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## **Prey size and predator density modify impacts by natural enemies towards mosquitoes**

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20 **Abstract**

21 1. Interactions between multiple predators can modify prey risk through its ontogeny and  
22 profoundly alter ecological community. Understandings of these biotic factors are important for  
23 robust quantifications of natural enemy effects on target species, yet their combined influence  
24 lacks investigation.

25 2. We used functional responses (FRs) to quantify the predatory impacts of *Notonecta glauca*  
26 (water boatman; *Ng*) and *Gammarus pulex* (river shrimp; *Gp*) towards four different larval instars  
27 of *Culex pipiens* in container-style habitats. Using conspecific pairs of predators, we examined  
28 multiple predator effects (MPEs) of both predator species across larval prey size, and applied prey  
29 preference tests to examine prey selectivity across predator-prey body size ratios.

30 3. Both predators were able to feed on *C. pipiens* larvae across their larval ontogeny, however, *Ng*  
31 consumed significantly greater larvae than *Gp*. Functional responses of *Ng* were typically Type  
32 IIs, whilst *Gp* trended towards sigmoidal Type IIIs. Predation by pairs of *Ng* and *Gp* showed  
33 independent MPEs towards first, third and fourth instar (except predation by *Gp* at higher densities  
34 of fourth instar) stages, while for second instar stages *Ng* showed synergistic MPEs and *Gp* showed  
35 antagonistic MPEs. Both predators preferred late instar mosquitoes (*Ng*: fourth instar; *Gp*: third  
36 instar). These preferences reflected predator:prey weight-length ratios, showing that relative sizes  
37 of predators and prey are important factors in prey selectivity.

38 4. Our results demonstrate that multiple predator effects, combined with intraspecific prey  
39 preferences, may modulate trophic interactions within ecosystems. Therefore, such effects should  
40 be increasingly considered to further understandings of agent efficacies.

41 **Keywords:** *Culex pipiens*, functional response, context-dependency; prey selectivity; container  
42 habitat

43

#### 44 **Introduction**

45         The strength of feeding interactions between consumers and resources is determined by  
46 multiple factors which must be quantified to understand ecological community dynamics and  
47 stabilities (McCann *et al.*, 1998; Berlow, 1999; Englund *et al.*, 2011; Barrios-O'Neill *et al.*, 2016,  
48 2019). As communities typically comprise multiple consumers (e.g. predators) which compete for  
49 shared resources (e.g. prey), predator-predator interactions may influence interaction strengths  
50 through emergent multiple predator effects (MPEs) as predator densities change (Sih *et al.*, 1998;  
51 Polis & Holt, 1992; Vance-Chalcraft & Soluk, 2005; Schmitz, 2009; Cuthbert *et al.*, 2019b).  
52 Foraging by both conspecific and heterospecific predator groupings may drive non-independent  
53 MPEs associated with non-trophic interactions (Vance-Chalcraft & Soluk, 2005). Whilst  
54 heterospecific MPEs may manifest differently compared to conspecifics owing to differences in  
55 morphologies and foraging tactics (Griffin *et al.*, 2015; Wasserman *et al.*, 2016), determinations  
56 of conspecific MPEs can provide important insights into interactions within relatively simple  
57 communities. Moreover, heterogeneity in predator-prey body size scaling is known to alter the  
58 strength of predatory interactions, with predators often less efficient at handling prey items that  
59 are relatively large or small (Rall *et al.*, 2012; Brose *et al.*, 2008, 2019). This, in turn, may drive  
60 differential prey selectivity patterns and impart size refuge effects (Dalal *et al.*, 2019a). However,  
61 how intraspecific prey characteristics such as size influence the strength of MPEs and direction of  
62 selectivity lacks examination. Understanding how such factors alter conspecific interaction

63 strengths is of particular importance for the assessment and selection of natural enemies for pests  
64 and disease vectors, as context-dependent inefficiencies may incur substantial economic and  
65 public health costs following release of a particular agent.

