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1 *Original Article*

2 **Additive multiple predator effects can reduce mosquito populations**

3 Ross N. Cuthbert^{1,2,*}, Amanda Callaghan², Arnaud Sentis³, Arpita Dalal⁴, Jaimie T. A. Dick¹

4

5 ¹Institute for Global Food Security, School of Biological Sciences, Queen's University

6 Belfast, Chlorine Gardens, Belfast BT9 5DL, Northern Ireland, United Kingdom

7 ²Ecology and Evolutionary Biology Section, School of Biological Sciences, Whiteknights,

8 University of Reading, Reading RG6 6AJ, England, United Kingdom

9 ³IRSTEA, Aix Marseille Univ., UMR RECOVER, 3275 route Cézanne, 13182 Aix-en-

10 Provence, France

11 ⁴Department of Ecology and Environmental Science, Assam University, Silchar 788011,

12 India

13

14 *Corresponding author: email, rcuthbert03@qub.ac.uk

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20

21 **Abstract**

22 1. Multiple predator interactions may profoundly alter ecological community dynamics and
23 can complicate predictions of simpler pairwise predator/prey interaction strengths. In
24 particular, multiple predator effects may lessen or enhance prey risk, with implications for
25 community-level stability. Such emergent effects may modulate natural enemy efficacy
26 towards target organisms.

27 2. In the present study, we use a functional response approach to quantify emergent multiple
28 predator effects among natural enemies towards the disease vector mosquito complex, *Culex*
29 *pipiens*. We quantify conspecific multiple predator-predator interactions of the cyclopoid
30 copepod *Macrocyclus albidus* (intermediate predator) by comparing multiple predator
31 consumption simulations, based on individual consumption rates, with multiple predator
32 consumption rates experimentally observed. Further, we examine the influence of the
33 presence of a predator at a higher trophic level, *Chaoborus flavicans*, on copepod group
34 predation.

35 3. Both predators displayed Type II functional responses, with *C. flavicans* consuming
36 significantly more prey than *M. albidus* individually. Overall consumption levels of
37 mosquitoes increased with greater predator density and richness. We did not detect
38 antagonistic or synergistic emergent multiple predator effects between conspecifics of *M.*
39 *albidus*, and the higher level predator did not reduce effects of the intermediate predator.
40 Accordingly, evidence for additive multiple predator interactions was found.

41 4. The lack of predator-predator interference between cyclopoid copepods and larval
42 chaoborid midges provides strong support for their combined application in mosquito
43 biocontrol. We propose increased examination of multiple predator effects in assessments of

44 natural enemy efficacies to better understand overall predatory effects within communities
45 and utilities in vector control.

46 **Keywords**

47 biological control; mosquito-borne disease; functional response; multiple predator effects;
48 cyclopoid; chaoborid

49

50 **Introduction**

51 Ecological communities typically comprise multiple consumers (e.g., predators) sharing
52 common resources (Sih et al., 1998; Barrios O'Neill et al., 2014; Wasserman et al., 2016).

53 Whilst community dynamics are often inferred on the basis of simple pairwise interaction
54 strengths (e.g., the effects of a predator on a prey population) (Schmitz, 2007, Tang et al.,
55 2014; Vázquez et al., 2015; Cuthbert et al., 2018d), biotic factors such as ontogenic shifts
56 (Bofill and Yee, 2019) and multiple predator-predator interactions (Sih et al., 1998; Bolker et
57 al., 2003; Wasserman et al., 2016) can also have profound implications for the outcomes of
58 predator-prey interactions. Utilisation of shared resources may often lead to competition
59 among predator species, and it is well established that these interactions may not combine
60 additively in the context of prey risk (Soluk, 1993; Sih et al., 1998; Barrios-O'Neill et al.,
61 2014). This can lead to erroneous inferences based on pairwise interaction strengths at the
62 community-level. Additionally, the presence of predators at a higher trophic level may alter
63 the efficacy of intermediate consumers through both density-mediation (i.e., direct predation)
64 and trait-mediation (i.e., non-consumptive effects) (Peacor and Werner, 2001; Werner and
65 Peacor, 2003). There is increasing evidence for the primary importance of trait-mediation as a
66 driver of trophic cascades within ecosystems (Gabowski, 2004; Schmitz et al., 2004; Trussell
67 et al., 2004). In particular, the impacts of aquatic predator cues in ecosystems may be

68 profound due to their immediacy and influence at the population-level, and simultaneously
69 across multiple life history stages (Peacor and Werner, 2001; Trussell et al., 2004, 2008;
70 Alexander et al., 2013).

