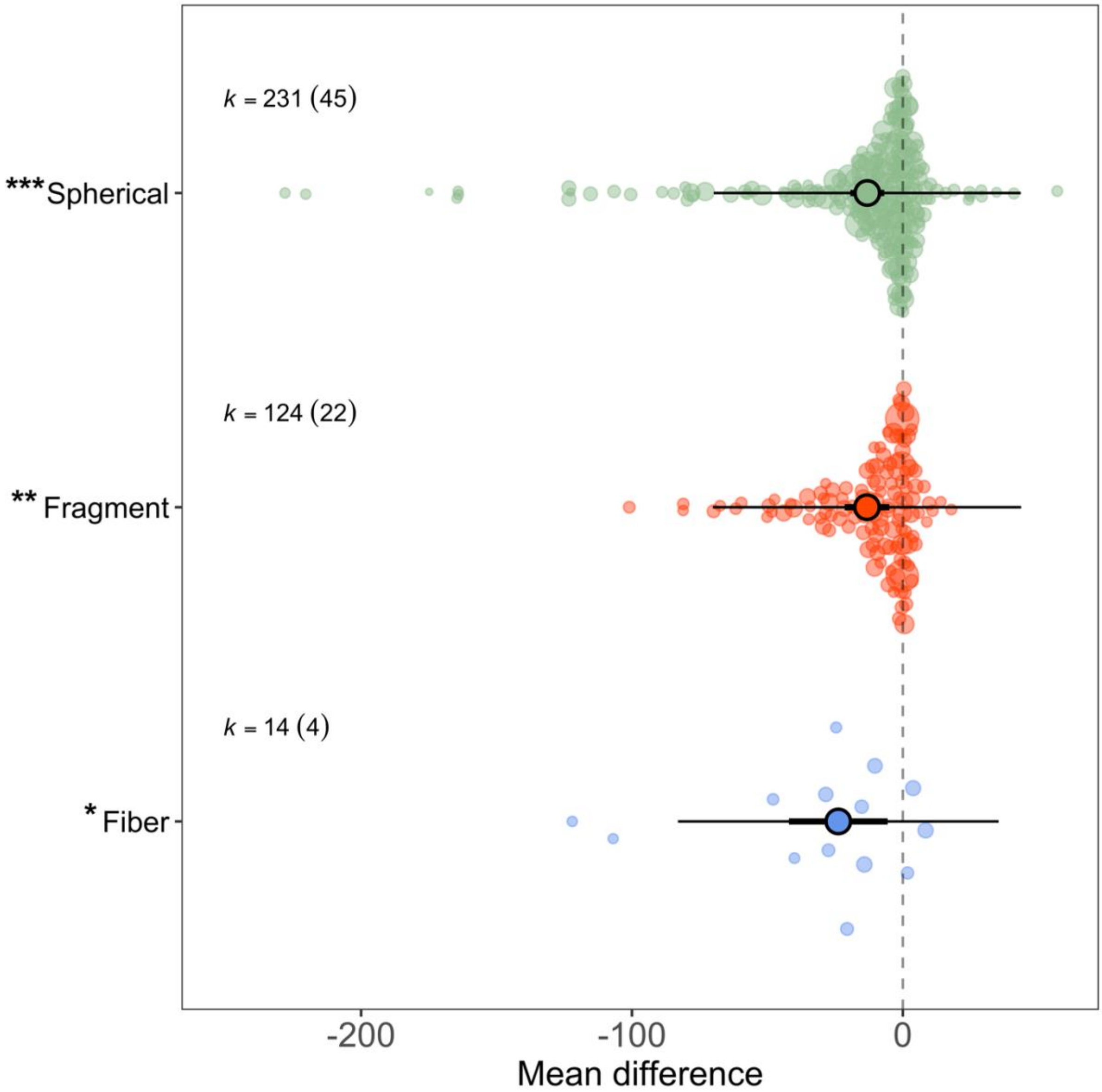


Fig. 7. Forest plot showing the mean differences in reproductive output for each particle shape category. Narrow lines represent prediction intervals, and black bold lines show 95% confidence intervals; ‘k’ indicates the number of data points, with the number in parentheses corresponding to the number of publications. Dashed line at zero represents the point where no effect is detected; *p < 0.05, **p < 0.01, ***p < 0.001.