66         The functional response (FR) can be used to assess the *per capita* efficiency of natural  
67 enemies through quantification of resource use under different resource densities (Solomon, 1949;  
68 O’Neil, 1990; Dalal *et al.*, 2019b). Functional response Types (i.e. Types I, II and III) and  
69 magnitudes (i.e. curve asymptotes) can be used to quantify trophic interactions strengths and  
70 potential community outcomes with respect to resource stability (Holling, 1966; Taylor, 1984;  
71 Juliano, 2001). Saturating Type II FRs are desirable in natural enemies given high rates of resource  
72 acquisition at low densities may cause target population extirpations. Functional responses are also  
73 powerful quantifiers of context-dependencies which alter interaction strengths (Dick *et al.*, 2014,  
74 2017; Barrios-O’Neill *et al.*, 2016), and have been more recently developed for assessments of  
75 MPEs (Barrios-O’Neill *et al.*, 2014; Wasserman *et al.*, 2016a; Sentis & Boukal, 2018). Multiple  
76 predator species can interact in three broad ways: (1) additively (i.e. independently), where prey  
77 risk is predictable from *per capita* effects; (2) synergistically, leading to prey risk enhancement  
78 (Losey & Denno, 1998; Sih *et al.*, 1998); or, (3) antagonistically, leading to prey risk reduction  
79 (Sih *et al.*, 1998; Vance-Chalcraft & Soluk, 2005). In the context of biological control, synergistic  
80 effects among natural enemies are desired as these may enhance impacts upon target species  
81 populations. Additive MPEs are also favoured as these indicate a lack of interference between  
82 predators.

83         Within predator-prey relationships, prey size refuge effects may also influence trophic  
84 dynamics and mediate population stability (Sih, 1987; Emmerson & Raffaelli, 2004; Sarwardi *et*

85 *al.*, 2012, 2013). In turn, these factors can lead to peak interaction strengths at intermediate  
86 predator-prey body size ratios (Barrios-O'Neill *et al.*, 2016; Brose *et al.*, 2019). Intraspecific  
87 variability in prey sizes may thus be an important determinant of predator preferences towards  
88 prey, and could allow for refuge effects as consumers are often unable to handle resources above  
89 or below specific size thresholds (Suchman & Sullivan, 1998; Yamaguchi & Kishida, 2016).  
90 Changing selectivity patterning across target prey ontogeny may therefore influence the efficacy  
91 of natural enemies for biocontrol applications, and particularly towards organisms which exhibit  
92 rapid ontogenic development, such as mosquitoes.

93 Mosquitoes cause unparalleled mortality and morbidity in human populations through  
94 effective transmission of pathogens and parasites which cause disease (ECDC, 2014; WHO, 2017).  
95 Environmental change, coupled with anthropogenic vectors, has led to increased exogenous  
96 mosquito incursions and the (re-)emergence of mosquito-borne diseases in many parts of the  
97 World (Lambrechts *et al.*, 2010; Medlock *et al.*, 2012; Schaffner *et al.*, 2013; Medlock & Leach,  
98 2015). Predatory biocontrol applications have been effective towards larval aquatic mosquito life  
99 stages (e.g. Kay & Nam, 2005), which comprises four prepupal instar classes. Whilst the strength  
100 of *per capita* interactions by predators has also been shown to differ across these larval stages  
101 (Cuthbert *et al.*, 2019a; Dalal *et al.*, 2019a, b), whether such effects are further influenced by  
102 predator density remains unexamined, despite the range of predators which target mosquitoes  
103 (Medlock & Snow, 2008).

104 In this study, we examined the predation by *Notonecta glauca* Linnaeus, 1758 (*Ng*)  
105 (Hemiptera: Notonectidae) and *Gammarus pulex* Linnaeus, 1758 (*Gp*) (Amphipoda: Gammaridae)  
106 towards larval *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) mosquitoes using a FR approach.

107 Both of these predator species are widespread and abundant in waterbodies in temperate regions,  
108 and have taxonomic analogues in tropical regions. *Notonecta glauca* is buoyant, but swims below  
109 the surface and clings to habitat structures (Streams, 1987). *Gammarus pulex*, on the other hand,  
110 is primarily benthic, but can also use the water column. Notonectids often dominate lentic  
111 ecosystems (Das & Gupta, 2012; Dalal & Gupta, 2018), profoundly affect ecological structuring  
112 (Blaustein, 1998), and are important predators of mosquito larvae (Ellis & Borden, 1970; Mondol  
113 *et al.*, 2017; Cuthbert *et al.*, 2019c; Dalal *et al.*, 2019b). Freshwater gammarids, on the other hand,  
114 often dominate lotic habitats and shallow stagnant pools/ditches (Meijering, 1991; Schwartz,  
115 1992). Compared to notonectids, the predatory impact of gammarids towards larval mosquitoes  
116 has been seldom considered (but see Schwartz, 1992; Roberts, 1995). These two predators were  
117 selected as they are numerically dominant in the study area and are representative of different  
118 aquatic habitat types (i.e. lentic and lotic) and zones (i.e. pelagic and benthic). Therefore,  
119 comparison of the chosen species allows for insights between common mosquito antagonists from  
120 different habitat types, and particularly given *Gp* is less likely to encounter larval mosquito prey  
121 in lotic habitats than *Ng* is in more lentic waterbodies. Furthermore, derivations of relative  
122 predator-prey body size effects on prey selectivities could provide insights into scaling generalities  
123 that influence the effects of multiple natural enemies. The present study thus aims to decipher  
124 whether: (1) predatory impacts vary between individuals of these two predator species towards  
125 different size classes of *C. pipiens* larvae; (2) whether emergent MPEs are exhibited between  
126 conspecific predatory pairs of these predators towards mosquito prey; (3) if either of the predators  
127 exhibit preference in selecting a particular size class of mosquito; and, (4) how robustly any prey  
128 preferences can be predicted using predator-prey body mass ratios.

