



**QUEEN'S
UNIVERSITY
BELFAST**

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

Dalal, A., Cuthbert, R., Dick, J., Sentis, A., Lavery, C., Barrios-O'Neill, D., Ortiz-Perea, N., Callaghan, A., & Gupta, S. (2019). Prey size and predator density modify impacts by natural enemies towards mosquitoes. *Ecological Entomology*. Advance online publication. <https://doi.org/10.1111/een.12807>

Published in:
Ecological Entomology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights
© 2019 The Royal Entomological Society. This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Open Access
This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: <http://go.qub.ac.uk/oa-feedback>

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 ± 0.07 mm; wet weight \pm SD, 0.02 ± 0.01
156 mg; second instar, length \pm SD, 2.10 ± 0.03 mm; wet weight \pm SD, 0.12 ± 0.02 mg; third instar,
157 length \pm SD, 3.90 ± 0.08 mm; wet weight \pm SD, 1.40 ± 0.01 mg; fourth instar, length \pm SD, 4.80
158 ± 0.06 mm; wet weight \pm SD, 2.20 ± 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 Akaike's Information Criterion (Δ AIC)
189 to select the FR models minimising information loss.

190 Following model selection *via* Δ 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 α 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 α_i were
223 converted into electivity indices (ε) 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

400 **Acknowledgements**

401 This work was funded by the Department of Biotechnology, India (F. N o. BT/IN/UK/DBT-
402 BC/2015-16) and British Council, UK (Application no. 228107673) for the programme “Newton-
403 Bhabha PhD placement programme” awarded to AD.

404

405 **References**

406 Akre, B.G. & Johnson., D.M. (1979) Switching and sigmoid functional response curves by
407 damselfly naiads with alternative prey available. *Journal of Animal Ecology*, **48**, 703-720.

408 Barrios-O'Neill, D., Kelly, R., Dick, J.T.A., Ricciardi, A., MacIsaac, H. & Emmerson, M.C.
409 (2016) On the context-dependent scaling of consumer feeding rates. *Ecology Letters*, **19**, 668–678.

410 Barrios-O'Neill, D., Kelly, R. & Emmerson, M.E. (2019) Biomass encounter rates limit the size
411 scaling of feeding interactions. *Ecology Letters* (in press). DOI: <https://doi.org/10.1111/ele.13380>

412 Barrios-O'Neill, D., Dick, J.T.A., Emmerson, M.C., Ricciardi, A., Mac- Isaac, H.J., Alexander,
413 M.E. & Bovy, J.C. (2014) Fortune favours the bold: a higher predator reduces the impact of a
414 native but not an invasive intermediate predator. *Journal of Animal Ecology*, **83**, 693–701.

415 Barrios-O'Neill, D., Dick, J.T.A., Emmerson, M.C., Ricciardi, A. & MacIsaac, H. (2015) Predator-
416 free space, functional responses and biological invasions. *Functional Ecology*, **29**, 377–384.

417 Berlow, E.L. (1999) Strong effects of weak interactions in ecological communities. *Nature*, **398**,
418 330–334.

419 Blaustein, L. (1998) Influence of the predatory backswimmer, *Notonecta maculata*, on invertebrate
420 community structure. *Ecological Entomology*, **23**, 246–252.

421 Bolker, B.M. (2010) bbmle: Tools for General Maximum Likelihood Estimation. R Package.
422 Available at www.cran.rproject.org.

423 Brose, U., Ehnes, R.B., Rall, B.C., Vucic-Pestic, O., Berlow, E.L. & Scheu, S. (2008) Foraging
424 theory predicts predator–prey energy fluxes. *Journal of Animal Ecology*, **77**, 1072-1078.

425 Brose, U., Archambault, P., Barnes, A.D., Bersier, L.F., Boy, T., Canning-Clode, J., *et al.* (2019)
426 Predator traits determine food-web architecture across ecosystems. *Nature Ecology and Evolution*,
427 **3**, 919–927.

428 Chesson, J. (1983) The estimation and analysis of preference and its relationship to foraging models.
429 *Ecology*, **64**, 1297-1304.

430 Cuthbert, R.N., Dick, J.T.A., Callaghan, A. & Dickey, J.W.E. (2018a) Biological control agent
431 selection under environmental change using functional responses, abundances and fecundities; the
432 Relative Control Potential (RCP) metric. *Biological Control*, **121**, 50–57.