Presence of DOM. Our analysis included plastic particles exposed to non-biotic media (344 data points; 62 studies), as well as particles exposed to DOM (25 data points; 4 studies). While exposure to MNP in media containing DOM or when particles were incubated in DOM containing media prior to exposure did not significantly reduce the reproductive output (MD = -12.22; SE = 7.14; 95% CI = [-26.23, 1.78]; z = -1.70; p = 0.08), exposure to MNP conducted in media without DOM significantly reduced *Daphnia* spp. reproduction (Fig. 8; mixed meta-regression model: MD = -13.66; SE = 2.78; 95% CI = [-19.13, -8.19]; z = -4.90; p< 0.001),

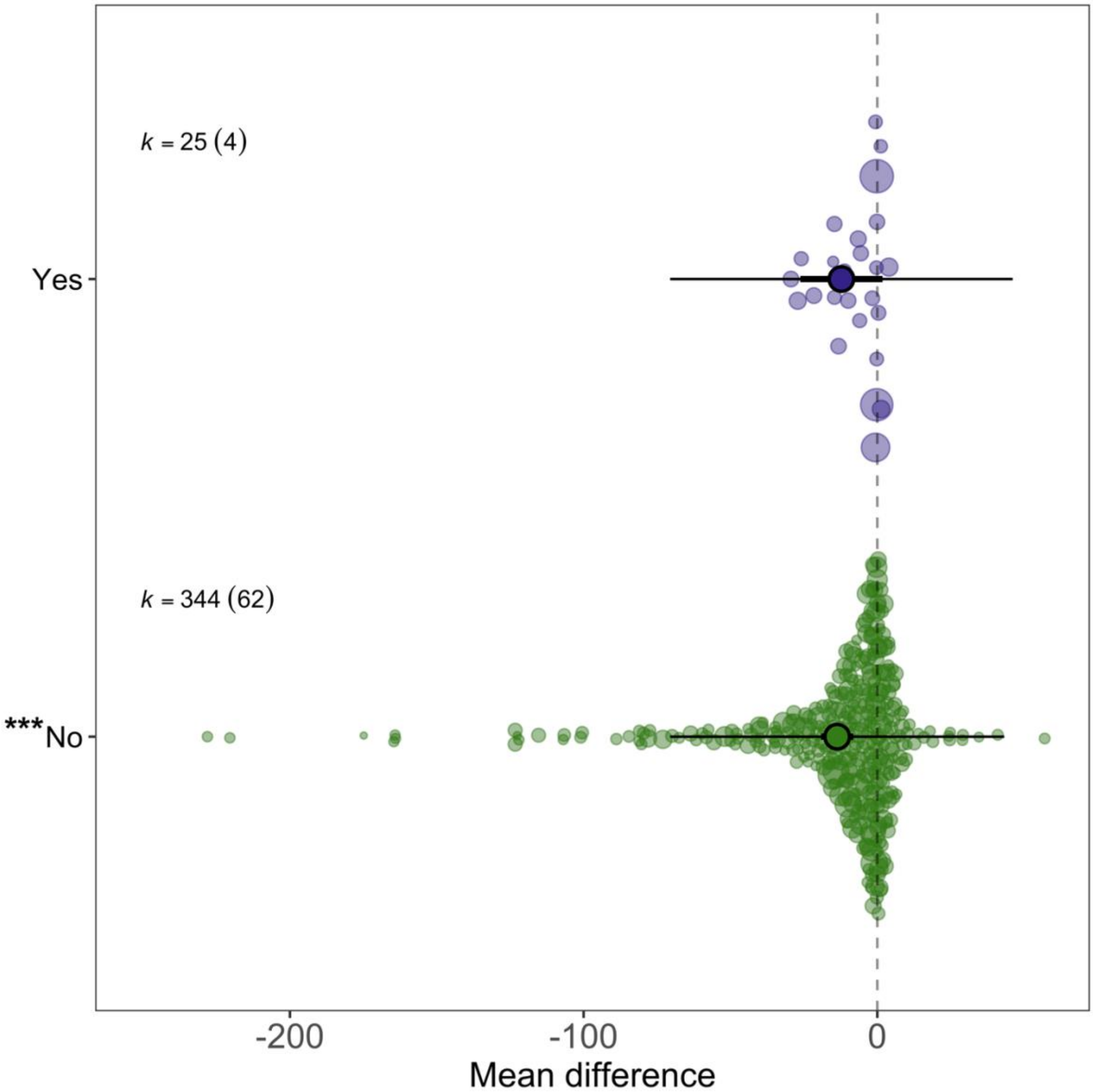


Fig. 8. Mean differences in reproductive output when exposure to MNPs was conducted in the media with DOM (yes) or without DOM (no). Narrow lines represent prediction intervals, and black bold lines show 95% confidence intervals; 'k' indicates the number of data points, with the number in parentheses corresponding to the number of publications. Dashed line at zero represents the point where no effect is detected; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

5 Discussion

Across all compiled studies, MNP reduced the number of offspring produced by *Daphnia* spp. by 13.6, on average, which represents a reduction of 20.8%. This effect increased with increasing MNP concentration, was stronger for particles categorized as microplastic than for those categorized as nanoplastic, and was further moderated by exposure duration, temperature, and particle shape category.