129

## 130 **Materials and methods**

### 131 *Collection and maintenance of organisms*

132           Between July and October 2016 in Northern Ireland, greater water boatmen (*N. glauca*;  
133 *Ng*) were collected from a pond at the Divis and Black Mountain National Trust site (54°36'7.46"N  
134 6° 1'51.84"W), while freshwater amphipods (*G. pulex*; *Gp*) were collected from the Minnowburn,  
135 a tributary of the River Lagan (54°32'54.32"N 5°57'8.48"W). *Notonecta glauca* were collected by  
136 dip netting while *Gp* were collected by kick sampling. The species were placed in separate plastic  
137 holding tanks with continuously aerated dechlorinated tap water at 18 °C ( $\pm$  2 °C) within a  
138 temperature controlled room on a 12:12 light:dark photoperiod, with water changed every 10 days.  
139 Predators were supplied with leaf litter, and fed on chironomid and mosquito larvae *ad libitum*.  
140 Their prey, larvae of the mosquito *C. pipiens*, were collected from Rubane, Northern Ireland  
141 (54°28'27.38"N 5°30'33.14"W) by sampling egg rafts from artificial containers that had been filled  
142 with a hay and yeast infusion to attract oviposition. Egg rafts were then transferred to the same  
143 laboratory at Queen's University Belfast. Upon hatching, mosquito larvae were placed in a plastic  
144 tray (L  $\times$  B  $\times$  H: 34  $\times$  26  $\times$  7 cm) filled with tap water and supplied crushed guinea pig pellets *ad*  
145 *libitum* to culture larvae to the required size class.

### 146 *Functional response experiments*

147           We used *Ng* and *Gp* (*Ng*, length  $\pm$  SD, 15.11  $\pm$  0.18 mm; breadth  $\pm$  SD, 4.87  $\pm$  0.17 mm;  
148 wet weight  $\pm$  SD, 153  $\pm$  0.004 mg; *Gp*, length  $\pm$  SD, 12.51  $\pm$  1.73 mm; breadth  $\pm$  SD [lateral side],  
149 2.57  $\pm$  0.42 mm; wet weight  $\pm$  SD, 50  $\pm$  0.02 mg) as focal predators. Predator treatments comprised

150 either single individuals of each species or two conspecifics of each species (i.e. 1 *Ng* or 1 *Gp*; 2  
151 *Ng* or 2 *Gp*), which were starved for 24 h prior to trials to standardize hunger levels. The use of  
152 mixed species groups was not feasible due to high interspecific predation. Trials were undertaken  
153 in polypropylene containers of 10 cm depth and 12 cm diameter, containing 950 mL dechlorinated  
154 tap water that had previously been aerated. We compared FRs towards the four instars of newly  
155 molted larval *C. pipiens* (first instar, length  $\pm$  SD,  $1.20 \pm 0.07$  mm; wet weight  $\pm$  SD,  $0.02 \pm 0.01$   
156 mg; second instar, length  $\pm$  SD,  $2.10 \pm 0.03$  mm; wet weight  $\pm$  SD,  $0.12 \pm 0.02$  mg; third instar,  
157 length  $\pm$  SD,  $3.90 \pm 0.08$  mm; wet weight  $\pm$  SD,  $1.40 \pm 0.01$  mg; fourth instar, length  $\pm$  SD,  $4.80$   
158  $\pm 0.06$  mm; wet weight  $\pm$  SD,  $2.20 \pm 0.01$  mg). These four instars of mosquito larvae were  
159 introduced separately at densities of 2, 4, 8, 16, 25, 50, 75 and 100 to the four different starved  
160 predatory units ( $n = 3$  per experimental group). Predators were allowed to feed for 24 h after which  
161 remaining live prey were counted. Batches of experimental trials were randomised with respect to  
162 prey density and predator treatment to eliminate time confounds. Controls consisted of prey  
163 without predators at each size class and density ( $n = 3$  per experimental group). Here, FRs are  
164 considered “phenomenological” rather than “mechanistic” in order to compare factorial treatments  
165 under matched experimental conditions (Jeschke *et al.*, 2002; Dick *et al.*, 2014).

