Science of the Total Environment 1003 (2025) 180735

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Plastic pollution and parasitism: Impact of nanoplastics on the transmission of a marine trematode parasite

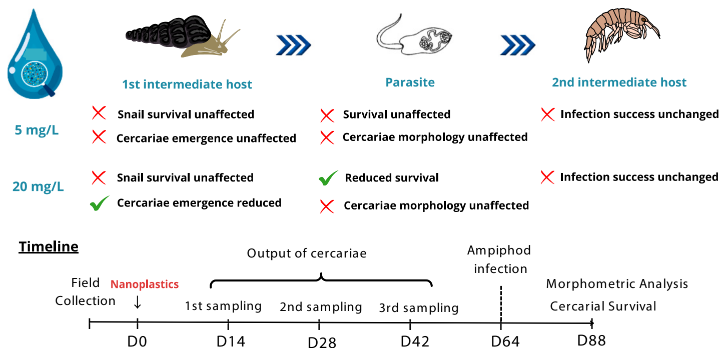
Lilou Mayeur [a], Robert Poulin [a,*], Chen-Hua Li [a]

[a] Department of Zoology, University of Otago, P.O. Box 56, Dunedin, 9054, New Zealand

HIGHLIGHTS

- Nanoplastics reduce emergence of infective stages (cercariae) in the trematode *Maritrema novaezealandensis*.
- High nanoplastic exposure shortens cercarial lifespan in this marine trematode.
- Cercarial morphology and infectivity remain stable despite nanoplastic exposure.
- Overall, parasite transmission stages are vulnerable to chronic nanoplastic pollution.
- The subtle effects of pollutants can have a cascading effect on marine diseases and food webs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Maritrema novaezealandensis
Marine pollution
Trematode transmission
Nanoplastics
Host-parasite interaction
Ecotoxicology

ABSTRACT

Factors constraining transmission success in parasitic systems remain poorly understood, especially in marine environments increasingly affected by anthropogenic disturbances. Among these, ocean pollution – an insufficiently recognized and inadequately controlled component of global change – poses emerging threats to biodiversity and ecological interactions. In particular, the impact of nanoplastics (NPs), pervasive and biologically active pollutants, on host–parasite dynamics remains largely unexplored. This experimental study examines the effects of NPs exposure on the trematode *Maritrema novaezealandensis*, a parasite with a complex life cycle involving the snail *Zeacumantus subcarinatus* and the amphipod *Paracalliope novizealandiae* as intermediate hosts. Infected snails were exposed for three months to different concentrations of NPs (0, 5, and 20 mg/L). We quantified cercarial (free-swimming infective stages) emergence from snails, assessed their survival and infectivity to amphipods, and investigated impacts on their morphological traits. A significant reduction in cercarial emergence was detected only after several weeks in the 20 mg/L treatment group, suggesting a delayed but cumulative inhibitory effect of high NP exposure. Cercarial survival was also significantly reduced at 20 mg/L, while no statistically significant differences were observed in morphological features or infectivity to amphipods. However, a trend toward reduced tail length at higher concentrations may reflect subtle functional impairments. While infectivity was maintained under short-term exposure, the reduced lifespan of cercariae may limit transmission opportunities in natural settings. These findings highlight the need to include assessment of parasite

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* Corresponding author.
E-mail address: robert.poulin@otago.ac.nz (R. Poulin).

https://doi.org/10.1016/j.scitotenv.2025.180735
Received 6 July 2025; Received in revised form 21 August 2025; Accepted 13 October 2025

transmission in ecotoxicological research, as even minor disruptions can cascade through food webs and affect ecosystem stability.

1. Introduction

The pollution of marine ecosystems is an escalating global concern, with plastic debris – particularly microplastics and nanoplastics – emerging as major environmental stressors due to their persistence, ubiquity, and capacity to interact at multiple biological levels (Morley et al., 2003; Ter Halle et al., 2017; Wagner and Reemtsma, 2019; Mavrianos et al., 2023; Yee et al., 2021; Joppien et al., 2022; Haney and Rochman, 2025). Nanoplastics (NPs), defined as plastic particles smaller than 100 nm (Mavrianos et al., 2023), originate either from the degradation of larger plastic debris or are intentionally manufactured for industrial and commercial applications (Yee et al., 2021; Ekvall et al., 2024; Webb et al., 2025) Due to their minute size and physicochemical properties, NPs possess a high potential for biological uptake, as they can traverse cellular membranes and biological barriers (Yee et al., 2021; Joppien et al., 2022). In mammals, for example, NPs have been shown to enter tissues via phagocytosis or endocytosis and can infiltrate microfold cells in Peyer's patches (Yee et al., 2021), raising concerns about their ability to accumulate and interfere with physiological functions. These concerns are magnified by recent findings showing that nanoplastics may adsorb various other pollutants and/or act as passive vectors for microbial pathogens. For example, protozoan parasites such as *Toxoplasma gondii*, *Cryptosporidium parvum* and *Giardia enterica* have been shown to adhere to microplastic surfaces, facilitating their persistence and transport in marine ecosystems (Zhang et al., 2022). This reveals an additional layer of risk, where plastic pollution may not only impact host physiology but also modify the dynamics of infectious disease transmission in aquatic environments.

