Vol. 153: 81–85, 2023
https://doi.org/10.3354/dao03721

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published March 23

NOTE

Microplastic exposure and consumption increases susceptibility to gyrodactylosis and host mortality for a freshwater fish

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ABSTRACT: Microplastics have been found in all surveyed ecosystems and in the diet of multiple species. Detrimental health impacts of microplastic consumption include reduced growth and fecundity, metabolic stress and immune alterations for both invertebrates and vertebrates. Limited information exists, however, on how disease resistance may be affected by microplastic exposure and consumption. Here, the impact of microplastic (0.01 and 0.05 mg l^{-1} of polypropylene) on fish host susceptibility to disease and mortality was assessed using the guppy *Poecilia reticulata*–gyrodactylid *Gyrodactylus turnbulli* system. Fish exposed to and/or consuming microplastic at both concentrations demonstrated significantly higher pathogen burdens over time compared with fish fed a plastic-free diet. Furthermore, microplastic (at both tested concentrations) was associated with increased mortality events for fish within all treatments, regardless of host infection status. This study adds to the growing body of evidence showing that microplastic pollution can be detrimental to fish welfare by reducing disease resistance.

KEY WORDS: Microplastic · Host–parasite interactions · Disease resistance · Fish welfare

1. INTRODUCTION

Microplastic pollution is one of the greatest environmental concerns of this century (Galloway et al. 2017, Rios Mendoza & Balcer 2020). This legacy contaminant has been found in all surveyed habitats (Wagner et al. 2014, Vince & Stoett 2018, Rios Mendoza & Balcer 2020, Rillig & Lehmann 2020) and in multiple animal tissues, but we are only now beginning to understand the impacts of its exposure and consumption on animal welfare (Horton et al. 2017, Triebskorn et al. 2019). Fish exposed to and consuming microplastic display transcriptional changes in immune genes and altered cellular immune responses (Limonta et al. 2019), which has the potential to impact disease susceptibility. Furthermore, different types of microplastics and their associated chemical additives can have interactive effects (Wang et al. 2018), making it much more challenging to disentangle the impacts of consumption on disease dynamics without needing unsustainably large live animal samples.

Masud et al. (2022) revealed that for three-spined sticklebacks *Gasterosteus aculeatus* from semi-natural habitats (managed ponds) with pre-existing monogenean infections, pristine polypropylene microplastic consumption significantly prolonged infections compared with fish not exposed to these plastics. Here, we assessed the impact of 2 concentrations of microplastic on host–parasite interactions

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using a tropical fish host that was immunologically naïve to our chosen parasite. We used a pristine polypropylene (i.e. with no chemical additives) microplastic, as it is the second most widely used plastic (Plastics Europe 2021). The host used here is the ecological and parasitological model, the Trinidadian guppy *Poecilia reticulata* (see Magurran 2005); the parasite is a common monogenean ectoparasite for this host, *Gyrodactylus turnbulli*.

2. MATERIALS AND METHODS

2.1. Host–parasite system

Size-matched female guppies *Poecilia reticulata* were used for this study (size range: 19–22 mm standard length), bred from a stock originally caught in the Lower Aripo River in Trinidad in 2012. All guppies were maintained in 70 l breeding tanks at 24 ± 0.5°C under a 12 h light:12 h dark photoperiod (lights on 07:00–19:00 h) and fed dry food flakes (Aquarian®) daily and freshly hatched *Artemia* nauplii every alternate day. To assess their susceptibility to disease, experimental infections utilized the Gt3 strain of *Gyrodactylus turnbulli*, isolated from a Nottingham aquarium shop in October 1997 (King & Cable 2007). To measure wet mass, all hosts were weighed on an electronic scale (0.01 mg accuracy; OHAUS®) prior to commencing treatment feeds (with and without microplastic exposure) and on Days 7 and 25 of the dietary exposure and infection trajectory.

2.2. Microplastic preparation and dietary exposure

Polypropylene pellets were placed in cryogenic vials (STAR LAB), dipped in liquid nitrogen, and subsequently ground using a pre-cleaned pestle and mortar (i.e. rinsed with acetone and then distilled water). After grinding, plastics were sieved in pre-cleaned stainless-steel metal sieves with 0.3 mm aperture to collect fragments. Therefore, all plastics used for this investigation were <0.3 mm — the majority microplastics, but due to the method of preparation, also including some nanoplastics.

