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A class of their own? Water-soluble polymer pollution impacting a freshwater host-pathogen system

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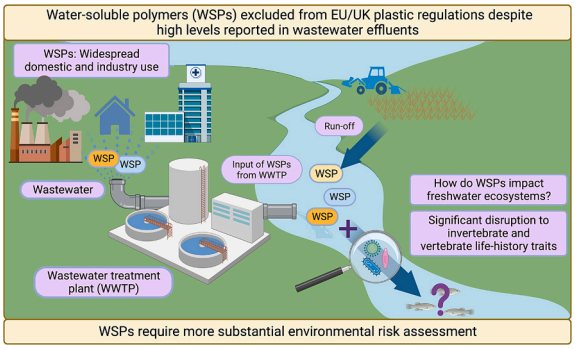
HIGHLIGHTS

- Water-soluble polymers (WSPs) pollute freshwater ecosystems but remain excluded from plastic regulations.
- Single and multi-stressor effects of WSPs and infection were investigated on a freshwater host-pathogen disease model.
- WSPs inhibited growth and altered the metabolic rate of fish, effects were time, polymer and dose-dependent.
- WSPs significantly enhanced parasite mortality and non-additive multi-stressor interactions were present.
- WSPs affected host and parasite life-histories, implying WSPs may alter interspecies interactions in freshwater ecosystems.

GRAPHICAL ABSTRACT



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ABSTRACT

While the inclusion of synthetic polymers such as primary microplastics within personal care products have been widely restricted under EU/UK Law, water-soluble polymers (WSPs) have so far slipped the net of global chemical regulation despite evidence that these could be polluting wastewater effluents at concentrations greatly exceeding those of microplastics. Polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) represent WSPs with common industry and household uses, down-the-drain disposal and a direct route to wastewater treatment plants, conveying high risk of environmental leaching into freshwater ecosystems. The current study is the first investigating the impacts of predicted environmental concentrations of these WSPs on life-history traits of two freshwater species also constituting a disease model (fish - *Poecilia reticulata* and parasite - *Gyrodactylus turnbulli*). Single effects of WSPs on fish as well as their interactive effects with infection of the ectoparasite were determined over a 45-day exposure. Generally, WSPs reduced fish growth and increased routine metabolic rate of fish implying a depleted energetic budget, however these effects were dose, exposure time and polymer dependent. Parasitic infection alone caused a significant reduction in fish growth and enhanced fish routine metabolic rate. In contrast, a non-additive effect on metabolic rate was evident in fish experiencing simultaneous infection and WSP exposure, suggesting a protective effect of the two WSPs for fish also exposed to a metazoan ectoparasite. Off-host parasite survival was significantly lowered by both WSPs; however, parasite counts of infected fish also exposed to WSP were not significantly different from the control, implying more complex mechanisms may

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underpin this stressor interaction. Distinct detrimental impacts were inflicted on both organisms implying environmental leaching of WSPs may be causing significant disruption to interspecies interactions within freshwater ecosystems. Additionally, these results could contribute to sustainable development in industry, as we conclude PVA represents a less harmful alternative to PVP.

1. Introduction

A decade of plastic pollution research has revealed that we are now in an environmental plastics crisis, with the 20th and 21st centuries coined the 'Plastics Age' (Bensaude-Vincent, 2022). Microplastics, defined as any insoluble synthetic polymer of 5 mm or less in any dimension, have held a pollution research spotlight for over a decade (ECHA, 2018). Aquatic environments are particularly vulnerable as they serve as reservoirs for the main microplastic leach routes: land run-off and wastewater effluent from combined sewer overflows and wastewater treatment plants (WWTPs), causing significant detriment to freshwater organisms in the form of bioaccumulation within food webs

Table 1
Summary of use, biodegradability, wastewater removal, predicted environmental concentrations (PEC) and toxicity of polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA).

WSP	Household uses	Industrial uses	WWTP removal	PEC	Toxicity	References
PVP	Excipient in food supplements and sweeteners (E1201) Pharmaceutical binder and coating Thickener, film forming and binding agent for cosmetics and toiletries *e.g.* stain prevention agent in toothpaste Single use contact lenses Denture fixative	Controlled-release fertilisers and pesticides Historical substitute for blood plasma Textile dispersing agent Wash-off adhesives Analytical chemistry uses *e.g.* nano-composite formation Releasing agent in disinfectants used widely in medicinal and veterinary practices	Commercially produced PVP non-biodegradable Readily biodegradable in pure forms and efficiently degraded in the presence of specific microbiota in aquatic environments however natural occurrence of these microorganisms is uncommon PVA based polymer blends and composite materials more resistant to biodegradation Some removal *via* sorption is probable	Sludge and aqueous phase delivery from WWTP 7.1 mg/L detected in wastewater effluent 0.16 mg/L detected 50 m downstream of WWTP outfall in the River Ruhr, Germany	Non carcinogenic, no chronic effects; WHO acceptable daily human adult intake 0–50 mg/kg bw Mild laxative effect observed in mammals administered 2500 mg/kg bw Non-significant decrease in heart rate of *Daphnia magna* exposed to 1, 5 and 10 mg/L, significantly decreased number of reproductive cycles when exposed to 5 mg/L for 21 days Hatching rate of *Pagrus major* (red seabream) embryos significantly decreased after acute exposure to 1000 mg/L PVP – LOEC	Haaf et al., 1985 Trimpin et al., 2001 Chiellini et al., 2003 Xiao et al., 2008 Antić et al., 2011 Julinová et al., 2012 Bergesch et al., 2017 Julinová et al., 2018 EFSA, 2020 Makhayeva et al., 2020 Mondellini et al., 2022
PVA	Washing machine and dishwasher capsule coatings Thickening and film-forming agent in cosmetics: Eye drops, peel off masks, deodorants, shampoos, lotions and makeup, sun creams	Nano-composites production Wash-off adhesives Various medical uses: Surgical threads, surgical glue, sheets and covers Lacquers and resins Paint thickening Food packaging humectant Textile coating agent	Slow biodegradation in aquatic environments Variable susceptibility to biodegradation depending on polymer configuration and composite form, which effect the biodegradability Specific PVA-degrading micro-organisms are not found in all ecosystems Predict roughly 29 % of manufactured PVA has the potential to contaminate aquatic ecosystems through wastewater and sludge fertiliser	Sludge and aqueous phase delivery from WWTP No recorded environmental concentration; based on US data on PVA usage and WWTP emissions - 0.4 mg/L for river PEC	Non carcinogenic, no chronic effects; WHO acceptable daily human adult intake 0–50 mg/kg bw No effects on *Danio rerio* or *D. magna* survival after 5 and 14-days exposure to 1 mg/L respectively Non-significant alterations in *Danio rerio* swimming behaviour at 1 μg/L Non-significant differences in *Daphnia magna* swimming behaviour observed at both 1 μg/L and 1 mg/L Significantly reduced reproductive success observed for *Daphnia magna* exposure to 5 mg/L for 21 days	Yamatsu et al., 2006 Keller et al., 2013 Gaaz et al., 2015 Julinová et al., 2018 Alonso-López et al., 2021 Rolsky and Kelkar, 2021 Nigro et al., 2022 Gökçe et al., 2022 Mondellini et al., 2022

causing inhibited life-history traits (Kershaw and Rochman, 2015; ECHA, 2018; Ha and Yeo, 2018). This has led to the implementation of action plans to mitigate plastic pollution at a global scale, such as the United Nations treaty on plastic pollution and at a more localised level, recent considerations to ban primary microplastic addition in products within the EU and UK (ECHA, 2019; UNEA, 2022). However, one major class of synthetic polymer, the water-soluble polymers (WSPs) has so far slipped through the net of global chemical regulation, as they lose their solid state after release to the environment, and therefore do not contribute to the identified threat posed by water-insoluble microplastics (ECHA, 2023).