Despite their relevance, empirical data on environmental NP exposure remain scarce. Existing studies are hindered by analytical limitations, and environmental concentrations are largely inferred from models or indirect detection methods (Wagner and Reemtsma, 2019). Although recent advances in NP detection have improved our understanding of their presence in aquatic environments (Cai et al., 2021), questions remain about the applicability of these techniques in complex environmental matrices (Wagner and Reemtsma, 2019). For instance, nanoscale plastic particles have been detected in remote marine regions such as the North Atlantic subtropical gyre (Ter Halle et al., 2017), or even in the Mediterranean Sea (Llorca et al., 2021), yet precise quantifications and standardized measurements are still lacking. Moreover, the ecological relevance of these particles is underscored by their demonstrated toxicity across a wide range of marine organisms, from algae and bivalves to zooplankton and fish (Zaki and Aris, 2022). Their accumulation at the base of the trophic chain may lead to trophic transfer toward higher organisms, including those consumed by humans (Geremia et al., 2023; Porter et al., 2023), reinforcing the importance of studying their ecological and health-related consequences.

A single gram of macroplastic can yield billions of NP particles (Yee et al., 2021), which have been shown to affect a wide range of physiological processes in aquatic organisms, including feeding, reproduction, immunity, and survival (Liu et al., 2020; Manzi et al., 2023; Mavrianos et al., 2023; Balsdon and Koprivnikar, 2024; Webb et al., 2025). Their pervasive effects span trophic levels due to their bioavailability and capacity to cross biological barriers. For instance, NPs have been observed in algae, where they impair growth by reducing chlorophyll content (Xu et al., 2024), as well as in zooplankton, key filter feeders at the base of the aquatic food web (Koelmans et al., 2015), thereby potentially destabilizing its entire structure (Ekvall et al., 2024; Haney and Rochman, 2025).

The ecological risks posed by NPs also extend to host–parasite interactions, a critical component of aquatic community dynamics that has received limited attention in the context of emerging pollutants (Morley et al., 2003; Mavrianos et al., 2023; Balsdon and Koprivnikar, 2024; Webb et al., 2025). Some studies have documented increased infection rates under NP exposure in zooplanktonic hosts (Manzi et al., 2023). In contrast, other studies suggest a reduction in trematode infection success due to mechanical or behavioral interference, as observed in amphibians exposed to polyester fibres (Buss et al., 2022). These diverging outcomes highlight the context-dependent nature of NP–parasite interactions.

Parasites, especially during their infectious free-living or early developmental stages, are vulnerable to environmental disturbances, including pollution (Morley et al., 2003; Sures et al., 2023). Pollutants may influence parasite transmission both directly—by impairing parasite viability—and indirectly—by modulating host susceptibility or immune responses. For example, larval parasites developing in or released from invertebrate hosts may experience altered development or infectivity under pollutant stress (Morley et al., 2003; Koelmans et al., 2015; Sures et al., 2023). These effects are particularly concerning given that most marine pollution, including plastics and chemical waste, originates from land-based sources and accumulates in coastal environments (Wagner and Reemtsma, 2019) – areas where complex host–parasite systems are concentrated.

The digenean trematode *Maritrema novaezealandensis*, endemic to New Zealand's coastal waters, offers a well-characterized and ecologically relevant model to investigate the effects of NP pollution on host–parasite dynamics (Keeney et al., 2007; Studer et al., 2010; Harland et al., 2015). Its life cycle involves three hosts: shorebirds as definitive hosts, the mud snail *Zeacumantus subcarinatus* as the first intermediate host, and crustaceans (including the amphipod *Paracalliope novizealandiae*) as second intermediate hosts (Martorelli et al., 2004; Keeney et al., 2007). Inside snails, the parasite undergoes asexual multiplication within the host's digestive-gonadal complex, producing thousands of genetically identical cercariae that regularly emerge from the snail into the environment (Keeney et al., 2007). These free-swimming larvae then infect amphipods and encyst as metacercariae. Transmission is completed when infected amphipods are consumed by shorebirds, within which the parasite matures into an adult. Eggs are shed via the bird's feces, contaminating the environment and enabling infection of snails, thus closing the transmission cycle. This model is particularly relevant given that its amphipod host, *Paracalliope novizealandiae*, plays a key role as prey for higher trophic levels in coastal food webs (Dauvin, 2024), making it a potential conduit for the biomagnification of plastic-associated stress across ecosystems.

Empirical studies have shown that *M. novaezealandensis* can cause snail castration and increased amphipod mortality, significantly impacting host population dynamics and reproductive output (Fredensborg et al., 2004, 2005), with cascading effects on food web structure and ecosystem stability. The parasite's transmission success is modulated by various environmental factors, including temperature (Studer et al., 2010; Mouritsen et al., 2018), ocean acidification (Harland et al., 2015), salinity fluctuations (Koprivnikar and Poulin, 2009), and exposure to ultraviolet radiation (Studer et al., 2012). The only previous information on the effects of NPs on trematode transmission comes from two studies in freshwater systems, one reporting no effect of NPs on cercarial survival and a reduced infection success when both host and parasites were exposed to NPs (Buss et al., 2022), and the other reporting an increased production of cercariae in snails exposed to high NP concentrations (Balsdon and Koprivnikar, 2024). Given these mixed results, the general behavioral and physiological impairment of snails caused by NP exposure including oxidative stress and impaired osmoregulation (Rodrigues et al., 2023), the key role of

M. novaezealandensis as a driver of community structure (Mouritsen et al., 2018), and its susceptibility to a range of other environmental stressors, the influence of NPs on its transmission success warrants investigation.