In a preliminary trial, guppies were clearly observed consuming microplastic when mixed with flake food and sprinkled on the water surface. For dietary exposure, fish were fed 2% bodyweight of flake food per day. Experimental fish were divided into 3 treatments, corresponding to 3 levels of microplastic: (1) 0.05 mg l^{-1}, n = 74; (2) 0.01 mg l^{-1}, n = 74; and (3) 0 mg l^{-1} controls, n = 74. These concentrations were chosen based on levels of microplastics detected in freshwater systems (reviewed in Fischer et al. 2016). Control fish (n = 74, not exposed to microplastic) were fed the same quantity of food as fish exposed to microplastic to ensure all hosts were given the same nutritional input. All experimental fish were isolated in 1 l containers for the duration of the experiment. Fish were fed once a day by sprinkling their diet on the water surface of each container. A 100% water change was conducted daily at 16:00 h to standardize timing of feeding. Fish were experimentally infected after 3 wk of dietary exposure, and the same feeding regimes continued throughout infection.

2.3. Experimental infections

From each of the 3 diet treatments, half the fish were experimentally infected. Briefly, this involved lightly anaesthetizing individual guppies with 0.02% MS-222 and infecting each fish with 2 gyrodactylid worms. Parasite transfer was conducted following standard methods of King & Cable (2007). Parasite infections were then monitored every 48 h for a maximum of 45 d by anaesthetizing fish and counting the total number of gyrodactylids on the surface of the fish over the entire infection trajectory.

2.4. Statistical analysis

All statistical analyses were conducted using RStudio v.2.1 (R Core Team 2020). To analyse the relationship between experimental treatments (microplastic exposure and controls) and disease resistance, the following parasite metrics were used: mean parasite intensity, maximum parasite count, duration of infection and area under curve (AUC). Here, mean parasite intensity is the arithmetic mean of the number of gyrodactylid worms per infected host within experimental treatments. This excludes hosts that either lost their parasites or died. Maximum parasite count is the total number of parasites on a single host at a single time point; specifically, the highest count recorded on any particular screening day during the entire monitored infection trajectory (i.e. 45 d in this study). Duration of infection is the length of time fish remained infected before either clearing infections or mortality occurred. To quantify total infection trajectory, the AUC was utilized and calculated with the

trapezoid rule. For analysing all parasite metrics, we utilized generalised linear models (GLMs). Fixed factors in the GLMs were the microplastic treatment (and controls) and standard length of fish, to determine if size was associated with the number of parasites found on hosts. To analyse maximum parasite count and mean parasite intensity (both count data), we utilized a GLM with negative binomial error family and square root link functions within the 'MASS' R package (Venables & Ripley 2002). This error family was chosen based on the lowest dispersion parameter, theta, to ensure there was no overdispersion. For AUC and duration of infection analysis (both continuous variables), we had to log- and square-root-transform the data respectively, as no error family or link function managed to generate normality or homoscedasticity of standardized residuals. Subsequently, for AUC analysis, a GLM with a gamma error family and a log link function were used, and a GLM with an inverse Gaussian error family and log link function was used for analysing duration of infection.

A GLM was also used to analyse mortality counts of fish exposed to microplastic (i.e. both infected and uninfected fish) with a Poisson error family and square root link function. A Kaplan-Meier survival analysis was also conducted using a Cox's proportional hazards model within the 'survival' package in R (Therneau & Grambsch 2000), which analysed the proportion of survival as a function of time to death. A generalised linear mixed model (GLMM) was fitted to the live wet mass and treatment to assess the impact of microplastic exposure on body condition. As the wet mass of the same individuals was measured at different time points, to prevent pseudoreplication, a GLMM was conducted using the 'lme4' package in R (Bates et al. 2015) with a Gaussian error family and log link function. The error family for the GLMM was chosen based on which model achieved the lowest Akaike's information criterion value (Thomas et al. 2013).

