

Nanoplastics modulate the outcome of a zooplankton–microparasite interaction

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Abstract

1. The accumulation and degradation of plastic waste in freshwater bodies poses a threat to aquatic biota. Microplastics (<5 mm) can transfer upwards in food chains and have been shown to induce deleterious effects on important players of freshwater ecosystems, including zooplankton.
2. A smaller category of microplastic particles, the so-called nanoplastics (≤100 nm) raise special concern due to their ability to act at sub-cellular and molecular levels. Despite growing knowledge of their effects on physiological traits of individual species, the way they affect interactions between species remains largely unexplored.
3. We studied the effects of nanoplastics on host–parasite interactions by exposing the zooplankton host *Daphnia galeata* × *longispina* to the parasitic yeast *Metschnikowia bicuspidata* without plastic and at two different concentrations of polystyrene nanoplastic beads (100 nm): 5 and 20 mg/L.
4. Both concentrations of nanoplastics increased the proportion of infected hosts; at the higher concentration, however, elevated rates of host mortality and impaired spore production cancelled out the parasite's advantage. Consequently, parasite success was greatest at the lower level of nanoplastic exposure.
5. Infection by *Metschnikowia* greatly reduced host lifespan and total offspring production (regardless of nanoplastic exposure), but only decreased the proportion of successfully reproducing hosts when *Daphnia* were additionally exposed to nanoplastics. Nanoplastics alone did not cause such a reduction in host fitness parameters: instead, the lower concentration increased lifetime offspring production by about 50%, suggesting hormesis.
6. Given that parasitism is a ubiquitous lifestyle in nature and that parasites can play important roles in the shaping and functioning of ecosystems, these results highlight the importance of including interactions between host and parasite species as alternative ecotoxicological endpoints to better assess the ecological consequences of plastic pollution.

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KEYWORDS

Daphnia, disease, hormesis, host–parasite, *Metschnikowia*

1 | INTRODUCTION

The contamination of waters by microplastics is receiving global attention. Plastics released in the environment break down into smaller pieces, leading to microplastics (with a size <5 mm) or to an even smaller fraction called nanoplastics (particles of a size ≤ 100 nm, referred to as NPs), which are then directly ingested by aquatic biota and can elicit an array of ecotoxicological effects (Wang et al., 2019). For instance, NPs raise special toxicological concerns due to their unique ability to penetrate lipid cell membranes and their potential to alter cellular functions (Liu, Xu, et al., 2021; Salvati et al., 2011; Trevisan et al., 2020), including gene expression (Liu, Cai, et al., 2020; Liu, Jiao, et al., 2020; Liu, Li, et al., 2021). NPs have been shown to cause oxidative stress and inhibit photosynthetic growth of phytoplankton (Bergami et al., 2017; Bhattacharya et al., 2010; Wan et al., 2015) and to reduce body size, fecundity, and survival of zooplankton (Cui et al., 2017; Kelpsiene et al., 2020; Lin et al., 2019).

Most studies have investigated the effects of microplastics and NPs at the species level. However, ecological interactions between species (e.g., competition, predation, parasitism) might represent additional important endpoints when inferring the consequences of plastic pollution on ecosystem functioning (Segner, 2011). For instance, exposure to microplastics or NPs can cause behavioural changes affecting the outcome of predator–prey interactions (de Sá et al., 2015; Mattsson et al., 2017; Seuront, 2018). Recent evidence suggests that host–parasite interactions can also be affected: a parasitic chytrid fungus displayed lower success infecting their phytoplankton hosts when exposed to NPs (Schampera et al., 2021), and similar results were found for trematodes infecting amphibians, when exposed to microplastics (Buss et al., 2021). Thus, it seems that microplastics and NPs have the potential to modulate biotic interactions in aquatic systems, a perspective that has been largely overlooked so far, but requires further attention. Parasitism represents a ubiquitous ecological interaction and the most widespread consumer lifestyle in nature (Lafferty et al., 2008). Host–parasite interactions also mediate a significant part of the trophic links in food webs (Amundsen et al., 2009) and can act as important drivers of co-evolution and diversification (Combes & Daniel, 2020). It is hence important to extend our knowledge on how emerging pollutants like NPs affect this ecological interaction.

According to the classic concept of the disease triangle, the outcome of an infection is determined by the reciprocal interaction between hosts, parasites, and their common external environment, in a variety of direct and indirect ways (McNew, 1960). First, environmental parameters such as pollution can suppress host immune defences and increase the host's susceptibility to infection. Unlike NPs, other forms of environmental pollution such as pesticides or salinisation are well studied in this respect. These have been shown to

alter host susceptibility to infection in amphibians (Buss et al., 2020; Gendron et al., 2003), fish (Fazio et al., 2013; Kreutz et al., 2010), or crustaceans (Coors et al., 2008; Merrick & Searle, 2019). Second, pollutants such as fungicides or bactericides may exhibit direct toxicity towards parasites, with the potential to suppress infection (Cuco et al., 2017; Ortiz-Cañavate et al., 2019) and increase host survival (Stockwell et al., 2015). Lastly, suboptimal host conditions might result in decreased host densities, subsequently reducing opportunities for parasite transmission (Lafferty & Holt, 2003).