This study aims to assess the effects of environmentally relevant concentrations of NPs on a key transmission stage of *M. novaezealandensis*. Specifically, we address three primary objectives: (i) evaluate the effect of NPs on *Z. subcarinatus* snail survival and cercarial multiplication within infected snails; (ii) assess the survival and morphology of cercariae emerging from infected snails exposed to NPs; and (iii) assess the effect of NP exposure of the snail host on the subsequent infectivity of cercariae to the amphipod second intermediate hosts (*P. novizealandiae*). By conducting a series of laboratory experiments across these life cycle stages, we aimed to elucidate whether and how NPs interfere with the ecological success of parasitic trematodes in coastal systems. This research contributes to the growing field of pollutant–parasite interactions and may inform future ecological risk assessments concerning NP pollution in marine environments.

2. Materials and methods

2.1. Snail collection

Two hundred mud snails, *Z. subcarinatus*, were hand-collected in January 2025, from Lower Portobello Bay, Otago Harbor (South Island, New Zealand), a site known for its high prevalence of trematode infections. Snails were fed ad libitum with *Ulva lactuca* (also collected from the same site) and maintained in plastic containers (24 × 18.5 × 12.5 cm) filled with 2 L of aerated seawater for 48 h to allow acclimatization. Following this resting period, snails were individually screened for trematode infections by placing them into the wells of a 24-well plate, each containing approximately 3 mL of seawater, and incubating them at 24 °C under continuous light for 24 h. Cercarial emergence was then assessed under a stereomicroscope. Uninfected snails and snails infected with *M. novaezealandensis* were retained for the study, whereas those infected with different trematode species were excluded.

2.2. Nanoplastic use

Fluorescent polystyrene/polymethacrylate particles (Micromer®-greenF; micromod Partikeltechnologie GmbH, Germany) at a nominal diameter of 100 nm (product code: 29-00-102) were used in this study. These consist of monodisperse spherical particles (density: 1.03 g/cm^3), composed of polystyrene, substituted polystyrenes, polystyrene copolymers, or polymethacrylates. The selected concentrations (0, 5, and 20 mg/L) were environmentally realistic and determined based on previous studies (Mavrianos et al., 2023; Manzi et al., 2023) that demonstrated significant effects of NPs on aquatic organisms. For instance, exposure at 50 mg/L was shown to markedly reduce host survival in freshwater *Daphnia* exposed to apicomplexan parasites, indicating potential toxicity at higher levels (Manzi et al., 2023). Accordingly, 20 mg/L was used here as a high exposure threshold, while 5 mg/L was considered a moderate, sublethal dose for comparative purposes (Mavrianos et al., 2023).

To ensure chemical equilibration between NPs and seawater, the solutions were poured into clean aerated plastic containers and kept at room temperature in the dark for 24 h before the introduction of *Z. subcarinatus*.

2.3. Experimental design

From the initial sample, 60 infected and 30 uninfected snails (shell length ranging from 11 to 16 mm) were selected. Each snail was individually marked with a numbered plastic tag (Queen Marking Kit, The Bee Works, Canada) and randomly assigned to one of the three concentration groups (0, 5, and 20 mg/L). Snails were maintained in cylindrical containers (11 cm high × 7 cm diameter) with 10 snails per container, each filled with 400 mL of aerated seawater with the designated NP concentration. Because snails can passively accumulate NPs in their tissues by ingestion of food on which NPs attach (Rodrigues et al., 2023), we expected NPs to reach the snails' digestive-gonadal complex and thus the cercariae developing there. For uninfected snails there was one container per concentration, and for infected snails there were two containers per concentration.

Throughout the experiments, snails were maintained in an auto-illuminated room with a 12 h light/12 h dark photoperiod, with ambient temperatures maintained between 12 °C and 16 °C. Environmental parameters such as temperature, pH, salinity, oxygenation (one air stone per container, all connected to a common pump), and food availability were kept constant across all containers, with the only variable being the NP concentration. General snail husbandry involved replenishing evaporated water (approx. 5–10 % of water volume per week) with seawater containing the appropriate NP concentration and feeding with *Ulva* sp. algae (5 g per container, added twice over the course of the experimental period). The experiment lasted three months, with measurements of different variables taken at different times (see Fig. 1), as explained below.

2.4. *Maritrema novaezealandensis* cercarial output, survival and morphology

The impact of NP exposure on cercarial multiplication and output was assessed on three occasions, at 14, 28 and 42 days after the start (Fig. 1), by quantifying the number of cercariae released from individual snails exposed to each NP concentration during biweekly incubation sessions over a six-week period. On each occasion, snails were individually incubated in wells of a 24-well plate containing approximately 3 mL of seawater (NP-free) at 24 °C under constant illumination for 24 h to stimulate cercarial emergence. The use of numbered plastic tags enabled the tracking of individual snails and allowed repeated measurements of cercarial emergence over time. Following each session, cercariae were counted under a microscope and snails were returned to their respective containers to continue the exposure protocol.

