



The influence of microplastics on trophic interaction strengths and oviposition preferences of dipterans

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1 *Short Communication*

2 **The influence of microplastics on trophic interaction strengths and**
3 **oviposition preferences of dipterans**

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21 Abstract

22 Microplastic (MP) pollution continues to proliferate in freshwater, marine and terrestrial
23 environments, but with their biotic implications remaining poorly understood. Biotic
24 interactions such as predation can profoundly influence ecosystem structuring, stability and
25 functioning. However, we currently lack quantitative understandings of how trophic
26 interaction strengths and associated behaviours are influenced by MP pollution, and how
27 transference of MPs between trophic levels relates to consumptive traits. We also lack
28 understanding of key life-history effects of MPs, for example, reproductive strategies such as
29 oviposition. The present study examines the predatory ability of non-biting midge larvae,
30 *Chaoborus flavicans*, towards larvae of *Culex pipiens* mosquitoes when the latter are exposed
31 to MPs, using a functional response (FR) approach. Transfer of MPs occurred from larval
32 mosquitoes to larval midges *via* predation. Microplastics transfer was significantly positively
33 related to predation rates. Predation by *C. flavicans* followed a Type II FR, with average
34 maximum feeding rates of 6.2 mosquito larvae per hour. These and other FR parameters
35 (attack rates and handling times) were not significantly influenced by the presence of MPs.
36 Further, *C. pipiens* adults did not avoid ovipositing in habitats with high concentrations of
37 MPs. We thus demonstrate that MPs can move readily through freshwater food webs *via*
38 biotic processes such as predation, and that uptake correlates strongly with consumption
39 rates. Further, as MPs do not deter adult mosquitoes from ovipositing, our experiments reveal
40 high potential for MP exposure and transference through ecosystems.

41 Key words

42 food chain; predator-prey; microplastics transference; functional response; *Chaoborus*; *Culex*

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45 **Introduction**

46 Microplastic (MP; < 5 mm in size) pollution is prolific in terrestrial and aquatic ecosystems
47 globally (Mason et al., 2018; Sighicelli et al., 2018; Wagner and Lambert, 2018). Whilst the
48 enormous scale of MP pollution is unequivocal, we currently have a poor understanding of
49 how MP presence influences interaction strengths between trophic groups (Wagner and
50 Lambert, 2018). Interaction strengths between predators and prey can profoundly impact the
51 infrastructure of ecosystems through the determination of predator population growth and
52 prey population stability (Paine, 1980; Gilbert et al., 2014). Thus, understanding factors that
53 influence predation are crucial to predicting ecosystem structure and functioning. Trophic
54 transfer *via* predation has been identified as a potentially major pathway through which MPs
55 can move through food webs (Batel et al., 2016; Chae et al., 2018; Nelms et al., 2018;
56 Provencher et al., 2018), however quantifications of how exposure to MP pollution influences
57 trophic interaction strengths are lacking, especially in highly vulnerable, understudied
58 freshwater environments (Blettler et al., 2018).

59 Functional responses (FRs) (Solomon, 1949; Holling, 1959) quantify resource use under
60 different resource densities and are powerful predictors of interaction strengths between
61 consumers and resources. Three FR types have been broadly characterised: Type I FRs are
62 filter-feeder specific (Jeschke et al., 2004), wherein consumption increases linearly with
63 resource densities; Type II FRs exhibit a decelerating intake rate, with high proportional
64 consumption at low resource densities potentially leading to resource extirpation; Type III
65 FRs are sigmoidal, characterised by low proportional consumption at low densities, thus
66 potentially facilitating refugia for prey (Holling, 1949). For predators and prey, FRs can
67 quantify how prey populations are regulated by predators across different context-
68 dependencies (e.g. Cuthbert et al., 2018). Shifts in the FR form and/or magnitude of
69 predators, for instance from destabilising Type II to stabilising Type III, are known to be

70 driven by environmental contexts (Alexander et al., 2012). However, we currently have little
71 quantitative bases to predict how pollutants such as MPs will influence predator-prey
72 dynamics. Furthermore, distributions of prey populations are often reliant on selective
73 processes relating to quantifications of risk and reward between habitat patches, especially
74 for reproductive decisions such as oviposition sites (Nonacs and Dill, 1990). Indeed, the
75 ability to avoid potentially harmful habitats can benefit the fitness of progeny and influence
76 the success of populations. However, there has been little research to quantify the influence
77 of MPs on selective behaviours, such as oviposition, which can drive species distributions
78 and influence biotic interactions (Goldstein et al., 2012; Majer et al., 2012).