433 Cuthbert, R.N., Dick, J.T.A. & Callaghan, A. (2018b) Interspecific variation, habitat complexity
434 and ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control.
435 *Biological Control*, **121**, 80-87.

436 Cuthbert, R.N., Callaghan, A. & Dick, J.T.A. (2018c) Dye another day: the predatory impact of
437 cyclopoid copepods on larval mosquito *Culex pipiens* is unaffected by dyed environments. *Journal*
438 *of Vector Ecology*, **43**, 334-336.

439 Cuthbert, R.N., Callaghan, A. & Dick, J.T.A. (2019a) Differential interaction strengths and prey
440 preferences across larval mosquito ontogeny by a cohabiting predatory midge. *Journal of Medical*
441 *Entomology*, **56**, 1428-1432.

442 Cuthbert, R.N., Callaghan, A., Sentis, A., Dalal, A. & Dick, J. T. (2019b) Additive multiple
443 predator effects can reduce mosquito populations. *Ecological Entomology*, (in press). DOI:
444 10.1111/een.12791

445 Cuthbert, R.N., Dalu, T., Wasserman, R.J., Callaghan, A., Weyl, O.L. & Dick, J.T. (2019c) Using
446 functional responses to quantify notonectid predatory impacts across increasingly complex
447 environments. *Acta Oecologica*, **95**, 116-119.

448 Dalal, A. & Gupta, S. (2018) Aquatic Insects as Pollution Indicator—A Study in Cachar, Assam,
449 Northeast India, in: V.P. Singh, V.P. *et al.* (Eds.), Environmental Pollution. Water Science and
450 Technology Library 77.

451 Dalal, A., Cuthbert, R.N., Dick, J.T. & Gupta, S. (2019a) Prey preferences of notonectids towards
452 larval mosquitoes across prey ontogeny and search area. *Pest Management Science*, (in press).
453 DOI: <https://doi.org/10.1002/ps.5556>

454 Dalal, A., Cuthbert, R.N., Dick, J.T. & Gupta, S. (2019b) Water depth-dependent notonectid
455 predatory impacts across larval mosquito ontogeny. *Pest Management Science*, **75**, 2610-2617.

456 Das, K. & Gupta, S. (2012) Seasonal variation of Hemiptera community of a temple pond of
457 Cachar District, Assam, northeastern India. *Journal of Threatened Taxa*, **4**, 3050-3058.

458 Dick, J.T., Alexander, M. E., Jeschke, J. M., Ricciardi, A., MacIsaac, H. J., Robinson, T. B.,
459 Kumschick, S., Weyl, O.L.F., Dunn, A.M., Hatcher, M.J., Peterson, R.A., Farnsworth, K.D. &
460 Richardson, D.M. (2014) Advancing impact prediction and hypothesis testing in invasion ecology
461 using a comparative functional response approach. *Biological Invasions*, **16**, 735-753.

462 Dick, J.T.A., Lavery, C., Lennon, J.J., Barrios-O'Neill, D., Mensink, P.J., Britton, R., Médoc, V.,
463 Boets, P., Alexander, M.E., Taylor, N.G., Dunn, A.M., Hatcher, M.J., Rosewarne, P.J., Crookes,
464 S., MacIsaac, H.J., Xu, M., Ricciardi, A., Wasserman, R.J., Ellender, B.R., Weyl, O.L.F., Lucy,
465 F.E., Banks, P.B., Dodd, J.A., MacNeil, C., Penk, M.R., Aldridge, D.C. & Caffrey, J.M. (2017)
466 Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts
467 of existing, emerging and future invasive alien species. *Journal of Applied Ecology*, **54**, 1259–
468 1267.

469 Ellis, R.A. & Borden, J.H. (1970) Predation by *Notonecta undulata* [Heteroptera : Notonectidae]
470 on larvae of the yellow-fever mosquito. *Annals of the Entomological Society America*, **63**, 963–
471 973.

472 Emmerson, M.C. & Raffaelli, D. (2004) Predator-prey body size, interaction strength and the
473 stability of a real food web. *Journal of Animal Ecology*, **73**, 399–409.

474 Englund, G., Ohlund, G., Hein, C.L. & Diehl, S. (2011) Temperature dependence of the functional
475 response. *Ecology Letters*, **14**, 914–921.