In their solution formulations, WSPs are invisible to the eye but universally used throughout multiple industries and households having a myriad of societal applications (Julinová et al., 2018). Their incorporation into masses of both commercially available and industrial products can be attributed to their favourable properties, including viscosity enhancement to thicken cosmetic products (Koltzenburg et al., 2014), surfactants for soaps and detergents, film-formation for commercial and industrial conditioners (Xiong et al., 2017; Huppertsberg et al., 2020), binding, coating and flavouring agent in food supplements and pharmaceuticals (Koltzenburg et al., 2014; EFSA, 2020), dispersing agents used within paints (Koltzenburg et al., 2014; Huppertsberg et al., 2020) as well as many other industrial applications (Table 1). Ironically, their primary use is direct application to WWTPs as flocculation agents, enabling the coagulation and separation of the solid sludge from the aqueous phase in domestic and industrial wastewater, including insoluble microplastic particles (Arp and Knutsen, 2019).

Unlike most other chemicals in Europe which are registered under the regulation on the registration, evaluation, authorisation, and restriction of chemicals (REACH), information on WSP production volumes is scarce and not freely available. Furthermore, information on their environmental fate and behaviour is absent despite their widespread domestic and industrial use, generalised, unmonitored disposal, and artificial nature. In contrast to the progress made in microplastic regulation, there are no current regulations or restrictions for WSPs, which are exempt under REACH Regulation (EC) No. 1907/2006 due to their solubility, despite evidence, albeit limited, that they may be contaminating aquatic ecosystems at considerably high concentrations when compared with insoluble microplastic pollution levels (Antić et al., 2011; Ziajahromi et al., 2016; Julinová et al., 2018). Only now are WSPs being described as emerging pollutants, when their predicted environmental concentrations exceed levels known for the most prominent microplastics in UK rivers (Ziajahromi et al., 2016; Nigro et al., 2022). Analytical methods to detect WSPs are still in their infancy, but there have been recent advances. These include the quantification of polyvinylpyrrolidone at up to 7 mg/L in wastewater effluent using pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS) (Antić et al., 2011). Worryingly, this example exceeds concentrations known for some of the most well studied insoluble polymers, where the most frequently studied polymer in ecotoxicological research, polyethylene, is estimated to have a worst-case scenario concentration of 0.27–1.4 mg/L in UK wastewater (Ziajahromi et al., 2016). Alongside detection in wastewater, the first study to quantify PEO at up to 100 μg/L in surface waters has been conducted using size-exclusion chromatography electrospray ionization-high-resolution mass spectrometry (SEC-ESI-HRMS) (Huppertsberg et al., 2020; Tisler et al., 2021; Pauelsen et al., 2023). These findings support the presence of even biodegradable WSPs in the environment.

Some WSPs are subject to rapid biodegradation rates once disposed of, particularly within WWTP where a microbial community is well established. Many common WSPs, including polyethylene glycol (PEG), have been detected polluting wastewater effluents at average concentrations of 7.4 μg/L in over 30 WWTPs in Germany (Freeling et al., 2019), despite being classed readily degradable and therefore low risk for contaminating and accumulating within freshwater ecosystems compared to other, more persistent insoluble polymers, such as polyethylene. Therefore, other common WSPs which are more resistant to microbial degradation such as polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) will theoretically have a much higher presence in wastewater effluent, yet their actual concentrations in wastewater are not yet well defined (Julinová et al., 2018; Alonso-López et al., 2021). Both PVP and PVA have been described as emerging contaminants in freshwater ecosystems due to their ubiquitous use in common household personal care products, but their effects in these systems are not known. In 2014, the US Food and Drug Administration reported 212 total cosmetic uses of PVA, with PVP alone having 799 total cosmetic uses (Burnett, 2017). This creates further concerns that these polymers may have a concentrated presence in wastewater effluent (Julinová et al., 2018). Table 1 summarises our current understanding of these two WSPs in the context of environmental fate and impact, including their uses, predicted environmental concentrations, and known toxicities.

It was estimated in 2021 that from the US alone, 1600 ± 500 metric tonnes per year (mtu/yr) of PVA was released into the environment *via* WWTPs, with a further 3500 ± 900 mtu/yr being released into the environment *via* land application as sludge fertiliser (Rolsky and Kelkar, 2021). With PVP detected in wastewater effluent at 7.1 mg/L and downstream from a WWTP outfall at 0.16 mg/L, it was determined that realistic environmental concentrations for the two polymers resided between 0.01 and 1 mg/L, hence these were the concentrations selected for testing within the current study (Antić et al., 2011; Keller et al., 2013; Julinová et al., 2018). Evidently, real concentrations of WSP pollution will be composed of multiple PVA and PVP polymers, differing in their molecular weight (MW), structural conformation, monomer identity *etc.* For this current study, however, we tested a singular standard for each polymer with a MW of 10,000 Da to create an initial investigation into polymer effects and allow comparisons in the environmental hazard assessment of these chemicals. At 10,000 Da PVA and PVP are considered relatively low MW. Current literature on the MW of other WSPs leaching into the environment imply that lower MWs are more likely to contaminate wastewater effluents, and these smaller sizes may pose more adverse risks due to higher bioavailability and tissue accumulation potential (Nigro et al., 2023; Pauelsen et al., 2023). Moreover, PVA and PVP exposure at this small size class have previously been shown to have adverse effects on aquatic organisms (Mondellini et al., 2022). Additionally, at the MW chosen for the current investigation, both polymers have surfactant properties, allowing for an environmental hazard comparison of two commonly used surfactants in industry on a well-established freshwater disease model, helping to inform industry for more sustainable chemical use, removing MW as a variable.

It is well documented that freshwater habitats exposed to high levels of environmental pollution, such as those in the vicinity of wastewater effluent sites, possess lower invertebrate diversity (Windsor et al., 2019; Schepker et al., 2020; Baturina et al., 2021). Stable interspecific interactions between communities within ecosystems are key to maintaining overall ecosystem health, with pollution interfering with these interactions (Malik et al., 2020). Parasitic disease occurrence has increased amongst freshwater vertebrate communities inhabiting polluted systems, through perturbing host-parasite interactions as a result of the differential sensitivities of hosts and their parasites (Shen and Zuo, 2020; Baines et al., 2021; Masud and Cable, 2023). Albeit a small pool of existing evidence for aquatic WSP toxicity, previous studies highlight that WSPs decreased fitness of *Daphnia magna,* decreasing lifespan and reproductive success, implying a heightened sensitivity of invertebrates to this chemical class (Gökçe et al., 2022; Mondellini et al., 2022). Host-pathogen interactions do not always result in disease, however understanding the impact of the combined effects of abiotic and biotic stressors such as WSPs and infection will improve our understanding of the impact of these chemicals on disease susceptibility and in turn, ecosystem health.