#### 166 *Prey preference experiments*

167 Prey preferences were examined by offering 100 larvae of each of the four *C. pipiens*  
168 instars simultaneously (i.e. 400 larvae overall) to the individual starved predators, *Ng* and *Gp* ( $n =$   
169 5 per experimental group), which were allowed to feed for 24 h. The numbers of remaining live  
170 prey within each prey group were then counted. Controls ( $n = 5$ ) were prey combinations in the  
171 absence of predators.

172 *Data analyses*

173 *Functional responses (FR)*

174 Logistic regression of the proportion of prey killed as a function of prey density was used  
175 to discern FR types (Juliano, 2001). Here, a significantly negative first order term indicates a Type  
176 II functional response, whilst a significantly positive first order term followed by a significantly  
177 negative second order term indicates a Type III FR. Conversely, a non-significant first and second  
178 order term could be considered a Type I FR. We used a generalised form of the FR, allowing for  
179 prey depletion over the allocated experimental time (Real, 1977; Rogers, 1972; Pritchard *et al.*,  
180 2017):

$$N_e = N_0(1 - \exp(bN_0^q(hN_e - T)))$$

182 (1)

183 where  $N_0$  is the initial prey density,  $N_e$  is the number of prey eaten,  $b$  is the search coefficient or  
184 capture rate, which, combined with the scaling exponent  $q$ , gives the density-dependent capture  
185 rate ( $bN_0^q$ ),  $h$  is the handling time and  $T$  is the total experimental time. In this model,  $q$  can be fixed  
186 at 0 (a Type II FR), 1 (a Type III FR) or instead optimized, providing a means to examine fits of  
187 either categorical (i.e. Type II or III) or continuous (i.e. flexible) forms. We used maximum  
188 likelihood estimation (Bolker, 2010) for model fitting and Aikaike's Information Criterion ( $\Delta$ AIC)  
189 to select the FR models minimising information loss.

190 Following model selection *via*  $\Delta$ AIC, we non-parametrically bootstrapped raw  
191 consumption data ( $n = 2000$ ) and refitted Equation 1 to construct 95% confidence intervals (CIs)

192 around FRs for each predator treatment combination. All FR analyses were performed with the  
193 “frair” package in R version 3.5.2 (Pritchard *et al.*, 2017; R Core Team, 2018). Visual overlaps of  
194 CIs were used to compare FRs among predator treatments across prey densities.

### 195 *Multiple predator effects (MPEs)*

196 For conspecific predator pairs, observed FRs were estimated by fitting Equation 1 to the  
197 experimental data on consumption rates by two conspecific predators at each prey density. To  
198 detect MPEs, we compared observed FRs to predicted FRs that were generated using a population-  
199 dynamic model of prey depletion over time, parametrised with FR estimates (capture rates and  
200 handling times) from single predator trials (McCoy *et al.*, 2012, Sentis & Boukal, 2018):

$$201 \quad \frac{dN}{dt} = - \sum_{i=1}^n f_i(N)P_i$$

202 (2)

203 where  $N$  is the prey density,  $P_i$  ( $i = 1, 2, \dots, n$ ) are the population densities of predators  $i$  and  $f_i(N)$   
204 is the FR of predator  $i$ . To generate predictions of expected prey survival in the conspecific  
205 predator experiments, initial values of  $N_0$  and  $P_i$  ( $i = 1, 2, \dots, n$ ) were set at the experimental initial  
206 prey and predator densities. The lower and upper CIs around the predictions were estimated with  
207 a global sensitivity analysis based on FR parameter estimates and their variance-covariance  
208 matrices (covariance is assumed to be zero when not known) to generate a number of random  
209 parameter sets using a Latin hypercube sampling algorithm (Soetaert & Petzoldt, 2010). For each  
210 parameter set, the expected prey survival was calculated using the ‘sensRange’ function in the R  
211 package ‘FME’ (Soetaert & Petzoldt, 2010). The 2.5% and the 97.5% quantiles of the survival

212 values obtained from these simulations were used as 95% CIs around the predictions. Predictions  
 213 from the population dynamic model were then compared to the observed values of conspecific  
 214 pairs *via* comparisons of FR CIs.

