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Impacts of polystyrene microplastics on *Daphnia magna*: a laboratory and a mesocosm study.

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Abstract

Most research into microplastics (MPs) in freshwaters has concentrated on measurements under controlled conditions without any link to the natural environment. Here we studied the effects of a 15 µm polystyrene MP on *Daphnia magna* survival, growth, and reproduction in the laboratory. We also exposed fifteen 25L freshwater mesocosms to a high concentration of the same MPs. Five were controls seeded with five species found in all ponds (mosquito, water flea, midge, spire shell and water mite), five identical but treated with 15 µm polystyrene MPs and five seeded with only mosquitoes and water fleas. The laboratory chronic toxicity test for both adults and neonate *Daphnia magna* revealed that effects were more related to the availability of food rather than the toxicity of MPs. In the mesocosms most of the MPs settled in the sediment after the first week of exposure. After four weeks the *D. magna* population decreased significantly in the MP mesocosms compared to the control mesocosms, although it subsequently recovered. There was no impact on other organisms added to the mesocosms, other than a difference in timing of lesser water boatman (*Corixa punctata*) colonisation, which colonised the control mesocosms in week 4 and the treated 4 weeks later. The detrivorous, sediment sifting, mayfly *Leptophlebia marginata* appeared in mesocosms in the fourth week of sampling and with significantly higher numbers in the MP treated mesocosm. Their activity had no significant impact on MPs in the water column, although numbers did increase above zero. The significant decline of *D. magna* suggests that their effect in a natural situation is unpredictable where environmental conditions and invertebrate communities may add additional stresses.

Introduction

Plastic pollution in aquatic habitats is a serious environmental issue worldwide that has galvanised businesses, the general public and governments into taking action. Much of the early research focussed on highly visible macroplastics in marine ecosystems with fewer than 4% of research papers on freshwater (Wagner and Lambert 2018). In recent years, interest has shifted towards freshwater ecosystems and, in particular, the impact of microplastics (MPs) (Wagner et al. 2014; Eerkes-Medrano et al. 2015; Wagner and Lambert 2018). Microplastics are diverse plastics, including polyethylene and polystyrene, whose fragments are smaller than 5 mm in size and are produced by the degradation of larger particles or are manufactured as microbeads for use in, for example, cosmetics and toiletries (Andrady, 2011; Imhof et al., 2013; Eriksen et al., 2014). Whilst bans on the use of MPs in toiletries have been in place for a number of years, the problems remain significant since there are many pollution routes and types of MP (Rochman et al, 2019).

There is no doubt that MP pollution is widespread, with a growing body of evidence to suggest that much higher MP concentrations are found in sediments compared to the water column. In Lake Taihu (China) the average number of MPs found in the water body was 3.4 - 25.8 MPs L^{-1}, while 11– 234.6 MPs kg^{-1} was found in the benthic sediment (Su et al., 2016). Similarly in Lake Chiusi (Italy) an average of 0.03 MPs L^{-1} were found in the surface water whereas 234 MPs kg^{-1} found in the sediment (Fischer et al., 2016). Higher levels of MPs have also been measured in river sediments including sediment of the River Thames, found to contain up to 660 MPs kg^{-1} (Horton et al., 2016). It is almost certain that the organisms living in these waters are ingesting MPs. However it is premature to generalise on whether the sediment or water column will have higher numbers of MPs since the data collected, as illustrated above, use very different methodologies.

Although there are numerous studies to investigate the occurrence and abundance of MPs in freshwater environments including rivers and lakes, relatively few have looked at the impact on the organisms being exposed (Sighicelli et al., 2018; Wagner and Lambert, 2018). Their size results in them being easily ingested by many aquatic organisms at various trophic levels and stages of development, including freshwater invertebrates (Cole et al., 2013; Scherer et al., 2017; Al-Jaibachi et al., 2018a; Aljaibachi and Callaghan, 2018; Liu et al., 2019). Microplastics can be carriers of toxic chemicals e.g. polychlorinated biphenols or plasticizers added during production and bacteria that can absorb onto their surface (Talsness et al., 2009). Therefore the behaviour of MPs in a pristine state may be very different from those released into the environment.