Parasites are known to influence the physiology and life-history traits of their hosts including growth (Roberts, 1989; Barber, 2007; Mladineo et al., 2020; Purivirojkul and Songsuk, 2020), metabolism

(Lemly and Esch, 1984; Cressler et al., 2013; Timi and Poulin, 2020), and mortality (Bakke et al., 2007; Casas-Mulet et al., 2021). Pollutants such as microplastics have been shown to exert analogous effects in single exposure scenarios on freshwater vertebrates, hence in a combined exposure with parasitic infection, an additive effect would be the assumed outcome of the multi-stressor interaction (Xia et al., 2020; Lu et al., 2022; Masud and Cable, 2023). The impact of WSPs on disease susceptibility has never been investigated before. Therefore, this study evaluated the effects of WSP exposure on the life-history traits of a vertebrate and invertebrate freshwater species, and investigated the host-parasite-pollutant interactions that arise in a combined exposure to WSPs and infection. We used the well characterised Guppy-*Gyrodactylus* disease model to investigate potential multi-stressor interactions. In doing so we determined whether the interactive effects of infection and WSPs are additive (combined effect equals the sum of the two stressors in isolation), synergistic (combined effect exceeds the sum) or antagonistic where the combined effect is lower than the additive expectation (Folt et al., 1999; Turschwell et al., 2022). Although direct toxicity of this polymer class has been assessed under OECD evaluation (acute toxicity test), to the best of our knowledge and for the concentrations chosen for this study, the effects of these polymers have never been assessed for a juvenile aquatic vertebrate exposed for over 21 days, or in a multi-stressor scenario. Therefore, this study assesses how exposure to two common WSPs; PVP and PVA impact fish growth, metabolism, and susceptibility to disease as well as the off-host survival of *G. turnbulli* (Fig. 1). Based on previous works investigating microplastic exposure on host-pathogen dynamics and the impact of shorter exposures to WSP on freshwater fauna (Mondellini et al., 2022; Masud and Cable, 2023), we predict chronic WSP exposure will inflict significant impacts on *P. reticulata* life-history manifested in inhibited growth and enhanced metabolic rate, we also expect an additive interaction of parasitic infection and WSP on infection dynamics and host responses, through increasing host susceptibility to disease.

2. Methods

2.1. Host-parasite system

Gyrodactylus turnbulli, is a monogenean ectoparasite, also known as a "Russian doll killer" because of their hyperviviparous reproduction and direct contagious dermal transmission (Bakke et al., 2007; Cable et al., 2011). Their primary host, the guppy (*Poecilia reticulata*) is a small, tropical fish which is native to the streams of South America, Tobago, and Trinidad (Houde, 2019). *P. reticulata* together with *G. turnbulli* provide an established host-pathogen system suitable for monitoring disease susceptibility non-destructively (*e.g.* Smallbone et al., 2016). *G. turnbulli*, isolated from a Nottingham aquarium shop in October 1997 were used to establish a parasite population maintained in culture using established methods in our laboratory (King and Cable, 2007; Reynolds et al., 2017). The current study utilised juvenile guppies (mean standard length 8 ± 2 mm) bred from a stock originally caught in the Lower Aripo River in Trinidad in 2012, and subsequently maintained under laboratory conditions for this study. All guppies were maintained in 70 L breeding tanks at 24 °C ± 0.5 °C under a 12 h light: 12 h dark photoperiod (lights on 07:00–19:00) and fed dry food flakes (Aquarian®) and freshly hatched *Artemia* nauplii *ad libitum* prior to this experiment. To assess their susceptibility and response to infectious disease, experimental infections were performed utilizing the *Gt3* strain of *G. turnbulli* (see Cable and van Oosterhout, 2007).

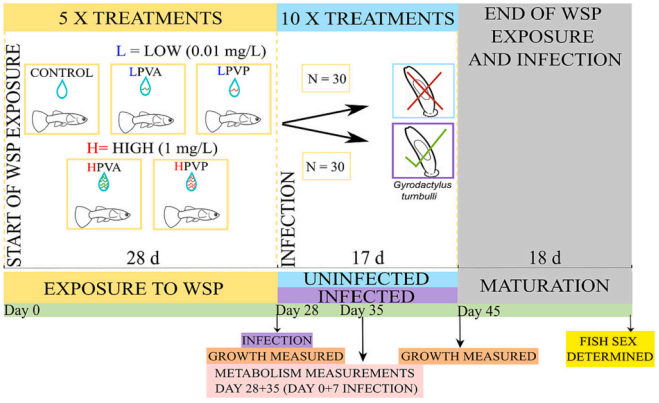

Fig. 1. Experimental design showing how *Poecilia reticulata* were exposed to individual stressors; Water-Soluble Polymer (WSP) or infection, or the combined stressors. Each treatment group was subdivided into infected and uninfected cohorts with *Gyrodactylus turnbulli* monitored every other day, while the uninfected fish were sham handled in the same manner. Host metabolism and growth were measured at two time points: one following single exposure to WSPs and the second representative of multi-stressor exposure to infection and polymer.

2.2. Preparation of PVA/PVP solutions and immersion exposure

Juvenile fish were randomly allocated to one of five treatment groups (Fig. 1): 1) Control, n = 60, 2) PVA low (LPVA; 0.01 mg/L), n = 60, 3) PVA high (HPVA; 1 mg/L), n = 60, 4) PVP low (LPVP; 0.01 mg/L), n = 60, and 5) PVP high (HPVP; 1 mg/L) n = 60 (Sigma-Aldrich). Each of the WSP treatments will herein be referred to as LPVA/LPVP and HPVA/HPVP, reflecting the low and high exposure concentrations for each polymer type. Sample sizes were chosen to achieve subsequent experimental infection sample sizes determined by power analysis (see Section 2.6). Both the PVA (CAS: 9002-89-5) and PVP (CAS: 9003-39-8) were purchased as solids from Sigma-Aldrich, both having a declared MW of 10,000 Da. PVA and PVP were each dissolved in autoclaved deionised water to create a concentrated stock solution for each. Although these polymers are not readily biodegradable, autoclaving ensured removal of any potential biodegrading microbiota in the solutions. These stock solutions were then used to dose static individual 500 mL fish tanks accordingly to achieve the low (0.01 mg/L) and high (1 mg/L) exposure concentrations for each polymer. A limitation of the current study resides with verifying the presence of polymers in the exposure tanks/animal tissue which we were unable to perform as analytical methods are still in development for these polymers, there is difficulty with detection limits and being able to quantify levels at the concentrations investigated.

A 100 % water change was conducted for all fish every alternate day at 4 pm to standardize food availability. Fish were fed every day, with either freshly hatched *Artemia* nauplii or dry food flakes (2 % body weight, Aquarian®) being administered on alternate days. For WSP exposure, juvenile fish were isolated into individual 500 mL tanks each with its own unique identifier number, and all but controls were exposed to either a low (0.01 mg/L) or high (1 mg/L) concentration of PVA or PVP using a pipette to directly dose each individual pot after every 100 % water change; these reflect concentrations 10-fold lower and higher than those found for PVP presence in freshwater environments 50 m downstream from wastewater effluent outflow, and for insoluble polymers, these levels of exposure are known to induce oxidative stress and inhibit the expression of genes relevant for growth and development in freshwater fish (Romano et al., 2020). Other than WSP exposure, control fish were maintained and handled in the same way as all other fish within this experiment.

