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Size Matters: The Effects of Polystyrene Nanoplastics on Parasite Transmission in the *Daphnia-Metschnikowia* Host–Parasite System

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ABSTRACT

1. The accumulation of micro- and nanoplastics (MNPs) poses a significant threat to freshwater ecosystems. Nanoplastics (NPs; < 1000 nm) are particularly concerning due to their ability to penetrate cellular membranes and disturb intracellular functions. While current research has predominantly focused on the toxicological impacts of MNP on individual species, their broader ecological effects, particularly on species interactions, remain poorly understood.
2. Prior studies have indicated that smaller NPs within the nano-size range generally cause more severe effects on individual organisms. However, the impact of varying NP sizes on species interactions has not been thoroughly explored. This study addresses this gap by examining the effects of polystyrene NP beads of two sizes (50 nm and 100 nm) and two concentrations (1 mg/L and 5 mg/L) on the infection dynamics of the fungal parasite *Metschnikowia bicuspidata* in two genotypes of the freshwater crustacean *Daphnia magna*.
3. Our results indicated that lower NP concentrations (1 mg/L) had no significant effects on either host or parasite fitness. Exposure to 50 nm NPs at 5 mg/L significantly diminished both the parasite's transmission success and the host's lifespan. Conversely, 100 nm NPs at the same concentration enhanced parasite fitness. Given that *M. bicuspidata* is a widespread and virulent parasite affecting various *Daphnia* species globally, alterations in infection dynamics due to NP pollution could have broader implications for *Daphnia* populations and freshwater food webs.
4. These findings highlight the critical need to incorporate species interactions into plastic pollution research and emphasise the importance of evaluating the effects of different NP sizes on ecological relationships to fully comprehend the ecological impact of MNP pollution.

1 | Introduction

Plastic production has surpassed that of almost all other human-made material, with only steel and cement being produced in greater quantities (Geyer, Jambeck, and Law 2017). In Europe, only around 30% of plastics get recycled (Geyer, Jambeck, and Law 2017). Over time, mechanical wear and UV degradation fragment plastic debris into smaller particles known as microplastics (MPs; < 5 mm) and nanoplastics (NPs; < 1 μm) (Gigault et al. 2021; Hartmann et al. 2019). These micro- and nanoplastics (MNPs) can enter the environment both as secondary particles from larger debris and directly in their original size, as they

are utilised in various industries, including clothing, cosmetics, and nanotechnologies (Hernandez, Yousefi, and Tufenkji 2017; Kagdada, Bhojani, and Singh 2023). While MPs have been relatively well-studied, NPs have long gone unnoticed due to detection challenges. However, recent advances in detection methods (Cai et al. 2021; Caldwell et al. 2022) have increased awareness of NP pollution. NPs have now been detected in diverse environments, including soil (Wahl et al. 2021), coastal beaches (Davranche et al. 2020), remote mountain ranges (Materić et al. 2020), lakes in uninhabited areas (Materić, Peacock, et al. 2022), and even in polar ice (Materić, Kjær, et al. 2022).

Due to their smaller size, NPs exhibit distinct physical properties and environmental interactions compared to MPs (Gigault et al. 2021). NPs are capable of penetrating cell membranes (Banerjee et al. 2022; Bhattacharya et al. 2010) and disturbing intracellular functions (Liu, Li, et al. 2021), which can lead to more severe effects. For instance, acute toxicity tests have demonstrated that only the 50 nm NPs, as opposed to 500 nm NPs and 5, 10 and 15 μm MPs, are capable of immobilising the crustacean *Daphnia magna* (Ma et al. 2016). Despite these findings, toxicological research has largely concentrated on larger MPs, primarily due to the technical challenges associated with studying the smaller NPs.

Daphnia, commonly known as water fleas, are extensively used to study the biological effects of contaminants, such as MNPs. In freshwater ecosystems, *Daphnia* are critical in linking primary producers with secondary consumers, such as fish and predatory invertebrates (Lampert 2011). These filter-feeders readily ingest MNPs (Frydkjær, Iversen, and Roslev 2017). Recent meta-analyses have reviewed numerous studies on the impact of MNPs on *Daphnia*, focusing on immobilisation and reproduction (Brehm et al. 2023; Funke, Webb, and Wolinska 2024). Both analyses found that *Daphnia* fitness is compromised in MNP-exposed treatments compared to particle-free controls. The risk of immobilisation increased with smaller particle sizes and, for some polymers, higher concentrations (Brehm et al. 2023). Additional research has shown that MNPs can reduce *Daphnia* body size (Besseling et al. 2014; Liu, Yu, et al. 2019), feeding rate (An et al. 2021; Rist, Baun, and Hartmann 2017), and alter swimming behaviour (Vaz et al. 2021), while also triggering the upregulation of genes related to stress response and detoxification (Fadare et al. 2020).

