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1 *Original Article*

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

3 Ross N. Cuthbert[1, 2,]*, Amanda Callaghan[2], Arnaud Sentis[3], Arpita Dalal[4], Jaimie T. A. Dick[1]

4

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

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

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

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

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

10 Provence, France

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

12 India

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

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Abstract

21

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 *Macrocyclops 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 synergyistic 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 Peaor, 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 simuntaneously

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) synergystically 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 reponses 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 quantatively compare ecological impacts under different resouce

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, synergystic

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

Materials and methods

Animal collection and maintenance

The intermediate predator, *M. albidus*, was collected from Glastry Clay Pit ponds, Northern

Ireland (54° 29' 18.5" N 5° 28' 19.9" W) using a polypropylene dipper. Copepods were

transported in source water to a laboratory at Queen's Marine Laboratory (QML), and

maintained in controlled laboratory conditions (25 ± 2 °C; $50 - 60$ % relative humidity; 16:8

light:dark regime) to stimulate proliferation. Gravid females were isolated from samples and

used to initiate pure cultures in accordance with the available literature (Suarez et al., 1992;

Marten and Reid, 2007). After nauplii emerged (larval stage), originating females were

dissected and identified to confirm the species. *Macrocyclops albidus* cultures were then

initiated in 10 litre tanks and fed *ad libitum* with *Paramecium caudatum* and *Chilomonas*

paramecium (Sciento, Manchester, England). These protozoan cultures were prepared in 2

litre flasks using autoclaved wheat seeds in the same laboratory conditions. The predator at a

higher trophic level, fourth instar *C. flavicans*, were purchased from a commercial supplier

(Northampton Reptile Centre, Northampton, England) and fed *ad libitum* with *Daphnia* sp.,

obtained from the same supplier. This predator was acclimatised to the insectary conditions

for five days prior to experimentation. The prey, newly hatched *C. pipiens* complex

mosquitoes, were obtained from the same insectary where a colony had been maintained.

Adult mosquitoes were kept in $32.5 \times 32.5 \times 32.5$ cm cages (Bugdorm, Watkins and

Doncaster, Leominster, England) and blood fed using defibrinated horse blood (TCS

Biosciences, Buckingham, England) through a membrane feeding system (Hemotek Ltd,

Accrington, England). Cotton pads soaked in a 10 % sucrose solution were provided for other

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.

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.

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 ΔAICc < 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   $$N_e = N_0(1 - \exp(a(N_e h - T)))$$

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   paramaeter estimates from single *M. albidus* and *C. flavicans* treatments:

221
$$\frac{dN}{dt} = -\sum_{i=1}^{n} f_i(N)P_i$$

222   (2)

223   where *N* is the prey population density, P_i (*i* = 1, 2, ..., *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 × *M. albidus*; 3 × *M. albidus* + 1 × *C.*

227   *flavicans*). Whilst multiple modelling approaches have been developed for predictions of

228   multiple predator effects, the population dynamic approach has been identifed 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; ΔAICc = 32.70).

246   The predator treatment had a significant effect on consumption of *C. pipiens* (χ^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 (χ^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

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 chaborids 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 chaborid 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 multipe 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 chaborids 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), chaborids ingest prey whole using prehensile attenae 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 analogoues

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 desireable 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 characterstics 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.

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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)
M. albidus ($\times$ 1)	-0.03, < 0.001	0.85, < 0.05	0.18, < 0.001	4.67
C. flavicans ($\times$ 1)	-0.01, < 0.05	0.95, < 0.001	0.02, < 0.05	57.68
M. albidus ($\times$ 3)	-0.03, < 0.001	1.94, < 0.001	0.07, < 0.001	27.82
C. flavicans ($\times$ 1) + *M. albidus* ($\times$ 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: $1 \times$ *Macrocyclops albidus* (Ma); $1 \times$ *Chaoborus flavicans* (Cf); $3 \times$ *Macrocyclops*

596 *albidus*; $3 \times$ *Macrocyclops albidus* + $1 \times$ *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 *Macrocyclops 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* ($\times$ 3) observed; dashed, 3 *M. abidus*

603 ($\times$ 3) predicted; c: solid, *M. albidus* ($\times$ 3) and *C. flavicans* ($\times$ 1) observed; dashed, *M. albidus*

604 ($\times$ 3) and *C. flavicans* ($\times$ 1) predicted). Shaded areas are 95 % confidence intervals.