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

Alberto Romero-Blanco · Adrián Remón-Elola · Álvaro Alonso

Received: 16 April 2021 / Accepted: 30 September 2021 / Published online: 14 October 2021 © The Author(s) 2021

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).
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 and 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 and 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 and 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 and 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 and 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 and 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 and 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 (± SD) shell length of 3.9 ± 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. 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, Geonaute). 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
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 sauces 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 \( \alpha \) 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 (±SD) air temperature in the climatic chamber was 18.6 ± 0.6 °C, and the relative humidity was 72.8 ± 8.3% (n = 137). Overall, the mean (±SD) water conductivity was 420.9 ± 37.04 microsiemens/cm, and the water temperature was 17.2 ± 0.3 °C (n = 3).
mean (±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 (χ² = 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 (χ² = 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). 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).

Table 1 Summary of the results of mixed ANOVA assessing the influence of MP treatments, time, and their interaction on the reaction time of P. 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 | F    | p            | Effect size |
|---------------------|--------------------|------|--------------|-------------|
| Within-subject      |                    |      |              |             |
| Time                | 9/270              | 80.4 | <0.00001     | 0.7         |
| Time×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 | F     | p         | 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 (χ² = 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
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 surfase 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.

Acknowledgements We want to acknowledge the comments of an anonymous reviewer who contributed to improve the quality of this manuscript. We also want to extend our sincere gratitude to INIA for providing microplastics for this study and to Daniel Antúnez for suggesting improvements for the English usage in this manuscript. Part of this work was carried out within the sabbatical period of Álvaro Alonso as a full professor in the University of Alcalá for the 2021-2022 academic year.

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

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This project was funded by Universidad de Alcalá (research projects CCG2013/EXP-054, CCG2016/EXP-054, and CCG2018/EXP-074), by the Ministerio de Economía, Industria y Competitividad of Spain (research projects INTERTOX RTI2018-096046-B-C21 (MCIU/AEI/FEDER, UE) and EXARBIN RTI2018-093504-B-I00 (MCIU/AEI/FEDER, UE)), and by the Youth Employment Initiative of the European Social Fund.

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.
Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits
use, sharing, adaptation, distribution and reproduction in any
medium or format, as long as you give appropriate credit to the
original author(s) and the source, provide a link to the Cre-
ative Commons licence, and indicate if changes were made. The
images or other third party material in this article are included
in the article’s Creative Commons licence, unless indicated
otherwise in a credit line to the material. If material is not
included in the article’s Creative Commons licence and your
intended use is not permitted by statutory regulation or exceeds
the permitted use, you will need to obtain permission directly
from the copyright holder. To view a copy of this licence, visit
http://creativecommons.org/licenses/by/4.0/.

References

Akindele, E. O., Ehlers, S. M., & Koop, J. H. E. (2019). First
empirical study of freshwater microplastics in West Africa
using gastropods from Nigeria as bioindicators. Limno-
logica, 78, 125708. https://doi.org/10.1016/j.limno.2019.
125708

Alonso, Á., & Camargo, J. A. (2003). Short-term toxicity of
ammonia, nitrite, and nitrate to the aquatic snail Potamopyr-
agus antipodarum (Hydrobiidae, Mollusca). Bulletin of
Environment Contamination and Toxicology, 70, 1006–
1012. https://doi.org/10.1007/s00128-003-0082-5

Alonso, Á., & Camargo, J. A. (2009). Long-term effects of
ammonia on the behavioral activity of the aquatic snail
Potamopyrgus antipodarum (Hydrobiidae, Mollusca). Archives
of Environmental Contamination and Toxicology, 56, 796–802.
https://doi.org/10.1007/s00244-008-9266-7

Alonso, Á., & Camargo, J. A. (2013). Nitrate causes deleteri-
ous effects on the behavior and reproduction of the aquatic
snail Potamopyrgus antipodarum (Hydrobiidae, Mollusca).
Environmental Science and Research, 20, 5388–5396. https://doi.org/10.1007/s11356-013-1544-x

Alonso, Á., & Castro-Díez, P. (2012). The exotic aquatic
mud snail Potamopyrgus antipodarum (Hydrobiidae, Mollusca): State of the art of a worldwide invasion. Aquatic
Sciences, 74, 375–383. https://doi.org/10.1007/s00027-012-0254-7

Arthur, C., Baker, J. E., & Bamford, H. A. (2009). Proceed-
ings of the International Research Workshop on the occurrence,
effects, and fate of microplastic marine debris, Sep-
tember 9–11, 2008. University of Washington Tacoma,
Washington DC, USA

