Assessment of the Effects of Environmental Concentrations of Microplastics on the Aquatic Snail *Potamopyrgus antipodarum*

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Abstract Microplastics are ubiquitous in aquatic ecosystems. They can be found at the surface, in the water column, and in sediments. Multiple negative impacts of microplastics on aquatic organisms have been reported, with most studies focusing on marine ecosystems. However, the effects of microplastics on freshwater ecosystems have been less studied, with a few studies focusing on benthic invertebrates. In this study, we exposed the New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843) to an environmental range of concentrations of polystyrene microparticles (size range from 0.01 to 514 µm at 100, 500, and 1000 mg microplastics/kg dry weight (dw) of sediment) and two supra-environmental concentrations (2000 and 4000 mg/kg dw sediment). The impacts of the exposure to microplastics on mortality, behavior, and reproduction were assessed at long-term exposure (31 days). Mortality and reproduction were not significantly affected by microplastics. On the contrary, most of the microplastic treatments altered the behavior, causing a significant increase in reaction time compared with controls (0 mg microplastics/kg dw sediment). The highest concentration (4000 mg/kg) did not have an impact on the reaction time over the experimental period compared with

controls. To our knowledge, this study is the first to assess the effects of microplastics on the behavior of the aquatic snail *P. antipodarum*. Our results showed that at environmental concentrations, the behavior of *P. antipodarum* was the most sensitive variable to the adverse effects of polystyrene microplastics.

Keywords Behavioral toxicology · Freshwater toxicology · New Zealand mud snail · Polystyrene · Reproductive toxicology

1 Introduction

Since mass production of plastics began in the 1940s, millions of tons of plastic are produced each year worldwide, and a significant amount ends up in aquatic ecosystems (Burns & Boxall, 2018; Cole et al., 2011). Besides, because of their physical and chemical properties, the complete breakdown of plastic in the environment can take centuries (Cole et al., 2011). Because of these issues, there is a significant increase in the number of plastics in aquatic ecosystems (León-Muez et al., 2020). This fact may increase the potentially harmful effects of plastics on the structure and the function of aquatic ecosystems (Burns & Boxall, 2018; Eerkes-Medrano & Thompson, 2018; Gouin et al., 2019). Additionally, the potential risk to human health has also risen in the last years (Burns & Boxall, 2018; Gouin et al., 2019).

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Due to several environmental factors, such as mechanical forcing or photochemical action, larger plastic items may disintegrate into smaller particles (<5 mm), which are known as microplastics (MPs) (Arthur et al., 2009; Thompson et al., 2004). MPs generated from these processes are known as secondary microplastics. However, this is not the only source of generation of MPs, as they are also directly manufactured (Au et al., 2015). These are known as primary microplastics.

MPs and plastics are ubiquitous contaminants that can be found in all compartments of aquatic ecosystems, from the water surface to the water column and sediments (Burns and Boxall, 2018). Most studies focus primarily on marine ecosystems (Burns & Boxall, 2018). Nevertheless, freshwater ecosystems are important reservoirs and transport routes of MPs and plastics (Klein et al., 2015). For instance, Lebreton et al. (2017) determined that plastics reach the oceans mainly through rivers.

One of the main threats of MPs is their small size, which may facilitate their uptake by a wide range of species through feeding activity, dermal uptake, and respiration (De Felice et al., 2019). As a consequence, MPs may translocate through the food chain from the most basic levels, thus posing a potential risk to biodiversity (Cole et al., 2011; Du et al., 2020). In addition, MPs usually contain organic contaminants added during their manufacture (Teuten et al., 2009). Moreover, their composition and relatively high surface area allow the adsorption of hydrophobic organic toxicants already present in the environment, constituting a potential source of bioaccumulation of toxicants in the food chains (Cole et al., 2011), although the evidence is inconclusive so far (Burns & Boxall, 2018).