71 Multiple predator effects can manifest in three ways: (1) additively, (2) synergistically or (3)
72 antagonistically. Whilst synergistic multiple predator effects result in enhancement of prey
73 risk to predation (Soluk and Collins, 1998; Losey and Denno, 1998; Sih et al., 1998),
74 antagonistic effects result in prey risk reductions (Sih et al., 1998; Vance-Chalcraft and
75 Soluk, 2005), both of which have implications that differ from additive multiple predator
76 effects inferred from pairwise predator-prey interaction strengths.

77 Functional responses quantify the relationship between resource (e.g., prey) density and
78 consumption rate (Holling, 1959), and have been applied extensively by ecologists as a
79 means of quantifying the density-dependence of *per capita* interaction strengths across
80 multiple fields (Abrams, 1982, Dick et al., 2014; Cuthbert et al., 2018d). Both functional
81 response form or Type (i.e., Types I, II, III), and their magnitude (e.g., curve asymptote), are
82 powerful indicators of the strength of interactions between trophic groups and, at least
83 theoretically, can aid inferences of population-level stability under different environmental
84 contexts (Murdoch and Oaten, 1975; Dick et al., 2014). Centrally, the attack rate (i.e., initial
85 curve slope) and handling time (i.e., curve asymptote, inversely maximum feeding rate) of
86 consumers can be used to quantitatively compare ecological impacts under different resource
87 densities and context-dependencies (e.g., Cuthbert et al., 2018b). However, the application of
88 functional responses to derivations of multiple predator effects has remained scarce and
89 produced equivocal results across different experimental systems (Soluk, 1993; Losey and
90 Denno, 1998; Barrios-O'Neill et al., 2014; Wasserman et al., 2016).

91 Functional responses have been a staple in quantifications of natural enemy efficacies
92 towards target organisms (Solomon, 1949; O'Neill, 1990; Van Drieche and Bellows, 2011;
93 Cuthbert et al., 2018c), but have again neglected multiple predator effects. Mosquitoes are
94 exposed to a broad range of predators across their life history (e.g., copepods, dragonflies,
95 spiders), and many important natural enemies have been identified across multiple habitat
96 types (Medlock and Snow, 2008; Shaalan and Canyon, 2009). In a biological control context,
97 synergism among predators is targetted as this may enhance effects upon target species
98 groups. On the other hand, antagonistic predator-predator interactions could reduce predatory
99 effects and alleviate target prey risk. In turn, this may heighten mosquito proliferations and
100 worsen disease risk. Additive effects are also desirable as this indicates a lack of interference
101 between predators. Copepods copepods (freshwater crustaceans) have been identified as
102 particularly efficacious agents for the population-level suppression of mosquitoes (Marten
103 and Reid, 2007; Cuthbert et al., 2018b). Their application has successfully eradicated
104 pathogens vectored by medically important mosquitoes at community-level scales (Kay and
105 Nam, 2005; Nam et al., 2012), and their predatory effects have been proven to be context-
106 independent to variables such as water clarity and habitat complexity (Cuthbert et al., 2018b,
107 c). Cyclopoids are a particularly diverse group with a worldwide distribution (Dussart and
108 Defaye, 2001), and often dominate lentic freshwater habitats where medically important
109 mosquitoes can breed. Furthermore, their ecological versatility facilitates application to
110 minute or ephemeral container-style habitats where many other predators cannot persist
111 (Marten and Reid, 2007).