2.3. Experimental infections

After 28 days exposure to their initial respective treatments, the additional stressor of infection was introduced. From each of the five treatments, 30 fish were randomly selected for experimental infection with *Gt3* strain of *Gyrodactylus turnbulli* parasites (Fig. 1). Parasite transfer was conducted following standard methods of King and Cable

(2007). This involved lightly anaesthetizing individual guppies with 0.02 % MS-222 briefly to allow each fish to become infected with two gyrodactylid worms by overlapping the caudal fins of a recipient fish and infected donor fish until the transfer of two worms has been observed *via* dissection microscopy. *G. turnbulli* are microscopic ectoparasites which attach only to the skin of fish, by anaesthetising fish and visually locating and counting the total number of gyrodactylid worms using microscopy every 48 h over the 17-day infection trajectory, parasite infections can be monitored and characterised through the determination of infection profiles, as this period at 24 ± 0.5 °C represents a typical complete infection trajectory of *G. turnbulli* on *P. reticulata* (see Schelkle et al., 2012). Once all the parasites were counted on an individual host, they were immediately returned to their exposure tanks. Sham infections and repeated anaesthesia were also carried out for control fish and non-infected treatment cohorts throughout the infection trajectory but without exposure to parasites. This ensured the same handling and period of anaesthesia was consistent across all treatments.

2.4. Fish growth

Fish ($n = 300$) were lightly anesthetised in 0.02 % MS-222 and their wet mass (0.001 g accuracy, OHAUS ®) and standard body length were measured on the first day of exposure (Day 0), the day of infection (Day 28) and the last day of infection and exposure period (Day 45; Fig. 1).

2.5. Respirometry

Metabolic rate was determined by measuring rates of respiration of 18 guppies per treatment at day 28 of exposure, and a multi-stressor reading of 9–12 guppies per treatment at day 35 exposure (and day 7 of infection; Fig. 1). Due to the addition of infected and uninfected cohort readings required for each treatment at the day 35 time point, sample size for this metabolism measurement was restricted by time as this effectively halved the readings possible for each treatment in one day. Guppies from each treatment were placed in 140 mL respirometer chambers. Food was withdrawn for 24 h before each fish was tested to ensure measurements were not influenced by thermal effects of food in the digestive tract. Mass specific routine metabolic rate (RMR) measurements were conducted in a respirometer (FireSting O2 meter, PyroScience, Aachen, Germany), where fish were run alongside a blank control. All water used for experimental purposes was autoclaved and temperature remained constant (24 ± 0.5 °C). The respirometer consisted of individual glass chambers (140 mL, sealed Duran™ square glass bottle with polypropylene screw cap, Fisher), each contained within the static instrument and were autoclaved and rinsed with ethanol prior to commencing each run in order to minimise background microbial respiration/algal photosynthesis levels. Oxygen concentration was then measured and recorded every second for 30 min, with 10 min acclimation and 20 min for recordings. Pilot runs with juveniles matching the size range used in this study revealed that 10 min is sufficient to allow acclimation, this period is also supported as a sufficient acclimation period in other studies measuring fish RMR (Chabot et al., 2016; Kır and Demirci, 2018). RMR was measured using the following equation:

$$\mathrm{RMR} = \Delta O_2 / fishmass(g) \times V_c$$

*V*c is the volume of the respirometer chamber and ΔO_2 is the rate of oxygen decline (Bonneaud et al., 2016) calculated as the slope of a linear regression. Fish wet mass was obtained to determine mass specific RMR.

2.6. Statistical analysis

All statistical analyses were conducted using RStudio version R-4.1.1. To determine a minimum effective sample size to detect effects of independent variables, we conducted *pwr.f2.test* power analysis within the MASS package in R (Cohen, 1988; Champely et al., 2018). This was done as all statistical modelling was based on Generalised Linear Models (GLMs) as well as the emmeans package in R. All final model selection was based on the lowest Akaike's information criterion (AIC) value (Bates et al., 2014), with the only exemption to this being quassi-Poisson models, in which case model selection was based on satisfying underlying assumptions of standardised residuals normality and homoscedastic variance. All analysis was based on raw data, and data transformation only occurred if model error structures and link functions could not satisfy model assumptions. Overall statistical analysis for growth and metabolism considered the interaction term for treatment and infection as predictor variables. For comparisons between treatment groups (Control, LPVA, LPVP, HPVA, HPVP at two different infection statuses; infected and uninfected), pairwise comparisons were extracted using emmeans *post hoc* test with multiple comparison adjustments (Tukey's honest significant difference (HSD)).

We size matched fish at the start of the experiment to ensure there was no significant differences between initial average standard lengths of fry between treatments (GLM with gaussian error family identity link function, $p > 0.05$). Length and weight gain over time were calculated by subtracting the starting standard length and weight (exposure day 0) from day 28 of exposure, corresponding to start of infection, and day 45, corresponding to day 17 of infection and the end of the experimental exposures to both WSP and infection. Models for growth analysis were run with a gaussian error structure and identity link function. Day 28 growth data was analysed using GLM with treatment as a predictive term for both length and weight gain, as this measurement was taken pre-infection this only included the control and four WSP treatments. Day 45 length and weight gain data was transformed using the Box-Cox method within the MASS package to meet model assumptions. Analysis of absolute growth for 45-day exposure to WSP included the multi-stressor GLM interaction of treatment with infection followed by *post hoc* analysis. Sex was included in the initial models but because it did not explain much variation and being non-significant, was removed from all but Day 28 weight gain GLMs (Whittingham et al., 2006; Thomas et al., 2017).

Day 28 and 35 of exposure were chosen to measure the relationship between routine metabolic rate (RMR) and treatment as these equated to the pre-infection and average peak parasite day (day 7 of infection) respectively. As with the growth data analysis, a GLM followed by *post hoc* analysis was used for Day 35 RMR to analyse the multi-stressor interaction (WSP*Infection) and identify differences between all predictor variables. Day 28 RMR of fish was transformed using the Box-Cox method within the MASS package and the GLM run with gaussian family and identity link. Data for day 35 RMR met model assumptions without transformation, the Gamma family with log link function were applied to optimise the fit of the GLM model. Sex was included in the initial models but because it did not explain significant variation, and being non-significant, was removed from the GLMs (Whittingham et al., 2006; Thomas et al., 2017).

For analysing parasite data, the following metrics were utilised: mean parasite intensity, area under curve (AUC), infection clearance time, peak parasite burden and the day this was reached (peak parasite day) and were all compared between treatments. AUC was calculated as a statistical measure for infection trajectories over time using the trapezoid rule (White, 2011) and was analysed using a GLM with a gaussian error family and identity link function. A generalised linear mixed model (GLMM) was applied to analyse the relationship between mean parasite intensity and experimental treatments. The glmmADMB package was utilised to allow for a zero-inflated mixed model to be performed using negative binomial family with log link function. Treatment and standard length were incorporated as fixed factors while Fish.ID was included as a random factor in the model to account for pseudo-replication as the same fish were screened on multiple days to determine parasite population dynamics on individual hosts. Finally, peak parasite day, peak parasite burden and infection clearance time were all analysed within a poisson GLM models using log link function to assess how treatment

affected infection dynamics. Initial poisson GLMs of peak parasite burden did not satisfy normality and homoscedastic variance assumptions, therefore a negative binomial GLM model was adopted instead. Sex and length were included in the initial models but were removed because they did not explain significant variation, length was only a significant predictor variable within the mean parasite intensity GLMM, and hence was removed from all other models (Thomas et al., 2017).