Previous studies have consistently demonstrated that within the nanoscale range, the adverse effect of plastic particles increases with decreasing size. For instance, 100 nm polystyrene beads were found to destroy the cell walls of unicellular green algae by adsorbing to their surfaces, an effect not observed with 1000 nm particles (Liu, Jiang, et al. 2019). Similarly, in mussels, the accumulation of 70 nm polystyrene beads in the digestive tract was significantly higher compared to 500 nm particles (Wang et al. 2021). In *Daphnia magna*, only polystyrene NPs with a diameter of 52 nm impacted survival, whereas larger particles (120–330 nm) did not (Mattsson et al. 2017). Another study on *D. magna* tested four sizes of polystyrene NPs (20, 40, 60 and 100 nm) and found that 20 nm particles were up to 12 times more toxic than 100 nm particles (Pochelon, Stoll, and Slaveykova 2021). Similarly, in the *Daphnia longispina* × *galeata* hybrid, the EC50 value for 50 nm polystyrene beads was approximately 20 times lower than that for 100 nm particles (de Souza Machado, Ghadernezhad, and Wolinska 2023). These findings indicate that NPs smaller than 60 nm are particularly toxic to *Daphnia*.

Despite extensive research on the effects of MNPs on individual species, their influence on species interactions remains poorly understood. MP exposure has been shown to alter the feeding behaviour of various aquatic invertebrates (An et al. 2021; Thomsen, Almeda, and Nielsen 2024), potentially destabilising trophic cascades (Pan et al. 2022). In addition to predator–prey dynamics, host–parasite interactions play a crucial role in trophic networks (Morton and Lafferty 2022). Parasites can influence host abundance (Tompkins et al. 2002), modify feeding behaviour (Mrugała, Wolinska, and Jeschke 2023), and serve as prey for non-host species (Johnson et al. 2010), thereby enhancing food web connectivity (Lafferty, Dobson, and Kuris 2006) and ecosystem resilience (Hatcher, Dick, and Dunn 2012). For instance, fungal parasites (chytrids) infecting dominant inedible filamentous cyanobacteria act as 'biological weapons' against toxic cyanobacteria blooms (Gerphagnon et al. 2015). Polystyrene beads (100 nm) have been shown to reduce chytrid infection in cyanobacteria by aggregating on cyanobacteria filaments, creating a physical barrier (Schampera et al. 2021). Similarly, polyester MP fibres (400 μm) decreased trematode infection in tadpoles (Buss, Sander, and Hua 2022). Conversely, exposure to polyethylene MP beads (100 μm) increased cercariae production by infected snails (Balsdon and Koprivnikar 2024). These observations underscore the complex and potentially unpredictable impacts of MNP pollution on host–parasite interactions (Balsdon and Koprivnikar 2024). They highlight the importance of considering the broader ecological consequences of plastic pollution, beyond the effects on individual species.

While substantial research has examined the impact of MNPs on *Daphnia* fitness (reviewed in Brehm et al. (2023), Funke, Webb, and Wolinska (2024), Samadi et al. (2022), Yin et al. (2023)), studies exploring their effects on *Daphnia*–parasite interactions remain limited. Existing research has shown that exposure to polystyrene NP beads (100 nm) significantly enhances infection rates by the yeast *Metschnikowia bicuspidata* (hereafter referred to as *Metschnikowia*) in both tested *Daphnia* taxa: the *D. longispina* × *galeata* hybrid, and *D. magna*, each represented by a single genotype (Manzi et al. 2023; Mavrianos et al. 2023). *Metschnikowia* is a highly virulent parasite that can kill its host within 2 to 3 weeks and substantially reduce host fecundity (Ebert, Lipsitch, and Mangin 2000; Manzi et al. 2020). It infects various *Daphnia* species across both temporary ponds and permanent lakes worldwide (Dallas, Holtackers, and Drake 2016; Hall et al. 2005; Wolinska et al. 2011). Investigating the effects of NP pollution on this host–parasite system could shed light on *Daphnia* population dynamics in contaminated environments. Specifically, if MNP pollution alters infection rates and subsequently affects *Daphnia* populations, it could have broader ecological implications, potentially affecting phytoplankton dynamics and fish populations (Sommer et al. 2012).