The planktonic crustacean *Daphnia* plays a key role in the trophic structure of aquatic food webs, being an important grazer of phytoplankton and serving as main prey for planktivorous fish (Lampert, 2011). Their high amenability to experimentation and rapid responses to environmental changes have contributed to elevate *Daphnia* as a widespread model in physiology, ecology, toxicology, and evolutionary biology (Miner et al., 2012; Reynolds, 2011; Seda & Petrusek, 2011). In their natural environment, *Daphnia* are frequently attacked by a number of microparasites (Ebert, 2005; Green, 1974; Wolinska et al., 2009) and within the last 20 years, *Daphnia* and their parasites have been involved in a number of ecological, evolutionary, and epidemiological questions (Decaestecker et al., 2007; Duffy et al., 2012; Ebert, 2008; Hall et al., 2007). Thus, using *Daphnia* and their wide array of microparasites as a model system may bring more insights into the consequences of plastic pollution on disease outcome. Such microparasites include the yeast *Metschnikowia bicuspidata* (hereafter referred to as *Metschnikowia*), a virulent parasite commonly infecting lake and pond *Daphnia* populations (Stirnadel & Ebert, 1997; Wolinska et al., 2011). As *Metschnikowia* reduces both the lifespan and fecundity of its *Daphnia* host (Cáceres et al., 2006; Hesse et al., 2012; Lohr et al., 2010), epidemics can have consequences for the entire food-web structure, such as reducing host density to levels where top-down control of phytoplankton is inhibited (Duffy, 2007) or increasing the host's vulnerability to fish predation (Duffy et al., 2005).

In spite of numerous experimental studies exploring the consequences of microplastic and NP exposure on *Daphnia* fitness (reviewed in Samadi et al., 2022) and a fairly recent surge of studies addressing the implications of NP contamination in the context of parasitic infections (Jimenez-Guri et al., 2021; Li et al., 2020; Schampera et al., 2021), it remains unknown whether, and if so how exposure to NPs affects infection outcomes in the popular *Daphnia*–microparasite system. Here, we report on the simultaneous exposure of *Daphnia galeata* \times *longispina* hybrids to a common parasitic yeast (*Metschnikowia*) and to NPs at two concentrations (5 and 20 mg/L). We evaluated the influence of NP exposure on the following determinants of parasite fitness: the proportion of parasite-inoculated hosts that survived long enough for the parasite to reproduce, the proportion of surviving hosts that became successfully infected, and

the number of parasite spores produced per successfully infected host. In addition, a combined metric was used to estimate the number of parasite transmission stages produced per encountered host, designated as the net spore output. Overall, we expected impaired success of infection under NP exposure, given that an upregulation of haemocytes was previously described in *Daphnia* exposed to plastic-contaminated media (Sadler et al., 2019). We also monitored how exposure to two stressors (NPs and parasites) could affect parameters of *Daphnia* fitness, expecting reduced lifespan and fecundity under the separate influence of both stressors, as well as synergistic negative effects under simultaneous exposure.

2 | METHODS

2.1 | Study organisms

2.1.1 | Parasite

The *Metschnikowia* parasite strain used in the experiment (METS_AMME_2008) was isolated from a *D. galeata* × *longispina* hybrid in 2008 from Ammersee, Germany. Parasite stock cultures were maintained on the host species *Daphnia magna* (genotype E17:07). *Metschnikowia* is a generalist parasite that naturally infects various *Daphnia* species (Stirnadel & Ebert, 1997) and infection of *D. magna* provides a high number of parasite spores, due to its large body size (Hesse et al., 2012). In nature, *Daphnia* become infected by ingesting *Metschnikowia* spores. The needle-shaped ascospores pierce through the gut epithelium, from which they can reach the haemolymph in the body cavity. Once there, the fungal development cycle triggers, and parasite multiplication leads to the accumulation of elongated asci throughout the body cavity, which ultimately leads to host death. Decomposition or mechanical damage to the carapace is necessary for mature ascospores to be released back into the water and infect other hosts (Green, 1974; Metschnikoff, 1884; Stewart Merrill & Cáceres, 2018).

2.1.2 | Host

A single genotype of the hybrid *D. galeata* × *longispina* was used (genotype AMME_51), originating from the same lake and location as the parasite and previously used for experimental infection assays with *Metschnikowia* (Manzi et al., 2020, 2022). Clonal cultures were kept in synthetic SSS-medium (Saebelfeld et al., 2017) at 19°C under a 12:12 hr light–dark photoperiod. *Daphnia* were fed three times a week (every second or third day) with the green alga *Acutodesmus obliquus* (maintained as continuous cultures at 19°C, under constant light). To obtain a large number of synchronised juveniles, feeding intensity was first set to 1 mg C/L of *A. obliquus* daily; the correlation between optical density (680nm) and carbon content was previously established and used to determine the appropriate feeding volumes. Ten glass jars filled with 200mL medium, each containing 15–20

adult *Daphnia*, were kept under these conditions for two generations. Every second day, juveniles were counted and removed until the onset of the experiment.

2.2 | Nanoplastic media

Spherical polystyrene particles with a nominal diameter of 100nm and tagged with fluorescent markers were purchased as a suspension in water with a concentration of 10 g/L (Micromod Partikeltechnologie GmbH, Germany, product code: 29-00-102, product name: micromer®-greenF). Detailed characterisation of these particles is provided in Schampera et al. (2021). Two concentrations of NPs were prepared in the SSS-medium as NP exposure treatments: 5 mg/L (i.e., low-NP) and 20mg/L (i.e., high-NP). These concentrations have been commonly applied in *Daphnia* studies investigating the toxicity of polystyrene NPs (e.g., Cui et al., 2017; Lin et al., 2019; Pochelon et al., 2021), as well as recent experiments using microplastics or NPs on freshwater host–parasite systems (Buss et al., 2021; Schampera et al., 2021). The zero-NP treatment was prepared without any addition of NPs. To allow for chemical equilibrium of the mixtures, the resulting media were incubated in experimental jars at 19°C in darkness for 24 hr, prior to transferring *Daphnia*.