79 In the present study, we thus examine whether MP exposure modulates interaction strengths
80 between predators and prey, and whether trophic MP transfer can be related to predation
81 rates. We then discern MP implications for ovipositional behaviour. Focal organisms were
82 larvae of the predatory non-biting midge *Chaoborus flavicans*, and filter-feeding larvae of the
83 mosquito complex *Culex pipiens*, which often co-occur in natural and artificial aquatic
84 habitats.

85 **Materials and methods**

86 *Experimental design*

87 Fluorescent 2 μm yellow-green carboxylate-modified polystyrene MPs (density 1.050 g cm^{-3} ,
88 88 excitation 470 nm; emission 505 nm, Sigma-Aldrich, UK) were used in all experiments.
89 Microplastics were stored as a stock suspension (2.5 mg mL^{-1}) and mixed as per Al-Jaibachi
90 et al. (2018a). *Chaoborus flavicans* (1.0 – 1.2 cm) larvae were purchased commercially
91 (Northampton Reptile Centre, UK) and acclimated for 6 days in a laboratory at the University
92 of Reading ($19 \pm 1 \text{ }^\circ\text{C}$, 16:8 light:dark) on a diet of *C. pipiens* larvae in 5 L dechlorinated tap-
93 water. Wild *C. pipiens* were collected from the Whiteknights campus of the University of

94 Reading (51°26'12.2"N, 0°56'31.2"W). Egg rafts of *C. pipiens* were sampled from artificial
95 container habitats and, upon hatching, fed *ad libitum* on crushed rabbit food pellets in the
96 same laboratory in 10 L dechlorinated tap-water. Gravid adult *C. pipiens* were collected
97 overnight using modified gravid box traps with a hay and yeast infusion used as bait (see
98 Townroe and Callaghan, 2014).

99 In experiment 1, in the laboratory (19 ± 1 °C, 16:8 light:dark), groups of 400 *C. pipiens*
100 larvae (0.15–0.20 cm) were exposed to one of two MP treatments (0 particles mL⁻¹, 100
101 particles mL⁻¹) in 500 mL arenas for 22 h, whilst predators were simultaneously starved.
102 Following treatments, *C. pipiens* larvae were rinsed in dechlorinated tap water and introduced
103 at 5 densities (2, 5, 10, 20, 40; $n = 5$ per treatment group) into 20 mL arenas containing 10
104 mL dechlorinated tap-water. After 2 h of prey acclimation, predatory *C. flavicans* were
105 introduced and allowed to feed for 2 h. Predators were then removed and remaining live prey
106 counted. Controls consisted of a replicate at each MP treatment and prey density in the
107 absence of predators. Individual predators and prey were frozen at -20 °C before
108 homogenisation and filtration using nucleopore track-etched membranes (Whatman, UK) of <
109 0.1 µm, with the MPs on filter membranes subsequently counted using an epi-fluorescent
110 microscope (Zeiss Axioskop, Germany).

111 In experiment 2, thirty wild gravid adult *C. pipiens* were transferred into each of six 30 cm³
112 cages in a laboratory (25 ± 1 °C, 16:8 light:dark). Mosquitoes were allowed to oviposit in one
113 of two paired 200mL arenas containing different MP treatments (0 particles mL⁻¹, 100
114 particles mL⁻¹), placed randomly in opposite corners of the cages, over 3 days. Egg rafts were
115 enumerated and removed daily.