While we expected the observed concentration dependency of effects, the increased negative impact of larger microplastic particles compared to the much smaller nanoplastic particles was surprising. Size has previously been shown to be negatively correlated with effect size (i.e. larger particles lead to smaller effects) for endpoints including *Daphnia* lifespan (Jeong et al., 2016) and reproduction (An et al., 2021), among others, which is usually explained by the increased surface to volume ratio of the smaller particles (Koelmans et al., 2022). In addition, nanoplastics are more often translocated into tissues and cells which may lead to adverse effects (Xu et al., 2019). At the same time, larger MNPs possess a larger volume. When considering that food uptake in these non-selective filter feeders follows a type I functional response (e. g., food consumption rate increases linearly with food abundance up to a threshold level at which it remains constant; Jeschke et al., 2004), and that the filtered volume is thus limited, the maximum amount of food that can be ingested along with MNPs will be lower for larger MNPs. The stronger effects reported for microplastic compared to nanoplastic particles may therefore be attributed to reduced food intake (i.e., food dilution effect; Ogonowski et al., 2016).

Similar to comparative studies on the toxicities of chemical pollutants (Völker et al., 2013), we found that sensitivity towards MNP might differ among different *Daphnia* species, with *D. carinata* being more sensitive than *D. magna* and *D. pulex*. However, in contrast to our findings, these studies on chemicals found *D. carinata* to be less sensitive than the other species (Phyu et al., 2004). A likely reason for this discrepancy is that species like *D. carinata* that occur not only in lakes but also in smaller ponds, may be more robust to particulate matter than pure pelagic species, as pond species are naturally exposed to higher concentrations of mineral particles (Hart, 1992). However, according to this hypothesis, *D. magna*, which usually dwells close to the ground stirring up and feeding on substrate, is expected to be more tolerant towards particulate matter compared to other species, a pattern that is not represented in our data. Similar to the few measurements for *D. galeata x longispina*, data for *D. carinata* are still scarce (only four data points from two studies) and more experiments designed specifically to test the difference between species are needed to draw more solid conclusions. For future experiments, it would also be interesting to test whether differences in sensitivity can also be found among different clones (i.e., within the same species; see for example Imhof et al., 2017).

For the compiled dataset, no moderating effect was observed for the age of the test individuals at the start of exposure. Although this might be a result of a strong bias in the data towards the testing of neonates, we found a moderating effect of exposure duration which is similarly biased towards 21-day exposure periods. While this consistency in experimental design is necessary to increase comparability among results across studies (see OECD, 2012), additional research is needed to specifically investigate how MNP effects on reproduction change with the test individuals' age and under extended exposure durations. Promising approaches include experiments measuring lifetime reproductive success (Betini et al., 2020) and studies assessing reproductive output across consecutive clutches (Imhof et al., 2017). The effect of temperature was also significant, and the negative slope indicates that MNP exposure at higher temperatures potentially reduces reproductive performance further. Again, considering the strong bias of studies towards testing at 20°C (following OECD, 2012), further experiments targeting specifically effects of MNP under increased temperatures (simulating multiple stressor situations under climate warming) are needed.

Biases in the compiled dataset are also visible in the traits of the tested MNP. While in total, a large number of different polymer types were tested, some polymers (e.g., PS with 231 data points) have a much higher representation than others (e.g., PET with only four data points). This uneven distribution limits the generalizability of the results for less-studied polymers. Additionally, nominally identical polymer particles might have different toxic effects (Ramsperger et al., 2022) depending on properties that are often not reported such as the particles' zeta potential or other measures of surface charge (Wieland et al., 2024), or different types and amounts of plastic associated chemicals (Ivleva, 2021). Similar to previous meta-analyses on MNP effects on *Daphnia* spp. (Brehm et al., 2023; Funke et al., 2024; Salomon et al., 2024) and other organisms (Ji et al., 2021), a bias towards testing spherical particles is visible also in our dataset with fibers still being tested only scarcely.