215 *Prey preferences*

216 Manly's selectivity index  $\alpha$  for conditions without prey replacement (Manly, 1974;  
 217 Chesson, 1983) was used to decipher preferences towards specific larval mosquito instar stages:

218

$$219 \quad \alpha_i = \frac{\ln((n_{i0}-r_i)/n_{i0})}{\sum_{j=1}^m \ln((n_{j0}-r_j)/n_{j0})}, i = 1, 2, \dots, m$$

220 (3)

221 where  $n_{i0}$  is the initial number of prey items and  $r_i$  is the number of consumed prey items by the  
 222 predator of prey type  $i$  and  $m$  is the number of prey types used in the experiment. Values of  $a_i$  were  
 223 converted into electivity indices ( $\varepsilon$ ) for displaying prey preference (Chesson, 1983):

$$224 \quad \varepsilon_i = \frac{m\alpha_i - 1}{(m-2)\alpha_i + 1}, i = 1, 2, \dots, m$$

225 (4)

226 Here, the indices for individual prey types ranged from -1 (prey absent in diet) to +1 (prey  
 227 representing 100% of diet), with a value of 0 corresponding to unselective feeding. Diet breadth  
 228 of a predator is defined as the number of prey types with electivity index larger than -1 (i.e. all  
 229 prey types consumed). Values of electivity indices for individual prey instars were compared with  
 230 values expected for no electivity using separate  $t$ -tests. We subsequently examined predator-prey

231 body length (R) and weight (R') ratios combined with consumption rates towards different prey  
232 instars by *Ng* and *Gp* to elucidate selectivity traits between predators.

233

## 234 **Results**

### 235 *Functional responses (FRs)*

236 As prey survival was 100% in control trials without predators, prey mortality in treatments  
237 with predators was deemed due to predation only. Individual *Ng* consumed significantly higher  
238 numbers of mosquito larvae than individual *Gp* overall (Fig. 1). *Ng* generally had higher search  
239 efficiencies (*b*) than *Gp* (see Table 1; Fig. 1), and displayed greater FR magnitudes (i.e. lower  
240 handling times, *h*; greater maximum feeding rates,  $1/hT$ ). Individual *Ng* showed a Type I FR for  
241 first instar prey, while for second, third and fourth instars this predator had a Type II response  
242 (Table 1). For *Ng* at low prey densities, FR CIs overlapped with the exception of first instar,  
243 indicating lower capture rates towards the first instar compared to other instars. The FR curves  
244 then split into two groups, wherein first and fourth instars, and second and third instars, overlapped  
245 from mid-high prey densities. Thus, FRs here at mid-high prey densities towards these two groups  
246 of prey were different, with second and third instars consumed more than first and fourth instars  
247 across this range. However, the FR CIs towards the first instar overlapped with all of the other  
248 instars under the highest prey densities, indicating greater similarities in feeding rates here (Fig.  
249 1a).

250 Individual *Gp*, on the other hand, showed Type III FRs towards first, second and third prey  
251 instars, while for fourth prey instars *Gp* displayed a Type I FR (Table 1). Functional response

252 curve CIs overlapped among the four prey instars at lower to mid prey densities, indicating  
253 similarities in capture rates. The extent of this overlap decreased with increasing prey densities,  
254 but the CI overlaps remained at the higher prey densities. Here, first and second instars were  
255 consumed more as compared to third and fourth instars at higher prey densities, with FR  
256 magnitudes between these prey type groups considerably different (Fig. 1b).

### 257 *Multiple predator effects (MPEs)*

258         Conspecific *Ng* predatory pairs demonstrated a Type II FR towards all larval mosquito  
259 instars, except for first instar prey, where a Type I FR was again found (Table 1; Fig. 2). Similar  
260 results were observed for the expected values of this species (Table 1). There was an emergent  
261 MPE towards second instar larvae, wherein the CIs of observed FRs were higher than predicted,  
262 indicating prey risk enhancement. Predation towards first, third and fourth instars showed no  
263 emergent MPE wherein CIs of observed and predicted FRs completely overlapped. For pairs of  
264 fourth instars, the overlap between observed and predicted CIs decreased with prey density, which  
265 indicates potential risk reduction at high prey densities. (Fig. 2).

266         Conspecific *Gp* predatory pairs demonstrated a Type III FR for all larval instars, except for  
267 third instar, for which a Type I FR was found (Table 1; Fig. 3). The predicted FRs for *Gp* pairs  
268 were Type III for all four instars, except for fourth prey instar stage which showed a Type I FR  
269 (Table 1). Here, emergent antagonistic MPEs were observed within conspecific *Gp* pairs for  
270 consumption of the second prey instar stage, with CIs of predicted FRs higher than observed FRs,  
271 showing prey risk reduction. On the other hand, first, third and fourth instar stages showed no  
272 emergent MPE where the CIs of observed and predicted FRs overlapped. Similar to conspecific  
273 *Ng*, divergences in CIs were observed from mid to high prey densities by conspecific *Gp*,

274 indicating increasing prey risk reduction for first, third and fourth instar stages as prey densities  
275 increased above 100 (Fig. 3).