The majority of research on the uptake and effect of MPs in freshwater organisms has been conducted in the laboratory which does not reflect the many variables found in the environment (Phuong et al., 2016; de Sá et al., 2018). Laboratory studies are nearly all undertaken on individual organisms which ignores the interactions that occur in the natural environment (Rosenkranz et al., 2009; Jemec et al., 2016). That said, these studies do give useful information on the uptake and ecotoxicity of polystyrene MPs in both laboratory and natural field conditions. Laboratory work on *D. magna* has shown that MPs can enter their gut system and show concentration-time dependent patterns (Nasser and Lynch, 2016; Ogonowski et al., 2016; Aljaibachi and Callaghan, 2018; Canniff and Hoang, 2018; Martins and Guilhermino, 2018). Similar results have been found in *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016), annelids (*Lumbriculus variegatus*), crustaceans (*Gammarus pulex*), ostracods (*Notodromas monacha*), mosquitoes (*Culex pipiens*), and gastropods (*Potamopyrgus antipodarum*) (Imhof et al. 2013; Al-Jaibachi, et al. 2018b). These studies are important since initial ingestion is more likely in lower trophic organisms which could enhance the transfer through the food chain (Anbumani & Kakkar 2018; Al-Jaibachi, et al. 2018a).

The relationship between laboratory results and the behaviour and interaction of MPs and invertebrates in the natural environment must be determined using more natural exposure methods. Microplastics entering a natural environment are unlikely to remain stationary but will instead be transported between environmental compartments (Lambert and Wagner, 2018). The fate and movement of MPs will depend on hydrology and vegetation (Lambert and Wagner, 2018) and in lakes is likely to depend on sediment disturbance. The abundance of MPs in most freshwater environments investigated highlights questions about their impact on the biota biodiversity, food chain, community composition and predator-prey interactions and the possibility to accumulate in the food chain or transfer ontogenically to different environments (Wright et al., 2013; Al-Jaibachi et al., 2018a; Cuthbert et al., 2019).

Here we investigated the chronic ecotoxicological impact of PS MPs size 15 μm in laboratory condition on adults and neonate *Daphnia magna* before taken it out into the field to study the abundance and impact on a community of freshwater invertebrates. *Daphnia magna* is a standard ecotoxicity model and shows a high sensitivity to toxicants (Pablos et al., 2015). They are also used as models of filter feeders in the freshwater environment and have been utilised to examine the uptake and depuration of MP sizes from 1 nm to 2 μm (Besseling, Wang, Lu, et al. 2014; Aljaibachi and Callaghan 2018). Work has also been directed to life-history effects and both the acute and chronic toxicity of MPs on *D. magna* (Ogonowski et al., 2016; Aljaibachi and Callaghan, 2018; Martins and Guilhermino, 2018) .

Freshwater mesocosms are widely recognised as supporting greater regional invertebrate diversity than most other freshwater ecosystems in the UK and across Europe and can be rapidly colonised by variety of organism (Krebs and Davies, 2009; Céréghino et al., 2010).

The small mesocosms chosen to implement the experiment have been studied previously and have demonstrated their value in rapidly measuring the impact of environmental stressors on freshwater communities in a controlled but natural environment (Céréghino et al., 2008).

Fluorescent 15 μm PS MPs were chosen for studies into the ecotoxicological effect on *Daphnia magna* because of concerns regarding our ability to detect smaller MPs in the mesocosms. The impact of MPs on the population size and community were examined by manipulating the mesocosms so that at the start of the study had the same population size and composition. The animals used were all taken from the mesocosms where they had naturally colonised. They included *C. pipiens* and *D. magna* as well as predators and animals that dwell in the sediment. The mesocosms were monitored for 12 weeks. We hypothesized that MPs would sink to the sediment and be unavailable to animals in the water column with a consequent lack of effect on population size or community composition.

