

Differential interaction strengths and prey preferences across larval mosquito ontogeny by a cohabiting predatory midge

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Accepted Version

Cuthbert, R. N., Callaghan, A. and Dick, J. T. A. (2019) Differential interaction strengths and prey preferences across larval mosquito ontogeny by a cohabiting predatory midge. *Journal of Medical Entomology*, 56 (5). pp. 1428-1432. ISSN 1938-2928 doi: <https://doi.org/10.1093/jme/tjz059> Available at <https://centaur.reading.ac.uk/86278/>

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To link to this article DOI: <http://dx.doi.org/10.1093/jme/tjz059>

Publisher: Oxford University Press

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1 *Short Communication*

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3 **Differential Interaction Strengths and Prey Preferences across Larval Mosquito**
4 **Ontogeny by a Cohabiting Predatory Midge**

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21 **Abstract**

22 Understandings of natural enemy efficacy are reliant on robust quantifications of interaction
23 strengths under context-dependencies. For medically important mosquitoes, rapid growth
24 during aquatic larval stages could impede natural enemy impacts through size refuge effects.
25 The identification of biocontrol agents which are unimpeded by ontogenic size variability of
26 prey is therefore vital. We use functional response and prey preference experiments to
27 examine the interaction strengths and selectivity traits of larvae of the cohabiting predatory
28 midge *Chaoborus flavicans* (Meigen 1830) (Diptera: Chaoboridae) towards larval stages of
29 the *Culex pipiens* (Diptera: Culicidae) mosquito complex. Moreover, we examine the
30 influence of search area variation on selectivity traits, given its importance in consumer-
31 resource interactions. Chaoborids were able to capture and consume mosquito prey across
32 their larval ontogeny. When prey types were available individually, a destabilising Type II
33 functional response was exhibited towards late instar mosquito prey, whilst a more stabilising
34 Type III functional response was displayed towards early instars. Accordingly, search
35 efficiencies were lowest towards early instar prey, whilst, conversely, maximum feeding rates
36 were highest towards this smaller prey type. However, when the prey types were present
37 simultaneously, *C. flavicans* exhibited a significant positive preference for late instar prey,
38 irrespective of water volume. Our results identify larval chaoborids as efficacious natural
39 enemies of mosquito prey, with which they frequently coexist in aquatic environments. In
40 particular, an ability to prey on mosquitoes across their larval stages, coupled with a
41 preference for late instar prey, could enable high population-level offtake rates and negate
42 compensatory reductions in intraspecific competition through size refuge.

43 **Keywords**

44 biological control; functional response; prey selectivity; *Chaoborus flavicans*; *Culex pipiens*

45 **Introduction**

46 Predatory natural enemies can drive population-level suppression of medically important
47 vector species (Cameron and Lorenz 2013; Cuthbert et al. 2018a). However, a lack of aerial
48 dispersal capability limits the efficacy of many biological control agents by inhibiting
49 colonization of patchy aquatic habitats across landscapes (Cuthbert et al. 2019c). Given that
50 vector mosquito species successfully colonize minute, ephemeral aquatic habitats of
51 changeable volumes, an impediment to applications of common natural enemies is presented
52 (e.g. fish, Azevedo-Santos et al. 2017). Furthermore, refuge effects relating to prey size can
53 reduce the suppressive efficacy of biological control agents towards target populations across
54 ontogenic stages (Kesavaraju et al. 2007; Marten and Reid 2007). For example, species which
55 only effectively predate early instar larval stages may, paradoxically, alleviate resource
56 competition among individual mosquitoes in later instar stages within aquatic ecosystems, if
57 not all prey are consumed. In turn, this may produce better disease vectors *via* positive effects
58 on adult health and longevity (Juliano et al. 2014). Thus, the identification of biological
59 control agents which are able to effectively prey on mosquito prey across their larval
60 ontogeny is crucial, particularly given the rapidity at which mosquitoes can complete
61 development, and their capability to vector pathogens and parasites which cause disease
62 (Juliano 2007; Cuthbert et al. 2018b).