Several studies have reported the negative effects of MPs on the aquatic fauna. Some studies have found negative impacts at the sub-organism level, such as inflammation, liver stress, and disturbance of the liver's metabolic profiles, oxidative stress, lower levels of steroid hormones, blockage of enzyme production, and adsorption of toxicants (Auta et al., 2017; Lei et al., 2018; Yao et al., 2019). Other authors have reported the negative effects at the organism level, such as hindrance of movement due to the aggregation of MPs to the body surface, obstruction of the digestive tract, decreased hunger stimulus, delayed ovulation, reproductive failure, and reduced growth

rate (Auta et al., 2017; Du et al., 2020; Foley et al., 2018; Setälä et al., 2016). However, other studies have not found definitive evidence on the impacts of MPs on organisms (Burns & Boxall, 2018), suggesting that the susceptibility to MPs may be species-specific (Redondo-Hasselerharm et al., 2018). A meta-analysis concluded that zooplankton, an essential link in the aquatic food chains, is the most susceptible biota to MP exposure (Foley et al., 2018). This fact could have significant impacts on food webs and trophic cascades (Foley et al., 2018; Pace et al., 1999).

The ecotoxicological study of MPs is complex due to the diversity of compounds and sizes. However, some of the most widely used plastics in ecotoxicological tests are polystyrene, polyethylene, polypropylene, or polyvinyl chloride (Burns & Boxall, 2018), since they are widely used plastics for manufacturing throw-away products (do Sul et al., 2014). They are also the main sources of MPs in ecosystems (Du et al., 2020; Klein et al., 2015). In fact, Klein et al. (2015) found that 54% of MPs sampled in the Rhine and Main Rivers were polystyrene, and Ballent et al. (2016) observed that polystyrene was the second most common plastic in the samples of Lake Ontario.

Particle types and sizes are also important factors to consider, since they may influence the results obtained in ecotoxicological studies (Lei et al., 2018). For an appropriate environmental risk assessment, it is recommended to use MP types and sizes that mimic realistic scenarios. However, Burns and Boxall (2018) have warned of the use of smaller particle sizes than those detected in the environment (e.g., <131 µm) in many studies. On the other hand, authors often employed homogeneous spherical MPs in bioassays (Burns & Boxall, 2018). However, it would be more realistic to use irregular MP fragments and fibers as these types of MPs are the most abundant in environmental samples (Burns & Boxall, 2018; He et al., 2020; Phuong et al., 2016).

Depending on their density and that of the water, MPs can be buoyant, like many pure hydrocarbon polymers (e.g., polyolefins, with a density range of 900–960 kg/m³: polypropylene, low-density polyethylene, linear low-density polyethylene), or non-buoyant (major polymers containing elements other than hydrogen and carbon) (Reisser, 2015). However, the density of buoyant MPs may be increased through biofouling (Lobelle & Cunliffe, 2011), which facilitates MP deposition on sediments and their

bioavailability for benthic invertebrates (Haegerbaeumer et al., 2019; Redondo-Hasselerharm et al., 2018). In fact, it has already been proven that sediment-dwelling and benthic grazer invertebrates are capable of ingesting MPs and nanoplastics (do Sul & Costa, 2014; Imhof et al., 2013; Scherer et al., 2017; Setälä et al., 2016). Despite this, most studies analyze the effect of non-buoyant polymers on fish (Imhof & Laforsch, 2016) and a few the impacts of MPs on benthic invertebrates.

Previous studies have already reported negative impacts on some biological traits of benthic invertebrates (Au et al., 2015; Lei et al., 2018; Redondo-Hasselerharm et al., 2018). Among them, gastropods are often exposed to MPs through their diet (Akindele et al., 2019). They play an important ecological role in aquatic ecosystems due to their rich biodiversity and ubiquitous distribution (Oehlmann & Schulte-Oehlmann, 2003). For instance, they are important decomposers and contribute to a large amount of biomass in the food chain, as well as being prey for a large number of species (Oehlmann & Schulte-Oehlmann, 2003). Therefore, aquatic gastropods may serve as important vectors for the transmission of microplastics through the food chain (Naji et al., 2018). For the reasons stated above and others, such as their sensitivity to a wide range of environmental toxicants, aquatic gastropods are considered as suitable bioindicators of pollution (Alonso & Camargo, 2013; Oehlmann & Schulte-Oehlmann, 2003). One of the most widely used in ecotoxicology is the New Zealand mud snail *Potamopyrgus antipodarum* (Alonso & Camargo, 2009, 2013). This deposit-feeder invertebrate is globally distributed throughout freshwater ecosystems (Alonso & Castro-Díez, 2012), and therefore, it may be often exposed to MPs. In fact, it has already been shown to be capable of ingesting MPs (Imhof et al., 2013).