112 Despite their importance for the natural regulation of mosquitoes, there has hitherto been a
113 lack of consideration for the implications of con/heterospecific multiple predator interactions
114 for the efficacy of copepods in mosquito biocontrol. Such multiple predator interactions have
115 been shown to substantially affect predatory effects by other mosquito antagonists (e.g.,

116 Barry and Roberts, 2014; Brahma et al., 2015). Further, chaoborid larvae (flies with an
117 aquatic larval stage) are important predators in many fishless aquatic ecosystems and have
118 the capacity to influence zooplankton assemblages *via* predation (Yan et al., 1991; Arnott and
119 Vanni, 1993; Nyberg, 1998). Although comparatively less studied than copepods in a vector
120 control context, larval chaoborids have also been identified as effective predators of larval
121 mosquitoes (Borkent, 1980; Cuthbert et al., 2019b), and have the capacity to colonise
122 artificial container-style habitats where mosquitoes breed *via* aerial dispersal in their adult
123 stage (Sunahara et al., 2002). Owing to their strong predatory capacity towards zooplankton
124 such as cyclopoid copepods (Smyly, 1979), chaoborid-copepod interactions may result in
125 prey risk reductions for mosquitoes, given that the two predator groups are known to co-
126 occur and interact in freshwater habitats (e.g., Moore et al., 1994). Alternatively, synergistic
127 or additive effects would indicate utility in their joint use. Therefore, the present study uses a
128 functional response approach to quantify con- and heterospecific multiple predator effects
129 within and between the cyclopoid copepod *Macrocyclops albidus* (Jurine, 1820) (Cyclopoida:
130 Cyclopidae) and a predator at a higher trophic level, larvae of the chaoborid *Chaoborus*
131 *flavicans* (Meigen, 1830) (Diptera: Chaoboridae), using larvae of the *Culex pipiens* (Diptera:
132 Culicidae) mosquito complex as basal prey. Firstly, we quantify individual functional
133 responses of the two predators, before secondly quantifying and comparing predicted
134 multiple predator effects among conspecific copepod groups in the presence or absence of the
135 predator at a higher trophic level. Based on previous findings, we hypothesise that: (1) *C.*
136 *flavicans* will exhibit a higher consumption rate than *M. albidus*; (2) overall consumption
137 rates will generally increase with greater predator density and richness; (3) the consumption
138 rates by multiple conspecific copepods will combine independently in the absence of the
139 higher predator, whilst negative interactions between chaoborids and copepods will reduce
140 consumption rates for the prey.

141

142 **Materials and methods**

143 *Animal collection and maintenance*

144 The intermediate predator, *M. albidus*, was collected from Glastry Clay Pit ponds, Northern
145 Ireland (54° 29' 18.5" N 5° 28' 19.9" W) using a polypropylene dipper. Copepods were
146 transported in source water to a laboratory at Queen's Marine Laboratory (QML), and
147 maintained in controlled laboratory conditions (25 ± 2 °C; 50 – 60 % relative humidity; 16:8
148 light:dark regime) to stimulate proliferation. Gravid females were isolated from samples and
149 used to initiate pure cultures in accordance with the available literature (Suarez et al., 1992;
150 Marten and Reid, 2007). After nauplii emerged (larval stage), originating females were
151 dissected and identified to confirm the species. *Macrocyclus albidus* cultures were then
152 initiated in 10 litre tanks and fed *ad libitum* with *Paramecium caudatum* and *Chilomonas*
153 *paramecium* (Sciento, Manchester, England). These protozoan cultures were prepared in 2
154 litre flasks using autoclaved wheat seeds in the same laboratory conditions. The predator at a
155 higher trophic level, fourth instar *C. flavicans*, were purchased from a commercial supplier
156 (Northampton Reptile Centre, Northampton, England) and fed *ad libitum* with *Daphnia* sp.,
157 obtained from the same supplier. This predator was acclimatised to the insectary conditions
158 for five days prior to experimentation. The prey, newly hatched *C. pipiens* complex
159 mosquitoes, were obtained from the same insectary where a colony had been maintained.
160 Adult mosquitoes were kept in 32.5 × 32.5 × 32.5 cm cages (Bugdorm, Watkins and
161 Doncaster, Leominster, England) and blood fed using defibrinated horse blood (TCS
162 Biosciences, Buckingham, England) through a membrane feeding system (Hemotek Ltd,
163 Accrington, England). Cotton pads soaked in a 10 % sucrose solution were provided for other
164 sustenance. Egg rafts were extracted regularly from cages and placed into 3 litre larval bowls,