116 *Data analysis*

117 In experiment 1, the relationship between MP uptake *via* predation in *C. flavicans* and
118 number of prey killed was examined using a generalised linear model (GLM) assuming
119 Poisson error distribution. A GLM with quasi-Poisson error distribution was used to examine
120 overall prey killed with respect to the ‘MP treatment’ and ‘prey supply’ factors, owing to
121 residual overdispersion. Functional responses (FRs) were modelled using ‘*frair*’ in *R*
122 (Pritchard et al., 2017) with Rogers’ random predator equation (Rogers, 1972). Attack rate
123 and handling time parameters were non-parametrically bootstrapped to generate 95%
124 confidence intervals and compared according to MP treatment using the delta method
125 (Juliano, 2001). Handling time estimates were used to generate maximum feeding rates over
126 the total feeding period ($1/h$).

127 In experiment 2, total egg raft counts were analysed using a generalised linear mixed model
128 with negative binomial distribution between paired MP treatments, owing to residual
129 overdispersion. Cage number was included as a random slope and intercept to account for the
130 paired experimental design.

131 **Results**

132 In experiment 1, *C. pipiens* larvae exposed to 100 particles mL⁻¹ contained 5.8 ± 2.7 (mean \pm
133 SD) MPs, whilst prey not exposed to MPs did not contain MPs. Whilst MPs were not
134 detected in predators following consumption of unexposed prey, transference occurred in all
135 *C. flavicans* that killed MP-exposed *C. pipiens*. Microplastic transference from *C. pipiens* to
136 *C. flavicans* *via* predation was significantly positively related to the number of prey killed (t
137 = 1.972, $p = 0.049$).

138 Survival of prey was 100% in predator-free controls, and so all prey deaths were assumed to
139 be due to predation. Predation by *C. flavicans* did not differ significantly according to prey
140 MP exposure ($t = 0.959$, $p = 0.343$). Prey killed increased significantly with greater prey

141 supplies ($t = 4.938, p < 0.001$) and under both MP treatments given no significant interaction
142 ($t = 0.721, p = 0.472$). *Chaoborus flavicans* exhibited Type II FRs irrespective of prey MP
143 exposure, given that first order terms were significantly negative in both treatment groups
144 (Table 1). Attack rates (initial curve slopes) did not differ significantly between MP exposure
145 treatments ($z = 1.694, p = 0.090$), but trended towards being higher where larval *C. pipiens*
146 were exposed to MPs prior. Handling times did not differ significantly between MP
147 treatments ($z = 1.087, p = 0.277$), although, reciprocally, maximum feeding rates (curve
148 asymptotes) tended to be higher towards prey not exposed to MPs (Table 1). Confidence
149 intervals for attack rates and handling times overlapped overall (Table 1), and across all prey
150 densities between MP treatments (Figure 1), further indicating a lack of significant difference
151 in FRs.

152 In experiment 2, a total of 43 egg rafts were oviposited in MP-treated water and 38 egg rafts
153 in controls. There was no significant difference in oviposition between these treatment groups
154 ($z = 0.380, p = 0.704$) (Figure 2). High statistical power, and thus low probability for Type II
155 error, was found for both predation (power = 0.94) and oviposition (power = 0.93).

156 **Discussion**

157 Microplastics continue to proliferate across the biosphere, with ecological implications
158 frequently unknown (Carlos de Sá et al., 2018; Mason et al., 2018; Sighicelli et al., 2018;
159 Wagner and Lambert, 2018). In the present study, we further demonstrate active uptake of
160 MPs through filtering by larval mosquitoes (Al-Jaibachi et al., 2018a, 2018b). Furthermore,
161 we demonstrate that MPs can be transferred and retained trophically from filter feeding
162 organisms to higher predators, and that trophic transference relates to consumption rates.
163 Predation by larval *C. flavicans* towards larval mosquito prey was significant irrespective of
164 prior prey exposure to MPs. Neither search efficiency (attack rate) or time taken to subdue,

165 capture and digest prey (handling time) were significantly affected by prey MP exposure.

166 Whilst both the area of attack rate and handling time parameters have been shown to be
167 heavily context-dependent (e.g. Barrios-O'Neill et al., 2016; Cuthbert et al., 2018), here we
168 show that the presence of MP pollution does not elicit changes to predation rates. Therefore,
169 MPs are likely to be readily transferred to predators from prey in MP-polluted systems.