2.7. Ethics statement

All work was approved by the Cardiff University Animal Ethics Committee and conducted under UK Home Office licence PP8167141.

3. Results

3.1. Fish growth

Growth (weight and length gain) was measured prior to experimental infection on the 28th day of WSP exposure. WSPs significantly affected growth, where fish weight gain was significantly inhibited across all treatments (Fig. 2A) causing an average 12 % reduction in weight gain of juvenile fish after 28 days (GLM: LPVA; $t = -3.9$, SE = 0.001, $p = 0.0001$, LPVP; $t = -4.0$, SE = 0.001, p = 0.0001, HPVA; $t = -3.8$, SE = 0.001, $p = 0.0002$, HPVP; $t = -2.3$, SE = 0.001, $p = 0.02$). LPVP was the only treatment where fish were significantly smaller in length than the control fish (GLM: LPVA; $t = -0.4$, SE = 0.1, $p = 0.72$, LPVP; $t = -2.2$, SE = 0.1, $p = 0.03$, HPVA; $t = -0.1$, SE = 0.1, $p = 0.95$, HPVP; $t = 1.2$, SE = 0.1, $p = 0.22$).

Growth was measured again on day 45, the last day of WSP exposure. Juvenile *P. reticulata* within the control treatment had grown an average of 3.8 mm and gained 0.02 g in weight over the 45 days. Reduction in absolute growth at this time point was only significant in LPVP exposed fish (Fig. 2A), and the weight gain of juveniles within PVA treatments were no longer significantly different to control fish (*post hoc*: LPVA; $t = 0.8$, SE = 0.001, $p = 0.99$, LPVP; $t = 3.3$, SE = 0.001, p = 0.03, HPVA; $t = 1.3$, SE = 0.001, p = 0.95, HPVP; $t = 1.8$, SE = 0.001, $p = 0.73$). Although LPVP exposed fish had inhibited weight gain over the 45-day exposure period, HPVP did not inhibit *P. reticulata* weight gain. Length gain at day 45 amongst all WSP treated fish was not significantly different from the controls (*post hoc*: LPVA; $t = -0.83$, SE = 1.2, p = 0.99, LPVP; $t = 1.6$, SE = 1.3, $p = 0.87$, HPVA, $t = -0.58$, SE = 1.2, p = 0.99, HPVP; $t = -1.0$, SE = 1.2, p = 0.99).

Exposure day 45 also corresponded with the last day of the infection trial. Parasitic infection alone caused significant reduction of juvenile weight gain, decreasing average growth of fish by 10 % length and 20 % weight. Although this trend was consistent for all infected fish within WSP treatments, parasitic infection only caused significant reduction in weight gain for control and LPVA exposed fish (*post hoc*: Control; $t = 4.6$, SE = 0.001, p = 0.0002, LPVA; t = 3.3, SE = 0.001, $p = 0.03$, LPVP; $t = -1.8$, SE = 0.001, $p = 0.75$, HPVA; $t = 2.2$, SE = 0.001, $p = 0.44$, HPVP; $t = 1.2$, SE = 0.001, $p = 0.98$). However, combined exposure to infection and WSP revealed an interactive effect of PVP and infection with *G. turnbulli* on guppy growth over 45 days exposure (Fig. 2B). A significant antagonistic interaction was evident for weight gain in fish exposed to both PVP and infection (GLM: LPVA; $t = 1.0$, SE = 0.001, $p = 0.34$, LPVP; $t = 2.0$, SE = 0.001, $p = 0.048$, HPVA; $t = 1.7$, SE = 0.001, $p = 0.09$, HPVP; $t = 2.5$, SE = 0.001, $p = 0.014$).

3.2. Fish metabolism

Routine metabolic rate (RMR) was measured prior to experimental infection on day 28 of exposure to WSP and on the average peak of infection (post-infection day 7/day 35 WSP exposure). Metabolic rate of control fish averaged at 0.79 mg/O_2 g^{-1} h^{-1} on day 28, 0.42 mg/O_2 g^{-1} h^{-1} on day 35. After 28 days exposure to WSP, on average juvenile *P. reticulata* RMR was 52 % higher than that of controls. After 35 days

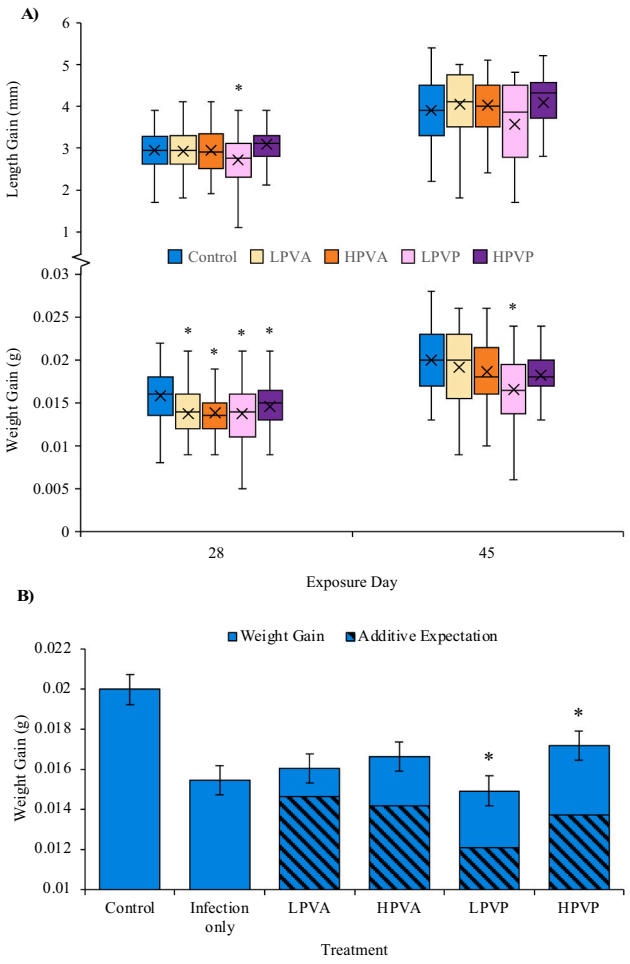

Fig. 2. A) Box and whisker plots (mean = 'X', upper and lower median quartiles = box, variability outside the quartiles = whiskers) of the total length (top row) and weight gain (bottom row) of juvenile *Poecilia reticulata* over the 45-day experimental period. Growth was measured after 28 ($n = 60$) and 45 days WSP exposure (control, $n = 27$; LPVA, $n = 29$; HPVA, $n = 29$; LPVP, $n = 26$; HPVP, $n = 29$). Significant differences in growth against control are indicated for each time point by asterisks (*) representing significance levels of $p < 0.05$. B) Bar chart (mean ± standard error) indicating the non-additive interaction between weight gain of juvenile *Poecilia reticulata* when infected with parasite *Gyrodactylus turnbulli* and the combined stressor treatments of WSP + parasitic infection (control, $n = 27$; infection, $n = 30$; LPVA + infection, $n = 29$; HPVA + infection, $n = 29$; LPVP + infection, $n = 29$; HPVP + infection, $n = 28$). The hatched bars indicate the expected additive interaction response with asterisks (*) indicating a significant antagonistic interaction ($p < 0.05$).

exposure, average RMR of WSP treated fish had increased to 270 % higher than control fish. Coincidently, infection alone had the same effect on fish RMR, with a 270 % increase in RMR compared to controls. The two stressors in combination had a reverse effect, only increasing fish RMR by 170 %; less than half of what was expected additively within the combined treatment, indicative of an antagonistic interactive effect of WSP and infection on fish routine metabolic rates.