2.3 | Experimental design and procedures

Daphnia female juveniles born under 48 hr from synchronised mothers were collected and used as experimental individuals. On experimental day 1, juveniles were randomly distributed across 150 individual glass jars filled with 5 mL of SSS-medium, following a full factorial design: 2 inoculation levels (inoculated with the parasite *Metschnikowia* or with a technical control) × 3 NP exposure levels (0, 5 and 20 mg/L) × 25 replicates.

On day 3 of the experiment, individual jars of the parasite treatment were inoculated with 1,000 *Metschnikowia* spores/mL. To do so, infected *D. magna* (genotype E17:07) from the parasite stock cultures were crushed in SSS-medium to obtain a suspension of spores. The spore concentration was determined by loading 10µL of the resulting suspension on an improved Neubauer counting chamber, and inoculation volume was calculated to achieve the desired final spore concentration. To account for any confounding effect in the preparation of the spore suspension (e.g., the presence of *Daphnia* tissue or bacteria), a technical control was prepared using uninfected *D. magna* (E17:07) and distributed to the control treatments. Hosts were infected as juveniles, as elevated success of infection (≥60%) was previously observed with *Daphnia* of a similar age (Cuco et al., 2017; Manzi et al., 2020; Paraskevopoulou et al., 2022).

Throughout the experiment (except for the parasite inoculation day) *Daphnia* were fed daily with 0.5 mg C/L of *A. obliquus*. On day 4, *Daphnia* were transferred to fresh medium, and the volume was increased from 5 to 10 mL per jar. From this point onwards,

Daphnia were transferred to new medium every 4 days. *Daphnia* were checked daily for mortality and offspring production (juveniles were counted and removed from the experimental jars). To later determine the proportion of successful infections and the number of parasite spores produced per infected individual, dead *Daphnia* from the parasite-inoculated treatments were preserved from day 11 onwards (i.e., starting from day 8 post-inoculation, as mature spores cannot be observed earlier; Stewart Merrill & Cáceres, 2018). The samples were fixed in 3.7% formaldehyde and stored at 4°C. *Daphnia* from the parasite-inoculated treatment that survived until the end of the experiment were also preserved. The identities of all preserved samples were blinded before analysis. The experiment was terminated on day 29, as no further mortality was observed for five consecutive days in any of the treatments.

2.4 | Data analysis

Data was analysed using R version 4.0.3 (R Core Team, 2020). Graphical outputs were produced using the *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2023) packages. Analyses of variances (ANOVAS) were performed with the *car* package (Fox et al., 2007), using type II sums-of-squares. ANOVA assumptions of normality and homoscedasticity of the residuals were verified by visual inspection of quantile-quantile plots and residuals against fitted values, respectively. To determine the proportion of variance explained by each factor, their respective sum of squares was divided by the total sum of squares (including those of their interaction and the residuals). Post hoc tests were performed to identify significant differences across treatments, using Tukey HSD test. *Daphnia* that died before day 3 (parasite inoculation day) were removed from all analyses, as these could not be assigned to either inoculation treatment (three individuals). Individuals that were lost due to handling errors were also removed (five individuals). This resulted in 72 (parasite-inoculated) and 70 (control) analysed individuals, respectively.

2.4.1 | Parasite fitness

Host viability (the proportion of inoculated hosts that survived until at least day 9 post-inoculation, when ascospores are usually first observed), the proportion of surviving hosts that became successfully infected, and parasite reproduction (spore yield upon host death per successfully infected host) were used to characterise parasite fitness. All parasite-inoculated individuals were included in the analysis for host viability; for the proportion of infected hosts, early deaths (i.e., individuals that died before day 9 post-inoculation) were excluded, and for parasite reproduction only successfully infected individuals were considered (see Figure S1 for the number of individuals included into each specific analysis). These three components were combined to estimate the parasite's net spore output (following the method introduced in Manzi et al., 2021). The net spore output was recorded as the number of spores produced per inoculated

host (similarly to parasite reproduction, except that non-viable or uninfected hosts were included as zero-values). This was done to account for the probability of hosts dying prior to the production of mature spores or resisting infection in a given treatment, which would reduce the average reproductive success of the parasite. Host viability and the proportion of infected hosts were analysed using binomial logistic regression with *NP concentration* as the explanatory variable. Parasite reproduction and net spore output were analysed using a general linear model with *NP concentration* as a fixed factor.

2.4.2 | Host fitness

Host lifespan, the proportion of individuals that reproduced at least once, and host fecundity (i.e., total number of juveniles among individuals that reproduced) were measured to characterise host fitness. Host lifespan was analysed using a linear model with *NP concentration* and *Infection* as fixed factors, including their interaction. In addition, host survival was analysed using Cox's proportional hazards regression, with *NP concentration* and *Infection* as covariates (see Figure S2, Table S1). Lifespan values were censored on the 29th day, representing the final day of the experiment during which some individuals were still alive. The proportion of individuals that reproduced was analysed using a binomial logistic regression with *NP concentration*, *Infection*, and their interaction as explanatory variables. Host fecundity was analysed using a linear model with *NP concentration* and *Infection* as fixed factors, including their interaction. Standardised mean differences (SMDs) were computed to estimate effect sizes, using the *SingleCaseES* package (Pustejovsky et al., 2022; Taddei et al., 2021). For each response variable, the low and high concentration groups were compared with the zero-NP level, separately for each infection treatment. This was done to evaluate the respective effect size of *NP concentration* on host fitness within both control and infected populations, as potentially large discrepancies in the proportion of variance explained by each factor (*Infection* vs. *NP concentration*) could overshadow the magnitude of NP effects. To analyse host fitness parameters, early deaths from the parasite-inoculated treatment (i.e., individuals whose infection symptoms could not be visually confirmed before death) were pooled together with successfully infected *Daphnia*, to ensure the comparability of host fitness variables across the parasite-inoculated and control treatments. Otherwise, excluding early deaths would have resulted in an overestimation of host lifespan in the parasite-inoculated treatments (for comparison, see right panel of Figures 2 and S3).