The aim of this study is to investigate whether smaller sizes of polystyrene NP beads, compared to previously tested 100 nm beads (Manzi et al. 2023; Mavrianos et al. 2023), induce more pronounced changes in the infection dynamics of the

Metschnikowia-Daphnia host–parasite system. To achieve this, *D. magna* (represented by two genotypes) was exposed to two concentrations (1 mg/L and 5 mg/L) of polystyrene NP beads of two sizes (50 nm and 100 nm), alongside parasite spores or a placebo inoculum. We assessed various measures of parasite and host fitness. Based on prior research with the same host–parasite system using 100 nm polystyrene beads (Manzi et al. 2023; Mavrianos et al. 2023), we anticipated an increase in parasite infection rate and reproduction in the presence of NPs. We expected the effects to be more pronounced with 50 nm beads compared to 100 nm beads, owing to the potentially greater stress imposed on hosts by the smaller particles.

2 | Material and Methods

2.1 | Host–Parasite System

Two genotypes of *Daphnia magna* were used as hosts in this study (clones E17:07 and NO-V-7, isolated from the UK and Norway, respectively). The *Daphnia* were reared in modified SSS medium (Saebelfeld et al. 2017), at 19°C under a 12:12 light–dark photoperiod, and fed every second day with 1 mg C/L of the green algae *Acutodesmus obliquus*. A single strain of the parasitic yeast *Metschnikowia bicuspidata* (METS_AMME_2008, isolated from Ammersee, Germany, in 2008) was used. This parasite is routinely maintained in vivo on the *D. magna* genotype E17:07. *Metschnikowia* infects its host when needle-shaped ascospores are ingested during feeding, subsequently crossing the gut barrier. The fungus then replicates in the hemolymph of the host (Figure S1) until the host dies from the infection, releasing new ascospores into the water column (Stewart Merrill and Cáceres 2018).

2.2 | Nanoplastics

The NP particles used in this study were spherical polystyrene beads with a green fluorescent marker (Micromod Partikeltechnologie GmbH, Germany, product name: micromer greenF) and a nominal diameter of 50 nm (product code: 29-00-501) or 100 nm (product code: 29-00-102). A detailed description of the 100 nm particles can be found in our previous work (Schampera et al. 2021). The stock solution (10 g/L) was stored at 4°C and then diluted in SSS-medium to prepare two test concentrations: 1 mg/L and 5 mg/L. The lower concentration of 1 mg/L was based on recent measurements of nanoplastic levels in European lakes (Materić, Peacock, et al. 2022) and represents a plausible, albeit elevated, concentration for natural environments. The higher concentration of 5 mg/L was selected to facilitate comparisons with previous studies on polystyrene 100 nm NPs and their effects on the *Daphnia-Metschnikowia* system (Manzi et al. 2023; Mavrianos et al. 2023) and to assess the potential impacts of high NP concentrations.

2.2.1 | Experimental Setup

The experiment involved two NP concentration levels (1 mg/L and 5 mg/L) and a control (0 mg/L), two NP size categories (50 nm and 100 nm), two *Daphnia* clones (E17:07 and NO-V-7), and two infection treatments ('Parasite' and 'No Parasite'). Each 'No Parasite' treatment had 10 replicates, while the 'Parasite' treatments had 20 replicates, resulting in a total of 300 experimental units. On day 1, 300 female juveniles, all born within a 24-h period, were transferred to individual experimental glass jars using glass pipettes. One juvenile that was injured during transfer was replaced on day 2. Each jar initially contained 5 mL of the designated NP treatment, a volume selected to maintain a high density of parasite spores. On day 6, parasite inoculation was conducted by homogenising 10 infected *Daphnia* from stock cultures in 2 mL of SSS-medium. The spore concentration was enumerated with a hemocytometer (Figure S1b) and a Zeiss Axioscope A1 Microscope with phase contrast (100× magnification). To achieve the target spore concentration of 3500 spores/mL, as used in previous studies (Manzi et al. 2021, 2023), the homogenate was adjusted. Experimental *Daphnia* were exposed to this spore suspension for 2 days, resulting in a final spore concentration of 3650 spores/mL. For 'No Parasite' treatments, 10 uninfected individuals from stock cultures were similarly crushed and added to control for any effects of *Daphnia* tissue in the medium. To promote infection, the light intensity was strongly reduced compared to the pre-experimental conditions (Shaw et al. 2020). *Daphnia* were fed daily with 1 mg C/L, except on the day of parasite inoculation.