Many studies focus on the effects of MPs on survival and reproduction. However, the behavior should also be a parameter to consider, as it is closely linked to fitness, survival, and physiological alterations (Hellou, 2011). Regarding *P. antipodarum*, there are no studies that assess the impacts of MPs on the behavior of this snail. As behavior may be altered at sub-lethal concentrations of compounds, causing deleterious effects on the organism's fitness (Alonso & Camargo, 2009; Scott & Sloman, 2004; Wang et al.,

2020), the assessment of behavior is important to obtain a broader and more realistic understanding of the influence of environmental concentrations of toxicants on organisms (Alonso & Camargo, 2009).

The aim of this study focuses on the assessment of polystyrene MPs on the aquatic mollusk *Potamopyrgus antipodarum*. To this end, we analyzed the effects of polystyrene microparticles on the mortality, behavior (the number of immobile animals, reaction time, and spatial distribution of individuals), and reproduction of this species. We hypothesized that MPs would cause negative effects on the studied parameters and that the increase of the MP concentration would intensify the deleterious effects.

2 Materials and Methods

2.1 Microplastic Acquisition

Polystyrene microparticles were provided by Miguel González Doncel from INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria). MPs were obtained by processing polystyrene test tubes without additives. These tubes were firstly ground by means of a hammer and finally pulverized by means of a ball mill. The particle size of polystyrene microplastics was analyzed through a Mastersizer 3000®. Microplastic sizes ranged from 0.01 to 514 µm. The most frequent particle size was 129 µm (19.45%), while 77.4% of the particles ranged between 42.9 and 214 µm.

2.2 Culture of *P. antipodarum*

Animals for this experiment were obtained from two 60-L glass aquaria with control water (moderately hard USEPA: 96 mg NaHCO₃, 60 mg CaSO₄·2H₂O, 4 mg KCl, 122.2 mg MgSO₄·7H₂O/L of deionized water) enriched with calcium carbonate (10 mg CaCO₃/L of deionized water) (United States Environmental Protection Agency, 2002). The density of the control water was ~1 g/cm³. We randomly selected 288 adults with a mean ($\pm$ SD) shell length of 3.9 $\pm$ 0.2 mm. Snails were distributed randomly in three acclimatization glass vessels (1.25 L) with control water. Individuals were subjected to an acclimatization period of 120 h in a climatic chamber at

18 °C (ANSONIC®VAC0732). After 48 h, 10 pellets (JBL, NovoPrawn, GmbH & Co. KG, Germany) were added to each glass vessel as food, and the water was renewed after 4 h. The activity of snails was measured (see “Sect. 2.4”) before translocating the animals to the different treatments.

2.3 Experimental Design

Six treatments were established (Fig. 1). All of them consisted of 51.3 g dry weight (dw) of sediment and 110 mL of control water. Washed river sand with a size range from 0.5 to 1 mm was used as sediment. Each treatment had a different concentration of MPs (Fig. 1). We selected three reported environmentally realistic nominal MP concentrations (He et al., 2020; Klein et al., 2015): 100, 500, and 1000 mg MP/kg dw of sediment, and two supra-environmental nominal concentrations of 2000 and 4000 mg MP/kg dw sediment plus a control (0 mg MP/kg dw sediment). Each treatment was replicated eight times with six randomly selected snails per replicate (Fig. 1). Glass vessels (volume 0.23 l; height 6 cm; diameter 8 cm) were used as experimental units. They were covered with perforated Petri dishes to reduce water evaporation. Also, three extra replicates with the previous characteristics but without MPs and snails were elaborated to measure the water temperature and water conductivity. Both parameters were measured with a conductivimeter (CM 35+, Crison Instruments) and a portable oximeter (OXI 45+, Crison Instruments) respectively at the end of each week. All replicates were kept at 18 °C throughout the experimental period in a climatic chamber (ANSONIC®VAC0732). These parameters were monitored with a thermometer twice a week and with a climatic recorder (PCE Instruments) every 3 h.