After ten weeks of exposure to varying NP concentrations (0, 5, and 20 mg/L), infected snails were again individually incubated (same conditions as above, but only for 12 h). After the 12 hour incubation, snails were transferred to fresh wells for 1 h to ensure the use of newly emerged (<1 h old) cercariae in survival assays. These freshly-emerged cercariae were pooled across snails from the same container and NP treatment using a 2 mL Pasteur pipette. Five replicates of 10 cercariae per container per NP treatment were placed in separate wells (total = 300 individuals) with 3 mL of NP-free seawater, and maintained at room temperature. The number of motile cercariae was then monitored hourly for 16 h (cercariae are non-feeding stages that do not live longer than 24 h). Cercariae were considered dead when no movement was observed, indicating loss of infectivity.

In parallel with the survival test, cercariae from each NP treatment were sampled for morphological analysis. Seven freshly emerged cercariae per treatment, randomly chosen among snail hosts, were mounted between a slide and coverslip and examined at 40× magnification under a light microscope. Digital images were captured, and morphometric measurements (body length, body width, tail length, tail width at base) were made using AmScope 3.7. These metrics were used to test for potential NP-induced morphological changes.

2.5. Amphipod collection and infection

Two days prior to the infection experiment, uninfected *P. novizealandiae* amphipods were collected from Hooper's Inlet (Otago Peninsula). This location is an ideal source of naïve amphipods, as amphipods from this location have never been found to harbor infections of *M. novaezealandensis* or any other metazoan parasite (Fredensborg et al.,

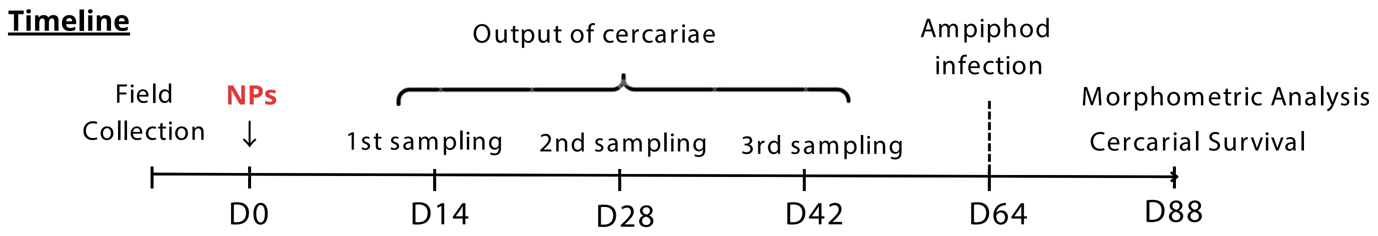

Fig. 1. Summary timeline of the experimental study along the 88-day duration. Exposure of infected snails to nanoplastic particles (NPs) started on day 0. Output of *Maritrema novaezealandensis* cercariae was tested on three occasions, experimental infections of amphipods on one occasion, and the experiment concluded with a final release of cercariae to quantify their survival and morphology.

2004). To ensure proper acclimation to laboratory conditions, the collected amphipods were kept for 48 h in a plastic container (24 × 18.5 × 12.5 cm) filled with 2 L of aerated seawater. To verify their uninfected status, a subsample ($n = 20$) was dissected and checked for parasites.

Following the procedure described above for tests of cercarial survival, cercariae <1 h old were pooled and used to infect uninfected amphipods. Ninety amphipods (30 for each NP concentration) were individually placed in 2 mL Eppendorf tubes containing ~1 mL of seawater and 10 cercariae. Exposures lasted 6 h at room temperature. After exposure, amphipods were transferred to clean containers (24 × 18.5 × 12.5 cm, 2 L seawater), one container per NP concentration treatment, and maintained for 72 h. They were then dissected under a stereomicroscope (10× magnification) to quantify metacercariae; these were yet to form a cyst but were clearly visible. Infectivity was calculated as the proportion of cercariae successfully established in an amphipod out of the 10 cercariae to which the amphipod was exposed.

2.6. *Data analysis*

All statistical analyses were conducted using R Statistical Software, version 4.2.2 (R Core Team, 2022). Prior to analysis, data from a few snails found to be co-infected by other trematode species were excluded. For each analysis, two explanatory variables (NP concentration and time) were used as the main fixed factors.

To analyze cercarial output (response variable), a Generalized Linear Mixed Model (GLMM) was fitted using the glmmTMB package (Brooks et al., 2017). NP concentration (0, 5, 20 mg/L), sampling time (D14, D28, D42; see Fig. 1), and shell length were included as fixed effects, with snail ID nested within containers as random effects to account for repeated measures on the same snails. Data from four infected snails were removed from the analysis as co-infection with other parasites was identified at one or more sampling time(s). One infected snail in the 5 mg/L concentration group was found dead at D42; therefore, we only included the cercarial output data at D14 and D28 for this snail. Normality assumptions were checked using Q-Q plots and Shapiro-Wilk tests. Type II Wald chi-square tests were conducted using the Anova() function from the car package (Fox and Weisberg, 2019) to assess the significance of fixed effects, and post-hoc tests were performed using emmeans (Lenth, 2025).

For cercarial survival, we applied a beta-regression GLMM with logit link (glmmTMB), using proportion of surviving cercariae as the response variable, and including concentration, time (hour post-emergence from the snail), and their interaction as fixed effects. Well ID was set as a random effect. Type II Wald chi-square tests were conducted using the Anova() function from the car package (Fox and Weisberg, 2019) to assess the significance of fixed effects, and post-hoc tests were performed using emmeans. Survival curves were visualized with ggplot2 (Wickham, 2016).