2. Materials and Methods

2.1. Preparation of microplastics (MPs)

Fluorescent 15 µm green carboxylate-modified polystyrene MPs (density 1.06 g cm^{-3}, excitation 470 nm; emission 505 nm, Sigma-Aldrich, UK) were used in all experiments. Microplastics were stored as a stock suspension (1%) and mixed as per Aljaibachi et al. (2018a). The number of PS particles from the stock solution were counted under the epi-fluorescent microscope at 10x magnification (Carl Zeiss Axioskop, Wetzlar, Germany). Each one milliliter of stock solution contained 5 x 10^6, MPs mL^{-1}. 2.2. *Daphnia* cultures

Daphnia magna were obtained from the Water Research Centre (WRC, Medmenham, UK) and cultured at the University of Reading for more than ten years prior to this experiment. Full details of culturing methods are given in (Hooper et al., 2006). *Daphnia* were maintained in Organization for Economic Co-operation and Development (OECD) reconstituted water (media) and fed yeast and *C. vulgaris* var Viridis following the methods of (Hayashi et al., 2008). New cultures of *Daphnia* were prepared with 15 neonates in 1,200 ml beakers filled with OECD media (the progeny of these neonates are the first brood). Juveniles were removed regularly from the culture and the media was changed once a week. The third brood produced by the original 15 neonates were used for experiments.

2.2 Uptake of microplastics with and without algae

Individual 18 day old *D. magna* were placed in 50 ml beakers filled with media and starved for 24 h prior to exposure. In a random design, animals were exposed to one of 4 concentrations of MP (2, 4, 8 and 16 x 10^5 ml^{-1}) with varying amounts of algae (Table 1) for 60 min. Each treatment was replicated three times. Animals were rinsed in distilled water to remove any MPs adhering to the outside and frozen at -20°C. Individual animals were homogenized using a glass Kontes Pellet Pestle (Fisher Sciences Loughborough, UK), in 500 µl distilled water in a 1.5 ml

Eppendorf tube. A further 500 μl distilled water was pipetted over to rinse the pestle. The homogenate was mixed using a whirlimixer and 500 μl removed and placed onto a nucleopore track-etched membrane (Whatman, UK) 10 μm with a white background. A manual air pump was used to filter the homogenate. The membrane was examined under an epi-fluorescent microscope (Zeiss Axioskop) at a magnification of 10x to count the MPs.

2.3 Adult Chronic Toxicity Tests

Third brood *D. magna* adults (18 days old) were placed individually into glass beakers filled with 50 mL of OECD reconstituted water (media) and exposed to one of six treatments ranging from only algae or only MPs and combinations of the two (Table 1), each with five replicates. Media and concentrations of MPs were renewed three times per week. In all treatments, life history characteristics (survival and reproduction) were monitored for 21 days. Neonates were counted daily and removed. Animals unable to swim after gentle stirring for 15 s were counted as dead. The experiment was run at 20 ± 2 ºC, light:dark 16:8 h.

2.4. Neonate Chronic Toxicity Test

A standard chronic toxicity test was conducted with reference to OECD guideline 211, with the exception that five individuals were used (OECD, 2012). Five individuals from third-brood neonates (< 24 h) were placed in 50 mL glass beakers and exposed to MPs and/or green algae *Chlorella vulgaris* (Table 2). Media and concentrations were renewed three times a week and life history characteristics (survival, reproduction and growth) were monitored daily for 21 days. Body length (from the top of the head to the base of the tail spine) was measured every other day under a stereomicroscope. The experiment was run at 20 ± 2 ºC, light : dark 16:8 h.

2.4. Study site and mesocosms

Thirty two mesocosms had previously been dug in the experimental grounds at the University of Reading, Berkshire, England (51°26'12.2"N, 0°56'31.2"W) in 2012. The mesocosms were laid out in a Latin square, with three metre intervals in three rows of eight mesocosms. Each mesocosm was a sunked bucket of diameter 48 cm depth 30 cm lined with a rubber pond liner. Fifteen of these mesocosms were randomly selected for use in this study.