165 and hatched larvae were fed *ad libitum* using ground guinea pig pellets until pupation (Pets at
166 Home, Newtownabbey, Northern Ireland), upon which they were transferred into the above
167 cages.

168 *Experimental design*

169 We starved non-ovigerous adult female *M. albidus* and fourth instar *C. flavicans* individually
170 for 24 hours before the experiment to standardise hunger levels. The experiment was
171 undertaken in plastic arenas of 42 mm diameter containing 20 mL of dechlorinated tap water
172 from an aerated source under insectary conditions (see above). To quantify functional
173 responses, we supplied prey densities of 2, 4, 8, 15, 30 or 60 first instar *C. pipiens*. (1.1 – 1.3
174 mm) per arena to four different predator treatments comprising adult female *M. albidus* (1.6 –
175 1.8 mm) and fourth instar larval *C. flavicans* (10.0 – 12.0 mm) over a six hour experimental
176 feeding period ($n = 3$ per experimental treatment group). The four predator treatments were:
177 one *M. albidus*; one *C. flavicans*; three *M. albidus*; or three *M. albidus* alongside one *C.*
178 *flavicans*. Quantifications of single predator functional responses enabled modelling of the
179 expected multiple predator consumption rates, which could then be compared to those
180 actually observed (see below). In the multiple predator species trials, where a copepod was
181 killed by a chaoborid, it was immediately replaced from a surplus supply (observed once).
182 We did not observe conspecific killing between copepods. Prey were allowed to settle for two
183 hours before the experiment was initiated through the addition of predators. After six hours,
184 the predators were removed and remaining live prey counted to derive those killed. Controls
185 consisted of three replicates at each prey density in the absence of any predator.

186 *Statistical analyses*

187 All statistical analyses were undertaken in R v3.5.1 (R Development Core Team, 2018). We
188 compared overall consumption with respect to the ‘predator’ (4 levels) and ‘prey supply’ (6

189 levels) factors using a generalised linear model (GLM) assuming a Poisson distribution and
190 log link as residuals were not overdispersed relative to degrees of freedom. An information
191 theoretic approach was followed *via* model averaging to select the model which minimised
192 information loss (Burnham and Anderson, 2002; Bartoń, 2015). The relative importance of
193 each model term was discerned, based on the sum of AICc weights. Models with $\Delta\text{AICc} < 2$
194 were considered interchangeable (Burnham and Anderson, 2002). The significance of factors
195 in the top model was discerned using likelihood ratio tests, with Tukey tests used for *post-hoc*
196 comparisons of significant effects.

197 Functional response analyses were undertaken using the ‘frair’ package (Pritchard et al.
198 2017). For observed consumption under each predator treatment, we determined functional
199 response types using logistic regression of prey killed as a function of prey density
200 (continuous predictor). A significantly negative first order term is indicative of a Type II
201 response, whereas a significantly positive first order term followed by a significantly negative
202 second order term indicates a Type III response (Juliano, 2001). To account for prey
203 depletion during the experiment, we fitted Rogers’ random predator equation for conditions
204 without prey replacement (Rogers, 1972; Trexler et al., 1988; Juliano, 2001):

$$205 \qquad N_e = N_0(1 - \exp(a(N_e h - T)))$$

206 (1)

207 where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is
208 the handling time and T is the total experimental period. The *Lambert W* function was
209 implemented due to the recursive nature of the Random predator equation (Bolker, 2008). We
210 non-parametrically bootstrapped ($n = 2000$) the a and h parameter estimates to produce 95 %
211 confidence intervals around observed functional response curves. This process enabled
212 results to be considered at the population-level, as oppose to the sample-level, negating the

213 additional use of more formal statistical tests (e.g., Barrios O’Neill et al., 2014). Moreover,
214 using the initial a and h parameter estimates, we calculated the functional response ratio (a/h)
215 for each predator treatment (see Cuthbert et al. 2019a). This approach balances information
216 from both parameters, with both high values of a and low values of h conducive to ecological
217 impact.