On day 8, the volume in each jar was increased to 15 mL. Every fourth day, *Daphnia* were transferred to new jars with fresh medium to minimise algae, waste, and NP agglomeration. The control and NP medium were prepared 24 h prior to each transfer to allow for chemical equilibrium. Jars were inspected daily for dead individuals and juveniles, which were promptly removed. From day 14 onwards, dead *Daphnia* from the 'Parasite' treatments were transferred to Eppendorf tubes with 500 µL of jar media. Each sample was preserved with 50 µL of formaldehyde to a final concentration of 3.7% for subsequent parasite spore counts and stored at 4°C until analysis. The experiment concluded on day 43, at which point all surviving *Daphnia* were similarly collected. Three individuals that died due to handling error (one stuck on the jar side and two during transfer) were excluded from the analyses.

2.2.2 | Parasite Fitness Parameters

All fixed *Daphnia* samples were processed in a blind manner. Each sample was crushed and loaded onto a hemocytometer for counting mature spores. Mature spores were first detected on day 10 post-inoculation, aligning with previous findings (Manzi et al. 2023; Mavrianos et al. 2023). *Prevalence of infection* was recorded as a binary value: "0" for samples with no visible spores and "1" for samples with visible spores. Note that individuals dying before day 10 post-inoculation were excluded from the prevalence dataset to ensure accurate measurement of infection outcome. *Net spore output*, indicative of parasite reproduction (Manzi et al. 2020), was calculated as the total number of mature spores inside each dead *Daphnia*. This measure includes all pre-transmission deaths and counts uninfected hosts as zero, providing a comprehensive assessment of the parasite's transmission success. It reflects both the likelihood of host death

before parasite reproduction and the host's resistance to infection within each treatment.

2.2.3 | Host Fitness Parameters

Host lifespan was recorded as the number of days each *Daphnia* survived, with the maximum lifespan capped at day 43 when the experiment concluded, as some uninfected individuals were still alive. The "Infected" category included all *Daphnia* with confirmed infections and those that died before day 10 post-inoculation, the earliest day when successful infection could be confirmed. This approach ensured comparability of host fitness variables across all treatments, preventing the overestimation of host lifespan in the parasite treatment due to the exclusion of individuals that died before infection confirmation.

2.2.4 | Data Analyses

All analyses were performed using R Statistical Software, version 4.2.2. Graphical representations were created using the "ggplot2" package (v3.4.0). Analyses of variances were conducted using the "car" package (v3.1.1) with type II sums-of-squares. To manage the model's otherwise imbalanced structure, "NP concentration" and "NP size" were combined into a new variable, "Concentration-Size", with five levels: "ZERO", "LOW-SMALL", "LOW-BIG", "HIGH-SMALL", "HIGH-BIG". For models with multiple variables and their interactions, the best-fit model was selected based on the Akaike Information Criterion (AIC). Post hoc tests (Tukey HSD) were used to determine significant differences between the levels of the "Concentration-Size" variable. For binary logistic regressions, post hoc tests were conducted using the "multcomp" package (v1.4.20).

The *prevalence of infection*, defined as the proportion of infected hosts among those that survived at least until day 10 post-inoculation, was analysed using a binary logistic regression. The explanatory variables included "Clone", "Concentration-Size", and their interaction. *Net spore output*, representing the total spore yield per inoculated host (with early deaths and uninfected hosts counted as zero), was analysed similarly but using a linear model. *Host lifespan* was also analysed with a linear model, incorporating "Clone", "Concentration-Size" and "Infection" (a factor with two levels: "Infected" and "No Parasite") as exploratory variables, along with their interactions. Significant effects of "Clone" and "Infection" led to additional analyses for each combination of these two treatments, with "Concentration-Size" as the exploratory variable, resulting in four distinct analyses.