Before adding the water, MPs were included in each treatment (Fig. 1). However, since the polystyrene density ranges from 0.96 to 1.05 g/cm³ (Lambert & Wagner, 2018), some particles did not sink and attach to the glass walls of test vessels. Therefore, we devised an air pumping system to detach MPs from the walls and to force them into the water column and subsequently on the sediment (Fig. 1). This increased its bioavailability for snails since MPs were finally distributed throughout the replicates (in the water surface, in the water

column, and on sediments). For this purpose, we used portable air pumps (Eheim 400, Sera Precision Air 55R plus, and Sera Precision Air 275R) and silicone tubes (Fig. 1). Pipette tips with parafilm were installed at the end of the tubes, and they were inserted into the middle of the water column to avoid disturbing the sediment. This procedure was carried out a week prior to the experiment. The laboratory temperature (including the maximum and minimum temperatures) was monitored with a thermometer.

2.4 Monitored Parameters

We monitored the sensitivity of the New Zealand mud snail to MPs for 31 days through three indicators that have been previously used as endpoints in *P. antipodarum* (Alonso & Camargo, 2013; Romero-Blanco & Alonso, 2019): (1) *Mortality* was evaluated as the cumulative number of dead adults in each treatment at the end of the experimental period. A snail was considered dead when its reaction time (see below in this subsection) exceeded 150 s and if no reaction was observed after touching its operculum (Romero-Blanco & Alonso, 2019). All dead animals were removed after monitoring. (2) *Behavior* was assessed by three variables: the number of immobile animals, the reaction time, and the spatial distribution of individuals in the experimental units. Immobility was assessed through the cumulative percentage of immobile snails in each treatment. Immobile adults were considered when their reaction time exceeded 150 s (see below reaction time) and movement inside the shell was detected. We followed Alonso and Camargo's (2009) method to assess the reaction time: first, the retraction of the soft body was stimulated by picking individuals up by the central part of the shell with forceps. Then, individuals were quickly pulled out from the water and reintroduced with the operculum facing the bottom of the glass vessel. The time employed to start the normal movement was measured with a chronometer (Onstart 100, Geonate). The normal movement was considered when the individual pulled its soft body out of the shell and started to slide using its foot. The spatial distribution of individuals was assessed by calculating the percentage of adults located on several parts of the experimental units: animals totally or partially buried in the sediment, animals on the sediment, animals on

Fig. 1 Diagram showing the different stages of the experimental procedure

the wall, and animals hanging from the water surface as a consequence of surface tension. This variable

has an important biological significance since it can provide clues about the ability of *P. antipodarum* to

avoid contaminated sediments (DeLange et al, 2016; Lefcort et al, 2004; West & Ankley, 1998). These observations were made twice a week for 31 days. Animals that moved outside of the water column were not considered. (3) *Reproduction* was assessed by three variables: the total number of neonates, the cumulative number of live neonates (i.e., sliding animals), and the cumulative number of dead neonates (i.e., animals without movement) per live adult. After monitoring, neonates were removed from the experiment. The last three variables were studied 1 day per week for 1 month. The monitoring of all these variables was done through a stereomicroscope fitted with a cold light source (Motic MLC-150C) with a 50% light intensity.

After each monitoring, animals were fed ad libitum with milled pellets (JBL, NovoPrawn, GmbH & Co. KG, Germany). Every 2 weeks, the water of all treatments was renewed through an iron-tipped saucers injector.