218 Following McCoy et al. (2012) and Sentis and Boukal (2018), we used a population-dynamic
219 approach to generate predictions of multiple predator functional responses using a and h
220 parameter estimates from single *M. albidus* and *C. flavicans* treatments:

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

222 (2)

223 where N is the prey population density, P_i ($i = 1, 2, \dots, n$) are the population densities of
224 predators i and $f_i(N)$ is the functional response of predator i (i.e., Eqn. 1). This generated a
225 ‘null’ model of emergent multiple predator effects, which was then compared directly to
226 observed multiple predator functional responses (i.e., $3 \times M. albidus$; $3 \times M. albidus + 1 \times C.$
227 *flavicans*). Whilst multiple modelling approaches have been developed for predictions of
228 multiple predator effects, the population dynamic approach has been identified as more robust
229 and precise than other methods (Sentis and Boukal, 2018). Predictions of prey consumption
230 were generated for all experimental initial prey densities, with Eqn. 2 integrated over the total
231 experimental time (see Sentis and Boukal, 2018). We generated 95 % confidence intervals
232 around predictions using global sensitivity analysis which integrated the 95 % confidence
233 intervals of the a and h parameter estimates from single predator treatments, alongside their
234 covariance-variance matrix. This generated a number of parameter sets using a Latin
235 hypercube sampling algorithm (Soetaert and Petzoldt, 2010). Equation 2 was integrated for

236 each set over time to generate multiple predator consumption predictions with 95 %
237 confidence intervals derived from the 2.5 % and 97.5 % quantiles of the simulated survival
238 values. Actual and predicted functional responses were then compared on the basis of
239 confidence interval overlaps across prey densities.

240

241 **Results**

242 Prey survival in controls exceeded 99.5 % and ‘natural’ prey mortality was thus not corrected
243 for in trials with predators. The ‘predator’ and ‘prey supply’ terms were retained in the top
244 model of prey consumption (both relative variable importance = 1). The interaction term
245 between these factors was removed (relative variable importance < 0.01; $\Delta\text{AICc} = 32.70$).
246 The predator treatment had a significant effect on consumption of *C. pipiens* ($\chi^2 = 102.69$, df
247 = 3, $p < 0.001$) (Fig. 1). Three *M. albidus* consumed significantly more mosquito prey than
248 single *M. albidus* ($p < 0.001$), but consumption by three copepods did not differ significantly
249 from single *C. flavicans* ($p > 0.05$). Single *C. flavicans* consumed significantly more prey
250 than single *M. albidus* ($p < 0.001$), and consumption by individual chaoborids did not differ
251 significantly from that of *C. flavicans* when present alongside three *M. albidus* ($p > 0.05$).
252 However, *C. flavicans* consumption alongside three *M. albidus* was significantly greater than
253 either single or triple *M. albidus* treatment levels (both $p < 0.001$). Prey consumption also
254 increased significantly under greater prey supplies ($\chi^2 = 307.89$, $df = 5$, $p < 0.001$).

255 All functional responses were categorised as Type II, owing to significantly negative linear
256 coefficients (Table 1). Whilst attack rates were relatively similar between individual *M.*
257 *albidus* and *C. flavicans*, handling times were considerably reduced for *C. flavicans* (Table
258 1). Accordingly, individual *C. flavicans* exhibited a substantially higher functional response
259 ratio compared to individual *M. albidus*. Attack rates were also similar between con- and

260 heterospecific multiple predator groups, whilst handling times were considerably lower in the
261 latter group, containing both *M. albidus* and *C. flavicans* (Table 1). Therefore, the functional
262 response ratio was highest under the heterospecific multiple predator group, indicating
263 greater effects on mosquito prey by copepods in the presence of the higher chaoborid
264 predator.