The mesocosms had been naturally colonised by macroinvertebrates over the previous five years. These were all removed including the sediments by passing the mesocosm water through a sieve (dimensions 6 x 12 cm; 250 µm pore size) and placing contents onto a white plastic sampling tray (25 x 35 x 5 cm) with some water.

2.4.1 Preparation and sampling of the mesocosms community

Ten of the 15 mesocosms were randomly selected for this experiment. Previous analysis of abundances during a pilot state determined that five species could be reintroduced in the same numbers into each mesocosm, in numbers that reflected the natural populations at the time (species and numbers in Table 2). Each mesocosm was filled with 25L of rain water then the level marked to allow refilling each week to maintain the water level. Five randomly selected mesocosms were left untreated as a control. Another five were treated with 500 µl of the original washed MP stock (5,000,000 MPs mL^{-1}) as a final concentration of 100 MPs mL^{-1}. The mesocosms were then left for one week to allow for any disturbance and stress to organisms caused by setting up the experiment.

Weekly sampling then followed using a standardised technique; using a mesocosm net of approx. 60mm x 120mm, with a small enough mesh size to collect both the zooplankton such as *D. magna* and other invertebrates such as mosquito larvae (*Culex* spp.). The net was swept through the water using a figure of 8 motion four times 10-15 cm below the surface of the water. Samples were then placed in a 1 litre plastic bottle and removed to identify macroinvertebrates in the laboratory using a stereo microscope and number of keys (Croft, 1986; Greenhalgh and Ovenden, 2007; Dobson et al, 2013). Identified organisms were counted and then returned to the mesocosm from which they came. All members of each species were individually counted, except for *D. magna*, numbers of which were estimated by counting the number of individuals in 1mL, and then multiplying this by the number of mL in the sample due to the very high numbers of individuals. This process was repeated once per week over 12 weeks, with the initial set up on and addition of the MPs on the 12th June 2017, first data collection on the 19th June 2017, and the final samples taken on the 29th August 2017. Samples were taken between 10am and 12pm weekly.

*2.4.2 Distribution of microplastics in mesocosm*s

Five of the 15 mesocosms not used in section 2.4.1 were treated as before but to each was added 2 kg of soil from the area around the mesocosms along with 25 L of rain water. These were set up to specifically measure the distribution of the MPs in the pond over time, not the animals. Nevertheless approximately equal numbers of *D. magna* and *Culex* larvae were added to the mesocosms since they were the dominant organisms in the mesocosms. The mesocosms were then treated with 500 µl of stock MP solution as detailed in section 2.4.1. The mesocosms were re-filled with rain water to 25 L weekly after samples were taken.

The mesocosms were left undisturbed for a week, then samples were taken weekly from the 22nd June 2017 until the 10th August 2017. Five 1mL water samples were taken from each of two depths (5cm under water surface and 5cm above sediment), using a 1 mL and then water samples were mixed together before being filtered onto a nucleopore track-etched membrane (Whatman, Kent, UK) <10 µm, by using a glass vacuum filter holder connected to a manual air pump.

Approximately 5g of sediment was collected using a spatula and stored in a 5 mL plastic tube. Half of this sediment was spread directly onto a glass microscope slide to count the MPs under an epi-fluorescent microscope.

2.5. Statistical analysis

Generalized linear model (GLM) and *post hoc* Tukey's comparisons of laboratory life history data were undertaken using SPSS 21 (SPSS, 2012),. Growth rate data were analysed using UNIANOVA (mixed model), followed by post-hoc pairwise comparisons (growth rate × treatments × time).

Probit analysis was conducted for the chronic toxicity tests (mortality rate for adults and neonates) and response curves for different concentrations were produced as a scatter plot using (Minitab V. 17).

The abundance of MPs in the mesocosms were analysed using (GLM). Analysis assumed a quasi-Poisson error distribution as counts were found to be over dispersed compared to degrees of freedom.