476 European Centre for Disease Prevention and Control (ECDC) 2014. *Rapid Risk Assessment: Zika*
477 *Virus Infection Outbreak, French Polynesia, February 14, 2014*. Stockholm: ECDC. Available at
478 : [http://www.ecdc.europa.eu/en/publications/Publications/Zika-virus-French-Polynesia-rapid-](http://www.ecdc.europa.eu/en/publications/Publications/Zika-virus-French-Polynesia-rapid-risk-assessment.pdf)
479 [risk-assessment.pdf](http://www.ecdc.europa.eu/en/publications/Publications/Zika-virus-French-Polynesia-rapid-risk-assessment.pdf).

480 Fischer, S., Pereyra, D. & Fernández, L. (2012) Predation ability and non-consumptive effects
481 of *Notonecta sellata* (Heteroptera: Notonectidae) on immature stages of *Culex pipiens* (Diptera:
482 Culicidae). *Journal of Vector Ecology*, **37**, 245–251.

483 Griffin, J.N., Toscano, B.J., Griffen, B.D. & Silliman, B.R. (2015) Does relative abundance modify
484 multiple predator effects? *Basic and Applied Ecology*, **16**, 641-651.

485 Hellmann, C., Wissel, B. & Winkelmann, C. (2013) Omnivores as seasonally important predators
486 in a stream food web. *Freshwater Science*, **32**, 548-562.

487 Hoddle, M.S., van Driesche, R.G., Elkinton, J.S. & Sanderson, J.P. (1998) Discovery and
488 utilization of *Bemisia argentifolii* by *Eretmocerus eremicus* and *Encarsia formosa* (Beltsville
489 strain) in greenhouses. *Entomologia Experimentalis et Applicata*, **87**, 15–28

490 Hoffmann, M.P., Wright, M.G., Pitcher, S.A. & Gardner, J. (2002) Inoculative releases of
491 *Trichogramma ostrinae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn:
492 field biology and population dynamics. *Biological Control*, **25**, 249–258.

493 Holling, C.S. (1966) The functional response of invertebrate predators to prey density. *The*
494 *Memoirs of the Entomological Society of Canada*, **48**, 1–86.

495 Jeschke, J.M., Kopp, M. & Tollrian, R. (2002) Predator functional responses: discriminating
496 between handling and digesting prey. *Ecological Monographs*, **72**, 95–112.

497 Jeschke, J.M., Kopp, M. & Tollrian, R. (2004) Consumer-food systems: why Type I functional
498 responses are exclusive to filter feeders. *Biological Reviews*, **79**, 337-349.

499 Juliano, S.A. (2001) Nonlinear curve fitting: predation and functional response curves, in:
500 Scheiner, S.M., Gurevitch, J. (Eds.), *Design and analysis of ecological experiments*. Oxford
501 University Press, Oxford, pp. 178-196.

502 Juliano, S.A. (2007) Population dynamics. *Journal of the American Mosquito Control Association*,
503 **23**, 265–275.

504 Kay, B.H. & Nam, V.S. (2005) New strategy against *Aedes aegypti* in Vietnam. *Lancet*, **365**, 613–
505 617.

506 Klecka, J. & Boukal, D.S. (2012) Who eats whom in a pool ? A comparative study of prey
507 selectivity by predatory aquatic insects. *PLoS ONE*, **7**, e37741.

508 Lavery, C., Dick, J.T.A., Alexander, M.E. & Lucy, F.E. (2015) Differential ecological impacts of
509 invader and native predatory freshwater amphipods under environmental change are revealed by
510 comparative functional responses. *Biological Invasions*, **17**, 1761–1770.

511 Lambrechts, L., Scott, T.W. & Gubler, D.J. (2010) Consequences of the Expanding Global
512 Distribution of *Aedes albopictus* for Dengue Virus Transmission. *PLoS Neglected Tropical*
513 *Diseases*, **4**, 645.

514 Losey, J.E. & Denno, R.F. (1998) Positive predator-predator interactions: enhanced predation rates
515 and synergistic suppression of aphid populations. *Ecology*, **79**, 2143–2152.

516 Manly, B.F.J. (1974) A model for certain types of selection experiments. *Biometrics*, **30**, 281–294.

517 McCann, K.S., Hastings, A. & Huxel, G.R. (1998) Weak trophic interactions and the balance of
518 nature. *Nature*, **395**, 794–798.

519 McCoy, M.W., Stier, A.C. & Osenberg, C.W. (2012) Emergent effects of multiple predators on
520 prey survival: the importance of depletion and the functional response. *Ecology Letters*, **15**, 1449–
521 1456.