3. RESULTS

Fish hosts exposed to and consuming pristine polypropylene microplastics at the tested concentrations had significantly higher mean parasite intensities (GLM: 0.05 mg l^{-1} exposure: z = 2.4, SE = 0.63, p = 0.018; 0.01 mg l^{-1} exposure: z = 3.25, SE = 0.72, p = 0.001; Fig. 1A) compared with control fish not exposed to any plastics. For all other parasite metrics — maximum parasite count, duration of infections and AUC — no significant difference was detected compared with control fish (lowest p-value for maximum parasite count: p = 0.347; AUC: p = 0.097; duration of infections: p = 0.227). It is worth noting that though classed as insignificant, the lower concentration of microplastic exposure (0.01 mg l^{-1}) was close to significant in being associated with a higher AUC for total infection trajectories (p = 0.097) compared with infected fish not exposed to any microplastics in their diets. There was no significant association detected between the standard length of fish and number of parasites for all GLMs (lowest p-value for all models run: p = 0.539).

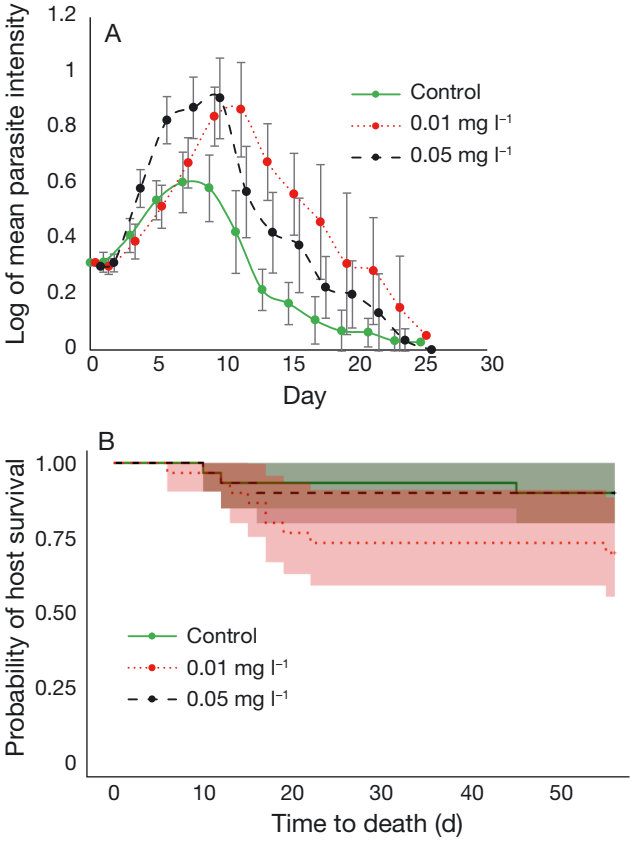

Fig. 1. (A) Mean (±SE) parasite intensity trajectory of control hosts *Poecilia reticulata* and those exposed to 0.01 and 0.05 mg l^{-1} microplastic and subsequently infected with *Gyrodactylus turnbulli*. (B) Survival plot with 95% confidence intervals showing marginal non-significant increase in the proportion of infected fish hosts dying when exposed to 0.01 mg microplastic l^{-1}

When analysing mortality counts of fish, all fish (regardless of infection status) had significantly higher mortality counts when exposed to microplastics compared with control fish that were not infected or exposed to microplastics (see Table 1 for statistical outputs). However, when analysing the proportion of

Table 1. Generalised linear model (GLM) analysis of fish mortality count within each experimental treatment (n = 30 fish per treatment) of the current study. Shown are the respective treatment categories, mortality counts and associated GLM outputs. Note that control fish, not exposed to any polypropylene (PP) microplastic or infection, are the baseline against which all other treatments are compared. NA: not applicable

Treatment	Mortality count	*z*	p
Control	0	NA	NA
Control + infection	3	2.4	0.014
0.01 mg l^{-1} PP	2	2	0.045
0.05 mg l^{-1} PP	3	2.4	0.014
0.01 mg l^{-1} PP + infection	9	3.7	0.0001
0.05 mg l^{-1} PP + infection	5	3.1	0.001

fish survival as a function of time to death, hosts consuming microplastic at 0.01 mg l^{-1} had the greatest proportion of deaths, though this was not significant compared with controls (Coxph: Coef = 1.17, z = 1.76, p = 0.077; Fig. 1B; number of deaths in 0.01 mg l^{-1} treatment = 9 out of 30 fish). No difference was seen in the proportion of deaths between control-infected fish not fed any microplastic and those exposed to 0.05 mg l^{-1} and subsequently infected (Coxph: Coef: 0.01, z = 0.01, p = 0.987; Fig. 1B; number of deaths in control and 0.05 mg l^{-1} treatment identical = 3 out of 30 fish). No impact of any treatment was found to significantly impact the wet mass of fish, and the majority of fish increased in mass during the experiment (lowest p-value for GLMM: 0.124).