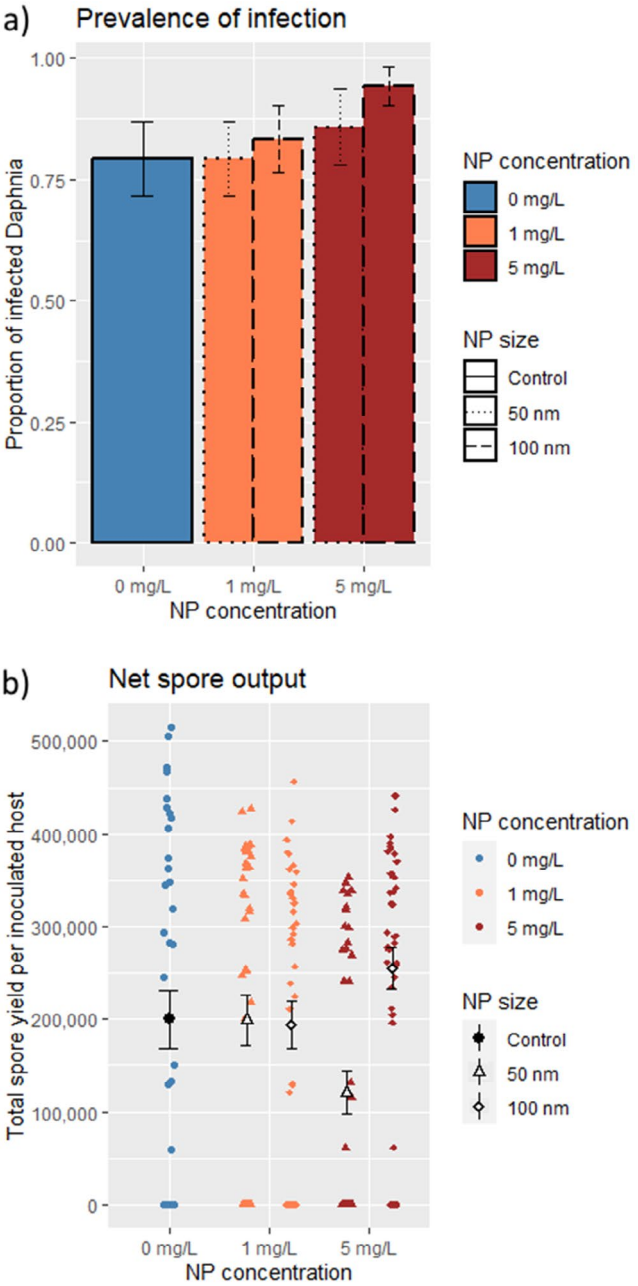

FIGURE 1 | Effects of exposure to polystyrene nanoplastics of varying sizes and concentrations on the *Daphnia magna—Metschnikowia bicuspidata* host–parasite system. The data are presented across four "Concentration-Size" experimental groups and a "zero NP" control. (a) Prevalence of infection, and (b) Net spore output. Error bars represent the standard error of the mean. Data from two *Daphnia* clones were pooled for these analyses.

3 | Results

The *prevalence of infection* was high across all treatments, averaging 81%, with no significant differences observed between *Daphnia* clones or among the "Concentration-Size" experimental groups (Figure 1a, Table 1). *Net spore output*, representing the total spore yield per inoculated host (including uninfected hosts and early deaths), showed no significant differences between the clones. However, there were notable differences among the "Concentration-Size" experimental groups (Figure 1b, Table 1). Post hoc analysis revealed that the "HIGH-SMALL" group exhibited a significantly lower *net spore output* compared to the "HIGH-BIG" group (Table S1).

Host lifespan was significantly influenced by "Clone", "Infection", and "Concentration-Size" (Figure 2, Table 2). On average, clone E17:07 survived 4.3 days longer than clone

TABLE 1 | Results of ANOVA (χ^2 test or *F*-test) assessing the effects of "Clone" and "Concentration-Size" on various fitness parameters of the parasite (*Metschnikowia bicuspidata*).

Response variable	Distribution (link function)	Group tested	Explanatory variable	Statistics (df;n)	*p*-value
Prevalence of infection	Binomial (link: logit)	All	Clone	$\chi^2_{(1;144)} = 0.031$	$p = 0.861$
		All	Concentration-Size	$\chi^2_{(4;144)} = 4.38$	$p = 0.358$
		All	Clone × Concentration-Size	$\chi^2_{(4;144)} = 2.313$	$p = 0.679$
Net spore output	Normal	All	Clone	$F_{(1;198)} = 3.601$	$p = 0.059$
		All	Concentration-Size	$F_{(4;198)} = 3.365$	$p = \mathbf{0.011}$
		All	Clone × Concentration-Size	$F_{(4;198)} = 0.491$	$p = 0.742$

Note: Significant *p*-values (≤ 0.05) are marked in bold.