265 Individually, functional responses of *M. albidus* and *C. flavicans* did not overlap above prey
266 densities of approximately eight (Fig. 2a), and so functional responses (i.e., predation rates)
267 of *C. flavicans* were significantly greater than *M. albidus* at higher densities (i.e.,
268 significantly lower handling times and higher maximum feeding rates). The functional
269 response curve of three *M. albidus* was well-predicted from the individual functional
270 response curve (Fig. 2b). Here, predicted confidence intervals overlapped with those
271 observed across all prey densities, indicative of non-significant differences and thus additive
272 multiple predator effects in copepods. Similarly, for three *M. albidus* in the presence of
273 individual *C. flavicans*, predicted and observed functional response confidence intervals
274 overlapped under all prey densities (Fig. 2c). Again, this indicated that multiple predator
275 consumption rates were well-predicted from individual predator treatments.

276

277 **Discussion**

278 Robust quantifications of multiple predator interactions are crucial to understanding
279 interaction strengths at the community-level (Sih et al., 1998; Bolker et al., 2003; Wasserman
280 et al., 2016; Sentis and Boukal, 2018). In a natural enemy context, predator-predator
281 interactions may alter the efficacy of biological control agents, with implications for
282 organism population and economic or sanitary risk. In the present study, we first quantified
283 the interaction strengths of two widespread natural enemies towards the disease vector *C.*

284 *pipiens* mosquito complex. Larval chaoborids and cyclopoid copepods are known to co-occur
285 and engage in predatory interactions in freshwater environments (Moore et al., 1994), and
286 these groups are well-adapted to temporary aquatic systems which mosquitoes also colonise
287 (Sunahara et al., 2002; Marten and Reid, 2007). Accordingly, both groups have importance as
288 natural enemies for mosquitoes across various aquatic environments. Both the cyclopoid
289 copepod *M. albidus* and larval chaoborid midge *C. flavicans* displayed predatory Type II
290 functional responses towards larval mosquito prey, although the functional response
291 magnitude (i.e., maximum feeding rate) was significantly higher in *C. flavicans*. Predation by
292 conspecific copepod groups was additive, with observed multiple predator consumption well-
293 predicted from simulations based on the functional response of individuals. Similarly, the
294 presence of the predator at a higher trophic level, *C. flavicans*, did not result in significant
295 differences in observed predation compared to expectations based on single predator groups.
296 Accordingly, the predator at a higher trophic level did not result in alterations to copepod-
297 mosquito interaction strengths in the present study, and multiple coexisting copepods did not
298 cause reductions to mosquito prey risk *via* interference. Our results thus indicate that these
299 predator species can be used in combination and that copepod density can be increased
300 without affecting their *per capita* effects on mosquito populations.

301 Overall consumption rates of chaoborids were significantly higher than cyclopoid copepods
302 in the present study. This is not surprising owing to their larger size and different prey
303 handling strategy. Whilst both focal predator species are ambush consumers that detect prey
304 through hydromechanical cue reception (Riessen et al., 1984; Hwang and Strickler, 2001;
305 Cuthbert et al., 2018a), chaoborids ingest prey whole using prehensile antennae and mandibles,
306 with initial ingestion occurring in the thoracic crop before subsequent regurgitation (Riessen
307 et al., 1984). This enables many individual prey items to be simultaneously packed into the
308 crop, and enables a relatively high predatory effect compared to other trophically analogous