Exposure to WSP for 28 days elevated metabolic rates significantly in fish exposed to PVA (GLM: LPVA; t = 2.2, SE = 0.02, $p = 0.03$, HPVA; $t = 3.0$, SE = 0.02, $p = 0.004$) and LPVP (GLM: $t = 2.4$, SE = 0.02, $p = 0.02$), but not those exposed to HPVP (GLM: t = 1.2, SE = 0.02, $p = 0.23$). Fish metabolism was measured again on day 35 of exposure (corresponding to day 7 infection), where a shift in the effect of WSP was observed for fish metabolism (Fig. 3A). HPVP now had the largest effect, significantly enhancing RMR (*post hoc*: $t = -9.6$, SE = 0.2, $p < 0.0001$), followed by HPVA (*post hoc*: $t = -7.4$, SE = 0.2, p < 0.0001) and LPVA (*post hoc*: $t =$

−4.4, SE = 0.2, $p = 0.002$), with LPVP having no significant impact (*post hoc*: $t = -1.1$, SE = 0.2, $p = 0.99$).

Multi-stressor exposure to WSP and infection had an interactive effect on fish RMR across all treatments (Fig. 3B), whereby an antagonistic effect on fish RMR was observed for all combined treatments (GLM: LPVA; $t = 3.1$, SE = 0.3, $p = 0.003$, LPVP; t = 2.4, SE = 0.3, $p = 0.02$, HPVA; $t = 7.4$, SE = 0.3, p < 0.0001, HPVP; $t = 7.8$, SE = 0.3, p < 0.0001). Infection with *G. turnbulli* caused significantly increased RMR in controls (*post hoc*: $t = 6.5$, SE = 0.2, $p < 0.0001$), however this increase was not seen across all WSP treatments. Infection of the LPVP exposed fish also caused higher RMRs than their equivalent uninfected cohort (*post hoc:* LPVP; $t = 3.4$, SE = 0.2, p = 0.03). In contrast, infected fish within the LPVA treatment had a non-significant increase in RMR (*post hoc*: LPVA; $t = 2.3$, SE = 0.2, $p = 0.42$), but a significantly decreased RMR was evident in infected fish exposed to HPVA and HPVP when compared to their uninfected cohorts exposed to WSP for 35 days (*post hoc*: HPVA; $t = -4.0$, SE = 0.2, $p = 0.005$, HPVP; $t = -4.5$, SE = 0.2, $p = 0.0008$).

3.3. Host-parasite interactions

Host standard length significantly affected mean parasite intensity only (GLMM: z = 2.01, SE = 0.1, $p = 0.045$). WSP exposure did not significantly impact parasite intensity (GLMM: LPVP; z = −1.19, SE = 0.2, p = 0.23, HPVP; z = −0.39, SE = 0.2, $p = 0.67$, HPVA; z = −0.67, SE = 0.2, $p = 0.5$), although infected fish exposed to LPVA had reduced parasite burdens compared to control fish which was marginally non-significant (GLMM: z = −1.68, SE = 0.2, $p = 0.09$, supplementary Fig. S1). WSP exposure did not affect AUC, maximum parasite count nor time taken for juvenile *P. reticulata* to clear their infections ($p > 0.05$). However, there were significant differences in the peak day of infection between treatments. Control fish reached their peak parasite burden on day 7 of infection, whereas LPVA exposed fish achieved peak burden within 5 days (GLM: z = −2.3, SE = 0.6, p = 0.02), LPVP also had a marginally non-significant peak burden on day 5 of the infection trial (GLM: z = −1.8, SE = 0.6, $p = 0.07$).

There were no significant differences in fish survival between treatments as no fish mortality occurred. However, WSPs did significantly impact off-host survival of the parasite *Gyrodactylus turnbulli.* A significantly higher proportion of parasites within the WSP (1 mg/L) treatments died compared with control worms, the most premature parasite deaths occurred in the PVP treatment ($p < 0.0001$). All parasites in the WSP treatments died within 26 h, whereas worms in the control treatment survived for an additional 3 h. PVP exposed worms died earliest, with 46 % dying within 20 h, compared to 25 % in PVA and control. (Coxph: PVA; Coef = 0.19, Z = 3.6, PVP; Coef = 0.35, z = 6.53, p < 0.0001, Fig. 4).

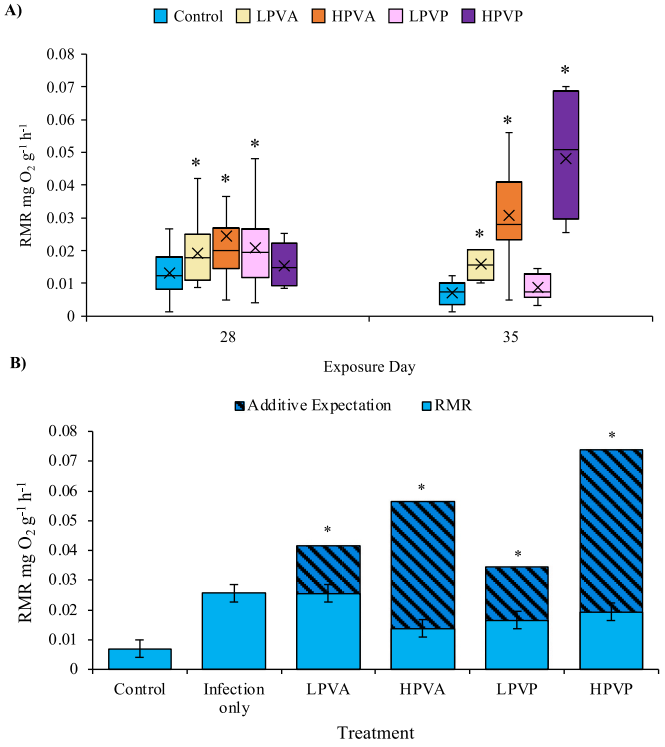

Fig. 3. A) Box and whisker plots (mean = 'X', upper and lower median quartiles = box, variability outside the quartiles = whiskers) of routine metabolic rate of juvenile *Poecilia reticulata* exposed to WSP only for 28 ($n = 18$) and 35 days, ($n = 9$). Significant differences against control are indicated for each time point by asterisks (*) representing significance levels of $p < 0.05$. B) Bar chart (mean ± standard error) indicating the non-additive interaction between routine metabolic rate of juvenile *Poecilia reticulata* after a 35 day exposure to polyvinyl alcohol (PVA) or polyvinylpyrrolidone (PVP) and infection with *Gyrodactylus turnbulli* for 7 days (control, n = 18; infection, n = 9; LPVA + infection, n = 9; HPVA + infection, n = 9; LPVP + infection, n = 12; HPVP + infection, n = 9). The hatched bars indicate the expected additive interaction response with asterisks (*) indicating a significant antagonistic interaction (p < 0.05).