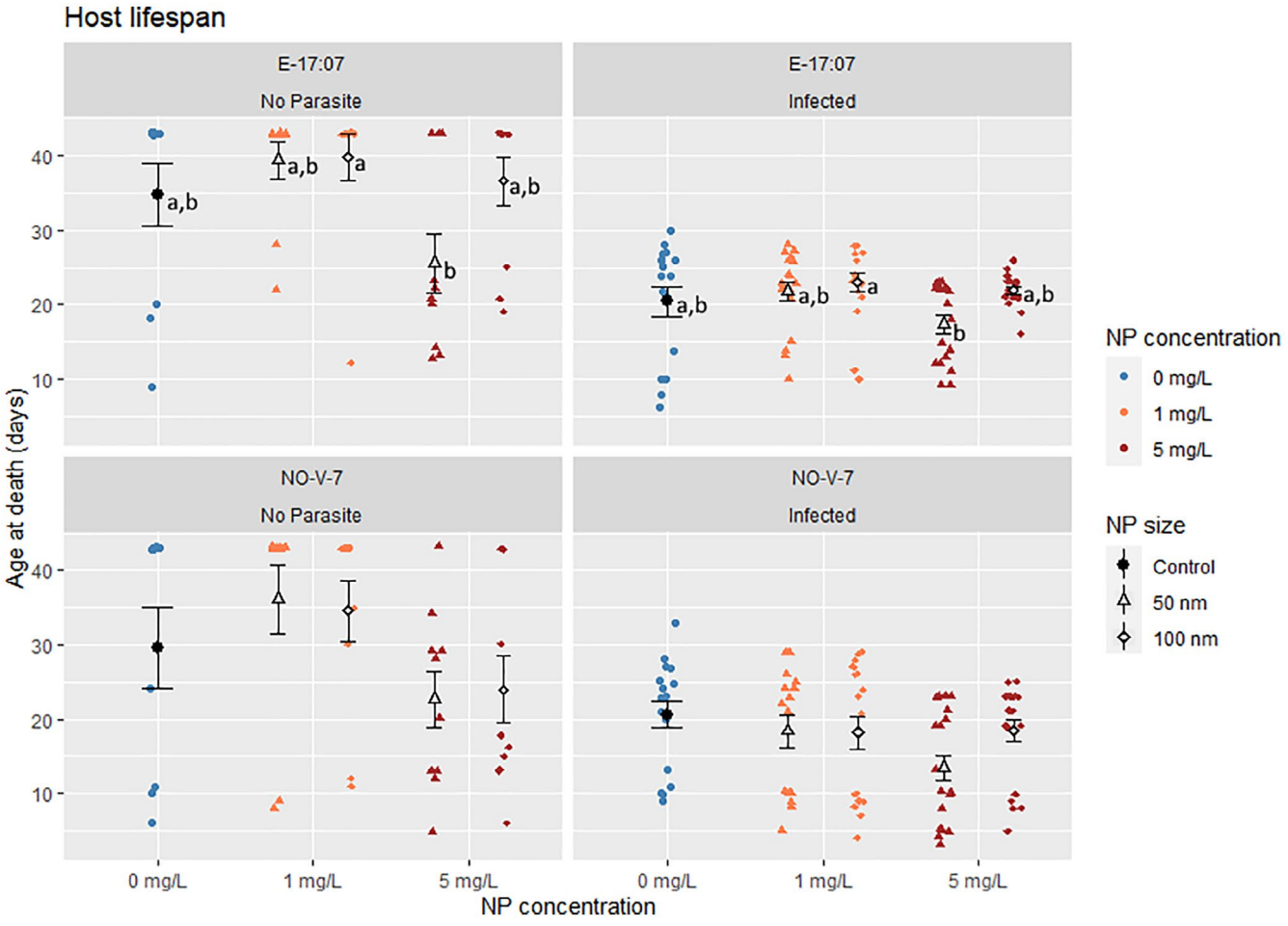

FIGURE 2 | Effects of exposure to polystyrene nanoplastics of varying sizes and concentrations on the lifespan of *Daphnia magna*. Data are shown for four "Concentration-Size" experimental groups and a "zero NP" control, separately for each *Daphnia* clone and infection treatment. Error bars represent the standard error of the mean. Statistically significant differences between groups are indicated by different letters ($p \leq 0.05$; post hoc Tukey HSD test).

NO-V-7. Infection reduced lifespan by an average of 13 days. Post hoc test revealed that the "HIGH-SMALL" group had a significantly shorter lifespan compared to all other groups (Table S2). When analysed separately for each combination of clone and infection treatment, clone E17:07 exhibited the shortest lifespan in the "HIGH-SMALL" group, regardless of whether the treatment was with or without infection (Figure 2).

4 | Discussion

This study aimed to evaluate the effects of polystyrene nanoplastics (NPs) on the *Daphnia–Metschnikowia* host–parasite system, focusing on whether smaller NPs (50 nm) have a more pronounced effect than larger NPs (100 nm) at environmentally relevant concentrations. Our findings revealed that exposure

TABLE 2 | Results of an ANOVA (χ^2 test or *F*-test) assessing the effects of "Clone", "Infection" and "Concentration-Size" on host (*Daphnia magna*) lifespan.