63 Coexisting predatory dipterans have been identified as potentially efficacious natural
64 enemies for controlling mosquito populations in their aquatic larval stages (Borkent 1980;
65 Griswold and Lounibos 2005; Cuthbert et al. 2019a, b). In particular, capabilities for natural
66 aerial dispersal in adult stages may promote simultaneous colonization of ephemeral aquatic
67 habitats by predatory dipteran larvae, helping to reduce mosquito abundances. The present
68 study thus uses functional responses (resource use under different resource densities; Holling
69 1959) and prey preferences to quantify interaction strengths of larvae of the predatory non-

70 biting midge *Chaoborus flavicans* (Meigen 1830) (Diptera: Chaoboridae) towards larvae of
71 the medically important *Culex pipiens* (Diptera: Culicidae) mosquito complex across their
72 larval ontogeny. Chaoborids are known to colonise various types of aquatic habitats where
73 mosquitoes breed, including artificial containers (e.g. Sunahara et al. 2002). Focusing on
74 container-style aquatic systems, we additionally examine whether differences in search area
75 further influence consumptive traits of this focal ambush predator, given the importance of
76 search area in mediating consumer-resource interaction strengths (Uiterwaal and DeLong
77 2018). We hypothesise that: (1) chaoborids will exhibit a higher magnitude functional
78 response towards early instar mosquitoes; and, (2) a consumptive preference will be exhibited
79 towards early instar mosquito stages compared to late instars, owing to their smaller size,
80 irrespective of search area differences.

81 **Materials and Methods**

82 Animal collection and maintenance

83 Fourth instar larval *Chaoborus flavicans* were purchased commercially (10 – 12 mm;
84 Northampton Reptile Centre, Northampton). These predatory chaoborids were maintained at
85 11 ± 1 °C and under a 12 h:12 h light and dark regime until experimentation in a laboratory at
86 Queen's Marine Laboratory (QML), Portaferry. Chaoborids were fed *ad libitum* with larval
87 mosquitoes. Predators were isolated and starved for 24 h prior to the experiments
88 individually. The prey, larvae of the *C. pipiens* mosquito complex, were obtained from a
89 colony maintained at QML, Portaferry, reared as per Cuthbert et al. (2018a). Prey larval
90 stages used in experiments were multi-generational.

91 Experimental design

92 In the functional response experiment, early (first instar, 1 – 2 mm) or late (fourth instar, 4 –
93 5 mm) larval mosquito prey were introduced separately at five densities (2, 4, 8, 16 and 32; *n*

94 = 6 per experimental group) in 50 mL arenas of 65 mm diameter containing dechlorinated tap
95 water from a continually aerated source. These size classes correspond to *C. pipiens* complex
96 instar stages reared in similar conditions in other studies (e.g. Cuthbert et al. 2018b; Dalal et
97 al. 2019). Two hours later, to allow for prey to acclimatize, predatory chaoborids were
98 introduced individually and allowed to feed undisturbed for 24 h, after which predators were
99 removed and live prey remaining were enumerated. Pilot studies indicated that this
100 acclimation time was appropriate for prey to settle within the experimental arenas. Controls
101 consisted of 6 replicates at each prey density and prey size class in the absence of predators.

102 In the preference experiment, early and late instar mosquito prey (stages/sizes as
103 before) were introduced simultaneously ($n = 15$ per prey type) to containers of either 50 mL
104 or 200 mL volume with dechlorinated tap water, of 65 mm and 115 mm diameter,
105 respectively. After settling as before, predatory chaoborids were introduced individually and
106 allowed to feed for 24 h, before remaining live prey of each size class were counted for each
107 volume treatment. Treatments were replicated 6 times, and controls consisted of six predator-
108 free replicates.

109 Statistical analyses

110 Data analyses were performed using R v3.4.4 (R Core Development Team 2018). In the
111 functional response experiment, overall prey consumption was analysed using generalized
112 linear models assuming a Poisson error distribution with log link. Model averaging was used
113 to identify models which minimized information loss using second order Akaike's
114 Information Criterion (AICc) (Burnham and Anderson 2002; Bartoń 2015). Tukey's tests
115 were used for post hoc comparisons of significant effects (Lenth 2016).