4. Discussion

Like microplastics, WSPs are a diverse chemical class of polymers which are largely synthetic, mass produced and used ubiquitously across most modern industries, where the majority of WSPs produced globally are used in wastewater treatment processes and the detergent industry (Fortune Business Insights, 2021). As a result, there is mounting concern that they have a significantly high presence within wastewater effluent and therefore are an emerging contaminant in freshwater systems (Julinová et al., 2018). Our results imply exposure to environmentally predicted concentrations of WSPs may be negatively impacting aquatic vertebrate life-history traits through enhanced energetic cost and inhibited growth. We also highlight a heightened sensitivity of invertebrates to WSPs in the current study, due to the significantly enhanced mortality rate seen for *Gyrodactylus turnbulli*. WSPs are dubbed 'relatively harmless' compounds, having been investigated exclusively in conventional chemical testing methods receiving LD_{50} scores between 15,000–100,000 mg/kg for small mammals (DeMerlis and Schoneker, 2003; Kurakula and Koteswara Rao, 2020). That is until 2022, where both Gökçe et al. (2022) and Mondellini et al. (2022) found chronic effects of PVA at 5 mg/L on *Daphnia magna*, where Mondellini et al. (2022) also found PVP to exert similar effects, significantly reducing reproductive success of *D. magna*. Nigro et al. (2022) testing the effects of a low-level exposure of PVA on a freshwater fish, *Danio rerio,* found no significant differences on swimming behaviour and a neurotoxicity enzymatic biomarker, however more recently, Nigro et al. (2023) found significant effects of PVP on *D. rerio* swimming behaviour, where hypoactivity was observed. These studies implied an enhanced sensitivity of invertebrates to these polymers at low levels of exposure, as well as inferring that chronic exposures may have cumulative detrimental effects for aquatic vertebrates, not initially obvious within acute exposures. The current study is the first account of predicted environmental concentrations of PVA and PVP showing significant detrimental impacts on invertebrate and vertebrate life-history traits, manifested in significantly decreased survival of the invertebrate parasite, *G. turnbulli* as well as inhibited growth at an enhanced energetic cost to juvenile *Poecilia reticulata,* at levels as low as 0.01 mg/L.

Differential sensitivities to the two types of WSP used in the current study were evident for *P. reticulata*. After 28 days exposure to WSP all juveniles experienced inhibited growth implying enhanced susceptibility of *P. reticulata* to this pollutant class in earlier stages of development, as although significant reduction in the growth of juvenile fish exposed to PVA was detected at day 28, this effect had dissipated at 45 days of

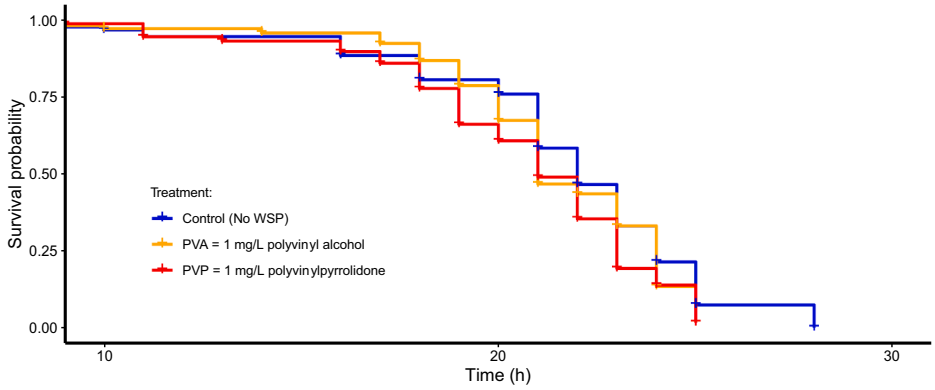

Fig. 4. The probability distribution of *Gyrodactylus turnbulli* off-host survival when exposed to water-soluble polymers polyvinyl alcohol and polyvinylpyrrolidone ($n = 40$, $p < 0.0001$).

exposure. In contrast, PVP effects remained, and potentially worsened, with increased exposure time. PVP consistently inhibited growth throughout the duration of this study, which together with the significantly enhanced routine metabolic rate for PVP exposed fish at day 45 but not 28, may imply that PVP effects may worsen with prolonged exposure. While the growth of *P. reticulata* at later developmental stages was unaffected by PVA, this delayed effect of PVP on juvenile guppies is concerning. Guppies can rapidly adapt to altered biotic conditions; this has previously been shown for other ubiquitous stressors such as oil pollution (Rolshausen et al., 2015). However, the significant energic costs of PVP exposure for fish were not evident unless experiencing prolonged exposure, where enhanced metabolic rate is indicative of increased stress levels and energy demands for growth. Similar effects have been shown to be caused by microplastic exposure in aquatic vertebrate species where interrupted lipid metabolism and resultant disruption to energy reserves occurs for European bass, and in zebrafish, plastic exposure caused inhibited ATP production, resulting in a significantly reduced energy budget (Brandts et al., 2018; Dimitriadi et al., 2021). Due to its binding and complexing properties, PVP could form flocs with particulate feed, condensing solid matter pre-ingestion or inside the gut of fish post-ingestion, causing a reduced surface area and lowered nutritional uptake resulting in reduced growth, functioning in a similar way to slow-release drugs and fertilisers in which WSPs are usual constituents (Kranz and Wagner, 2006; Antić et al., 2011; Chen et al., 2018). Longer exposure investigations into PVP are imperative to appropriately assess risk of this potential emerging pollutant in freshwater environments. While the current study provides evidence that PVA could present a less harmful alternative polymer for surfactant use in industry, alternative agents should be considered to replace PVP.

Routine metabolic rate of *P. reticulata* was enhanced in all but one WSP treatment at each time point. Exposure to xenobiotics triggers an overproduction of reactive oxygen species (ROS) causing oxidative stress in exposed organisms leading to the activation of defensive responses (Dawood et al., 2020). PVA and PVP are known to increase ROS production in aquatic fauna, meaning the energy required for basal metabolism is much higher due to partitioning of energy reserves for protective, energy expensive physiological pathways (Mondellini et al., 2022). Hence enhanced RMR is likely to imply an enhanced ROS production in fish. At both concentrations, PVA exposure caused enhanced metabolic rate in fish at both 28 days and 45 days, this complements the findings of Mondellini et al. (2022), where exposure to PVA caused an increase in ROS production in *D. magna*, implying enhanced stress. Fish exposed to 0.01 mg/L PVP for 45 days and 1 mg/L PVP for 28 days did not show evidence of enhanced stress or energy demand despite experiencing significant reduction in growth, as the RMR of these fish was not significantly different from control. In the few existing chronic toxicological assessments of PVP toxicity, no significant effects were seen for *Daphnia* exposed to concentrations below 5 mg/L for 21 days (Mondellini et al., 2022). There are such low safety concerns, that EFSA have approved PVP as a food additive for several years, determining there is no need for numerical values for acceptable daily intake (EFSA, 2010, 2020). More recently however, Nigro et al. (2023) demonstrated PVP causes significant impairment of swimming performance in *Danio rerio* embryos not only at the high concentration also applied in the current study (1 mg/L), but also at 0.001 mg/L, suggesting the potential for PVP to be acting as a neuroactive substance. This may explain the unexpected low RMR measured for fish exposed to LPVP for 45 days and HPVP for 28 days. Low RMR may reflect hypoactivity of juvenile guppies as seen in Nigro et al. (2023), as fish experiencing decreased mobility would have a reduced energy demand.