309 invertebrates (Dodson, 1972; von Ende and Dempsey, 1981; Cuthbert et al., 2019b). On the
310 other hand, *M. albidus* has chewing mouthparts, using mandibles to tear prey items into
311 digestible pieces (Fryer, 1957). Accordingly, it is likely that lower handling time constraints
312 facilitate higher offtake rates towards mosquito prey by chaobiorids as compared to cyclopoid
313 copepods. Nevertheless, despite significant differences in handling times between the two
314 predators, attack rates were more similar between the species, which, combined with Type II
315 functional responses, corresponds to high levels of ecological impact at low prey densities.
316 Indeed, in the context of biological control, Type II functional responses are desirable as
317 they are, at least theoretically, associated with population-destabilising effects on target
318 organisms (Cuthbert et al., 2018d). The functional response ratio has recently been identified
319 as a novel approach to balance information from both functional response parameters in
320 predictions of ecological impact (Cuthbert et al. 2019a). Here, functional response ratios
321 peaked under the heterospecific multiple predator group. Accordingly, greater predator
322 diversities were conducive to the highest ecological impacts in the present study, with the
323 presence of *C. flavicans* bolstering the predatory effects of conspecific copepod groups on
324 larval mosquitoes.

325 The predictability of multiple copepod functional responses from individual interaction
326 strengths suggests that, at least under densities used in the present study, this group of species
327 does not respond to the presence of conspecifics in a predation context. This lack of predator-
328 predator interference corroborates with the reported efficacy of cyclopoid copepods in
329 mosquito population management, wherein a high numerical response facilitates high
330 predatory effects when copepods reach high abundances (Marten and Reid, 2007; Nam et al.,
331 2012; Cuthbert et al. 2018d). Owing to their sit-and-wait prey capture strategy, this lack of
332 predator-predator interference is not surprising, with multiple predator effects shown to be
333 dependent on the particular behavioural characteristics of species (Wasserman et al., 2016). In

334 a similar vein, the ambush-style capture strategy of chaoborids likely also limited interference
335 between predators in the present study, with intraguild predation only observed in one
336 instance. Furthermore, whilst chaoborids often forage in pelagic water zones, many cyclopoid
337 copepods, including *M. albidus*, are benthic foragers, therefore potentially enabling spatial
338 separation between the two predator types, which further limits antagonistic interactions (see
339 Schmitz, 2007). Applications of natural enemies which occupy different parts of waterbodies
340 could enhance mosquito control *via* reductions in predator free space, yet these effects may
341 be further altered by habitat characteristics such as water volume. Experimental arena size
342 can directly influence the strength of predator-prey interactions (e.g., Uiterwaal and DeLong,
343 2018), and it is therefore probable that choice of volume can influence experimental
344 quantifications of multiple predator effects in aquatic systems through, for example,
345 modulating predator-predator encounter rates. Given the particular tendency for *C. pipiens* to
346 spend time at the water surface (Yee et al., 2004), effects of different experimental water
347 depths may be especially profound in mediating interaction strengths. Nevertheless,
348 comparative studies which examine feeding rates phenomenologically (see Jeschke et al.,
349 2002) across standardised experimental conditions provide useful insights into predatory
350 interactions, whilst omitting search area as a confounding variable. Further, as the present
351 study used relatively small arena sizes, it is unlikely that larger volumes would alter the
352 additive nature of predator-predator interactions through changes to encounter rates.

353 Although not significantly different to single chaoborid treatments, consumption levels
354 towards mosquitoes tended to peak under the combined copepod and chaoborid predator
355 treatment group in the present study. Therefore, both increased abundances and diversity of
356 predators resulted in the greatest interaction strength towards medically important
357 mosquitoes. Alongside general studies on the biological control of crop pests (Snyder et al.,
358 2003, 2006, 2008; Finke and Denno, 2004), a number of studies have examined the combined