### 276 *Prey preference*

277 Both predator species consumed all four instars of larval mosquitoes; however, there were  
278 substantial differences in prey preferences (Table S1). The fourth instar stage ( $t = 13.55$ ,  $P < 0.01$ )  
279 was the preferred prey size for *Ng*, followed by the third instar stage ( $t = 5.52$ ,  $P < 0.05$ ), whereas  
280 first instar prey were avoided ( $t = 22.38$ ,  $P < 0.01$ ). There was no significant preference or  
281 avoidance for second instars ( $t = 2.63$ , NS), although there was a tendency for avoidance (Fig. 4)  
282 On the other hand, *Gp* significantly preferred third instars ( $t = 15.22$ ,  $P < 0.01$ ), significantly  
283 avoided the first instar ( $t = 3.99$ ,  $P < 0.05$ ) and tended to avoid both the second ( $t = 1.58$ , NS) and  
284 fourth ( $t = 2.09$ , NS) instars of *C. pipiens* larvae (Fig. 4).

285 Figure 5 shows that the predator-prey body length ratio (R) as well as body weight ratio  
286 (R') were similar for both the predators with respect to their preferred prey (i.e. body length ratio,  
287 *Ng* for fourth instar = 0.617, *Gp* for third instar = 0.624; body weight ratio, *Ng* for fourth instar =  
288 1.848, *Gp* for third instar = 1.560), with the consumption peaks aligning with their respective  
289 preferred prey items across both length and weight ratios.

290

### 291 **Discussion**

292 The present study examined the predatory impacts of two aquatic invertebrates, *N. glauca*  
293 (*Ng*) and *G. pulex* (*Gp*), against larval prey of the medically important mosquito *C. pipiens*.  
294 Comparative FRs were used to quantify emergent MPEs between conspecific pairs of each

295 predator species, and assess whether the strength and direction of MPEs differed according to  
296 mosquito prey size. Moreover, the influence of prey size for prey selectivity traits by the predators  
297 was examined and related to relative predator-prey body sizes. Predatory efficiencies of *Ng* were  
298 considerably higher than *Gp*, with the former tending to display destabilizing Type II FRs and the  
299 latter Type III FRs. The strength and direction of MPEs differed between the predators among  
300 prey sizes. For *Ng*, predator-predator interactions were mostly independent, combining additively  
301 to increase prey risk with predator density. Further, evidence for synergistic MPEs was displayed  
302 towards second instar prey by *Ng*. Contrastingly, *Gp* MPEs tended to manifest more  
303 antagonistically, with significantly negative MPEs towards second instar mosquito prey.  
304 Preferential selection towards mosquito prey also differed between the predators across larval  
305 ontogeny, with *Ng* preferring the fourth instar size class, and *Gp* the third instar class. In turn, these  
306 selective propensities were shown to align with the relative scaling of predator-prey body sizes.  
307 Thus, we demonstrate that MPEs and preferences differ across ontogenic prey stages, with  
308 potential implications for the control of vector mosquitoes by natural enemies during their aquatic  
309 life history.

310 Type II FRs are common within invertebrate predator-prey pairings in laboratory settings  
311 (e.g. Sentis et al., 2012, 2017; Lavery *et al.*, 2015; Barrios-O'Neill *et al.*, 2015, 2016; Wasserman  
312 *et al.*, 2016b; Cuthbert *et al.*, 2018a, b, c). Whilst the relevance of laboratory experiments to  
313 empirical food webs should be treated with caution, the container-based systems used in our  
314 experiments are broadly representative of artificial container habitats which *C. pipiens* can  
315 colonise. Therefore, in such habitats, our experiment revealed marked interspecific differences in  
316 predatory impact, conspecific MPEs and selectivity traits. Here, both individual and conspecific

