

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

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

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

28 **Introduction**

29 Plastic pollution in aquatic habitats is a serious environmental issue worldwide that has
30 galvanised businesses, the general public and governments into taking action. Much of the
31 early research focussed on highly visible macroplastics in marine ecosystems with fewer than
32 4% of research papers on freshwater (Wagner and Lambert 2018). In recent years, interest
33 has shifted towards freshwater ecosystems and, in particular, the impact of microplastics
34 (MPs) (Wagner et al. 2014; Eerkes-Medrano et al. 2015; Wagner and Lambert 2018).
35 Microplastics are diverse plastics, including polyethylene and polystyrene, whose fragments
36 are smaller than 5 mm in size and are produced by the degradation of larger particles or are
37 manufactured as microbeads for use in, for example, cosmetics and toiletries (Andrady, 2011;
38 Imhof et al., 2013; Eriksen et al., 2014). Whilst bans on the use of MPs in toiletries have been
39 in place for a number of years, the problems remain significant since there are many pollution
40 routes and types of MP (Rochman et al, 2019).

41

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

53

54 Although there are numerous studies to investigate the occurrence and abundance of MPs in
55 freshwater environments including rivers and lakes, relatively few have looked at the impact
56 on the organisms being exposed (Sighicelli et al., 2018; Wagner and Lambert, 2018). Their
57 size results in them being easily ingested by many aquatic organisms at various trophic levels
58 and stages of development, including freshwater invertebrates (Cole et al., 2013; Scherer et
59 al., 2017; Al-Jaibachi et al., 2018a; Aljaibachi and Callaghan, 2018; Liu et al., 2019).
60 Microplastics can be carriers of toxic chemicals e.g. polychlorinated biphenols or plasticizers
61 added during production and bacteria that can absorb onto their surface (Talsness et al.,
62 2009). Therefore the behaviour of MPs in a pristine state may be very different from those
63 released into the environment.

64

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

78 more likely in lower trophic organisms which could enhance the transfer through the food chain
79 (Anbumani & Kakkar 2018; Al-Jaibachi, et al. 2018a).

80

81 The relationship between laboratory results and the behaviour and interaction of MPs and
82 invertebrates in the natural environment must be determined using more natural exposure
83 methods. Microplastics entering a natural environment are unlikely to remain stationary but
84 will instead be transported between environmental compartments (Lambert and Wagner,
85 2018). The fate and movement of MPs will depend on hydrology and vegetation (Lambert
86 and Wagner, 2018) and in lakes is likely to depend on sediment disturbance. The abundance
87 of MPs in most freshwater environments investigated highlights questions about their impact
88 on the biota biodiversity, food chain, community composition and predator-prey interactions
89 and the possibility to accumulate in the food chain or transfer ontogenically to different
90 environments (Wright et al., 2013; Al-Jaibachi et al., 2018a; Cuthbert et al., 2019).

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

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

103 The small mesocosms chosen to implement the experiment have been studied previously and
104 have demonstrated their value in rapidly measuring the impact of environmental stressors on
105 freshwater communities in a controlled but natural environment (Céréghino et al., 2008).
106 Fluorescent 15 µm PS MPs were chosen for studies into the ecotoxicological effect on *Daphnia*
107 *magna* because of concerns regarding our ability to detect smaller MPs in the mesocosms. The
108 impact of MPs on the population size and community were examined by manipulating the
109 mesocosms so that at the start of the study had the same population size and composition. The
110 animals used were all taken from the mesocosms where they had naturally colonised. They
111 included *C. pipiens* and *D. magna* as well as predators and animals that dwell in the sediment.
112 The mesocosms were monitored for 12 weeks. We hypothesized that MPs would sink to the
113 sediment and be unavailable to animals in the water column with a consequent lack of effect
114 on population size or community composition.