Cercarial morphology (body and tail measurements) was compared across treatments using Kruskal–Wallis tests (kruskal.test), due to small sample sizes and non-normal distributions. Boxplots and descriptive statistics were generated with ggplot2 and Rmisc (Hope, 2022).

Infectivity of cercariae to amphipods was assessed by modeling the number of cercariae that successfully infected each amphipod using a negative binomial GLMM (glmmTMB), with NP concentration as a fixed effect. Model diagnostics supported this approach due to overdispersion of count data. Type II Wald chi-square tests were conducted using the Anova() function from the car package (Fox and Weisberg, 2019) to assess the significance of fixed effects. Infection patterns were visualized using boxplots.

3. Results

Despite effects on parasite emergence and survival (see below), no signs of acute NP toxicity were observed in the snail host *Z. subcarinatus*. Mortality remained low (1–2 snails/concentration) and evenly distributed across all treatments, including the control group. Snails exhibited normal activity and feeding behavior throughout the 3-month experiment, suggesting that NP exposure at the tested concentrations did not induce overt physiological or behavioral stress.

3.1. *Output of Maritrema novaezealandensis cercariae*

Our first objective was to evaluate the relationship between NP concentration (0, 5, and 20 mg/L) to which snail hosts are exposed, and cercariae production in *M. novaezealandensis* across different sampling times (D14, D28, and D42) from the start of exposure to NPs.

The analysis revealed a significant interaction between NP concentration and sampling time affecting cercarial output ($\chi^2 = 13.13$, $p = 0.011$, Table 1). While no significant differences were observed at the early time points (Day 14 and Day 28), a marked decline in cercarial production was detected at Day 42 in snails exposed to the highest NP concentration (20 mg/L). At this final timepoint, cercarial output at 20 mg/L was significantly lower than in the 0 mg/L control group (post-hoc test, $p = 0.035$), indicating a delayed inhibitory effect of prolonged exposure to a high-concentration of NPs (Fig. 2).

3.2. *Cercarial survival*

The survival of *M. novaezealandensis* cercariae was monitored over a 16-hour period for individuals issued from snails maintained long-term at three NP concentrations (0, 5, and 20 mg/L). At each time point, the proportion of surviving cercariae was calculated per well. Wald chi-square tests (Type II) of the beta-regression GLMM model revealed significant main effects of time ($\chi^2 = 403.86$, $p < 0.001$) and NP

Table 1
Results (type II Wald chi-square tests) of a Generalized Linear Mixed Model (GLMM) testing the effects of concentration of nanoplastic particles (NP), exposure time and snail shell length on the number of *Maritrema novaezealandensis* cercariae produced by snail hosts.

Variables	χ^2 (df)	*p*-Value
NP concentration	5.32 (2)	0.070
Sampling time	5.16 (2)	0.076
Shell length	2.60 (1)	0.107
NP concentration × Sampling time	13.13 (4)	0.011

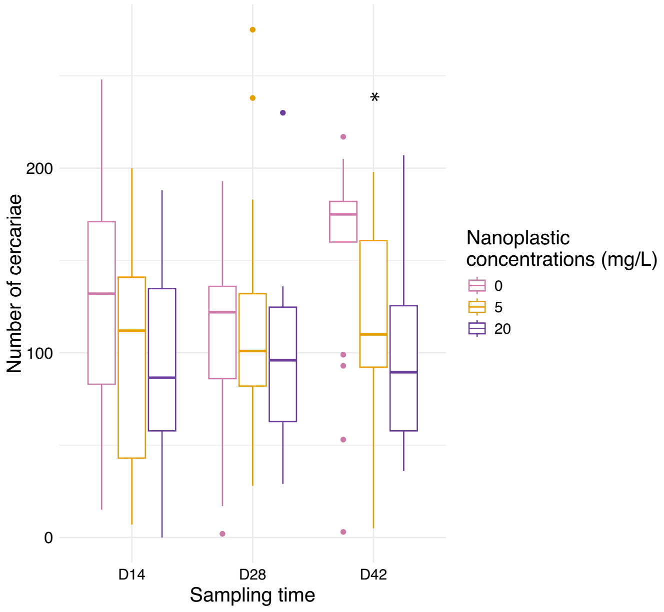

Fig. 2. Cercarial output of *Maritrema novaezealandensis* at different nanoplastic concentrations (0, 5, and 20 mg/L) over time (D14, D28, D42). Boxplots represent the distribution of cercariae numbers per snail. Significant decline in cercarial production was detected at D42. * $p < 0.05$.

Table 2
Results (type II Wald chi-square tests) of a beta-regression Generalized Linear Mixed Model (GLMM) testing the effects of and concentration of nanoplastic particles (NP) and time since emergence from the snail on the survival of *Maritrema novaezealandensis* cercariae.

Variables	χ^2 (df)	*p*-Value
NP concentration	10.56 (2)	0.005
Time	403.86 (1)	$2.2e^{-16}$
NP concentration × time	1.73 (2)	0.422

concentration ($\chi^2 = 10.56$, $p = 0.005$) on cercarial survival (Table 2). However, the interaction between time and concentration was not statistically significant ($p = 0.42$), indicating that the rate of mortality over time was similar across treatments. The analysis showed that cercariae originating from snails exposed to NPs exhibited reduced survival over a 16-hour period, with the most pronounced mortality observed at the 20 mg/L concentration. Across all treatments, survival declined steadily throughout the observation period, with a notable inflection point around 10 h (Fig. 3).