522 Medlock, J.M. & Snow, K.R. (2008) Natural predators and parasites of British mosquitoes – a
523 review. *Journal of the European Mosquito Control Association*, **25**, 1–11.

524 Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H. & Van
525 Bortel, W. (2012) A review of the invasive mosquitoes in Europe: ecology, public health risks,
526 and control options. *Vector- Borne and Zoonotic Diseases*, **12**, 435-447.

527 Medlock, J. M. & Leach, S.A. (2015) Effect of climate change on vector-borne disease risk in the
528 UK. *The Lancet Infectious Diseases*, **15**, 721-730

529 Meijering, M.P.D. (1991) Lack of oxygen and low pH as limiting factors for *Gammarus* in Hessian
530 brooks and rivers. *Hydrobiologia*, **223**, 159–169.

531 Mills, N.J. & Lacan, I. (2004) Ratio dependence in the functional response of insect
532 parasitoids: evidence from *Trichogramma minutum* foraging for eggs in small host patches.
533 *Ecological Entomology*, **29**, 208–216.

534 Mondol, R.P., Chandra, G., Bandyopadhyay, S. & Ghosh, A. (2017) Effect of temperature and
535 search area on the functional response of *Anisops sardea* (Hemiptera: Notonectidae) against
536 *Anopheles stephensi* in laboratory bioassay. *Acta Tropica*, **166**, 262-267.

537 Murdoch, W.W. (1969) Switching in general predators: experiments on predator specificity and
538 stability of prey populations. *Ecological Monographs*, **39**, 335-354.

539 Murdoch, W.W. & Oaten, A. (1975) Predation and population stability. *Advances in Ecological*
540 *Research*, **9**, 1–131.

541 O’Neil, R.J. (1990) Functional response of arthropod predators and its role in the biological control
542 of insect pests in agricultural systems, in: Dunn, P.E., Baker, R.R. (Eds.), *New directions in*
543 *biological control: alternatives for suppressing agricultural pests and diseases*. Alan R. Liss, Inc.,
544 New York, pp. 83–96.

545 Papáček, M. (2001) Small aquatic and ripicolous bugs (Heteroptera: Nepomorpha) as predators
546 and prey: The question of economic importance. *European Journal of Entomology*, **98**, 1-12.

547 Polis, G.A. & Holt, R.D. (1992) Intraguild predation: the dynamics of complex trophic
548 interactions. *Trends in Ecology & Evolution*, **7**, 151–154.

549 Pritchard, D.W., Paterson, R.A., Bovy, H.C. & Barrios-O’Neill, D. (2017) Frair: an R package for
550 fitting and comparing consumer functional responses. *Methods in Ecology and Evolution*, (online).

551 R Core Team (2018) R: A language and environment for statistical computing. R Foundation for
552 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

553 Rall, B.C., Brose, U., Hartvig, M., Kalinkat, G., Schwarzmüller, F., Vucic-Pestic, O., *et al.* (2012)
554 Universal temperature and body-mass scaling of feeding rates. *Philosophical Transactions of the*
555 *Royal Society B: Biological Sciences*, **367**, 2923–34.

556 Real, L.A. (1977) The kinetics of functional response. *The American Naturalist*, **111**, 289–300.

557 Rindone, R.R. & Eggleston, D.B. (2011) Predator-prey dynamics between recently established
558 stone crabs (*Menippe* spp.) and oyster prey (*Crassostrea virginica*). *Journal of Experimental*
559 *Marine Biology and Ecology*, **407**, 216–225.

560 Roberts, G.M. (1995) Salt marsh crustaceans, *Gammarus duebeni* and *Palaemonetes varians* as
561 predators of mosquito larvae and their reaction to *Bacillus thuringiensis subsp. Israelensis*.
562 *Biocontrol Science and Technology*, **5**, 379 – 385.

563 Rogers, D. (1972) Random search and insect population models. *Journal of Animal Ecology*, **41**,
564 369–383.

565 Sarwardi, S., Mandal, P.K. & Ray, S. (2012) Analysis of a competitive prey-predator system with
566 a prey refuge. *Biosystems*, **110**, 133–148.

567 Sarwardi, S., Mandal, P.K. & Ray, S. (2013) Dynamical behaviour of a two-predator model with
568 prey refuge. *Journal of Biological Physics*, **39**, 701–722.