115 **2. Materials and Methods**

116 *2.1. Preparation of microplastics (MPs)*

117 Fluorescent 15 μm green carboxylate-modified [polystyrene](#) MPs (density
118 1.06 g cm^{-3} , [excitation](#) 470 nm; emission 505 nm, Sigma-Aldrich, UK) were used in all
119 experiments. Microplastics were stored as a stock suspension (1%) and mixed as per [Aljaibachi](#)
120 [et al. \(2018a\)](#). The number of PS particles from the stock solution were counted under the epi-
121 fluorescent microscope at 10x magnification (Carl Zeiss Axioskop, Wetzlar, Germany). Each one
122 milliliter of stock solution contained 5×10^6 , MPs mL^{-1} .

122 *2.2. Daphnia cultures*

123 *Daphnia magna* were obtained from the Water Research Centre (WRC, Medmenham, UK)
124 and cultured at the University of Reading for more than ten years prior to this experiment.
125 Full details of culturing methods are given in (Hooper et al., 2006). *Daphnia* were maintained
126 in Organization for Economic Co-operation and Development (OECD) reconstituted water
127 (media) and fed yeast and *C. vulgaris* var *Viridis* following the methods of (Hayashi et al.,
128 2008). New cultures of *Daphnia* were prepared with 15 neonates in 1,200 ml beakers filled
129 with OECD media (the progeny of these neonates are the first brood). Juveniles were
130 removed regularly from the culture and the media was changed once a week. The third brood
131 produced by the original 15 neonates were used for experiments.

132 *2.2 Uptake of microplastics with and without algae*

133

134 Individual 18 day old *D. magna* were placed in 50 ml beakers filled with media and starved for
135 24 h prior to exposure. In a random design, animals were exposed to one of 4 concentrations
136 of MP ($2, 4, 8$ and $16 \times 10^5 \text{ ml}^{-1}$) with varying amounts of algae (Table 1) for 60 min. Each
137 treatment was replicated three times. Animals were rinsed in distilled water to remove any MPs
138 adhering to the outside and frozen at -20°C . Individual animals were homogenized using a glass
139 Kontes Pellet Pestle (Fisher Sciences Loughborough, UK), in 500 μl distilled water in a 1.5 ml

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

145 2.3 Adult Chronic Toxicity Tests

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

153 2.4. Neonate Chronic Toxicity Test

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

161 2.4. Study site and mesocosms

162 Thirty two mesocosms had previously been dug in the experimental grounds at the University
163 of Reading, Berkshire, England (51°26'12.2"N, 0°56'31.2"W) in 2012. The mesocosms were

164 laid out in a Latin square, with three metre intervals in three rows of eight mesocosms. Each
165 mesocosm was a sunked bucket of diameter 48 cm depth 30 cm lined with a rubber pond liner.
166 Fifteen of these mesocosms were randomly selected for use in this study.

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

171 *2.4.1 Preparation and sampling of the mesocosms community*

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

181

182 Weekly sampling then followed using a standardised technique; using a mesocosm net of
183 approx. 60mm x 120mm, with a small enough mesh size to collect both the zooplankton such
184 as *D. magna* and other invertebrates such as mosquito larvae (*Culex* spp.). The net was swept
185 through the water using a figure of 8 motion four times 10-15 cm below the surface of the
186 water. Samples were then placed in a 1 litre plastic bottle and removed to identify

187 macroinvertebrates in the laboratory using a stereo microscope and number of keys (Croft,
188 1986; Greenhalgh and Ovenden, 2007; Dobson et al, 2013). Identified organisms were counted
189 and then returned to the mesocosm from which they came. All members of each species were
190 individually counted, except for *D. magna*, numbers of which were estimated by counting the
191 number of individuals in 1mL, and then multiplying this by the number of mL in the sample
192 due to the very high numbers of individuals. This process was repeated once per week over 12
193 weeks, with the initial set up on and addition of the MPs on the 12th June 2017, first data
194 collection on the 19th June 2017, and the final samples taken on the 29th August 2017. Samples
195 were taken between 10am and 12pm weekly.