The weekly abundance of invertebrate groups was analysed in R v3.4.2 (R Development Core Team, 2017). Generalized Linear Model (GLM) was used assuming a quasi-Poisson error distribution since they were not normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$).

Rainfall and air temperature data were obtained from the University of Reading Atmospheric Observatory and analysed by correlation analysis package in R v3.4.2 against MPs number in the mesocosm water column.

3. Results

3.1. Uptake of increasing concentrations of MPs

Ingestion of MPs by *D. magna* without algae increased significantly as MP concentration increased ($F_{3,32}$=14.12, p< 0.001, Fig S1). The same was true wherever MPs> algae ($F_{3,32}$=29.20, p< 0.001). When *D. magna* were exposed to equal amounts of MP and algae (MP=algae), there was no increase in ingestion with increasing concentration ($F_{3,32}$=0.415, p=0.743). The mean number of MPs ingested by *D. magna* exposed to algae>MPs significantly decreased as algal concentration increased ($F_{3,32}$=148.63, p < 0.001).

3.2 Adult Chronic toxicity test

Low availability of algae significantly increased adult mortality (X^2(5, n=30) =17.4, p=0.004) (Fig. 1). The presence of MPs had no impact, positively or negatively on survival, either with low (U= 10, p=0.317) or high algal concentrations (U=10, p=0.513).

3.3. Reproduction Test

A 21-day reproduction test of adult *D. magna* revealed significant differences in the mean number of offspring between treatments (X^2(5, n=30) = 216.1, p= 0.001) (Fig. 2). This was because treatments with low food were associated with low numbers of offspring (S1 Table 1).

3.4. Neonate chronic toxicity test

Mortality tests were significantly different between treatments exposed to low and high algae concentrations, irrespective of the presence of MPs (X^2(5, n=30) =17.79, p = 0.003) (Fig. 3).

3.5. Reproduction test following neonate exposure to MPs

A 21-day reproduction test of adult *D. magna* revealed significant differences in the mean number of offspring between treatments (X^2(5, n=30) = 1032, p>0.001, Fig. 4). This was because low amounts of algae were associated with a reduction in reproduction: MPs had no impact (S1 Table 2).

3.6 Growth Rate

There were highly significant differences in growth rate between the treatments ($F_{45,283}$=3.455, p< 0.001) (Fig. 5). Growth rate was higher in treatments with high levels of algal food (S1 Table 3).

3.7 Distribution of microplastics between the water and sediment in mesocosms

Significantly more MPs were measured in sediment compared to water over time ($F_{(2,68)}$=59.4, p <0.001) (Fig. 6). The number of MPs in the water body remained constant over time, with no evidence of a change in number ($F_{(1,33)}$=0.33, p= 0.567) (Fig. 6). The abundance of MPs in the water column showed no correlation with increase in air temperature, correlation = - 0.06 ; $F_{(1,5)}$= 0.018, p= 0.898 and a non-significant negative with rainfall correlation of = - 0.70; $F_{(1,5)}$= 0.1361, p= 0.727.

3.8.Effects of microplastics on species abundance

Daphnia magna numbers fluctuated between weeks but overall there were no significant differences between controls and mesocosms exposed to MPs at the end of 12 weeks (Fig. 7) (Z=0.918, p=0.36). However, on a week by week basis, there were some highly significant differences, with lower *D. magna* numbers in the MP treated mesocosms in the first half of the experiment (SI Table 4).

Similarly overall abundances of other macroinvertebrates showed no effect of treatment over the 12 weeks (*C. pipiens* Z=-1.055, p=0.29; *P. antipodarum* Z= 1.596; P = 0.110; *Hydrachnidia* Z= 0.005; P = 0.996; *C. plumbeus* Z= -1.168, P = 0.24). Abundances of macroinvertebrates that had independently colonised the mesocosms suggested an impact on only one species, the mayfly *Leptophlebia* spp. which started to appear from the fourth week of sampling and was significantly dominant in mesocosms treated with MPs $X^2(1) = 5.62$, p =0.018 (Fig. 8 A). *Corixa punctata* (Lesser water boatman), which also appeared in the fourth week of sampling in the control mesocosm was not affected by MP treatmentX^2 (1) = 0.683, p =0.40) (Fig. 8 B). Despite the lack of overall significant differences, there were clearly significant differences between treatments in various mesocosms in certain weeks (Tables SI 5-7).

