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

Running title: Multiple predator effects differ across prey size

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Abstract

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

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

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

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

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

Introduction

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

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

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

Within predator-prey relationships, prey size refuge effects may also influence trophic 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 lead 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.

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

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

Prey preference experiments

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

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

(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 (ΔAIC)
189 to select the FR models minimising information loss.

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

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

Multiple predator effects (MPEs)

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

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

(2)

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

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

Prey preferences

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

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

(3)

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

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

(4)

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

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

Results

Functional responses (FRs)

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

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

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

Multiple predator effects (MPEs)

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

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

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

Prey preference

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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Table and figures captions

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

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

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

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

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

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