Au, S. Y., Bruce, T. F., Bridges, W. C., & Klaine, S. J. (2015).
Responses of Hyalella azteca to acute and chronic micro-
plastic exposures: Effects of microplastic exposure on
Hyalella azteca. Environmental Toxicology and Chemis-
try, 34, 2564–2572. https://doi.org/10.1002/etc.3093

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribu-
tion and importance of microplastics in the marine envi-
ronment: A review of the sources, fate, effects, and poten-
tial solutions. Environment International, 102, 165–176.
https://doi.org/10.1016/j.envint.2017.02.013

Ballent, A., Corcoran, P. L., Madden, O., Helm, P. A., & Long-
staffe, F. J. (2016). Sources and sinks of microplastics in
Canadian Lake Ontario nearshore, tributary and beach
sediments. Marine Pollution Bulletin, 110, 383–395.
https://doi.org/10.1016/j.marpolbul.2016.06.037

Brennecke, D., Ferreira, E. C., Costa, T. M. M., Appel, D., da
Gama, B. A. P., & Lenz, M. (2015). Ingested microplas-
tics (>100μm) are translocated to organs of the tropical
fiddler crab Uca rapax. Marine Pollution Bulletin, 96,
491–495. https://doi.org/10.1016/j.marpolbul.2015.05.001

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M.,
& Thompson, R. C. (2008). Ingested microscopic plastic
translocates to the circulatory system of the mussel Mytil-
us edulis (L.). Environmental Science and Technology,
42, 5026–5031. https://doi.org/10.1021/es800249a

Burns, E. E., & Boxall, A. B. A. (2018). Microplastics in the
aquatic environment: Evidence for or against adverse
impacts and major knowledge gaps: Microplastics in the
environment. Environmental Toxicology and Chemistry,
37, 2776–2796. https://doi.org/10.1002/etc.4268

Cole, M., Lindeque, P., Fileman, E., Halsband, C., & Galloway,
T. S. (2015). The impact of polystyrene microplastics on
feeding, function and fecundity in the marine copepod
Calanus helgolandicus. Environmental Science and Tech-
nology, 49, 1130–1137. https://doi.org/10.1021/acs.
504525u

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011).
Microplastics as contaminants in the marine environment:
A review. Marine Pollution Bulletin, 62, 2588–2597.
https://doi.org/10.1016/j.marpolbul.2011.09.025

Cong, Y., Jin, F., Tian, M., Wang, J., Shi, H., Wang, Y., & Mu,
J. (2019). Ingestion, egestion and post-exposure effects
of polystyrene microspheres on marine medaka (Oryzias
melastigma). Chemosphere, 228, 93–100. https://doi.org/
10.1016/j.chemosphere.2019.04.098

De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo,
N., Bacchetta, R., Ortenzi, M. A., & Parolini, M. (2019).
Polystyrene microplastics ingestion induced behavioral
effects to the cladoceran Daphnia magna. Chemosphere,
231, 423–431. https://doi.org/10.1016/j.chemosphere.
2019.05.115

Lange De, H. J., Sperber, V., & Peeters, E. T. H. M. (2006).
The impact of polystyrene microplastics on predatory
performance and efficiency, and possible influ-
ences of developmental conditions. Marine Envi-
ronmental Pollution, 156, 359–362. https://doi.org/10.
1016/j.envpol.2014.196, 359–362. https://doi.org/10.
1021/es504525u

de Sá, L. C., Luís, L. G., & Guilhaumin, L. (2015). Effects
of microplastics on juveniles of the common goby (Poma-
toschistus microps): Confusion with prey, reduction of the
predatory performance and efficiency, and possible influ-
ence of developmental conditions. Environmental Pollu-
tion, 196, 359–362. https://doi.org/10.1016/j.envpol.
2014.10.026

do Sul, J. A. I., & Costa, M. F. (2014). The present and future
of microplastic pollution in the marine environment. Envi-
ronmental Pollution, 185, 352–364. https://doi.org/10.
1016/j.envpol.2013.10.036

