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**

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Introduction

Microplastic (MP; < 5 mm in size) pollution is prolific in terrestrial and aquatic ecosystems globally (Mason et al., 2018; Sighicelli et al., 2018; Wagner and Lambert, 2018). Whilst the enormous scale of MP pollution is unequivocal, we currently have a poor understanding of how MP presence influences interaction strengths between trophic groups (Wagner and Lambert, 2018). Interaction strengths between predators and prey can profoundly impact the infrastructure of ecosystems through the determination of predator population growth and prey population stability (Paine, 1980; Gilbert et al., 2014). Thus, understanding factors that influence predation are crucial to predicting ecosystem structure and functioning. 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 et al., 2018; 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).

Functional responses (FRs) (Solomon, 1949; Holling, 1959) quantify resource use under different resource densities and are powerful predictors of interaction strengths between consumers and resources. Three FR types have been broadly characterised: Type I FRs are filter-feeder specific (Jeschke et al., 2004), wherein consumption increases linearly with resource densities; Type II FRs exhibit a decelerating intake rate, with high proportional consumption at low resource densities potentially leading to resource extirpation; Type III FRs are sigmoidal, characterised by low proportional consumption at low densities, thus potentially facilitating refugia for prey (Holling, 1949). For predators and prey, FRs can quantify how prey populations are regulated by predators across different context-dependencies (e.g. Cuthbert et al., 2018). Shifts in the FR form and/or magnitude of 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 ± 1 °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 cm^3

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.

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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**

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