2.5 Statistical Analyses

To assess the influence of treatments on mortality, the cumulative percentage of immobile adults, and the production of the total, live, and dead neonates per live adult, non-parametric Kruskal–Wallis tests were performed. A post hoc test (Dunn’s Kruskal–Wallis multiple comparisons test with Bonferroni correction)

was applied when significant differences were obtained to analyze which treatments caused the differences compared with controls. A mixed ANOVA was used to assess the influence of treatments and time on reaction time and the spatial distribution of adults. Reaction time was log-transformed (natural logarithm) to achieve homogeneity of variances and normality. The Greenhouse–Geisser approach was applied for the analysis of the spatial distribution of adults since the sphericity assumption was not met. When significant differences were obtained, the Student *t* pairwise test with Bonferroni correction was applied to find out which time periods caused the differences. To analyze which treatments led to the differences compared with controls, the Dunnett test was performed. In this case, an α of 0.01 was established to minimize type I errors. All the statistical analyses were carried out with the R software (R Core Team, 2021).

3 Results

The mean ($\pm$ SD) air temperature in the climatic chamber was 18.6 ± 0.6 °C, and the relative humidity was $72.8 \pm 8.3\%$ ($n=137$). Overall, the mean ($\pm$ SD) water conductivity was 420.9 ± 37.04 microsiemens/cm, and the water temperature was 17.2 ± 0.3 °C ($n=3$). The

Fig. 2 Mean ($\pm$ SD) of the cumulative percentage of immobile adults of each treatment at the end of the experimental period ($n=8$ replicates per treatment). The y axis has been scaled from 0 to 6% to better visualize the data. The control treatment has been named “Ct.” No significant differences were found

between treatments and control ($p>0.05$; Dunn’s Kruskal–Wallis multiple comparisons test with Bonferroni correction). The artwork was created with the R software (R Core Team, 2021)

Fig. 3 Differences between the mean of the reaction time (in seconds) of each MP treatment with the control. Control is represented by a dashed line. Positive values show a slower activity than control and vice versa ($n=8$ replicates per treatment). Treatments, time, and their interaction significantly affected

the reaction time ($p<0.05$; mixed ANOVA). Asterisks indicate significant differences between MP treatments and control ($p<0.01$; Dunnett test). The standard deviation has been removed for clarity. The artwork was created with the R software (R Core Team, 2021)

mean ($\pm SD$) laboratory temperature during the aeration period was 20.4 ± 1.03 °C, with a maximum of 22 ± 0.8 °C and a minimum of 19.8 ± 0.4 °C ($n=6$).

3.1 Mortality and Behavior of Adult Snails

The cumulative mortality of adults at the end of the experiment was negligible in all treatments ($\chi^2=4.05$, $p=0.54$; Kruskal–Wallis). Only five animals out of 288 died throughout the entire experiment: three of them in the treatment of 100 mg MP/kg dry dw sediment, 1 in the treatment of 500 mg MP/kg dry dw sediment, and 1 in the treatment of 2000 mg MP/kg dry dw sediment. No snails died in the control treatment.

The cumulative percentage of immobile adults is shown in Fig. 2. Significant differences were found between treatments ($\chi^2=13.74$, $p<0.05$; Kruskal–Wallis). However, no significant differences were found in the post hoc test between treatments and the control ($p>0.05$; Dunn's Kruskal–Wallis multiple comparisons with Bonferroni correction). In any case, the percentage of immobile adults was very low (less than 10%) (Fig. 2).

The influence of treatments and time on the reaction time of *P. antipodarum* is shown in Fig. 3. Treatments, time, and the interaction between them significantly affected the reaction time ($p<0.05$; mixed

ANOVA) (Table 1). All treatments except the treatment of 4000 mg MP/kg dw sediment resulted in a significant increase in the reaction time of snails during the experimental period comparing with controls ($p<0.01$; Dunnett test) (Table 1, Fig. 3). Time caused a significant increase in the reaction time of all treatments and controls ($p<0.05$; Student *t* pairwise with Bonferroni correction) (Table 1, Fig. 3).