do Sul, J. A. I., Costa, M. F., & Fillmann, G. (2014). Micro-
plastics in the pelagic environment around oceanic islands
of the Western Tropical Atlantic Ocean. Water, Air,
and Soil Pollution, 225, 1–13. https://doi.org/10.1007/s11270-014-2004-z
Du, J., Xu, S., Zhou, Q., Li, H., Fu, L., Tang, J., Wang, Y., Peng, X., Xu, Y., & Du, X. (2020). A review of microplastics in the aquatic environmental: Distribution, transport, ecotoxicology, and toxicological mechanisms. Environmental Science and Pollution Research, 27, 11494–11505. https://doi.org/10.1007/s11356-020-08104-9
Eerkes-Medrano, D., & Thompson, R. (2018). Occurrence, fate, and effect of microplastics in freshwater systems. In Zeng, E. (Eds.), Microplastic contamination in aquatic environments (pp. 95–132). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-813747-5.00004-7
Field, A. P., Miles, J., & Field, Z. (2012). Discovering statistics using R. Sage.
Foley, C. J., Feiner, Z. S., Malinich, T. D., & Höök, T. O. (2018). A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Science of the Total Environment, 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046
Gouin, T., Becker, R. A., Collot, A., Davis, J. W., Howard, B., Inawaka, K., Lampi, M., Ramon, B. S., Shi, J., & Hopp, P. W. (2019). Toward the development and application of an environmental risk assessment framework for microplastic. Environmental Toxicology and Chemistry, 38, 2087–2100. https://doi.org/10.1002/etc.4529
Haegerbauer, A., Mueller, M.-T., Fueser, H., & Traunspurger, W. (2019). Impacts of micro- and nano-sized plastic particles on benthic invertebrates: A literature review and gap analysis. Frontiers in Environmental Science, 7, 17. https://doi.org/10.3389/fenvs.2019.00017
He, B., Goonetilleke, A., Ayoko, G. A., & Rintoul, L. (2020). Abundance, distribution patterns, and identification of microplastics in Brisbane River sediments, Australia. Science of the Total Environment, 700, 134467. https://doi.org/10.1016/j.scitotenv.2019.134467
Hellou, J. (2011). Behavioural ecotoxicology, an “early warning” signal to assess environmental quality. Environmental Science and Pollution Research, 18, 1–11. https://doi.org/10.1007/s11356-010-0367-2
Imhof, H. K., Ileva, N. P., Schmid, J., Niessner, R., & Laforsch, C. (2013). Contamination of beach sediments of a subalpine lake with microplastic particles. Current Biology, 23, R867–R868. https://doi.org/10.1016/j.cub.2013.09.001
Imhof, H. K., & Laforsch, C. (2016). Hazardous or not – Are adult and juvenile individuals of Potamopyrgus antipodarum affected by non-buoyant microplastic particles? Environmental Pollution, 218, 383–391. https://doi.org/10.1016/j.envpol.2016.07.017
Klein, S., Worch, E., & Knepper, T. P. (2015). Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany. Environmental Science and Technology, 49, 6070–6076. https://doi.org/10.1021/acs.est.5b00492
Lambert, S., & Wagner, M. (2018). Microplastics are contaminants of emerging concern in freshwater environments: An overview. In Wagner, M., & Lambert, S. (Eds.), Freshwater microplastics (pp. 1–23). Springer International Publishing. https://doi.org/10.1007/978-3-319-61615-5
Lebreton, L. C. M., van der Zwart, J., Damsteeg, J.-W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world’s oceans. Nature Communications, 8, 15611. https://doi.org/10.1038/ncomms15611
Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K. M., & He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. Science of the Total Environment, 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103
Lefcort, H., Abbott, D. P., Cleary, D. A., Howell, E., Keller, N. C., & Smith, M. M. (2004). Aquatic snails from mining sites have evolved to detect and avoid heavy metals. Archives of Environmental Contamination and Toxicology, 46, 478–484. https://doi.org/10.1007/s00244-003-3029-2
León-Muez, D., Peñalver-Duque, P., Ciudad, C., Muñoz, M., Infante, O., Güemes Santos, S., Parrilla Giráldez, R., & Serrano, L. (2020). Primer muestreo de microplásticos en arroyos y ríos de la España peninsular. Ecosistemias: Revista Cientifica y Tecnica De Ecologia y Medio Ambiente, 29, 2087. https://doi.org/10.7818/ECOS.2087
Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Huang, Y., & Zhao, Y. (2019). Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran Daphnia pulex. Chemosphere, 215, 74–81. https://doi.org/10.1016/j.chemosphere.2018.09.176
Lol обесп, D., & Cunillé, M. (2011). Early microbial biofilm formation on marine plastic debris. Marine Pollution Bulletin, 62, 197–200. https://doi.org/10.1016/j.marpolbul.2010.10.013
Muyssen, B. T. A., & Janssen, C. R. (2004). Multi-generation cadmium acclimation and tolerance in Daphnia magna Straus. Environmental Pollution, 130, 309–316. https://doi.org/10.1016/j.envpol.2004.01.003