3 | RESULTS

3.1 | Parasite fitness

Host viability (i.e., the proportion of hosts that survived at least 9 days post-inoculation, giving the parasite a chance to reproduce) decreased with increasing NP concentrations: respectively 100%,

92%, and 74% of inoculated individuals survived until day 9 post-inoculation from the zero-NP, low-NP, and high-NP treatments (Figure 1a, Figure S1; $\chi^2_{[2;69]} = 10.06$, $p < 0.001$, Table 1). Among these viable hosts, the proportion of successfully infected individuals increased under NP exposure: respectively 44%, 73%, and 82% of viable hosts became infected in the zero-NP, low-NP, and high-NP treatments (Figure 1b; $\chi^2_{[2;61]} = 7.67$, $p = 0.022$, Table 1). By contrast, parasite reproduction was about three times lower in the high-NP treatment, compared to the low-NP and zero-NP treatments (Figure 1c; NP concentration explained 39% of the variance, $F_{[2;38]} = 12.14$, $p < 0.001$, Table 1). This was also true when parasite reproduction was corrected by the host's lifespan post-inoculation (see Figure S4). While the net spore output per inoculated host was lowest in the high-NP treatment (Figure 1d; $F_{[2;69]} = 6.35$, $p = 0.003$, Table 1), it did not differ significantly from the zero-NP treatment ($p = 0.404$; see Table S2).

3.2 | Host fitness

On average, *Daphnia* lifespan was reduced by about 12 days in the parasite-inoculated treatment, across all NP concentrations (Figure 2a; Infection explained 52% of the variance, $F_{[1;113]} = 139.56$, $p < 0.001$, Table 2). *Daphnia* exposed to high-NP concentrations died about 5 days earlier than *Daphnia* exposed to low-NP concentrations, across both infection treatments (Figure 2a; NP concentration explained 6% of the variance, $F_{[2;113]} = 7.63$, $p < 0.001$, Table 2). Within the control group, no mortality was observed under low-NP exposure, while individuals from the zero-NP treatment started dying from day 17 onwards (Figure S2). The Cox regression analysis

showed that both Infection and NP concentration significantly influenced *Daphnia* survival (Figure S2, Table S1; $p < 0.001$). Notably, individuals from the Infected treatments experienced a 7-fold increase in the probability of dying throughout the experiment (Table S1, $\exp[\text{coef}] = 7.026$). In the uninfected group, there was a medium effect size of low-NP exposure on host lifespan (SMD = 0.54; Table 3). The SMD value for high-NP concentration was 4.34 in the infected group, which is indicative of a large effect size (Andrade, 2020), despite the post hoc test indicating no significant difference for this contrast ($p = 0.43$; Table S2).

The proportion of individuals that reproduced was lower for infected *Daphnia*, but only under low- and high-NP treatments (Figure 2b; significant NP concentration \times Infection interaction, $\chi^2_{[2;113]} = 6.14$, $p = 0.046$, Table 2). All control individuals, but only 67% of infected individuals reproduced under low-NP exposure; similarly, this proportion was reduced from 79% to 55% in the high-NP treatment. Effect sizes on this parameter were mostly small, although there was a medium effect size of high-NP exposure in the infected treatment (SMD = -0.61; Table 3).

Host fecundity was strongly reduced in the infection treatment: across all NP concentrations, control individuals produced on average 10.4 (95% confidence interval [CI] ± 0.09) offspring, whereas infected ones produced on average 2.4 (95% CI ± 0.28) (Figure 2c; Infection explained 61% of the variance, $F_{[1;87]} = 171.14$, $p < 0.001$, Table 2). There was also a significant effect of the NP concentration \times Infection interaction on host fecundity ($F_{[2;87]} = 4.52$, $p = 0.014$, Table 2): within the control group, *Daphnia* produced a higher number of offspring under low-NP exposure, with an average of 12.5 (95% CI ± 1.09) offspring, against 8.6 (95% CI ± 1.7) and 9.9 (95% CI ± 1.45) in the zero- and high-NP treatments, respectively (Tukey