4. DISCUSSION

In the current study, we reveal the impact of pristine polypropylene microplastic on fish disease resistance and mortality. Fish consuming polypropylene microplastic (at 0.01 and 0.05 mg l^{-1}) showed evidence of reduced resistance to gyrodactylid infections and increased host mortality levels at both tested concentrations of plastic, regardless of infection status.

This study and our related investigation on threespined sticklebacks *Gasterosteus aculeatus* (see Masud et al. 2022) highlight that exposure to and consumption of pristine polypropylene can negatively impact disease resistance. Even though the stickleback experiment was conducted at much lower temperatures than the current study, and we acknowledge that temperature does impact infection dynamics (see Stewart et al. 2018), sticklebacks maintained their gyrodactylid infections for much longer when consuming microplastic (polypropylene at 0.05 mg l^{-1}) compared with fish not exposed to microplastics. It is also worth noting that the guppies in the current study were immunologically naïve to gyrodactylosis, unlike the sticklebacks which did have prior exposure to these ectoparasites, and it is known that prior exposure impacts subsequent disease responses (e.g. Cable & van Oosterhout 2007). How the changes in disease resistance seen in this study on guppies relate to underlying cellular and genetic immune responses is beyond the scope of this study, and studies on the impact of microplastics on fish immunity are limited. Zebrafish that consume microplastic show downregulation in key genes involved in innate immunity, specifically epithelium integrity (Limonta et al. 2019). This might suggest that for zebrafish there is reduced pathogen control at epithelial barriers and rising chances of infections at mucosal sites. The zebrafish study did not, however, investigate disease resistance (Limonta et al. 2019). In this study, the pathogen investigated is an ectoparasite that forages on the mucous and epithelial cells of fish (see Bakke et al. 2007). Therefore, if, as suggested by the zebrafish study, downregulation of immune genes at epithelial barriers is occurring in guppies, this would explain the increased severity of gyrodactylosis in fish consuming microplastic. While this study was conducted under controlled laboratory conditions, it does highlight broader implications for managed and wild fish stocks that are exposed to microplastics, which may lead to higher parasite burdens (e.g. Pennino et al. 2020)

Fish mortality was also impacted by microplastic consumption. All fish exposed to microplastic (regardless of their infection status) had elevated mortality counts compared with control fish that were not infected or exposed to any microplastics. Interestingly, when only focussing on infected fish, compared with fish that were infected but not exposed to any microplastics (i.e. control infections), only hosts exposed to the lower microplastic concentration of 0.01 mg l^{-1} revealed marginally increased mortality when analysing proportion of death as a function of time; it is not clear why this is so. Most studies have shown a significant effect of microplastic on fish mortality in juveniles, linked to stunted growth, cellular toxicity and DNA damage (Pannetier et al. 2020), whereas the link between microplastic consumption and mortality is less apparent in adult fish (Guven et al. 2018). However, as shown in the current study, microplastics on their own and by reducing disease resistance can impact adult fish mortality.

To conclude, this study has shown that microplastic polypropylene exposure and consumption at 2 concentrations (0.01 and 0.05 mg l^{-1}) can reduce fish resistance to parasitic disease and increase mortality levels. Future research will need to pair infection and mortality data presented here with underlying transcriptomics to understand the underlying mechanisms behind the observed functional changes to host–parasite dynamics.

Acknowledgements. The present work was part of N.M.'s Cardiff University unpublished PhD thesis (2021). We thank Nick Matthews for technical support. All work was approved by the Cardiff University Animal Ethics Committee and conducted under UK Home Office licence PPL 303424. We thank the reviewers for their assistance with our paper.

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Editorial responsibility: Lori Gustafson, Fort Collins, Colorado, USA
Reviewed by: E. C. Pope and 1 anonymous referee

Submitted: September 6, 2022
Accepted: January 20, 2023
Proofs received from author(s): March 3, 2023