4. Discussion

Microplastic pollution in freshwater environments is a global challenge to ecosystem and human health, and the long-term effect are still poorly understood (Horton et al., 2017; Rochman et al., 2019). Most studies have focused on laboratory experiments to examine the uptake and toxicity of MPs in freshwater invertebrates with limited results from fields studies (Wagner and Lambert, 2018). Here, for the first time we examine the abundance and chronic ecotoxicological effects of 15 μm polystyrene MPs on freshwater organisms in the laboratory and in small mesocosms.

Laboratory chronic toxicity tests with *D. magna* adults and neonates exposed to two 15 μm polystyrene MP concentrations (100 and 800 MPs/mL) revealed that mortality was linked to algal food availability, not exposure to MPs, despite MP ingestion. This suggests some selectivity in eating algae over MPs, something that has been demonstrated previously. *Daphnia* exposed to primary MPs or kaolin, with low and high food concentrations, revealed life history trait changes solely linked to food concentration, not MPs (Ogonowski et al., 2016).

Our previous research using 2 μm polystyrene MPs was designed to look specifically at the impact of food, using MPs of approximately the same size of the algal cell with algal concentrations chosen based on the minimum and maximum normal daily feeding of *Daphnia* (Aljaibachi and Callaghan, 2018). When exposed to a single concentration of 2 μm MPs *Daphnia* almost immediately ate them in large quantities in proportion to their concentration, a finding replicating that of Pavlaki et al., (2014). However we found that *Daphnia* given algae with MPs were quite selective, preferentially eating algae over 2 μm MPs (Aljaibachi and Callaghan, 2018), a result found elsewhere with *Daphnia* selectively feeding on phytoplankton rather than clay particles (DeMott, 1986).

A number of studies have shown that MPs fed to laboratory organisms have practically no impact in the confines of the systems used (Schür et al., 2019; Wang et al., 2019). Reproduction tests for adults and neonates showed a similar effect in that food availability had an impact, but MPs did not. A similar result was found with *Daphnia* exposed to primary MPs or kaolin, with low and high food concentrations, where *Daphnia* life history trait differences were linked to food concentration, not MPs (Ogonowski et al., 2016).

Given that the MPs used were pristine and had not been in contact with any toxins which might adhere to their surface, we can say with confidence that, in themselves, these particles have no important effect on laboratory *Daphnia*. It could simply be the case that they are too large to be ingested by neonates, but the experiments here took neonates through to adults, with no effect and animals definitely ingested MPs.

Studies on different types of MPs and smaller sizes of MP have found toxic effects. Deposit-feeding marine lugworms, *Arenicola marinara*, fed on plasticised polyvinylchloride under laboratory conditions at concentrations found in the environment suffered depleted energy reserves which were probably linked to a reduction in feeding and an inflammatory response

(Wright et al., 2012). Likewise, marine mussels *Mytilus edulis* fed factory clean high-density polyethylene up to 80 μm in size displayed toxic effects including a strong inflammatory response and was related to cellular uptake (von Moos et al 2012). One explanation for the lack of effect in our research is that the polystyrene is less toxic than other MPs (Wright et al., 2012; von Moos et al., 2012). A study on 20 μm polystyrene MPs in the marine copepod *Calanus hegolandicus* also found no effect on egg production or survival (Cole et al 2015). Looking at these and other studies, a theme has emerged in that different plastics and MP sizes are being used in different studies, generating conflicting results. MPs cannot be treated as though they are one type of stressor and conclusions based on simple experiments and approaches are probably not informative. There is an argument for a systematic analysis of MP size, type, concentration, test organism and exposure method (de Sa et al 2018). . We would also argue that studies of effects should not be confined to laboratory systems. Laboratory experiments have little relation to natural systems where external factors can play a role in the disturbance and abundance of MPs, including the presence of competitors, predators and temperature and rainfall.