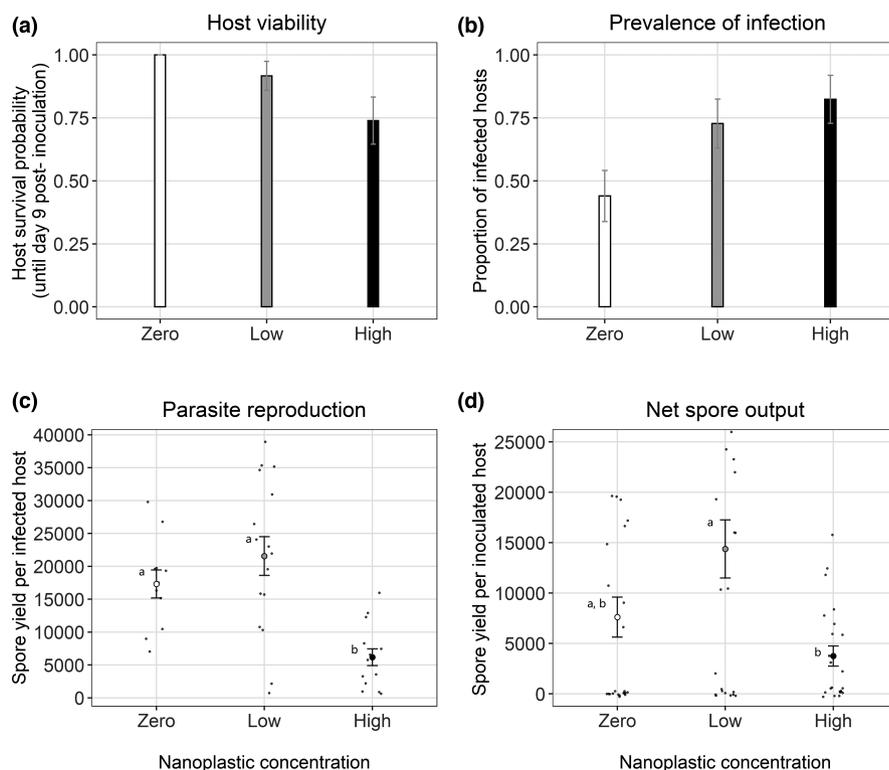


FIGURE 1 Parasite fitness traits at three NP concentrations: zero (0 mg/L), low (5 mg/L), and high (20 mg/L). (a) The proportion of inoculated *Daphnia* individuals that survived until day 9 post-inoculation (i.e., the earliest day when parasite spores were detected). (b) The proportion of surviving *Daphnia* individuals that were successfully infected by the parasite. (c) Spore yield per successfully infected host. (d) Net spore output per inoculated host. Error bars represent the standard error of the mean. Different groups of letters denote statistical significance (< 0.05), as evidenced by Tukey HSD test.

TABLE 1 ANOVA (F-test or χ^2 test) testing for fixed effects of nanoplastic (NP) concentration on parameters influencing parasite success (host viability, proportion of infected hosts, parasite reproduction, and net spore output).

Response variable	Distribution (link function)	Explanatory variables	Statistic (degrees of freedom)	p-value	% Variance explained
Host viability	Binomial (link: logit)	NP concentration	$\chi^2_{(2;69)} = 10.06$	<0.001	11.58
Proportion of infected hosts	Binomial (link: logit)	NP concentration	$\chi^2_{(2;61)} = 7.67$	0.022	11.81
Parasite reproduction	Normal	NP concentration	$F_{(2;38)} = 12.14$	<0.001	38.98
Net spore output	Normal	NP concentration	$F_{(2;69)} = 6.35$	0.003	15.55

Note: Only data from the parasite-inoculated treatment was included here. Significant p-values (<0.05) are highlighted in bold.

HSD test; Figure 2c, Table S2). There was a large effect size of low-NP exposure on host fecundity in the uninfected group, with an SMD value of 0.97 (Table 3).

4 | DISCUSSION

Even though the effects of NPs on individual species have been thoroughly investigated, their effects on ecological interactions between species remain largely unknown. Considering the important role played by parasites in ecosystem structure and functioning (Amundsen et al., 2009), parasitism is one such interaction that seems crucial to include when examining the ecological consequences of plastic pollution.

4.1 | Parasite fitness

In the *Daphnia*–*Metschnikowia* system, three conditions need to be met to grant parasite transmission upon encounter with a susceptible host: (1) the parasite needs to enter and establish within the host (i.e., reach the haemolymph via the gut); (2) the host has to live long enough for the parasite to complete its infection cycle (production of mature ascus generally takes >8 days in this system); and (3) the parasite must successfully develop from its initial infectious stage to its final transmittable stages. Respectively described as *infection*, *pre-transmission mortality*, and *parasite development* in a model by Stewart Merrill and Johnson (2020), these three conditions may be differentially affected by exposure to NPs, potentially shifting parasite transmission and disease dynamics.

First, entrance and colonisation of the host are crucial for a successful infection. We observed a higher proportion of individuals becoming successfully infected in both NP treatments, in comparison to controls. One possible mechanism for increased infection risk lies in host behavioural changes that affect the chances of encountering a parasite. For instance, exposure to copper is known to increase filtering rate, and thus parasite uptake in *Daphnia* (Civitello et al., 2012). Such behavioural changes have been observed on *D. magna* exposed to polystyrene microplastics, including alterations of their swimming activity (i.e. increased distance and velocity) and phototactic behaviour, both of which could affect encounter with parasite spores in the sediment or water column (De Felice

et al., 2019). Interestingly, ingestion of microplastics can obstruct the digestive tract of *Daphnia* (An et al., 2021; De Felice et al., 2019), probably leading to nutritional stress (Liu et al., 2022). While filtering rates were shown to be boosted in nutritionally stressed individuals (Lampert & Brendelberger, 1996), resulting in enhanced exposure to parasite spores (Dallas et al., 2016), previous findings rather suggest that food acquisition and foraging rates in *Daphnia* would be reduced under NP exposure (Rist et al., 2017). Alternatively, accumulation of nanoparticles in the gut is known to cause inflammatory responses (Pirsaheb et al., 2020; Silva et al., 2021), which could facilitate the passage of spores through the damaged epithelium. While the host seemed more prone to develop infection when exposed to NPs, this was counter-balanced by two offsetting effects: a strong decrease in spore production (exclusively at 20 mg/L), as well as elevated levels of pre-transmission mortality, which occurred at both concentrations.