317 groups of *Ng* showed linear Type I FRs towards first instars, whilst *Gp* displayed a Type I response  
318 for fourth instars as individuals and third instars in conspecific groups, where feeding rates linearly  
319 increased. Although Type I FRs are mechanistically confined to filter-feeders (Jeschke *et al.*,  
320 2004), this FR form has also been observed in two parasitoid genera, *Trichogramma* and  
321 *Eretmocerus* (Hoddle *et al.*, 1998; Hoffmann *et al.*, 2002; Mills & Lacan, 2004). Yet, in our  
322 experiment, this likely reflected insufficient prey supplies, which may otherwise have driven an  
323 asymptotic consumptive decline as in Type II and Type III FRs. Both individual and conspecific  
324 groups of *Ng* displayed Type II FRs towards all later instar mosquito prey types, conducive to  
325 higher predation pressure at low prey densities. This indicates that predation by *Ng* has the  
326 potential for high impact at low densities of these instars. Type II FRs, in theory, negate refuge  
327 effects at low prey densities, and therefore may promote mosquito extirpations from specific  
328 systems (Taylor & Collie 2003, Rindone & Eggleston, 2011). On the other hand, *Gp* mostly  
329 displayed Type III FRs in both individual and conspecific groups. In contrast to Type II FRs, Type  
330 III FRs are associable with low-density refugia for prey (Murdoch & Oaten, 1975; Williams &  
331 Martinez, 2004), and, empirically, a Type III FR may be driven by switching behaviours where  
332 the predator encounters alternative prey (see Murdoch, 1969; Akre & Johnson, 1979). Thus,  
333 compared to *Ng*, *Gp* may better allow for mosquito population persistence *via* low density refugia  
334 in container-based habitats.

335 Overall, the present study found *Ng* to be a much more voracious predator than *Gp* towards  
336 mosquito larvae, with the latter generally displaying lower capture rates, longer handling times  
337 and lower maximum feeding rates. Notonectids are well-known to be voracious predators in  
338 aquatic ecosystems (Papáček, 2001; Schaefer & Panizzi, 2000), which aligns with our results,

339 whilst *Gp* exhibit more omnivorous feeding strategies (Hellmann *et al.*, 2013). The higher capture  
340 rates of *Ng* compared to *Gp* further again indicate higher predation pressure on mosquito larvae  
341 by this species at low prey densities. For *Ng*, capture rates were particularly high for larger, late  
342 instars of *C. pipiens* (third and fourth instar stages). *Notonecta glauca* also displayed high  
343 maximum feeding rates towards second and third instars as a result of reduced handling times  
344 towards these prey types compared to fourth instar prey. Similar consumptive traits have been  
345 observed for the congeneric species *Notonecta sellata* (see Fischer *et al.*, 2012) and multiple  
346 *Anisops* species (Dalal *et al.*, 2019b). *Gammarus pulex*, on the other hand, exerted highest capture  
347 rates towards fourth instar prey as individuals and third instar prey in conspecific groups. These  
348 results, coupled with the observed prey preferences, suggest that this larval mosquito stage is most  
349 impacted by gammarid predators at low densities. However, handling times for *Gp* were generally  
350 shortest for the smallest prey classes, driving greater maximum feeding rates. Whilst the present  
351 study used size-matched adults of both predators, intraspecific differences in predator size may  
352 also drive differences in prey size selectivities, and thus shifts in prey preferences associated with  
353 ontogenic size changes in predators requires further examination.

354         The presence of conspecific and heterospecific consumers (e.g. higher predators) often  
355 drives prey risk reductions (i.e. *via* antagonistic interactions) (Barrios-O'Neill *et al.*, 2014;  
356 Wasserman *et al.*, 2016a). However, there has been a lack of investigation into whether emergent  
357 MPEs differ according to prey ontogeny, despite the importance of relative predator-prey body  
358 size scaling for interaction strengths (Barrios-O'Neill *et al.*, 2016; Brose *et al.*, 2019). The present  
359 study identified interspecific differences in MPEs between conspecific pairs, with *Ng* MPEs  
360 tending to combine additively or synergistically, and *Gp* generally displaying antagonistic

361 interactions. Prey size also influenced the strength of MPEs within predator species, with  
362 significant synergy displayed towards second instar prey in *Ng* and antagonism significantly  
363 shown towards second instar prey by *Gp*. Intraguild predation was not observed during the  
364 treatments in the conspecific pairs, which therefore leads to an assumption of interference between  
365 the predators which, in turn, reduces target mosquito prey risk. Whilst these results show an  
366 important context-dependency for the nature of MPEs, further research is required to decipher the  
367 biological bases for the patterns exhibited, such as key behavioural differences among prey stages.  
368 Further, determining MPEs across prey sizes under heterospecific groupings could better represent  
369 non-trophic interactions at the community level than in the present study. Nevertheless, given that  
370 *Ng* and *Gp* typically occupy lentic and lotic waters, respectively, the potential for these species  
371 to interact in the real world may be limited. Moreover, for mass-release or promotion of specific  
372 natural enemies, understandings of conspecific predator-predator interactions is of particular  
373 importance given that biocontrol programs often focus on individual agent species.