The mesocosm experiment was conducted during a year with an extremely hot and dry summer and evaporation of water from the mesocosms was an issue; water had to be added to maintain the volume. This was an issue in both control and treated mesocoms and there was no evidence that this mixed the MPs up into the water column. Temperature and rainfall were also not significantly correlated with MP numbers in the water column. After the second week of the experiment MPs fell to the bottom of the mesocosms, leaving almost none in the water column. This has also been shown in larger, more natural systems such as lakes (Su et al., 2016).

Daphnia magna population numbers were lower in mesocosms exposed to MPs compared to the controls for the first seven weeks of exposure. Although MPs fell to the sediment after two weeks, *Daphnia* would have been exposed to high levels of MPs initially. Any negative impact would be evident in the first few weeks but disappear in a new generation (after 21 days) as the MPs effectively disappeared. This is in line with transgenerational research where *D. magna* exposed to pristine microspheres (mixed sizes of 1–5 μm) recovered if they were placed into clean water, although they suffered effects on mortality, reproduction and the population growth rate up to third generations post exposure (Martins and Guilhermino, 2018).

The population abundance of other species in the mesocosms was variable. The mosquito *C. pipiens* fluctuated in number over 12 weeks in both control and treated mesocosms but it is well known that mosquito populations are very variable and seasonal (Ortiz-Perea et al., 2018; Townroe and Callaghan, 2015). *Culex pipiens* was not significantly affected by the presence of MPs which agrees with research on *Culex* mosquitoes showing that 15 MPs had no effect on mortality or growth rate (Al-Jaibachi et al, 2018).

Although not added at the start of the experiment, the mayfly *Leptophlebia* spp rapidly colonised the mesocosms with significantly more in MP-treated mesocosms. Since *Leptophlebia* species are detritivores (Sweeney et al, 1986), there would have been more food availability in treated ponds from *D. magna* deaths in the first 6 weeks. This may have resulted in more individuals surviving and being collected during sampling. A second species to colonise ponds was the lesser water boatman, *Corixa punctata*. This is a potential competitor for food since it has a diet of algal cells but there was no significant difference in numbers between mesocosm treatments.

The abundance of MPs in most freshwater environments investigated highlights questions about their impact on the biota biodiversity, food chain, community composition and predator-prey interactions and the possibility to accumulate in the food chain or transfer ontogenically to different environments. Trophic transfer via predation has been identified as a potentially major pathway through which MPs can move through food webs (Batel et al., 2016; Chae and An, 2017; Nelms et al., 2018; Provencher et al., 2018), however quantifications of how exposure to MP pollution influences trophic interaction strengths are lacking, especially in highly vulnerable, understudied freshwater environments (Blettler et al., 2018). aibachi, et al. 2018a; Al-Jaibachi, et al. 2018b; Rillig 2012; Wright et al. 2013) we demonstrate that MPs can be transferred and retained trophically from filter feeding organisms to higher predators, and that trophic transference relates to consumption rates. Predation by larval *C. flavicans* towards larval mosquito prey was significant irrespective of prior prey exposure to MPs. Neither search efficiency (attack rate) nor time taken to subdue, capture and digest prey (handling time) was significantly affected by prey MP exposure. Whilst both the area of attack rate and handling time parameters have been shown to be heavily context-dependent e.g. (Barrios-O'Neill et al., 2016; Cuthbert et al., 2018b), here we show that the presence of MP pollution does not elicit changes to predation rates. Therefore, MPs are likely to be readily transferred to predators from prey in MP-polluted systems.