569 Schaefer, C.W. & Panizzi, A.R. (2000) Heteroptera of Economic Importance (CRC, Boca Raton,
570 FL).

571 Schaffner, F., Medlock, J.M. & Van Bortel, W. (2013) Public health significance of invasive
572 mosquitoes in Europe. *Clinical Microbiology and Infection*, **19**, 685-692.

573 Schmitz, O.J. (2009) Effects of predator functional diversity on grassland ecosystem function.
574 *Ecology*, **90**, 2339–2345.

575 Schwartz, S.S. (1992) Benthic predators and zooplanktonic prey: predation by *Crangonyx*
576 *shoemakeri* (Crustacea; Amphipoda) on *Daphnia obtusa* (Crustacea; Cladocera). *Hydrobiologia*,
577 **237**, 25-30.

578 Sentis, A., G emard, C., Jaugeon, B. & Boukal, D.S. (2017) Predator diversity and environmental
579 change modify the strengths of trophic and non-trophic interactions. *Global Change Biology*, **23**,
580 2629–2640.

581 Sentis, A., Hemptinne, J.L. & Brodeur, J. (2012) Using functional response modeling to investigate
582 the effect of temperature on predator feeding rate and energetic efficiency. *Oecologia*, **169**, 1117-
583 1125.

584 Sentis, A. & Boukal, D.S. (2018) On the use of functional responses to quantify emergent multiple
585 predator effects. *Scientific Reports*, **8**, 11787.

586 Sih, A. (1987) Prey refuge and predator–prey stability. *Theoretical Population Biology*, **31**, 1–12.

587 Sih, A., Englund, G. & Wooster, D. (1998) Emergent impacts of multiple predators on prey. *Trends*
588 *in Ecology & Evolution*, **13**, 350–355.

589 Soetaert, K. & Petzoldt, T. (2010) Inverse modelling, sensitivity and Monte Carlo analysis in R
590 using package FME. *Journal of Statistical Software*, **33**, 1-28.

591 Solomon, M.E. (1949) The natural control of animal populations. *Journal of Anim Ecology*, **18**,1–
592 35.

593 Streams, F. A. (1987) Within-Habitat Spatial Separation of two *Notonecta* Species: Interactive vs.
594 Noninteractive Resource Partitioning. *Ecology*, **68**, 935-945.

595 Suchman, C. & Sullivan, B. (1998) Vulnerability of the copepod *Acartia tonsa* to predation by the
596 scyphomedusa *Chrysaora quinquecirrha*: effect of prey size and behavior. *Marine Biology*, **132**,
597 237.

598 Taylor, R.J. (1984) Predation. Chapman and Hall, London.

599 Taylor, D.L. & Collie, J.S. (2003) Effect of temperature on the functional response and foraging
600 behavior of the sand shrimp *Crangon septemspinosa* preying on juvenile winter flounder
601 *Pseudopleuronectes americanus*. *Marine Ecology Progress Series*, **263**, 217–234

602 Vance-Chalcraft, H.D. & Soluk, D.A. (2004) Is prey predation risk influenced more by increasing
603 predator density or pre- dator species richness in stream enclosures? *Oecologia*, **139**, 117–122.

604 Vance-Chalcraft, H.D. & Soluk, D.A. (2005) Multiple predator effects result in risk reduction for
605 prey across multiple prey densities. *Oecologia*, **144**, 472–480.

606 Wasserman, R.J., Alexander, M.E., Dalu, T., Ellender, B.R., Kaiser, H. & Weyl, O.L.F. (2016a)
607 Using functional responses to quantify interaction effects among predators. *Functional Ecology*,
608 **30**, 1988–1998.

609 Wasserman, R.J., Alexander, M.E., Weyl, O.L.F., Barrios-O'Neill, D., Froneman, P.W. & Dalu,
610 T. (2016b) Emergent effects of habitat complexity and temperature on predator-prey interactions.
611 *Ecosphere*, **7**, e01239.

612 Williams, R.J. & Martinez, N.D. (2004) Stabilization of chaotic and nonpermanent food-web
613 dynamics. *The European Physical Journal B*, **38**, 297–303.

614 World health organization (2017) Factsheet: Vector borne disease. World health organization.

615 Yamaguchi, A. & Kishida, O. (2016) Antagonistic indirect interactions between large and small
616 conspecific prey *via* a heterospecific predator. *Oikos*, **125**, 271–277.

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 α , 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 α 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.

644