359 efficacy of multiple antagonists towards larval mosquitoes (Nilsson and Soderstrom, 1988;
360 Culler and Lamp, 2009; Barry and Roberts, 2014; Brahma et al., 2015; Bofill and Yee, 2019).
361 Yet, there has been a lack of application of functional responses in quantifications of multiple
362 predator effects towards mosquitoes, despite the inherent density-dependence of consumer-
363 resource interactions (Holling, 1959). Whilst antagonistic predator-predator interactions have
364 been reported in previous studies, our results suggest that the promotion of cyclopoids and
365 chaoborids for the control of medically important mosquitoes could further enhance
366 biological control efforts *via* additive effects. However, the *per capita* interaction strength of
367 chaoborids was far higher to that of copepods, with the latter group contributing relatively
368 little to combined interactions. Importantly, both focal predator types in the present study are
369 capable of colonising a broad range of lentic aquatic habitats where vector mosquitoes breed,
370 including ephemeral container-style habitats (Sunahara et al., 2002; Marten and Reid, 2007).
371 In particular, aerial dispersal by chaoborids during their adult stage could enable effective
372 colonisation of discrete habitat patches which other predators cannot reach. Equally, many
373 cyclopoid copepods are able to enter periods of diapause during phases of temporary drying
374 between hydroperiods, reducing the potential for exploitation of predator-free habitats by
375 mosquito colonists (Marten and Reid, 2007). Indeed, diapausing copepods are known to
376 survive for months in the absence of water (Frisch, 2002).

377 In conclusion, the predatory effect of cyclopoid copepods is not significantly altered by either
378 the presence of conspecifics or predators at a higher trophic level. Both predator types
379 exhibited additive functional responses towards common mosquito prey, conducive to high
380 predictability from individual interaction strengths and a lack of interference among
381 consumers. Accordingly, their combined promotion in aquatic habitats could further assist in
382 mosquito population management strategies through bolstering of predatory effects. Such
383 insights into multiple predator interactions offer improved quantifications of the overall

384 effects of natural enemies upon target species, further informing as to the efficacy of selected
385 biological control agents before their release into empirical systems.

386

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389

390 **Contribution of authors**

391 RC conceived the designed the experiment. RC performed the experiment. RC analysed the
392 data. RC and AS did the modelling. AC, AS, AD and JD contributed materials and analysis
393 tools. RC wrote the first draft of the manuscript, and all authors contributed substantially to
394 revisions. The authors declare that they have no conflicts of interest.

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583 **Table 1.** First order term results from logistic regression to determine functional response
 584 types, alongside rounded parameter estimates and functional response ratios resulting from
 585 Rogers' random predator equation.

Predator treatment	First order term, p	Attack rate (a), p	Handling time (h), p	Functional response ratio (a/h)
<i>M. albidus</i> ($\times 1$)	-0.03, < 0.001	0.85, < 0.05	0.18, < 0.001	4.67
<i>C. flavicans</i> ($\times 1$)	-0.01, < 0.05	0.95, < 0.001	0.02, < 0.05	57.68
<i>M. albidus</i> ($\times 3$)	-0.03, < 0.001	1.94, < 0.001	0.07, < 0.001	27.82
<i>C. flavicans</i> ($\times 1$) + <i>M. albidus</i> ($\times 3$)	-0.03, < 0.001	1.69, < 0.001	0.02, < 0.001	79.99

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594 **Fig. 1.** Larval *Culex pipiens* consumption under four observed predator treatments across all
595 densities: 1 × *Macrocyclus albidus* (Ma); 1 × *Chaoborus flavicans* (Cf); 3 × *Macrocyclus*
596 *albidus*; 3 × *Macrocyclus albidus* + 1 × *Chaoborus flavicans*. In the boxplots, the horizontal
597 bar displays the median, the box gives the interquartile ranges and the whiskers show the
598 largest and smallest values up to 1.5 times the interquartile range.

599

600 **Fig. 2.** Functional responses of *Macrocyclus albidus* (Ma) and *Chaoborus flavicans* (Cf)
601 towards larval *Culex pipiens*, individually (a: solid, *M. albidus*; dotted, *C. flavicans*), or in
602 con/heterospecific predatory units (b: solid, *M. albidus* (× 3) observed; dashed, 3 *M. albidus*
603 (× 3) predicted; c: solid, *M. albidus* (× 3) and *C. flavicans* (× 1) observed; dashed, *M. albidus*
604 (× 3) and *C. flavicans* (× 1) predicted). Shaded areas are 95 % confidence intervals.