It is often argued that particles that have been exposed to the environment could change their properties in a way that influences their toxicity (Behera & Das, 2023; Waldschläger et al., 2020). Although exposure to DOM is not entirely understood, studies suggest that particles with eco-corona or biofilm are more likely to be internalized by cells (Ramsperger et al., 2020), and higher rates of internalization may lead to stronger biological effects. However, these cell-based studies used murine macrophage cell lines which differ drastically from endothelial cells. Moreover, *Daphnia* presents a peritrophic membrane coating the midgut. This membrane protects the gut from potential injuries caused by the naturally occurring particles ingested during water filtering (Quaglia et al., 1976). Thus, when the membrane is undamaged, only MNPs smaller than its pore size (e.g. *Daphnia magna* ~ 130 nm) can enter in contact with the epithelial cells (Heinlaan et al., 2011), the point of potential cellular uptake.

Our meta-analysis is inconclusive regarding effects of the presence of DOM, as treatments with particles exposed to DOM had a broader CI and non-significant result. DOM has been previously shown to mitigate toxic effects of MNP on *Daphnia* survival (Fadare et al., 2019; Salomon et al., 2024) and on other species (e.g., *Artemia salina*; (Kamalakannan et al., 2024). This suggests that while incubation in biotic environments leads to eco-corona formation, it also attenuates the toxic effects of MNPs, possibly by altering the particles' bioavailability (Liu et al., 2022) or by serving as an additional food source (Amariei et al., 2022). The mitigating effect of eco-corona presence and the harsher effects of larger size particles could both be explained as a response of *Daphnia* to food availability. Alterations of *Daphnia* reproductive success caused by MNPs are a combination of nutrition uptake impairment and toxic effects. Therefore, food concentration during experimental exposure should be considered when discussing MNP toxicity.

In our meta-analysis, we compiled 369 data points from 64 studies, which about doubles the number of data points and studies aggregated in a previous meta-analysis published by Funke et al. (2024; 158 data points from 32 studies). Unexpectedly however, the effect size estimate from our analysis shows a wider CI (width: 10.86; range: -19.03 to -8.17) than the CI reported in Funke et al. (2024; width: 7.86, range: -14.44 to -6.58). Considering that microplastics research currently shifts towards testing a more diverse set of MNP traits (e.g. fibers and more diverse sets of polymers) and given the significant effects of several moderators in our analysis, we anticipate that the wider CI reflects a higher heterogeneity and thus higher variance not only in the measured effect sizes but also in MNP trairs compared to the dataset of Funke et al. (2024). In addition, the average effect of MNP on *Daphnia* reproduction estimated by our analysis (offspring number on average reduced by 13.6) is higher than the effect size estimated by Funke et al. (2024; offspring number reduced by 10.51). The increase of recent studies testing MNP properties that lead to higher toxicities (e.g. fibers, polymer types like TW particles; (Carrasco-Navarro et al., 2021; Qiao et al., 2019) has likely led to this shift. For future meta-analyses, we would expect that the generalized hazard (i.e., averaged over all MNP traits) of MNPs, as estimated from literature values might still change. This could either increase with greater representation of MNP traits associated with higher toxicity or decrease, for example, depending on the species. Considering MNP traits in future analyses is thus vital to get an understanding of the trait-dependent hazard and risk that MNPs pose on the environment.

Even though our analysis accounts for many moderators, our results suggest that other unexplored moderators or interactions may additionally contribute to the observed variability (see high residual heterogeneity in the full model). A better characterization and reporting of MNP properties and the experimental setup are thus essential to derive more accurate effect sizes. Additional moderators that might influence effects include, for instance, the food concentration during MNP exposure , the particles' surface properties (e.g., zeta-potential, other surface charge measure, or a detailed characterization of the eco-corona, if present), and an in-depth analysis of plastic-associated chemicals (Diepens & Koelmans, 2018; Zimmermann et al., 2020).

6 Conclusion

Our meta-analysis compiles 369 data points from 64 studies investigating effects of MNP on *Daphnia* spp. reproduction. Based on these data, we show that, across all tested MNPs, reproduction decreased on average by 13.6 neonates per adult – a 20.8% reduction compared to the control. The variance in the data is however high and additional factors that are often not measured or not reported likely influence measured outcomes further. These include additional factors pertaining to the experimental setup, like the food concentration during exposure, and a more in-depth characterization of MNPs including measurements of surface properties (surface charge, zeta-potential, properties of the eco-corona) and a detailed analysis of plastic-associated chemicals.

Supplementary Information

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Author's contributions

MMM and CL acquired funding. MMM supervised the study. MMM and ALAV conceptualized the study. ALAV conducted literature search and data extraction. LL validated and corrected the extracted data. ALAV and MMM performed the statistical analyses and visualized the results. ALAV drafted the initial manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Availability of data and materials

All raw data are provided in the supplemental material. Additionally, all data and code will be made openly available on github and Zenodo upon acceptance for publication.

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