374 The present study also demonstrated differences in prey selectivity patterns towards  
375 mosquito prey by *Ng* and *Gp*. Such intraspecific prey selectivity can have important size refuge  
376 implications for coexisting larval mosquito stages. *Notonecta glauca* selected the largest prey  
377 instar with greatest energy levels (fourth instar). A previous study found *Ng* to be highly selective  
378 in more complex food webs, and to prefer *Culex* sp. (mosquito) over six alternative prey species  
379 (*Asellus aquaticus*, *Chironomus* sp., *Cloeon dipterum*, *Daphnia* sp., *Lymnaea stagnallis* and *Rana*  
380 *arvalis*) (Klecka & Boukal, 2012). On the other hand, in the present study, *Gp* selected the second  
381 most energy-rich option (third instar). Importantly, such positive selection for late instar prey may  
382 offset reductions in competition associated with preferential consumption of early instar stages,

383 and thus the focal predators may effectively reduce adult mosquito recruitment (Juliano, 2007).  
384 Given our results align fully with relative predator-prey body masses and lengths for both predator  
385 species, this suggests that prey selectivity patterns are predictable based on the physical  
386 characteristics of predator-prey participants. Thus, such effects should be considered by  
387 practitioners involved in the prediction of natural enemy efficacies in complex ecological  
388 communities.

389 In conclusion, this study demonstrated differential predatory impacts of two aquatic natural  
390 enemies towards larvae of the vector mosquito *C. pipiens*. Our results suggest that notonectids are  
391 particularly voracious predators of larval mosquitoes in container-style aquatic environments,  
392 where they may be introduced/promoted. Multiple predator effects and prey selectivities were  
393 found to differ both inter- and intraspecifically, with prey size having a marked effect on the nature  
394 of trophic and non-trophic interactions. Selectivity patterns were well-predicted by predator-prey  
395 body size ratios, further suggesting the importance of size scaling for trophic interactions and  
396 natural enemy impacts. Whilst the present study furthers our understandings of context-  
397 dependencies which may alter community dynamics and natural enemy efficacies, future work is  
398 required to extend the results into field-based systems under more natural conditions.

399

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404

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617

618 **Table and figures captions**

619 **Table 1.** Functional response (FR) types, estimates of search efficiency ( $b$ ) and handling time ( $h$ )  
620 for single individual or conspecific pairs of *Notonecta glauca* ( $Ng$ ) and *Gammarus pulex* ( $Gp$ )  
621 towards four instars of *Culex pipiens* larvae, alongside their significance levels. Lowest AIC value  
622 were considered for the selection of FR types.

623 **Fig. 1.** Functional responses of single predators (a) *Notonecta glauca* and (b) *Gammarus pulex*  
624 towards four instars of *Culex pipiens* larvae. Shaded areas are 95% confidence intervals from  
625 bootstrapping ( $n = 2000$ ). Points are raw observations. Note differences in y axes scaling.

626 **Fig. 2.** Multiple predator effect (MPE) of conspecific pairs of *Notonecta glauca* ( $Ng$ ) towards four  
627 instars of *Culex pipiens* larvae. Solid lines (red) are the observed values and dashed lines (blue)  
628 are the predicted values. Shaded areas are 95% confidence intervals. Points are raw observations.

629 **Fig. 3.** Multiple predator effect (MPE) of conspecific pairs of *Gammarus pulex* ( $Gp$ ) towards four  
630 instars of *Culex pipiens* larvae. Solid lines (red) are the observed value and dashed lines (blue) are  
631 the predicted values. Shaded areas are 95% confidence intervals. Points are raw observations.

632 **Fig. 4.** Electivity index values (mean  $\pm$  SE), based on Manly's  $\alpha$ , for (a) *Notonecta glauca* and (b)  
633 *Gammarus pulex* against four larval instars of *Culex pipiens*. Positive value indicate prey  
634 preference and negative value indicate prey avoidance. The asterisk (\*) mark indicates where the  
635 electivity values of the prey instars are significantly different from zero ( $P < 0.05$ ).

636 **Fig. 5.** Numbers of prey consumed against  $\log_{10}$  (predator-prey body length ratio+1) for (a)  
637 *Notonecta glauca* ( $Ng$ ) and (b) *Gammarus pulex* ( $Gp$ ), alongside  $\log_{10}$  (predator-prey body weight  
638 ratio+1) of (c) *Notonecta glauca* ( $Ng$ ) and (d) *Gammarus pulex* ( $Gp$ ). Means are  $\pm$  SE.

639

640 **Supplementary file**

641 **Table S1.** Values of Manly's  $\alpha$  by individual predators, *Notonecta glauca* and *Gammarus pulex*,  
642 derived from preference tests considering four larval instars of *Culex pipiens*, and their  
643 corresponding raw consumption values.

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