196 2.4.2 Distribution of microplastics in mesocosms

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

204

205 The mesocosms were left undisturbed for a week, then samples were taken weekly from the
206 22nd June 2017 until the 10th August 2017. Five 1mL water samples were taken from each of
207 two depths (5cm under water surface and 5cm above sediment), using a 1 mL and then water
208 samples were mixed together before being filtered onto a nucleopore track-etched membrane
209 (Whatman, Kent, UK) <10 µm, by using a glass vacuum filter holder connected to a manual
210 air pump.

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

214 2.5. *Statistical analysis*

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

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

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

225 The weekly abundance of invertebrate groups was analysed in R v3.4.2 (R Development Core
226 Team, 2017). Generalized Linear Model (GLM) was used assuming a quasi-Poisson error
227 distribution since they were not normally distributed, as assessed by Shapiro-Wilk's test ($p <$
228 0.05).

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

232 3. Results

233 3.1. Uptake of increasing concentrations of MPs

234 Ingestion of MPs by *D. magna* without algae increased significantly as MP concentration
235 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
236 (MP=algae), there was no increase in ingestion with increasing concentration ($F_{3,32}=0.415,$
237 $p=0.743$). The mean number of MPs ingested by *D. magna* exposed to algae > MPs
238 significantly decreased as algal concentration increased ($F_{3,32}=148.63, p < 0.001$).
239

240

241 3.2 Adult Chronic toxicity test

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

245 3.3. Reproduction Test

246 A 21-day reproduction test of adult *D. magna* revealed significant differences in the mean
247 number of offspring between treatments ($X^2(5, n=30) = 216.1, p= 0.001$) (Fig. 2). This was
248 because treatments with low food were associated with low numbers of offspring (S1 Table
249 1).

250 3.4. Neonate chronic toxicity test

251 Mortality tests were significantly different between treatments exposed to low and high algae
252 concentrations, irrespective of the presence of MPs ($X^2(5, n=30) = 17.79, p = 0.003$) (Fig. 3).
253

253

254 3.5. Reproduction test following neonate exposure to MPs

255 A 21-day reproduction test of adult *D. magna* revealed significant differences in the mean
256 number of offspring between treatments ($X^2(5, n=30) = 1032, p > 0.001$, Fig. 4). This was

257 because low amounts of algae were associated with a reduction in reproduction: MPs had no
258 impact (S1 Table 2).

259

260 3.6 Growth Rate

261 There were highly significant differences in growth rate between the treatments ($F_{45,283}=3.455$,
262 $p < 0.001$) (Fig. 5). Growth rate was higher in treatments with high levels of algal food (S1
263 Table 3).

264 3.7 Distribution of microplastics between the water and sediment in mesocosms

265 Significantly more MPs were measured in sediment compared to water over time ($F_{(2,68)}=59.4$,
266 $p < 0.001$) (Fig. 6). The number of MPs in the water body remained constant over time, with
267 no evidence of a change in number ($F_{(1,33)}=0.33$, $p= 0.567$) (Fig. 6). The abundance of MPs in
268 the water column showed no correlation with increase in air temperature, correlation = - 0.06 ;
269 $F_{(1,5)}= 0.018$, $p= 0.898$ and a non-significant negative with rainfall correlation of = - 0.70;
270 $F_{(1,5)}= 0.1361$, $p= 0.727$.

271

272 3.8. Effects of microplastics on species abundance

273 *Daphnia magna* numbers fluctuated between weeks but overall there were no significant
274 differences between controls and mesocosms exposed to MPs at the end of 12 weeks (Fig. 7) (
275 $Z=0.918$, $p=0.36$). However, on a week by week basis, there were some highly significant
276 differences, with lower *D. magna* numbers in the MP treated mesocosms in the first half of the
277 experiment (SI Table 4).