Neither the treatments nor the interaction between treatments and time showed a significant effect on any of the variables of the spatial distribution of *P. antipodarum* (percentage of individuals totally buried,

Table 1 Summary of the results of mixed ANOVA assessing the influence of MP treatments, time, and their interaction on the reaction time of *Potamopyrgus antipodarum*. The time was the within-subject factor (10 observations made during 31 days after acclimatization), the treatment (Ct, 100, 500, 1000, 2000, and 4000 mg MP/kg dry dw sediment) was the inter-subject factor, and the reaction time (in seconds) was the response variable

Source of variation	Degrees of freedom	<i>F</i>	<i>p</i>	Effect size
Within-subject				
Time	9/270	80.4	<0.00001	0.7
Time $\times$ treatment	45/270	2	<0.001	0.2
Between subjects				
Treatment	5/30	4.3	<0.01	0.1

Table 2 Summary of the results of mixed ANOVA assessing the influence of treatments, time, and their interaction on the spatial distribution of adults. The time was the within-subject factor (8 observations made during 31 days after acclimatization), the treatment (Ct, 100, 500, 1000, 2000, and 4000 mg MP/kg dw sediment) was the inter-subject factor, and the spatial distribution (percentage of adults totally buried, partially buried, on the wall, on the sediment, and hanging from the water) was the response variable. Mixed ANOVA was repeated for each response variable

Source of variation	Degrees of freedom ^a	<i>F</i>	<i>p</i>	Effect size
Totally buried				
Within-subject				
Time	4.8/203.1	27.7	< 0.00001	0.31
Time × treatment	24.2/203.1	0.8	0.7	0.07
Between subjects				
Treatment	5/42	1.04	0.4	0.04
Partially buried				
Within-subject				
Time	5.5/229.2	8.02	< 0.00001	0.14
Time × treatment	27.3/229.2	0.6	0.9	0.06
Between subjects				
Treatment	5/42	0.2	0.9	0.003
On the wall				
Within-subject				
Time	4.8/200.2	46.2	< 0.00001	0.4
Time × treatment	23.8/200.2	1.1	0.4	0.07
Between subjects				
Treatment	5/42	0.3	0.9	0.01
On the sediment				
Within-subject				
Time	5.2/220.04	8.6	< 0.00001	0.15
Time × treatment	26.2/220.04	0.9	0.7	0.08
Between subjects				
Treatment	5/42	2.4	0.05	0.04
Hanging from the water				
Within-subject				
Time	3.6/152.8	2.6	< 0.05	0.05
Time × treatment	18.2/152.8	0.9	0.6	0.09
Between subjects				
Treatment	5/42	1.8	0.1	0.02

^aDegrees of freedom (degrees of freedom of numerator/degrees of freedom of denominator) were corrected for sphericity using the Greenhouse–Geisser approach (Field et al., 2012)

partially buried, on the sediment, on the walls, and hanging from the water) ($p > 0.05$; mixed ANOVA) (Table 2). In contrast, the time had a significant effect on the spatial distribution ($p < 0.05$; mixed ANOVA) with a significant decrease in the percentage of totally and partially buried adults (Greenhouse–Geisser adjusted $p < 0.00001$; Student *t* pairwise with Bonferroni correction) and a significant increase on the number of individuals located on the wall and on the sediment (Greenhouse–Geisser adjusted $p < 0.05$; Student *t* pairwise with Bonferroni correction) (Fig. 4). However, no significant differences were found in the post hoc test for the percentage of adults hanging from the water ($p > 0.05$; Student *t* pairwise with Bonferroni correction) (Fig. 4).

3.2 Neonate Production

No significant differences were found in the total, live, and dead number of neonates produced per live adult between treatments ($\chi^2 = 4.26$ – 7.05 , $p > 0.05$; Kruskal–Wallis) (Fig. 5). Neonate production was very low in all treatments (Fig. 5).