116 Functional response analyses were performed using the 'frair' package (Pritchard et
117 al. 2017). Logistic regression was used to categorize functional response types, wherein a

118 Type II functional response is evidenced by a significantly negative first order term, and a
119 Type III functional response by a significantly positive first order term followed by a
120 significantly negative second order term (Juliano 2001). A generalized form of the functional
121 response was implemented, assuming no prey replacement (Real 1997; Pritchard et al. 2017):

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

123 (1)

124 where N_e is the number of prey eaten, N_0 is the initial prey density, b is the search coefficient,
125 h is the handling time, q is the scaling exponent and T is the total time available. The scaling
126 exponent q can be fixed at 0 where a categorical Type II functional response is evidenced.
127 Where functional responses types are equivocal, q can be optimized to provide flexibility in
128 functional response fits, where $q > 0$ indicates movement towards a sigmoid Type III curve.
129 Suitability of models was examined using AIC. Locally Weighted Scatterplot Smoothing
130 (LOWESS) lines were also plotted (9/10 smoother span) to further illustrate functional
131 response forms. Functional response curves were then non-parametrically bootstrapped to
132 generate 95 % confidence intervals.

133 In the preference experiment, generalized linear mixed models assuming a Poisson
134 error distribution and log link were used to examine the influence of prey size and water
135 volume on consumption (Bates et al. 2015). Owing to repeated measures, each replicate was
136 included as a random effect, with prey type treated as a within-subject variable. Model
137 averaging was implemented during model selection based on AICc.

138 Manly's selectivity index between prey types was employed to account for prey
139 depletion over the experimental period (Manly 1974; Chesson 1983).

$$\alpha_i = \ln((n_{io} - r_i)/n_{io}) / \sum_{j=1}^m \ln((n_{jo} - r_j)/n_{jo})$$

141 (2)

142 where a_i is Manly's selectivity index for prey type i , n_{i0} is the number of prey type i available
143 at the start of the experiment, r_i is the number of prey type i consumed, m the number of prey
144 types, r_j is the number of prey type j consumed and n_{j0} the number of prey type j available at
145 the start of the experiment. The value of a_i ranges from 0 to 1, with 0 indicating complete
146 avoidance and 1 indicating complete positive selection. In a two prey system, values > 0.5 are
147 indicative of preference towards the focal prey type.

148 **Results**

149 Over 99.5 % of larval mosquito prey survived in control groups, and thus prey deaths were
150 attributed to predation in both experiments. In the functional response experiment,
151 significantly greater numbers of early instar prey were consumed than late instar prey ($\chi^2 =$
152 9.07 , $df = 1$, $p = 0.003$), and greater numbers of prey were consumed where more prey were
153 available ($\chi^2 = 80.95$, $df = 4$, $p < 0.001$). There was a significant interaction between these
154 terms ($\chi^2 = 11.55$, $df = 4$, $p = 0.02$), with consumption between the two prey size classes more
155 similar at low densities as compared to high densities (Figure 1; Figure 2).

156 Chaoborids displayed an equivocal functional response form towards early instar
157 mosquito prey (Table 1). A flexible model with scaling exponent q fixed at 1 was, however,
158 shown to minimise information loss. On the other hand, a significant Type II functional
159 response was demonstrated towards late instar mosquito prey, and thus q was fixed at 0 here
160 (Table 1). These functional response forms were further evidenced by LOWESS lines, with
161 proportional early instar consumption initially rising before subsequently decreasing at higher
162 prey densities, whilst, contrastingly, late instar proportional consumption was consistently
163 reduced across all increasing densities (Figure 2). Search coefficients by chaoborids tended to
164 be lower towards early instar mosquito prey, further evidenced by inflection and divergence
165 between prey types at low densities (Table 1; Figure 1). Handling times, however, were also

166 significantly reduced towards early instar prey than later instar prey, causing higher
167 maximum feeding rates towards this smaller prey type (Table 1; Figure 1).