Response variable	Distribution (link function)	Group tested	Explanatory variable	Statistics (df;*n*)	*p*-value
Host lifespan	Normal	All	Clone	$F_{(1;275)} = 14.09$	$p < \mathbf{0.001}$
		All	Infection	$F_{(1;\,275)} = 124.081$	$p < \mathbf{0.001}$
		All	Concentration-Size	$F_{(4;\,275)} = 7.172$	$p < \mathbf{0.001}$
		All	Infection × Concentration-Size	$F_{(4;\,275)} = 2.41$	$p = \mathbf{0.049}$
		E-17 No-parasite	Concentration-Size	$F_{(4;50)} = 2.808$	$p = \mathbf{0.036}$
		NO-V-7 No-parasite	Concentration-Size	$F_{(4;49)} = 1.848$	$p = 0.137$
		E-17 Infected	Concentration-Size	$F_{(4;88)} = 2.813$	$p = \mathbf{0.03}$
		NO-V-7 Infected	Concentration-Size	$F_{(4;88)} = 2.078$	$p = 0.091$

Note: Only significant interactions are included in the table. Additionally, separate analyses were conducted on datasets split by clone and infection treatment. Significant *p*-values (≤0.05) are highlighted in bold.

to 50 nm NPs at 5 mg/L notably diminished both parasite and host fitness. In contrast, 100 nm NPs at the same concentration increased parasite fitness. Lower NP concentrations (1 mg/L) did not show significant effects on either host or parasite fitness. These results highlight the differential impacts of NP size and concentration on ecological interactions and underscore the complexity of plastic pollution's effects on freshwater ecosystems.

4.1 | Effect of NP Exposure on Parasite

Parasite fitness is intrinsically linked to the availability of susceptible hosts. In a polluted environment, the survival of hosts until the point of parasite transmission is critical for the parasite's success. Our results demonstrate that exposure to 50 nm NP particles at 5 mg/L significantly reduced *Daphnia* survival, thereby limiting parasite fitness by decreasing the number of potential hosts available for infection.

Interestingly, we did not observe a significant increase in the prevalence of infection with NP exposure, contrasting with previous studies on the *Daphnia–Metschnikowia* system (Manzi et al. 2023; Mavrianos et al. 2023). Infection rates in our control groups were notably high, with E17:07 at 73.3% and NO-V-7 at 85.7%, compared to lower rates reported in earlier studies (14% in Manzi et al. (2023), and 44% in Mavrianos et al. (2023)). This elevated baseline infection likely constrained the potential for further increases in prevalence. Although the proportion of infected individuals was slightly higher in the 100 nm NP treatment at 5 mg/L, this difference was not statistically significant (Figure 1a). The high baseline infectivity in our study might be attributed to experimental conditions, such as reduced light levels used to promote infections (Shaw et al. 2020), rather than a minor increase in spore concentration (3650 vs. 3460 spores/mL in Manzi et al. (2023)). *Metschnikowia*'s increased infectivity under darker conditions (Shaw et al. 2020) could account for some variation compared to studies conducted under standard lighting. Additionally, NP exposure might have reduced *Daphnia* feeding rates (Rist, Baun, and Hartmann 2017), potentially leading to lower ingestion of parasitic spores. This reduction in feeding could have counterbalanced any negative effects of NPs on the host immune system (Liu, Xu et al. 2021).

Net spore output (reflecting the total spore yield including early deaths and hosts that successfully defended themselves) was elevated in the 100 nm NP treatment at 5 mg/L and decreased in the 50 nm NP treatment at the same concentration (Figure 1b). The former aligns with previous studies that reported an increase in net spore output with 5 mg/L of 100 nm NPs in both *D. magna* and *D. galeata* × *longispina* taxa (Manzi et al. 2023; Mavrianos et al. 2023). The observed increase in spore output with 100 nm NPs contrasts sharply with the reduction seen with 50 nm NPs, highlighting the differential effects of NP size on parasite dynamics. This suggests that smaller NPs may be more toxic to both the host and parasite, leading to reduced parasite fitness, while larger NPs may enhance parasite reproduction.

4.2 | Effect of NP Exposure on Host

As anticipated, infection with *Metschnikowia* significantly reduced host lifespan (Manzi et al. 2020). However, our study revealed that also NP exposure had a marked impact on host survival, even at the relatively low concentration of 5 mg/L, compared to other studies. This effect was particularly pronounced with 50 nm NPs, supporting the hypothesis that smaller particles are more toxic. These findings align with previous research indicating that NPs smaller than 60 nm are particularly toxic to *Daphnia* (de Souza Machado, Ghadernezhad, and Wolinska 2023; Pochelon, Stoll, and Slaveykova 2021). The increased toxicity of smaller NPs can be attributed to their enhanced ability to penetrate cellular membranes compared to larger particles. This facilitates more profound disruptions at the cellular level, including alterations in protein structures, interference with essential cellular processes, and damage to critical

organelles such as mitochondria (Fu et al. 2018; Hollóczki and Gehrke 2019; Liu, Li, et al. 2021; Trevisan, Uzochukwu, and Di Giulio 2020). Such disruptions can impair metabolic functions, reduce energy availability, and ultimately lead to a shortened lifespan, highlighting the severe impact of smaller NPs on host fitness.