Second, decreased longevity of the host directly correlates with the transmission of *Metschnikowia*. If the host dies before the parasite can produce its final ascus stage, this effectively leads to infection failure and a null spore output. Considered a major component of host competence (the ability of a host to transmit a given parasite, or a parasite's within-host R_0 ; Stewart Merrill & Johnson, 2020), this potential barrier to parasite transmission has been referred to as pre-transmission mortality, a phenomenon that can occur shortly after exposure (LaFonte & Johnson, 2013) or at some point between infection and transmission (Nemeth et al., 2011). Both instances probably contributed to the reduction in host viability (our equivalent for pre-transmission mortality) observed in the treatments with combined exposure to NPs and the parasite. If the host does survive beyond that point and the parasite starts to multiply, its reproductive output will be positively correlated with host body size (Hesse et al., 2012), and thus likely to increase with host age. To determine whether the strong effects of NPs on spore yield were due to altered lifespan—as opposed to intrinsic processes influencing parasite multiplication—we additionally assessed spore yield as a function of host lifespan post-inoculation. Although the reduced spore yield observed in the high NP treatment was independent of reduced lifespan, suggesting less efficient multiplication of the parasite at a concentration of 20 mg/L, the expected pattern of a higher spore yield with longer host lifespan was found in the low NP treatment (which led to the highest reproductive output for the parasite). Hence, negative effects of NPs on host lifespan may impact parasite performance

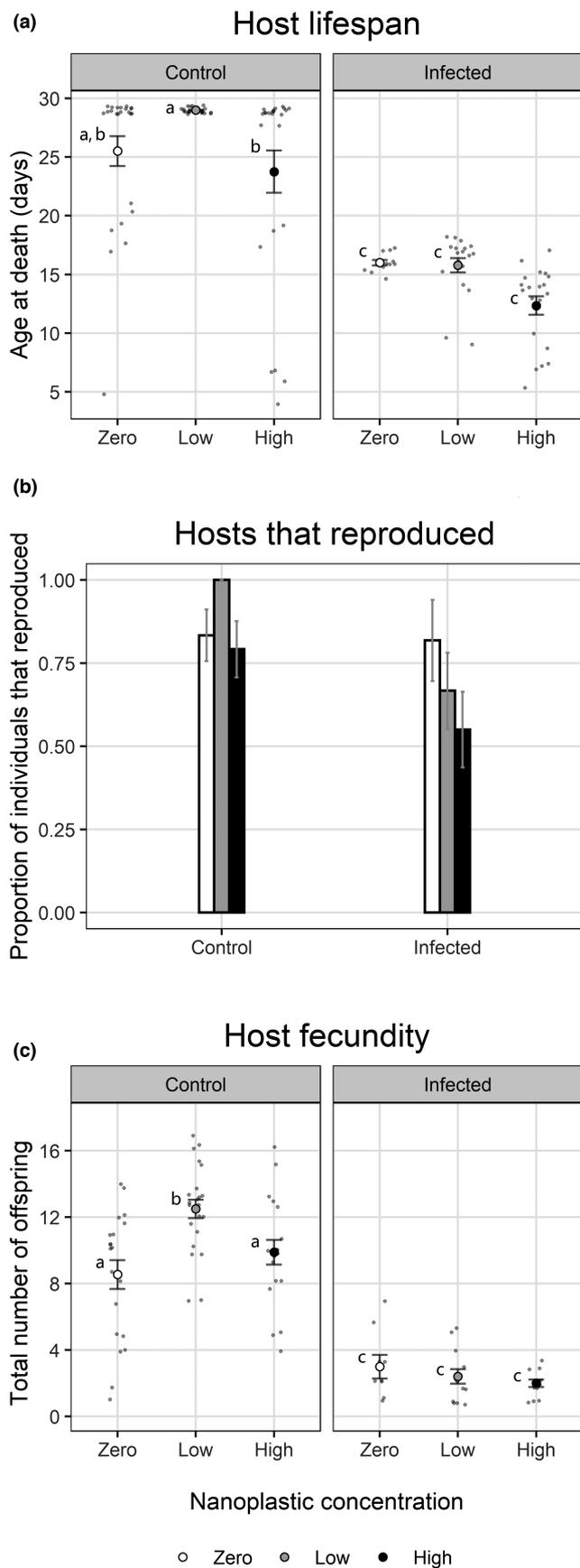


FIGURE 2 Host fitness traits in the Control and parasite-inoculated (*Infected*) groups at three NP concentrations: zero (0 mg/L), low (5 mg/L), and high (20 mg/L). (a) Age at death of individual *Daphnia*. (b) Proportion of *Daphnia* that reproduced. (c) Total number of offspring produced in the lifetime of a *Daphnia* individual. The *Infected* group depicts successfully infected individuals and those that died before day 9 post-inoculation (as infection status could not be reliably assessed before that day). Error bars represent the standard error of the mean. Different groups of letters denote statistical significance (<0.05), as evidenced by Tukey HSD test.