4 Discussion

The survival of *P. antipodarum* was not affected by different concentrations of MPs, suggesting that this species could survive in aquatic ecosystems with wide ranges of MP concentrations. Other studies have

Fig. 4 Mean ($\pm$ SD) of the proportion of totally buried adults (A), partially buried adults (B), adults on the wall (C), adults on the sediment (D), and adults hanging from the water (E) ($n=8$ replicates per treatment). The control treatment has been

named “Ct.” The time caused significant differences ($p < 0.05$; mixed ANOVA). The artwork was created with the R software (R Core Team, 2021)

Fig. 5 Mean ($\pm$ SD) of the total number of neonates per alive adult at the end of the experimental period ($n=8$ replicates per treatment). The control treatment has been named “Ct.” No significant differences were found between treatments ($p > 0.05$; Kruskal–Wallis). The artwork was created with the R software (R Core Team, 2021)

also reported an absence of lethal effects of MPs on benthic macroinvertebrates. For example, Redondo-Hasselerham et al. (2018) found no mortality of different concentrations of irregular polystyrene micro-particles at long-term exposures on the amphipods *Gammarus pulex* and *Hyallela azteca*, the isopod

Asellus aquaticus, the mollusk *Sphaerium corneum*, and the oligochaeta *Tubifex* spp. Weber et al. (2018) also found similar results in individuals of *G. pulex* exposed to polyethylene terephthalate (PET) MPs. Likewise, Imhof and Laforsch (2016) exposed *P. antipodarum* to a mixture of irregular non-buoyant

MPs, including polystyrene, and observed a negligible mortality in all treatments. In contrast, Liu et al. (2019) found 100% mortality in the cladoceran *Daphnia pulex* exposed to 400 mg/L polystyrene microspheres. This suggests that the microplastic availability and feeding habits can be critical in the number of particles that can be ingested, which may determine their negative effects (Au et al., 2015). For example, Setälä et al. (2016) and Scherer et al. (2017) observed that deposit-feeders and surface and sub-surface feeders ingest fewer MPs than pelagic animals. Therefore, *P. antipodarum* might have not ingested enough MPs to show negative impacts on its survival. It could also have been ameliorated through the ingestion avoidance of MP. In the case of *P. antipodarum*, Imhof et al. (2013) demonstrated that it is capable to ingest MPs with sizes up to 235 µm. Furthermore, due to the wide distribution of MPs in our replicates (on the water surface, in the water column, and on the sediment) and the size range used in our study (0.01 to 514 µm), it is likely that *P. antipodarum* could have ingested MPs.

Behavior was the parameter most affected by the exposure to MPs. All treatments with MPs except the treatment with the highest concentration (4000 mg MP/kg dw of sediment) tended to increase the overall reaction time of the animals comparing with controls. Several authors also found that MP treatments negatively affected the behavior of the marine fish *Sebastes schlegelii* and the common goby *Pomatoschistus microps*, increasing the foraging time and decreasing the swimming speed, the movement range, or the predatory performance (de Sá et al., 2015; Yin et al., 2018). These effects increased over time, suggesting that chronic exposure of *P. antipodarum* to irregular polystyrene microparticles may have a negative impact on its behavior. Consequently, access to resources and avoidance of predators could be impaired if *P. antipodarum* suffers the slowing down of its activity.

Probably, the effects of MPs on the reaction time of *P. antipodarum* occur through their intake. However, there is little information about the fate of MPs once inside the body. Several authors have reported blockage of the digestive tract and/or damage to digestive tissues by MPs, and their translocation to cells and tissues with subsequent physiological damage and food impairment for various species, such as the crab *Uca*

rapax or the mussel *Mytilus edulis* (Brennecke et al., 2015; Browne et al., 2008; von Moos et al., 2012). Therefore, it is likely that MPs followed similar pathways in *P. antipodarum*. Another possibility is that MPs may have caused damages to internal or external tissues (Wright et al., 2013). Since we exposed snails to a heterogeneity of MP shapes and sizes, sharp particles may have inflicted them abrasions and injuries that disturbed their normal movement.