168 In the prey preference experiment, significantly greater numbers of late instar prey
169 were consumed than early instar prey overall ($\chi^2 = 4.06$, $df = 1$, $p = 0.04$). There was no
170 significant difference in overall prey consumption between container water volumes ($\chi^2 =$
171 0.07 , $df = 1$, $p = 0.79$) and the preference for late instar prey was evident across both volume
172 types, owing to a non-significant interaction term ($\chi^2 = 0.13$, $df = 1$, $p = 0.72$). Selectivity
173 indices were thus higher towards late instar mosquito prey compared to early instar prey
174 irrespective of the container volume treatment (Figure 3).

175 **Discussion**

176 In the present study, larval *C. flavicans* were able to effectively capture and consume both
177 early and late instar larval *C. pipiens* prey. However, there were considerable differences in
178 interaction strengths according to target prey size/ontogeny. Whilst chaoborids exhibited a
179 significantly higher maximum feeding rate (functional response asymptote) towards the
180 smaller early instars, the search efficiency (functional response initial slope) towards this
181 prey type was reduced compared to larger late instar mosquito prey. These consumptive
182 propensities are also reflected in the differential functional response forms observed, with
183 predation towards late instar prey categorized as a Type II form, wherein proportional prey
184 consumption at low prey densities was high. Conversely, a Type III functional response was
185 exhibited towards early instar prey, owing to low prey consumption rates at low prey
186 densities. Given that Type III functional responses can be stabilizing to populations due to
187 density-dependent refugia (Murdoch and Oaten 1975), predatory chaoborid larvae may
188 impart a degree of stability to early instar mosquito prey populations. Nevertheless, as early
189 instar larval mosquito prey progress through subsequent ontogenic stages, an increased search

190 efficiency and selective preference may enable high consumption of remaining large prey.
191 Thus, any early instar low-density prey refuge imparted by chaoborids may be offset by
192 intensified interaction strengths towards later instar stages at low densities. In turn, this could
193 negate intraspecific competition alleviation in larval mosquitoes, which has been suggested to
194 produce better vectors (Juliano et al. 2014).

195 Here, larval chaoborids exhibited a significant positive preference for late instar
196 mosquito prey, despite the longer handling time required for this prey type. This selective
197 propensity was prevalent irrespective of search volume, which has been shown to strongly
198 influence consumer-resource interactions in other experimental systems (Uiterwaal and
199 Delong 2018). Chaoborids are ambush predators which capture nearby prey through
200 hydromechanical cue reception (Riessen et al. 1984), and are known to impact mosquito
201 larval abundances in container-style aquatic habitats (Sunahara et al. 2002). It is probable that
202 larger prey are more prevalent to this ambush predator, driving a selective preference and
203 higher search efficiency through strong hydromechanical signalling. Given that positive
204 selection of late instar larval mosquito prey is favourable for the biological control of
205 medically important populations (Juliano 2007), use of predatory natural enemies which
206 positively select late instar prey, naturally coexist with mosquitoes and which are also
207 capable of aerial dispersal could assist in population-level suppression.

208 Although multi-generational larval mosquito prey were utilised in the present study,
209 chaoborids have also been shown to be efficacious predators of wild-caught larval
210 mosquitoes under different environmental contexts (Cuthbert et al. 2019b). Furthermore, wild
211 *C. pipiens* oviposition has been shown to be undeterred by the presence of larval chaoborids,
212 which may further enhance their predatory impacts (Cuthbert et al. 2019b). We thus propose
213 that the promotion of chaoborids in environments, or deliberate introduction, could assist in
214 mosquito population management strategies. However, examinations of prey selectivity traits

215 across larval chaoborid ontogenic variations are also required, alongside integrations of
216 predator population-level responses to resource availability (i.e. numerical response) under
217 different environmental contexts. Overall, the combined testing of *per capita* interaction
218 strengths alongside prey preferences offers great utility in the quantification of natural enemy
219 efficacies under context-dependencies.