Lastly, provided that completion of the parasite's life cycle is ensured, environmental conditions such as food availability or temperature may still modulate the parasite's reproductive output in a quantitative manner (Manzi et al., 2020; Pulkkinen & Ebert, 2004). Here, the parasite's spore yield was strongly reduced at the higher NP concentration. A plausible explanation would be that suboptimal parasite growth indirectly results from the impairment of resource acquisition that has been described in *Daphnia* exposed to microplastics and NPs (An et al., 2021; Rist et al., 2017). Whether it be a direct consequence of improper feeding or a distinct phenomenon induced by NP ingestion, most studies challenging *Daphnia* with NPs also report important reductions in somatic growth and, consequently, body length (Guilhermino et al., 2021; Trotter et al., 2021; Zimmermann et al., 2020). Seeing as the spore yield of *Metschnikowia* seems to correlate positively with host size at the time of death (Hesse et al., 2012), it is thus conceivable that hosts exposed to 20 mg/L of NPs would have reached smaller sizes than the other treatments (this was not verified in the present experiment). Interestingly, microplastics were shown to induce an upregulation of haemocytes in *Daphnia* (Sadler et al., 2019), which is the primary mechanism of defence against *Metschnikowia* infections (Metschnikoff, 1884; Stewart Merrill & Cáceres, 2018). Assuming that NPs could enter the haemolymph prior to parasite settlement, an earlier activation of the immune system might affect subsequent infections in a similar manner to the phenomenon of immune priming caused by sequential infections (Rodrigues et al., 2010; Syller & Grupa, 2016). NPs could also promote additional mechanisms of host immunity, such as phenoloxdase activity, which may not provide defence against *Metschnikowia* or other parasites (Mucklow et al., 2004; Mucklow & Ebert, 2003) but could contribute to a mobilisation of host resources that would otherwise be used by the parasite for its growth.

Despite a strong negative effect of the high-NP treatment on *Metschnikowia*'s spore yield, we found that the parasite's net output per inoculated host did not differ significantly between the control and high-NP treatment. This suggests that the respective effects of NPs influencing parasite fitness in this treatment, either positively (proportion of infected) or negatively (host mortality, spore production) cancelled each other out. By contrast, the lower concentration tested in this experiment only resulted in neutral to positive effects for the parasite. Consequently, the overall reproductive success of the parasite was higher at 5 mg/L than at 20 mg/L. However, it is necessary to consider how this finding relates to natural conditions. It is at present difficult to delineate

by either negating parasite development (via unusual levels of pre-transmission mortality) or decreasing the parasite's reproductive output.

TABLE 2 ANOVA (F-test or χ^2 test) testing for fixed effects of nanoplastic (NP) concentration, Infection, and their interaction on life history parameters of the host (host lifespan, proportion of individuals that reproduced and host fecundity).

Response variable	Distribution (Link function)	Explanatory variables	Statistic (Degrees of freedom)	p-value	% Variance explained
Host lifespan	Normal	NP concentration	$F_{(2;113)} = 7.63$	<0.001	5.65
		Infection	$F_{(1;113)} = 139.56$	<0.001	51.68
		NP×Infection	$F_{(2;113)} = 1.10$	0.335	0.82
Proportion of hosts that reproduced	Binomial (link: logit)	NP concentration	$\chi^2_{(2;113)} = 3.77$	0.152	3.06
		Infection	$\chi^2_{(1;113)} = 7.73$	0.005	6.80
		NP×Infection	$\chi^2_{(2;113)} = 6.14$	0.046	2.21
Host fecundity	Normal	NP concentration	$F_{(2;87)} = 6.63$	0.002	4.73
		Infection	$F_{(1;87)} = 171.14$	<0.001	61.02
		NP×Infection	$F_{(2;87)} = 4.52$	0.014	3.23

Note: Data for successfully infected hosts were pooled together with early deaths to improve their comparability with the control treatment. For host fecundity, only those individuals that reproduced at least once were included in the analysis. Significant p-values (<0.05) are highlighted in bold.

Response	Infection treatment	Contrast	SMD	CI	Magnitude
Host lifespan	Control	Zero–Low	0.54	[0.13,0.96]	medium
	Control	Zero–High	-0.27	[-0.95,0.41]	small
	Infected	Zero–Low	-0.26	[-1.79,1.26]	small
	Infected	Zero–High	-4.34	[-6.95,-1.75]	high
Proportion of hosts that reproduced	Control	Zero–Low	0.42	[0.02,0.83]	small
	Control	Zero–High	-0.11	[-0.68,0.47]	small
	Infected	Zero–Low	-0.35	[-1.11,0.415]	small
	Infected	Zero–High	-0.61	[-1.40,0.175]	medium
Host fecundity	Control	Zero–Low	0.98	[0.40,1.56]	high
	Control	Zero–High	0.33	[-0.23,0.90]	small
	Infected	Zero–Low	-0.25	[-0.95,0.45]	small
	Infected	Zero–High	-0.43	[-1.08,0.22]	small

TABLE 3 Effect sizes (95% confidence intervals) testing host fitness responses for nanoplastic concentration (low or high), as compared with the baseline group (zero).

Note: Contrasts between NP treatments are provided within each Infection treatment. Standardised mean differences (SMDs) are considered small around absolute values of 0.2–0.5, medium around 0.5–0.8 and large at 0.8 or greater (highlighted in bold). CI, confidence interval.

relevant NP concentrations in natural waters, as no standard analytical method is able to detect NP particle numbers in environmental samples (Besseling et al., 2019; Gaylarde et al., 2021). However, Besseling et al. (2019) argue that fragmentation of spherical microplastic particles (<5 mm) into 100 nm NP particles may lead to particle concentrations that are ultimately $>10^{14}$ times higher than current estimates for environmental microplastic concentrations. As a point of comparison, microplastic pollution in freshwater habitats can sometimes reach 100–500 particles/L of water (Li et al., 2018; Lu et al., 2021). While the concentrations of 5 mg/L and 20 mg/L were chosen to maximise comparability with previous studies (see Section 2.2), these are estimated to equal 9.1×10^{12} and 3.64×10^{13} particles/L respectively (conversion based on Leusch & Ziajahromi, 2021), which may be considered high for natural ecosystems, but still plausible in heavily polluted sites.