Interestingly, the treatment with the highest MP concentration (4000 mg MP/kg dw sediment) had no impacts comparing with the control. Liu et al. (2019) obtained similar results with *Daphnia pulex* exposed to nanoplastics regarding its body length at 7 days and time to the first clutch. They speculate that this species may enhance tolerance to high concentrations of nanoplastics. It is possible that this was the case for *P. antipodarum* individuals subjected to the highest concentration treatment. Similar observations were made by Muyssen and Janssen (2004) on the production of neonates in *Daphnia magna*. Lei et al. (2018) also found that the highest concentrations of polyethylene and polypropylene MPs had less impact on the survival rates of the benthic nematode *Caenorhabditis elegans* than lower concentrations. One possible interpretation is that *P. antipodarum* adjusts its behavioral activity to cope with high concentrations of MPs, probably by means of ingestion avoidance of MPs. Several studies have shown that there is a positive relationship between the intake of MPs and their concentration (Redondo-Hasselerharm et al., 2018; Setälä et al., 2016). Therefore, we speculate that the highest MP concentration animals satiated earlier, and subsequently, they might have reduced their consumption of MPs, suffering a smaller impact on their reaction time. On the contrary, lower concentrations would not cause satiety on snails, so they might have continued feeding even though accumulating negative effects on their movement behavior. Satiation may happen through the blockage of the digestive tract by MP particles (Wright et al., 2013).

All treatments showed significant changes in the spatial position of individuals over time. Snails tended to leave the sediment and spread out along the walls in all treatments and controls. This suggests that the lower parts of the replicates accumulated more stressing conditions than the upper parts. This avoiding behavior may have been caused by an

oxygen shortage at the benthos by the accumulation of food debris. However, the absence of negative impacts from MPs suggests that *P. antipodarum* can acclimate to a wide range of MP concentrations. These results coincide with previous studies that have reported the developed tolerance that *P. antipodarum* shows to a wide range of abiotic conditions (Alonso & Camargo, 2003; Romero-Blanco & Alonso, 2019).

The poor neonate production in all treatments (including controls) did not allow us to clearly determine if the reproduction was affected by MPs. The low levels observed could be due to the underestimation of the number of neonates identified. Since *P. antipodarum* neonates present a transparent shell, their location in the sediment is difficult. Nevertheless, it is plausible that MPs did not affect the neonate production, as shown by Imhof and Laforsch (2016). In contrast, other reproductive parameters of *P. antipodarum* can be affected by exposure to high doses of MPs, such as shell development (Imhof & Laforsch, 2016). Negative impacts on fertility by MP consumption have been demonstrated by other authors in the marine copepod *Calanus helgolandicus* (Cole et al., 2015), the freshwater amphipod *Hyallela azteca* (Au et al., 2015), the cladoceran *Daphnia pulex* (Liu et al., 2019), and the marine fish *Oryzias melastigma* (Cong et al., 2019).

Some of our results showed large standard deviations. This could be due to the wide size ranges (0.01 to 514 µm) and shape heterogeneity of the MPs used in our study, factors that may influence the hazardousness of MPs (Imhof & Laforsch, 2016; Wright et al., 2013).

5 Conclusions

This study shows that behavioral parameters of *P. antipodarum* are sensitive to MP exposure. Behavior is an effective endpoint to understand how MPs affect the fitness of this species, and therefore, it is an appropriate endpoint to assess the impacts of MPs on benthic invertebrates. In addition, this study reported the deleterious effects of realistic environmental concentrations of MPs on the behavior of *P. antipodarum*.

This work reproduces a realistic scenario through environmental MP concentrations that can be found in freshwater ecosystems worldwide. It is important to highlight that realistic concentrations of MPs alone and without any other added toxicant can cause an impairment in the behavior of this species. Consequently, chronic exposure to MPs may affect the fitness and survival of *P. antipodarum*. Furthermore, in natural ecosystems, these adverse effects may be worsened with the presence of other pollutants or other types of stressors.

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Author Contribution AR-B: conceptualization, formal analysis, investigation, methodology, writing—original draft, and visualization. AR-E: formal analysis, validation, and investigation. AA: conceptualization, methodology, validation, writing—review and editing, supervision, project administration, and funding acquisition.

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Data Availability Raw data are available in the Figshare online repository: <https://doi.org/10.6084/m9.figshare.16558521.v1>.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication All authors consent to the publication of this article.

Conflict of Interest The authors declare no competing interests.

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