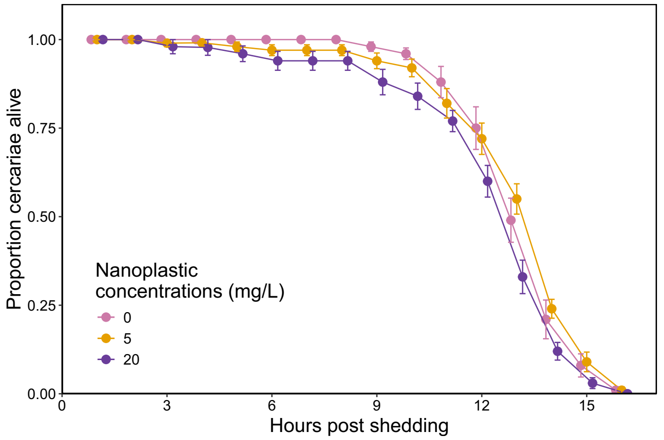

Fig. 3. Survival trajectories of *Maritrema novaezealandensis* cercariae, showing that cercariae exposed to the 20 mg/L condition consistently exhibited lower survival probabilities, while the 0 and 5 mg/L groups followed similar patterns.

3.3. Morphometric analysis

The effect of exposure of the snail host to different NP concentrations (0, 5, and 20 mg/L) on cercarial morphology was evaluated for different traits. Kruskal–Wallis rank sum tests revealed no significant differences across concentrations for body length ($\chi^2 = 1.10$, df $= 2$, $p = 0.58$), body width ($\chi^2 = 2.60$, df $= 2$, $p = 0.27$), tail length ($\chi^2 = 2.79$, df $= 2$, $p = 0.25$), or tail width ($\chi^2 = 0.42$, df $= 2$, $p = 0.81$). However, a slight tendency toward shorter tails was noted at 5 and 20 mg/L (Table 3).

3.4. Amphipod infection by cercariae

To assess whether exposure of the snail host to NPs affected the infectivity of *M. novaezealandensis* cercariae, the number of cercariae that successfully infected amphipods was compared among the three concentrations of NPs after exposure of amphipods to identical numbers of cercariae. The model revealed no statistically significant effect of NP concentration on cercarial infectivity (Type II Wald chi-square test: $\chi^2 = 1.73$, $p = 0.42$). The median number of cercariae per amphipod remained similar across all exposure groups (Fig. 4).

4. Discussion

Plastic pollution of aquatic ecosystems, and its impact on ecological processes, is a growing concern (Porter et al., 2023; Ekvall et al., 2024; Haney and Rochman, 2025). However, experimental investigations of potential effects on host-parasite interactions have been limited to date (e.g., Buss et al., 2022; Mavrianos et al., 2023; Manzi et al., 2023; Balsdon and Koprivnikar, 2024). The present study examined the impact of NP exposure on a key transmission stage, i.e. cercariae, in the life cycle of the marine trematode *M. novaezealandensis*. This parasite infects a broad range of intertidal crustaceans, such as amphipods, isopods, shrimps and crabs (Koehler and Poulin, 2010), and its sensitivity to environmental factors has been shown to modulate its relative impact on different host species and modify their community structure (Mouritsen et al., 2018). It was hypothesized that NPs would disrupt parasite transmission by altering cercarial emergence, survival, morphology and/or infectivity. The results partially supported this hypothesis: while NP exposure had very little effect on parasite morphology or infectivity, it significantly reduced cercarial emergence and lifespan at higher concentrations (20 mg/L). These findings raise important ecological considerations, which we discuss below.

4.1. Reduced emergence suggests cumulative stress effects

Balsdon and Koprivnikar (2024) reported an increase in cercarial emergence in freshwater snails fed a diet high in NPs. Our findings are

Table 3
Mean (± standard error) measurements of body and tail dimensions of *Maritrema novaezealandensis* cercariae produced in snail hosts exposed to increasing nanoplastic concentrations (0, 5, and 20 mg/L) for several weeks. No statistically significant differences were found among groups for any of the four morphological traits.

	Body length (µm)	Body width (µm)	Tail length (µm)	Tail width (µm)
0 mg/L	109.16 ± 6.26	56.06 ± 1.13	66.16 ± 6.25	11.66 ± 0.92
5 mg/L	111.34 ± 6.20	52.84 ± 1.10	56.13 ± 3.08	12.31 ± 0.64
20 mg/L	104.28 ± 5.28	52.56 ± 2.84	57.04 ± 2.27	11.76 ± 0.68