Naji, A., Nuri, M., & Vethaak, A. D. (2018). Microplastics contamination in molluscs from the northern part of the Persian Gulf. Environmental Pollution, 235, 113–120. https://doi.org/10.1016/j.envpol.2017.12.046
Oehlmann, J., & Schulte-Oehlmann, U. (2003). Molluscs as bioindicators. In B. A. Markert, A. M. Breure, & H. G. Zechmeister (Eds.), Bioindicators and biomonitors - Principles, concepts and applications (pp. 577–635). Elsevier.
Pace, M. L., Cole, J. J., Carpenter, S. R., & Kitchell, J. F. (1999). Trophic cascades revealed in diverse ecosystems. Trends in Ecology & Evolution, 14, 483–488. https://doi.org/10.1016/S0169-5347(99)01723-1
Phuon, N. N., Zalouk-Vergnoux, A., Poirier, L., Kamari, A., Châtel, A., Mouneyrac, C., & Lagarde, F. (2016). Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environmental Pollution, 211, 111–123. https://doi.org/10.1016/j.envpol.2015.12.035
R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/
Redondo-Hasselerharm, P. E., Falahudin, D., Peeters, E. T. H. M., & Koelmans, A. A. (2018). Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environmental Science and Technology, 52, 2278–2286. https://doi.org/10.1021/acs.est.7b05367
Reisser, J. W. (2015). Buoyant plastics at sea: Concentrations and impacts. Doctoral dissertation, retrieved from https://api.research-repository.uwa.edu.au/portalfiles/portal/8149594/Reisser_Julia_2015.pdf
Romero-Blanco, A., & Alonso, A. (2019). Tolerance assessment of the aquatic invasive snail Potamopyrgus antipodarum to different post-dispersive conditions: Implications for its invasive success. NeoBiota, 44, 57–73. https://doi.org/10.3897/neobiota.44.31840
Scherer, C., Brennholt, N., Reifferscheid, G., & Wagner, M. (2017). Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Science and Reports, 7, 17006. https://doi.org/10.1038/s41598-017-17191-7
Scott, G. R., & Sloman, K. A. (2004). The effects of environmental pollutants on complex fish behavior: Integrating behavioral and physiological indicators of toxicity. Aquatic Toxicology, 68, 369–392. https://doi.org/10.1016/j.aquatox.2004.03.016
Setälä, O., Norkko, J., & Lehtiniemi, M. (2016). Feeding type affects microplastic ingestion in a coastal invertebrate community. Marine Pollution Bulletin, 102, 95–101. https://doi.org/10.1016/j.marpolbul.2015.11.053
Teuten, E. L., Saquing, J. M., Knappe, D. R. U., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S., Yamashita, R., Ochi, D., Watamuki, Y., Moore, C., Viet, P. H., Tana, T. S., Prudente, M., Boonyatumanond, R., Zakaria, M. P., Akhvong, K., et al. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences, 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284
Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., & Russell, A. E. (2004). Lost at sea: Where is all the plastic? Science, 304, 838–838. https://doi.org/10.1126/science.1094559
United States Environmental Protection Agency (2002). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency, Washington DC, USA, 335
von Moos, N., Burkhardt-Holm, P., & Köhler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. Environmental Science and Technology, 46, 11327–11335. https://doi.org/10.1021/es302332w
Wang, X., Liu, L., Zheng, H., Wang, M., Fu, Y., Luo, X., Li, F., & Wang, Z. (2020). Polystyrene microplastics impaired the feeding and swimming behavior of mysid shrimp Neomysis japonica. Marine Pollution Bulletin, 150, 110660. https://doi.org/10.1016/j.marpolbul.2019.110660
Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., & Wagner, M. (2018). PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate Gammarus pulex. Environmental Pollution, 234, 181–189. https://doi.org/10.1016/j.envpol.2017.11.014
West, C. W., & Ankley, G. T. (1998). A laboratory assay to assess avoidance of contaminated sediments by the freshwater oligochaete Lumbriculus variegatus. Archives of Environmental Contamination and Toxicology, 35, 20–24. https://doi.org/10.1007/s002449900343
Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. Environmental Pollution, 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031
Yao, P., Zhou, B., Lu, Y., Yin, Y., Zong, Y., Chen, M.-T., & O’Donnell, Z. (2019). A review of microplastics in sediments: Spatial and temporal occurrences, biological effects, and analytic methods. Quaternary International, 519, 274–281. https://doi.org/10.1016/j.quaint.2019.03.028
Yin, L., Chen, B., Xia, B., Shi, X., & Qu, K. (2018). Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). Journal of Hazardous Materials, 360, 97–105. https://doi.org/10.1016/j.jhazmat.2018.07.110

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.