220 **Acknowledgements**

221 We acknowledge funding from the Department for the Economy, Northern Ireland.

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303 Table 1. Results from logistic regression considering proportional prey consumption as a
 304 function of prey density, and functional response parameter estimations alongside associated
 305 significance levels.

Prey stage	First order term (Type II), p	First order term (Type III), p	Second order term (Type III), p	Search coefficient (b), p	Handling time (h), p	Maximum feeding rate ($1/h$)
Early instar	-0.02, 0.09	0.08, 0.14	-0.003, 0.08	0.16, < 0.001	0.07, < 0.001	14.48
Late instar	-0.06, < 0.001	-0.08, 0.19	0.0005, 0.76	0.89, 0.005	0.23, < 0.001	4.36

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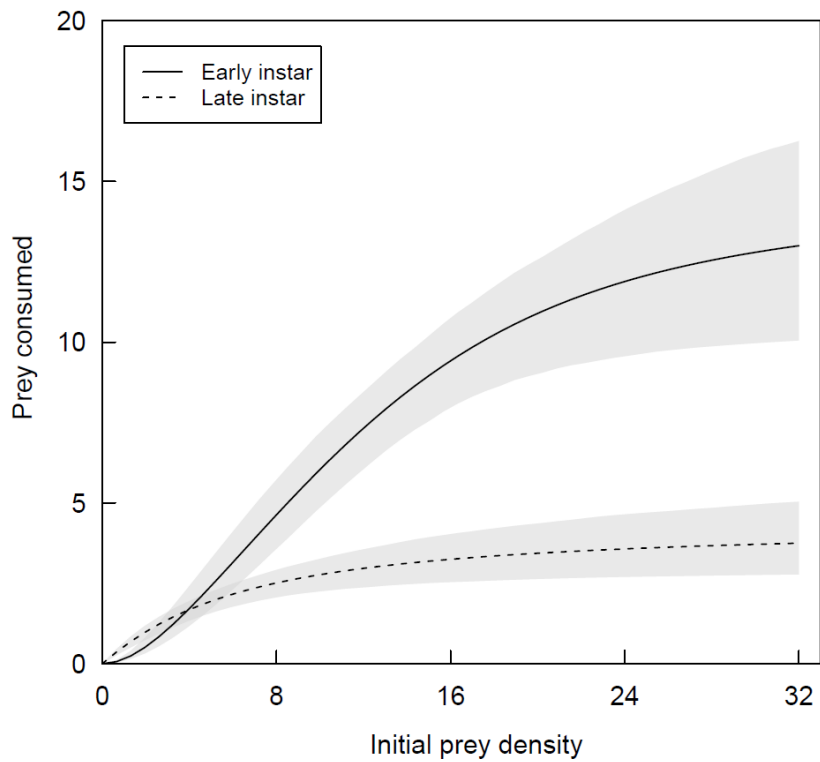
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313 Figure 1. Functional responses of larval *Chaoborus flavicans* towards early and late instar
314 larval *Culex pipiens* prey. Shaded areas are bootstrapped ($n = 2000$) 95 % confidence
315 intervals ($n = 6$ per experimental group).