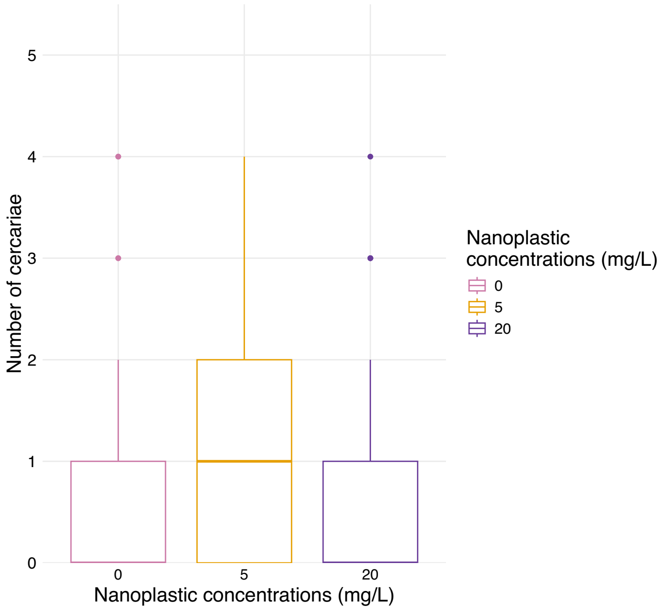

Fig. 4. Boxplots showing the number of *Maritrema novaezealandensis* cercariae successfully infecting amphipod hosts (*Paracalliope novizealandiae*) after exposure to increasing concentrations of nanoplastics (0, 5, and 20 mg/L). No significant difference in infection success was observed among treatments.

quite the opposite. The decline in cercarial emergence observed at Day 42 under 20 mg/L NP exposure suggests a delayed yet biologically meaningful effect of chronic pollutant stress on parasite transmission. Contrary to previous expectations of early or dose-dependent suppression, our findings highlight a more nuanced response in which the inhibitory effect becomes apparent only after extended exposure, a common pattern in ecotoxicological studies. This pattern supports the hypothesis that NPs can exert sublethal, cumulative physiological stress over time, ultimately impairing the parasite's ability to reproduce within its molluscan host. Two non-exclusive mechanisms may explain this finding. First, NPs could exert indirect effects via the molluscan host, altering its metabolism by activating costly tissue repair mechanisms and thus limiting the energy available for intramolluscan parasite development (Balsdon and Koprivnikar, 2024; Fredensborg et al., 2005). Second, direct toxicity via physical or cellular-level damage to the trematode's sporocysts (the within-snail stage responsible for asexually producing cercariae) may reduce their capacity to produce cercariae, as observed in other pollution-parasite systems (Gilbert and Avenant-Oldewage, 2017). Histological studies would help clarify the relative contribution of these mechanisms.

Interestingly, while chronic stress is sometimes associated with increased cercarial output due to host immunosuppression (Fredensborg et al., 2005), our findings suggest an inhibitory threshold effect. This aligns with studies showing that NPs and other stressors (e.g., temperature, pollutants) suppress trematode reproduction only above certain concentrations or levels, often without causing direct host mortality (Morley et al., 2003; Koprivnikar and Poulin, 2009).

4.2. Nanoplastics shorten cercarial lifespan

Unlike the findings of Buss et al. (2022), in our study exposure of snails to NPs negatively impacted the survival of their emerging cercariae, with mortality being most pronounced at 20 mg/L. This lower survival is particularly significant given the non-feeding and short-lived nature of cercariae, whose transmission success relies on their ability to rapidly locate a host. Survival began to decline noticeably around the 10-hour mark across all experimental conditions, with similar temporal trajectories, indicating that NP exposure primarily exacerbates overall mortality rates rather than shifting the onset of mortality.

Mechanistically, NPs must enter cercariae as they develop within their snail host. The ensuing reduction in their viability may stem from oxidative stress, tegumental damage, or impaired osmoregulation, all of which have been documented in snails and other aquatic invertebrates exposed to NPs (Koelmans et al., 2015; Liu et al., 2021; Rodrigues et al., 2023), and even in primary producers such as diatoms and microalgae, where antioxidant defenses are upregulated in response to NP-induced ROS production (Xu et al., 2024). These observations are consistent with broader evidence indicating that NPs reduce survival in aquatic invertebrates through oxidative stress and immune system impairment (Liu et al., 2020; Ekvall et al., 2024; Xu et al., 2024). Although no gross morphological abnormalities were observed, reduced functional lifespan could severely impair transmission success, particularly in environments with low host density (Morley et al., 2003; Oberdörster et al., 2005).

4.3. Morphological stability does not exclude functional impairment

Morphometric analysis revealed no statistically significant alterations in total length, body size, or tail dimensions of cercariae across treatments. A slight, non-significant reduction in tail length was noted in NP-exposed groups, particularly at 20 mg/L. Although sample sizes were small and thus statistical power was limited, these findings suggest structural resilience. However, they do not preclude functional impairment. Cercarial tails are essential for motility and host finding, and even subtle reductions may affect swimming efficiency and infection dynamics. The absence of visible anatomical damage highlights the need to investigate microstructural and behavioral changes. Future research should incorporate ultrastructural assessments and assays of swimming performance or host-targeting behavior to better understand how NPs may impair function without altering form.

4.4. Infectivity appears unaffected by exposure to NPs

Despite the physiological stress observed, infectivity remained undiminished in cercariae produced in NP-exposed snails. This suggests that essential features for host penetration – such as secretory gland activity and tegument integrity (Morley et al., 2003) – were not compromised within the timeframe of this study. However, while the infection process may remain effective, reduced cercarial survival time could limit opportunities for host contact in natural settings, where the water volumes cercariae may have to travel are much larger, thus functionally decreasing transmission potential. Given that cercariae were exposed to amphipods for only 6 h in the infectivity assays – well short of the onset of substantial mortality observed around 10 hour post-emergence – it is likely that the infective period assessed remained within a window where viability was not yet critically compromised. As such, the absence of detectable differences in infectivity between groups may reflect the timing of the assay rather than a true lack of NP-induced functional impairment.