Dose-dependent effects were evident for PVP at the two concentrations chosen for the current study. While LPVP inhibited fish weight gain at both measured time points, HPVP did not significantly inhibit the weight gain of fish after 45 days exposure. This is comparable with the work of Mondellini et al. (2022) where PVP was found to significantly increase growth of *Daphnia* at 10 mg/L but did not impact growth at lower exposure concentrations. Albeit a different WSP, the same study also found significant inhibition in *Daphnia* growth when exposed to PVA at 5 mg/L but not at the higher tested concentration of 10 mg/L. This was also seen for zebrafish embryos exposed to different concentrations of PVP, where swim behaviour at specific light conditions was significantly altered at 0.001 mg/L exposure concentration but not at 0.5 mg/L (Nigro et al., 2023). For *Daphnia* these observed effects were attributed to the phenomenon of hormesis where at low exposure concentrations, harmful substances give stimulating, beneficial effects on exposed organisms (Sakai, 2006; Mondellini et al., 2022). Furthermore, in the current study at exposure day 45, there was no effect of LPVP on the metabolic rate of fish, meanwhile HPVP caused a significant increase in RMR of fish. These effects could imply a dose-dependent effect of PVP on ROS production in *P. reticulata*. While it is a limitation of the current study to have only investigated two concentrations of these polymers, once more accurate detection methods are defined, future work should focus on characterising the adverse effects of these polymers at the incremental concentrations within a range at which they are detected in differing environmental compartments not limited to surface water concentrations.

The combination of infection and pollutant stressors in this study had an unexpected interactive effect on *P. reticulata* growth and metabolic rate. Infected fish exposed to WSPs had higher weight gain than control infected fish, where there was a significant antagonistic interaction between infection and PVP on fish weight gain. Moreover, an antagonistic interaction was also evident between WSP and infection on the metabolic rate of fish. Exposure to the high concentrations of both WSPs resulted in a significant reduction in metabolic rate of fish also exposed to infection, the reverse effect of single exposure to the respective stressors. This surprise interaction between WSP and infection with

G. turnbulli could be explained by the action of the parasites on the host. Gyrodactylid parasites primarily feed on epidermal cells and mucus (Olafsdottir and Buchmann, 2004; Bakke et al., 2007). In some scenarios, PVA and PVP formulations can be used as surfactants and flocculating agents respectively, and could therefore be interacting with the fish's surface, perhaps integrating with the mucosal layer, preventing the ectoparasite *G. turnbulli* effectively attaching, moving, and feeding on the fish's surface. A fish mucus-WSP matrix may prevent *G. turnbulli* ingesting sufficient nutrients from the mucus layer, promoting the maintenance of mucosal goblet cells throughout infection, which otherwise deplete during *Gyrodactylus* infection (Shephard, 1994). Moreover, PVP is an ingredient used within STRESS COAT™; a commercial product within the aquaculture industry which forms a 'synthetic slime coating on fish' helping to reduce fish stress and promotes epidermal healing, which marries with the suggestions that WSPs reinforce the mucus layer providing a synthetic coating, preventing worms from feeding effectively on fish epidermis. By providing this synthetic barrier, WSPs may reduce the energy costs of infection with *G. turnbulli*, allowing for enhanced growth and reduced metabolic costs for *P. reticulata* than those exposed to infection only, hence the antagonism observed in the current study.

The low concentrations of WSP (0.01 mg/L) resulted in an earlier peak infection day for fish, likely due to enhanced mortality of the worms by WSP and largely unaffected disease susceptibility of hosts at these low levels of WSP exposure. However, at higher concentrations of WSP, lower parasite burdens could be expected due to enhanced mortality of the parasites, however this was not observed. This could convey an increased disease susceptibility of hosts at these levels of WSP exposure, signifying a trade-off between enhanced disease susceptibility of the host and heightened mortality rates of the parasites. At low levels, WSP exposure could benefit infected fish by reducing the survival of gyrodactylids. Generally, parasites are more susceptible to pollutants than their hosts (Blanar et al., 2009). Hosts in polluted environments have been known to be at a potential advantage compared to uninfected individuals; infected European Chub exposed to POPs, for example, exhibited reduced oxidative damage compared to fish not infected with parasitic worms (Molbert et al., 2020). However, disease dynamics for WSP exposed fish was concentration dependent, where parasite burdens of fish exposed to the higher concentrations of WSP were not significantly different from the control, despite the concentration of WSP being 100-fold higher. This could be attributed to the effect of WSP on *G. turnbulli* survival, which also complements the lower metabolic rate identified for fish experiencing the combined exposure to WSP and infection, implying reduced stress. Analogous to our findings, the effects of parasitism in polluted environments for fish hosts were seen to be dose-dependent by Molbert et al. (2021), where oxidative stress was enhanced in parasitised European Chub exposed to low PAH levels but was significantly reduced amongst infected fish when the exposure was increased 100-fold. The pollutant-host effect may be enhancing disease susceptibility of the host; however, the pathogen-stressor interaction could be reducing the parasites infection success and in turn, reducing host stress and energetic costs of infection.

5. Conclusion

This study adds to the emerging pool of evidence that the numerous chemical classes of water-soluble polymers require more substantial environmental risk assessment. Future studies could consider the effects of these polymers at the community level, as their chemical properties may be altering biotic interactions within food webs as implied in this study, manifested with interspecific species interactions such as parasitism, and perhaps the bioavailability of food. This study shows concentrations of WSP as low as 0.01 mg/L cause significant disruption to invertebrate and vertebrate life-history traits. Finally, parasites are an integral part of every food web, this is the first study investigating how this chemical class impacts a parasite, more work is needed to determine the complex interaction which may be influencing WSP impact on host stress and disease dynamics. Here, we highlight the significant detrimental impacts of polyvinyl alcohol and polyvinylpyrrolidone on vertebrate and invertebrate life-histories and exhibit the disruption potential of water-soluble polymers for biotic interactions within aquatic ecosystems. Evidently, these are just two chemical standard examples of the soluble polymer class; the molecular weight of 10,000 Da was selected to directly compare the behaviour of the polymers and as to not add further variable into the study. Future studies on how molecular weight might impact polymer behaviour would therefore be an interesting addition to the field. Nonetheless this research highlights the need for further environmental fate and impact studies for WSPs to determine whether polymers within this class require consideration within the recent EU Commission review on polymer registration. While negative impacts were highlighted in the current study, it was determined that PVP in this instance appeared to be more detrimental than PVA, which conveys the potential for appropriate hazard and exposure assessment to result in the favourable substitution of polymers in this vast chemical class. However, before this can be addressed, accurate determination of environmental concentrations and fate is needed to begin investigating widespread environmental impact.

CRediT authorship contribution statement

Charlotte Robison-Smith: conceptualisation, project administration, methodology, data analysis, writing - original draft preparation and editing; **Eve C. Tarring:** methodology, writing - review and editing; **Numair Masud**: supervision, writing - review and editing; **Benjamin D. Ward:** conceptualisation, writing - review and editing; **Jo Cable:** conceptualisation, supervision, writing - review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168086.

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