170 Larval *Culex* mosquitoes actively filter and retain MP particles, and MPs have been shown to
171 transfer ontogenically from larval to pupal stages, and then to the terrestrial adult mosquito
172 stage (Al-Jaibachi et al., 2018a, 2018b). As with mosquitoes, *C. flavicans* exhibits both
173 aquatic and terrestrial life stages, and thus the potential for ontogenic transference of MPs *via*
174 this species from aquatic to terrestrial environments is high, and could accordingly impact
175 terrestrial vertebrates. Strong potential for MPs to move further through food chains and
176 impact organisms has been demonstrated in other freshwater systems (Chae et al., 2018). In
177 the present study, as transference across trophic stages was positively related to predation
178 rates, uptake of MPs *via* predation may be related to intraspecific or intraindividual variations
179 in consumptive traits. Indeed, such variabilities are often naturally present within populations,
180 and could influence MP pollution impact (Alexander et al., 2015).

181 Oviposition by mosquitoes is selective across gradients of risk and reward (Pintar et al.,
182 2018). The present study demonstrates that gravid adult *Culex* mosquitos are not deterred
183 from ovipositing in aquatic habitats with MPs. Therefore, there is a high potential for larval
184 stages to be exposed to MPs throughout their aquatic life stages, before subsequently
185 transferring MPs trophically or ontogenically (Al-Jaibachi et al., 2018b). Although
186 concentrations of MPs in the present study were high (but see Fischer et al., 2016; Su et al.,
187 2016), the lack of effect of MPs on predation rates and oviposition suggests that similar
188 observations could occur in environments with lower MP concentrations.

189 It is probable that colonists are naïve to potential risks of MPs to fitness. However, Al-
190 Jaibachi et al. (2018a) found that MPs have little influence on the survival and fitness of
191 *Culex* mosquitoes across their life history. Whilst this is the first study to quantitatively link
192 MP transfer with predation rates, work is required to further explore potential trophic
193 accumulation of MPs, alongside behavioural implications, and to discern the potential for
194 aerial dispersal of MPs by freshwater insects aside from mosquitoes (Al-Jaibachi et al.,
195 2018b). Previous work has demonstrated the direct exploitation of MPs particles as
196 oviposition sites by insects aside from mosquitoes in aquatic systems (Goldstein et al., 2012;
197 Majer et al., 2012), and MPs are ingested by a range of other aquatic invertebrates (Nel et al.,
198 2018; Windsor et al., 2019). Here, as larval chaoborids ingest prey whole before regurgitating
199 undigested, solid material, examinations of physiological restrictions on MP retention are
200 required for this group (Moore 1988), particularly given their ecological importance in
201 freshwater environments (Riessen et al. 1984). Elucidations of environmental context-
202 dependencies which modulate MP uptake and transfer rates would additionally be of value,
203 alongside the time-dependent effects of depuration.

204 **References**

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306 **Tables**

307 Table 1. First order terms from the proportion of prey killed as a function of prey density
308 according to MP exposure treatments. Attack rate, handling time and maximum feeding rate
309 parameters from Rogers' random predator equation and bootstrapped ($n = 2000$) 95 %
310 confidence intervals (CIs).

311

312 **Figure legends**

313 Fig. 1. Functional responses of larval *Chaoborus flavicans* towards larval *Culex pipiens*
314 following exposure to different microplastic (MP) treatments (absent, 0 particles mL⁻¹;
315 present, 100 particles mL⁻¹). Shaded area represents bootstrapped ($n = 2000$) confidence
316 intervals.

317 Fig. 2. Number of egg rafts oviposited by *Culex pipiens* between paired treatments with
318 microplastics (MPs) absent (0 particles mL⁻¹) or present (100 particles mL⁻¹).

319 **Ethics**

320 Ethics committee approval was not required.

321 **Data accessibility**

322 Data files are available in online supplementary material.

323 **Author contribution**

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

329 **Competing interests**

330 We declare we have no competing interests.

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