Notably, some systems show increased infection or co-infection rates under NP exposure (Manzi et al., 2023), suggesting that parasite performance can be modulated positively or negatively depending on the species and context (see Balsdon and Koprivnikar, 2024). Our findings contrast with such reports, supporting the notion that parasite traits (e.g., swimming reliance, host-finding behavior) play a role in determining sensitivity to pollutants. Longer-term behavioral studies examining orientation, host detection, and penetration efficiency would help clarify the ecological implications of this apparent disconnect between viability and infectivity.

4.5. No acute toxicity detected in the molluscan host

The absence of significant mortality or behavioral impairment in *Z. subcarinatus* snails suggests that NP exposure at concentrations up to

20 mg/L for three months does not induce acute toxicity in this molluscan host. The very low and treatment-independent death rates, along with sustained activity and feeding behavior, support the interpretation that the observed decline in cercarial production stems from parasite-specific effects rather than compromised host viability.

However, since our experimental snails were naturally infected with *M. novaezealandensis*, known to cause parasitic castration (Fredensborg et al., 2005), we could not assess the impact of NP exposure on the snail's reproductive output. This is a notable limitation, as sublethal stress could affect fecundity or energy allocation in uninfected hosts. Future studies using uninfected snails and incorporating physiological endpoints (e.g., oxidative stress, respiration, reproduction) are needed to assess potential hidden costs of NP exposure. Furthermore, direct measurements of the quantity of NPs accumulated within snails and their distribution across snail tissues would also be highly informative.

4.6. Ecological implications and future directions

These findings align with previous reports showing that NPs can alter physiological traits in aquatic invertebrates, including survival, development, and reproduction (Liu et al., 2020; Porter et al., 2023; Ekvall et al., 2024; Balsdon and Koprivnikar, 2024). Beyond the host–parasite relation, our findings raise broader ecological and public health concerns. Parasites – despite being key regulators of biodiversity, population control, and food web dynamics – are often neglected in ecotoxicological assessments (Sures et al., 2023; Webb et al., 2025). Positive or negative impacts on their life cycles and transmission efficiency could have cascading ecosystem consequences (Morley et al., 2003; Harland et al., 2015; Mouritsen et al., 2018; Sures et al., 2023). While this study focused on a single trematode species, expanding the research to other parasitic taxa is essential to assess whether these patterns are generalizable. Furthermore, considering that amphipods are integral prey for fish, birds, and cephalopods in many benthic ecosystems (Dauvin, 2024), disruptions in their infection status or survival caused by NP exposure could propagate through food webs, affecting higher trophic levels and potentially altering predator–prey interactions. In addition, the possible influence of NPs on transgenerational plasticity in parasites—analogous to responses seen under thermal or acidification stress—remains an important question for future investigation.

An important future challenge will be to assess the influence of NPs on host-parasite interactions in a multi-stressor context (Sures et al., 2023). For instance, the negative effect of NPs on cercarial production in *M. novaezealandensis* uncovered in the present study might help to mitigate the predicted increase in cercarial output under global warming (Studer et al., 2010; Mouritsen et al., 2018). Rising seawater temperatures and increased NP pollution occur in parallel, along with multiple other environmental changes, making it difficult to predict the net resulting impact on parasite transmission. Also, our study used spherical polystyrene NPs with consistent size and surface properties, differing markedly from environmental NPs, which are chemically and morphologically heterogeneous (Wagner and Reemtsma, 2019). Aggregation, bioavailability, and chronic exposure effects may vary under more realistic conditions (Oberdörster et al., 2005). Developing standardized yet ecologically relevant reference materials is another key challenge for future research.

Despite these limitations, the present study demonstrates that NPs impair key stages of trematode transmission, i.e. the number of cercariae produced and their functional lifespan, without causing visible harm to the host, highlighting the potential for indirect ecological effects. Building on earlier studies of NPs and trematode parasitism (Buss et al., 2022; Balsdon and Koprivnikar, 2024), our study is an important and novel contribution to the emerging field of plastic parasitology. While our study focused on acute and relatively short-term responses, future experiments should explore chronic exposures, sublethal effects, and multi-generational consequences. Investigations into NP effects on parasite gene expression, immune evasion strategies, and host-parasite coevolution could provide valuable insights into the broader impacts of plastic pollution on parasitism in aquatic ecosystems. From a public health standpoint, our results underscore the need to consider NPs as a potential indirect driver of disease ecology. In particular, the impact of NP pollution on the dynamics of trematode species that infect humans, such as *Schistosoma mansoni*, warrants urgent investigation.

CRediT authorship contribution statement

Lilou Mayeur: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Robert Poulin:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Chen-Hua Li:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Funding

This research was not supported by any specific grant from agencies in the public, commercial or not-for-profit sectors.

Declaration of competing interest

The authors declare having no financial or other conflict of interest.

Acknowledgements

This work was conducted while LM was affiliated with the University of La Réunion and supported in part by the Erasmus+ program. We are grateful to the technical staff of the Zoology Department, University of Otago, for logistical support.

Data availability

All raw data and R codes used for the analyses in the present study are available from Figshare (DOI: https://doi.org/10.6084/m9.figshare.29474465).

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