4.2 | Host fitness

As expected, *Metschnikowia* infection severely impaired host fitness, reducing host lifespan and offspring production (Cáceres et al., 2006; Hesse et al., 2012). However, NPs alone did not reduce host fitness components, similarly as reported elsewhere (Rist et al., 2017). Rather, a lower dose of NPs appeared beneficial for the host, causing a slight boost to all fitness traits. This suggests hormesis, a commonly observed phenomenon characterised by a low-dose stimulation and a high-dose inhibition in dose–response models (Stanley et al., 2013). According to a recent review summarising the dose effect in microplastic and NP studies, 65 studies thus far support the idea of a hormetic dose–response model (Agathokleous et al., 2021). Evidence for hormesis has been recorded on several occasions in the *Daphnia*

genus (Campos, Piña, et al., 2012; Stanley et al., 2013; Zaluzniak & Nugegoda, 2006); notably, when *D. magna* was exposed to a plastic leachate, positive effects were observed on growth and reproduction (Xu et al., 2020). A similar hormetic effect of plastic particles has also been observed in crabs (Liu et al., 2019), algae (Gunasekaran et al., 2020), and oysters (Gardon et al., 2020). Among the more general mechanisms that have been suggested to underlie hormesis, leading hypotheses include protection against DNA damage, changes in gene expression and regulation, enhancement of immune function, and the fact that a given stressor may interact with different receptor subtypes, with higher affinity receptors promoting one metabolic change opposite to that of lower affinity subtypes (reviewed in Shi et al., 2016). Calabrese and Baldwin (2001) proposed that hormetic effects represent evolutionary-based adaptive responses to environmentally induced disruptions in homeostasis, while other studies suggest a potential involvement of epigenetics in the process (reviewed in Vaiserman, 2011). In the specific case of *Daphnia*, several mechanisms have been associated with reproduction enhancement, such as the promotion of a typical r-strategy (Zaluzniak & Nugegoda, 2006), as well as disruptions in lipid, carbohydrate and ecdysone metabolism (Campos et al., 2013; Stanley et al., 2013). Evidence also suggests that hormetically enhanced offspring production in *Daphnia* can be offset by adverse outcomes, such as increasing oxygen demands and a reduced tolerance to low levels of oxygen (Campos, Pina, & Barata, 2012).

Overall, our results indicate that the negative effects of infection exceeded the impacts of exposure to NP. However, while post hoc tests revealed that the lifespan of infected *Daphnia* did not differ significantly between the zero and high-NP treatments (Table S2), the size of this effect was still considered of high magnitude (Table 3). This suggests that exposure to high concentrations of NPs may still have relevant negative impacts in *Daphnia* populations frequently infected with *Metschnikowia*. According to Feckler et al. (2018), effect sizes can serve as an important tool for assessing the biological relevance of effects that do not match the criteria for statistical significance, particularly when trying to understand the impacts of multiple stressors and their interactions. Additionally, the hormetic response evidenced by increased fecundity in uninfected *Daphnia* exposed to low NPs was lost in infected populations. This also supports the point made by Morris et al. (2022) that the net impacts of stressors are usually best explained by the effect of the stronger stressor alone. These examples highlight the importance of considering the relative strength of different stressors when assessing their net impact on aquatic biota, as such findings may also be relevant for decision makers in the field of water management and conservation.

While our findings mostly corroborate the independent effects of *Metschnikowia* infection and microplastic particles on fitness parameters of *Daphnia*, we also provide novel estimations of this parasite's virulence in the context of NP contamination. Here, NPs and parasitic infection displayed synergistic, detrimental effects on

Daphnia. The combination of both stressors harmed the host especially in the high-NP treatment, where nearly half of the individuals did not reproduce. Such a phenomenon could be relevant for natural populations where NP concentrations are higher than usual (i.e., direct disposal sites), and could possibly lead to decreased host population densities. Given their role as grazers, changes in *Daphnia* densities could have important repercussions on primary production, water quality, and trophic interactions (Jeppesen et al., 1999; Lampert, 2011). Moreover, increased rates of host mortality prior to reproduction may affect the transmission of other types of parasites present in the environment, particularly vertically transmitted ones. For instance, other pollutants such as the insecticide carbaryl greatly reduced the fitness of *Hamiltosporidium magnivora*, a vertically transmitted microsporidium of *Daphnia* (Coors et al., 2008). Nevertheless, the findings reported here stem from a single clone of *D. galeata* × *longispina*; as such, future experiments expanding on host genotypes, but also species diversity are desirable to further generalise or nuance these observations (Barata et al., 2002; Imhof et al., 2017).

5 | CONCLUSION

We have shown, using a *Daphnia*-yeast model system, that altered environmental conditions caused by NP pollution can affect specific traits of infection in diverging ways. Specifically, the proportion of infected hosts was elevated under NP exposure, but, at the highest concentration, the parasite's advantage was offset by increased host mortality and a decreased number of transmission stages produced inside the host. In summary, this study highlights the need to consider ecological interactions between species when assessing the potential consequences of anthropogenic stressors in the environment. Given the important role of parasites in the shaping and functioning of ecosystems, we illustrate the importance of extending research on the consequences of microplastic and NP pollution beyond toxic effects on single organisms, and recommend incorporating host-parasite interactions as important ecotoxicological endpoints.

AUTHOR CONTRIBUTIONS

Conceptualisation: J.W. Developing methods: S.M., J.W., F.M., C.S., R.A., N.A. Conducting the research: S.M., N.A. Data analysis, preparation of figures and tables: S.M., F.M., R.A. Data interpretation, writing: S.M., F.M., J.W., R.A., C.S., N.A.

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DATA AVAILABILITY STATEMENT

The data supporting this study can be found at: doi: [10.5281/zenodo.5512138](https://doi.org/10.5281/zenodo.5512138)

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