278 Similarly overall abundances of other macroinvertebrates showed no effect of treatment over
279 the 12 weeks (*C. pipiens* $Z=-1.055$, $p=0.29$; *P. antipodarum* $Z= 1.596$; $P = 0.110$;

280 *Hydrachnidia* $Z= 0.005$; $P = 0.996$; *C. plumbeus* $Z= -1.168$, $P = 0.24$). Abundances of

281 macroinvertebrates that had independently colonised the mesocosms suggested an impact on
282 only one species, the mayfly *Leptophlebia* spp. which started to appear from the fourth week
283 of sampling and was significantly dominant in mesocosms treated with MPs $X^2(1) = 5.62, p$
284 $=0.018$ (Fig. 8 A). *Corixa punctata* (Lesser water boatman), which also appeared in the
285 fourth week of sampling in the control mesocosm was not affected by MP treatment $X^2(1) =$
286 $0.683, p = 0.40$ (Fig. 8 B). Despite the lack of overall significant differences, there were
287 clearly significant differences between treatments in various mesocosms in certain weeks
288 (Tables SI 5-7).

289 **4. Discussion**

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

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

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

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

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

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

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

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

349 *Daphnia magna* population numbers were lower in mesocosms exposed to MPs compared to
350 the controls for the first seven weeks of exposure. Although MPs fell to the sediment after two

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

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

363

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

372

373 The abundance of MPs in most freshwater environments investigated highlights questions
374 about their impact on the biota biodiversity, food chain, community composition and predator-
375 prey interactions and the possibility to accumulate in the food chain or transfer ontogenically

376 to different environments. Trophic transfer via predation has been identified as a potentially
377 major pathway through which MPs can move through food webs (Batel et al., 2016; Chae and
378 An, 2017; Nelms et al., 2018; Provencher et al., 2018), however quantifications of how
379 exposure to MP pollution influences trophic interaction strengths are lacking, especially in
380 highly vulnerable, understudied freshwater environments (Blettler et al., 2018). aibachi, et al.
381 2018a; Al-Jaibachi, et al. 2018b; Rillig 2012; Wright et al. 2013) we demonstrate that MPs can
382 be transferred and retained trophically from filter feeding organisms to higher predators, and
383 that trophic transference relates to consumption rates. Predation by larval *C. flavicans* towards
384 larval mosquito prey was significant irrespective of prior prey exposure to MPs. Neither search
385 efficiency (attack rate) nor time taken to subdue, capture and digest prey (handling time) was
386 significantly affected by prey MP exposure. Whilst both the area of attack rate and handling
387 time parameters have been shown to be heavily context-dependent e.g. (Barrios-O'Neill et al.,
388 2016; Cuthbert et al., 2018b), here we show that the presence of MP pollution does not elicit
389 changes to predation rates. Therefore, MPs are likely to be readily transferred to predators from
390 prey in MP-polluted systems.

391

392 To conclude, this research addresses a key knowledge gap, namely that little is known about
393 the ecological impacts of MPs in the freshwater natural environment. Most research to date has
394 focused on laboratory studies which don't take biotic and abiotic environmental changes into
395 account. *Daphnia* numbers were significantly reduced in MP treated mesocosms despite no
396 effect on other organisms, other than an increase in *de novo* colonisation, and no effect on
397 *Daphnia* life history parameters in the laboratory. The effects of 15 µm polystyrene MPs on
398 *Daphnia magna* survival, growth, and reproduction in the laboratory were similar to a parallel
399 study of ours using 2 µm polystyrene MPs. This showed that the availability of algal food was
400 far more important than any toxic impact of the MPs. This demonstrates that laboratory studies

401 can indicate effects only under the conditions set. Most of the MPs had settled in the mesocosm
402 sediment after the first week of exposure which was not possible in the laboratory since no
403 sediment was used. The study highlights a need to look at the availability of both food and
404 MPs in natural environments where a community of organisms are interacting.
405

406 Figure legends

407

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

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

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

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

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

423 **Figure 6** The mean number of MPs in the mesocosm sediment and water body \pm SE. In the
424 mesocosms.

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

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

430 **Ethics**

431 Ethics committee approval was not required.

432 **Data accessibility**

433 Data files are available in online supplementary material.

434 **Author contribution**

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

440 **Competing interests**

441 We declare we have no competing interests.

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445

446

447 **5. References**

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604

605 **Table 1 Concentrations of MPs (MPs ml⁻¹) and algae (μl) added to each treatment to study**
 606 **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

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

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

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

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