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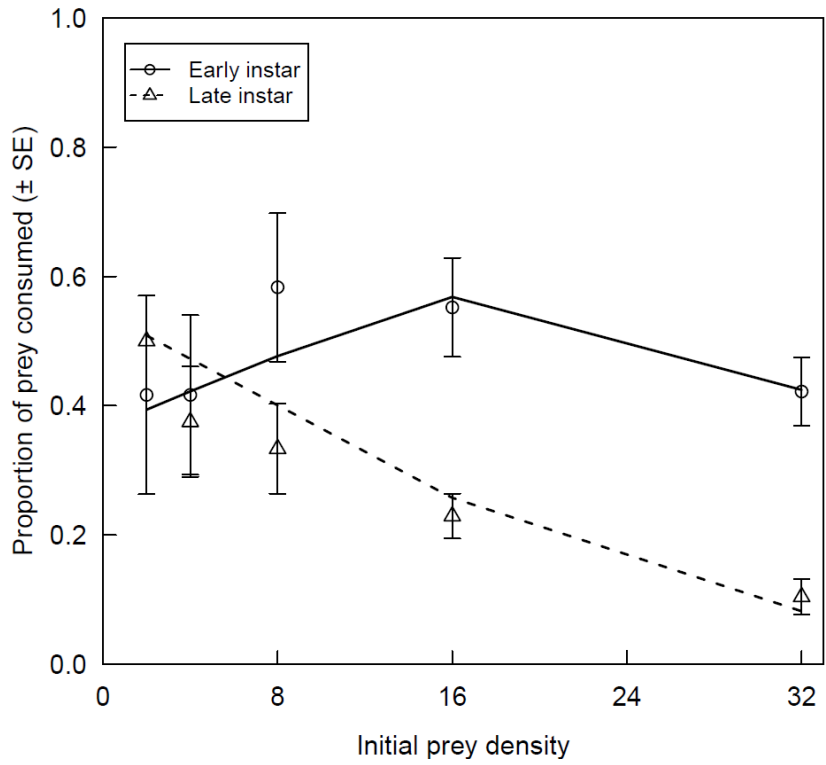
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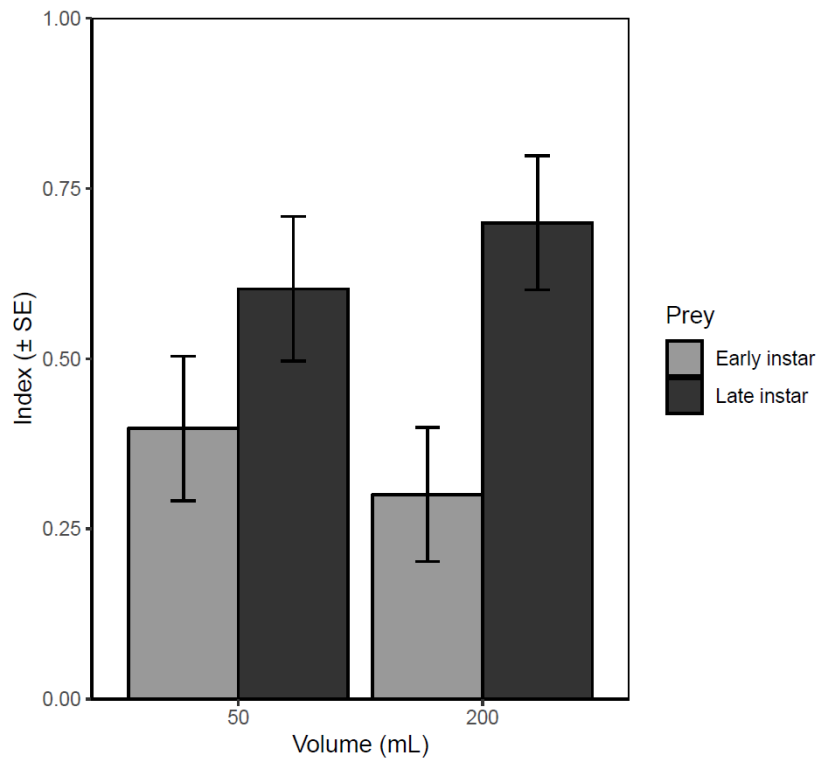
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326 Figure 2. Locally Weighted Scatterplot Smoothing (LOWESS) lines considering proportional
 327 prey consumption by larval *Chaoborus flavicans* as a function of larval *Culex pipiens* density
 328 between different prey sizes. Points are mean values ($n = 6$ per experimental group).

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333 Figure 3. Mean Manly's selectivity indices towards early and late instar *Culex pipiens* prey
 334 by larval *Chaoborus flavicans*. Values over 0.5 indicate active positive selection, whilst
 335 values below 0.5 suggest avoidance ($n = 6$ per experimental group).