To conclude, this research addresses a key knowledge gap, namely that little is known about the ecological impacts of MPs in the freshwater natural environment. Most research to date has focused on laboratory studies which don't take biotic and abiotic environmental changes into account. *Daphnia* numbers were significantly reduced in MP treated mesocosms despite no effect on other organisms, other than an increase in *de novo* colonisation, and no effect on *Daphnia* life history parameters in the laboratory. The effects of 15 µm polystyrene MPs on *Daphnia magna* survival, growth, and reproduction in the laboratory were similar to a parallel study of ours using 2 µm polystyrene MPs. This showed that the availability of algal food was far more important than any toxic impact of the MPs. This demonstrates that laboratory studies can indicate effects only under the conditions set. Most of the MPs had settled in the mesocosm sediment after the first week of exposure which was not possible in the laboratory since no sediment was used. The study highlights a need to look at the availability of both food and MPs in natural environments where a community of organisms are interacting.

Figure legends

Figure 1 Mortality of *Daphnia magna* for 21 days, expressed as a function of time, after chronic MP exposure in the laboratory under high and low food conditions.

Figure 2 Effects of combinations of high and low MPs and algae concentrations on the mean number of offspring of *Daphnia magna*. Error bars indicate ± 95% confidence intervals. Results obtained under laboratory conditions.

Figure 3 Mortality rate over 21 days for neonate *Daphnia magna* after exposure to different treatments of MPs and algae, under laboratory conditions.

Figure 4 *Daphnia magna* reproduction (neonate production) after 21 days exposure to a range of MP and algae treatments (algae (Low), algae (High), Algae = MP (Low), Algae = MP(High), Algae>MP, MP>Algae). Error bars indicate ± 95% confidence. Results obtained under laboratory conditions.

Figure 5 Effect of 21 days exposure to different combinations of MPs and algae (Algae (Low), Algae (High), Algae = MP(Low), Algae = MP(High), Algae >MP, MP> Algae) on the body length of *Daphnia magna*. Each point represents the mean of five replicates ± standard error (SE). Results obtained under laboratory conditions.

Figure 6 The mean number of MPs in the mesocosm sediment and water body ± SE. In the mesocosms.

Figure 7 Mean abundance of *Daphnia magna* in the mesocosms over the experimental period in relation to treatments. The error bars indicate the standard error (±SE) of the mean.

Figure 8 The mean abundance of (A) *Leptophlebia* spp. (mayfly larvae) and (B) *Corixa punctate* (lesser water boatman) in the mesocosms over the experimental period in relation to treatments. The error bars indicate the standard error ±SE of the mean.

Ethics

Ethics committee approval was not required.

Data accessibility

Data files are available in online supplementary material.

Author contribution

All authors provided substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; were involved in drafting the article or revising it critically for important intellectual content; approved the final version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests

We declare we have no competing interests.

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Table 1 Concentrations of MPs (MPs ml^{-1}) and algae (µl) added to each treatment to study chronic toxicity in *D. magna*.

Treatments	Algae concentrations (µl)	Microplastics concentrations (MPs ml^{-1})
Algae (Low)	100	0
Algae (High)	800	0
Algae=MPs (Low)	100	100
Algae=MPs (High)	800	800
Algae>MPs	800	100
MPs>Algae	100	800

Table 2 Classification and number of the species added to each mesocosms.

Species	Habitat and feeding	Classification	Number in each pond
Daphnia magna	Water column Filter feeder	Class: Branchiopoda Order: Cladocera Family: Daphniidae	1000
Culex pipiens	Water column and surface Filter feeder	Class: Insecta Order: Diptera Family: Culicidae	15

Chironomus *plumosus*	Sediment Filter feeder	Class: Insecta Order: Diptera Family: Chironomidae	30
Jenkins spire-shell *Potamopyrgus* *antipodarum*	Water surface and sides Herbivore	Class: Gastropoda Order: Littorinimorpha Family: Tateidae	15
Water mite *Hydrachnidia*	Water column Predator	Class: Arachnida Order: Trombidiformes Family: Hydrachnidiae	15