4.3 | Applied Concentrations

Our findings indicate that the toxicity of NPs, particularly smaller particles, varies with concentration, suggesting a potential threshold between 1 and 5 mg/L. The lower concentration tested (1 mg/L) was informed by findings from Materić, Kjær, et al. (2022), which reported polyethylene NP concentrations in Swedish freshwater systems averaging 563 μg/L, with a peak of 700 μg/L. While polystyrene NPs were not detected in these habitats, concentrations of 242 μg/L have been reported for polystyrene NPs in a periurban river in the UK (Sullivan et al. 2020). Although these levels exceed earlier estimates of environmental NP concentrations (Liu et al. 2020), they may still under-represent actual conditions, particularly in densely populated areas (Kunz et al. 2023). Given Sweden's low population density and the relative isolation of study sites from urban centres (Materić, Kjær, et al. 2022), the higher concentration tested (5 mg/L), while unlikely in current environments, could reflect potential future scenarios in heavily polluted regions. Furthermore, as microplastics degrade into nanoplastics, NP concentrations—measured by particle count per volume—could increase by up to 10^{14} times compared to current microplastic levels (Besseling et al. 2019). This dramatic increase highlights the growing concern over NP pollution, especially as detection technologies advance. Materić, Kjær, et al. (2022) found NPs in every stream and lake sampled within a forested catchment in Sweden, emphasising the widespread nature of NP contamination.

5 | Outlook and Conclusions

Our study offers important insights into the impact of nanoplastic (NP) pollution, specifically polystyrene bead particles, on host-parasite interactions between *Daphnia magna* and *Metschnikowia bicuspidata*. We observed that exposure to 5 mg/L of 50 nm NPs reduced parasite fitness, while 100 nm NPs at the same concentration actually enhanced it. These findings highlight the nuanced and size-dependent effects of NP pollution on ecological interactions.

Future research should explore the multi-generational impacts of NPs. Most current studies, including ours, focus on single-generation effects (reviewed in Funke, Webb, and Wolinska (2024)). However, there is growing evidence that NP exposure can adversely affect subsequent generations of *Daphnia* (Liu et al. 2020), including those not directly exposed (Nogueira et al. 2022). Understanding these long-term effects is essential, as NP pollution persists in the environment and could influence entire populations and ecosystems over time. Additionally, it is important to investigate whether prolonged NP exposure affects the transgenerational plasticity of parasites, similar to patterns observed with other stressors such as fungicides (Cuco et al. 2020) and temperature changes (Sun et al. 2022). Another key area to study would be the impact of NPs on host body growth, given that spore production is proportional to the size of *Daphnia*'s body cavity (Hesse et al. 2012). Reduced body size resulting from NP exposure (Besseling et al. 2014; Liu, Xu et al. 2021) could contribute to the lower spore production observed, potentially limiting parasite development and further explaining the variations in infection dynamics noted in our study.

While our findings offer a focused view on the effects of NPs in the *Daphnia-Metschnikowia* system, extending research to other common *Daphnia* parasites is crucial. Investigating how different NP sizes influence a broader range of host-parasite interactions will help determine whether our results are specific to *Metschnikowia* or indicative of broader trends in how NPs alter *Daphnia*'s susceptibility to pathogens.

Finally, our results suggest that NP pollution could have significant ecosystem-level consequences. Changes in *Daphnia* infection rates and overall fitness might lead to cascading effects in freshwater ecosystems. Given that *Daphnia* are crucial components of aquatic food webs, alterations in their health and population dynamics could impact primary producers like phytoplankton, as well as higher trophic levels such as fish. Exploring these broader ecological consequences will be key to predicting the full impact of NP pollution on aquatic ecosystems and developing effective management strategies.

Author Contributions

L.W., J.W., F.M.: conceptualization; L.W., F.M.: developing methods; L.W., J.W.: conducting the research; L.W.: data analysis; L.W.: preparation of figures and tables; L.W., J.W., F.M.: data interpretation; J.W., L.W., F.M.: writing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting this study can be found at: https://zenodo.org/records/13273333.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.