Interspecific variation, habitat complexity and ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control

1 **Interspecific variation, habitat complexity and ovipositional responses**

2 **modulate the efficacy of cyclopoid copepods in disease vector control**

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21 **ABSTRACT**

22 The use of predatory biological control agents can form an effective component in the

23 management of vectors of parasitic diseases and arboviruses. However, we require predictive

24 methods to assess the efficacies of potential biocontrol agents under relevant environmental

25 contexts. Here, we applied functional responses (FRs) and reproductive effort as a proxy of

26 numerical responses (NRs) to compare the Relative Control Potential (RCP) of three

27 cyclopoid copepods, *Macrocyclops albidus*, *M. fuscus* and *Megacyclops viridis* towards

28 larvae of the mosquito *Culex quinquefasciatus*. The effects of habitat complexity on such

29 predatory impacts were examined, as well as ovipositional responses of *C. quinquefasciatus*

30 to copepod cues in pairwise choice tests. All three copepod species demonstrated a

31 population destabilising Type II FR. *M. albidus* demonstrated the shortest handling time and

32 highest maximum feeding rate, whilst *M. fuscus* exhibited the highest attack rate. The

33 integration of reproductive effort estimations in the new RCP metric identifies *M. albidus* as

34 a very promising biocontrol agent. Habitat complexity did not impact the FR form or

35 maximum feeding rate of *M. albidus*, indicating that potentially population destabilising

36 effects are robust to habitat variations; however, attack rates of *M. albidus* were reduced in

37 the presence of such complexity. *C. quinquefasciatus* avoided ovipositing where *M. albidus*

38 was physically present, however it did not avoid chemical cues alone. *C. quinquefasciatus*

39 continued to avoid *M. albidus* during oviposition when both the treatment and control water

40 were dyed; however, when an undyed, predator-free control was paired with dyed, predator-

41 treated water, positive selectivity towards the treatment water was stimulated. We thus

42 demonstrate the marked predatory potential of cyclopoid copepods, utilising our new RCP

43 metric, and advocate their feasibility in biological control programmes targeting container-

44 style habitats. We also show that behavioural responses of target organisms and

45 environmental context should be considered to maximise agent efficacy.

46 Keywords: Functional response; Numerical response; Relative Control Potential; Copepod;

47 Mosquito; Oviposition

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49 **1. Introduction**

50   Mosquitoes continue to endanger public health globally through the transmission of

51 vector-borne diseases, which account for hundreds of thousands of deaths annually (World

52 Health Organisation, 2017). Transmission rates of arboviruses and the ecology of their

53 vectors are shifting rapidly under environmental change (Medlock and Leach, 2015; Benelli

54 and Mehlhorn, 2016), and at different spatiotemporal scales (Townroe and Callaghan, 2014;

55 Cunze et al. 2016). Furthermore, incursions and proliferations of highly invasive mosquito

56 species are occurring as a direct result of anthropogenic activity (e.g. Yee, 2016; Medlock et

57 al. 2017), with implications for public health and persistence of native species (Juliano, 2010;

58 Schäffner et al. 2013). Thus, the development of effective strategies to assist or augment the

59 control of vectors of mosquito-borne disease is critical. These strategies in turn rely on rapid

60 and reliable assessments of the efficacy of biocontrol agents under relevant environmental

61 contexts.

62   *Culex quinquefasciatus* Say, 1823 is a member of the *C. pipiens* complex, which are

63 vectors of diseases such as West Nile virus, Sandbis virus, St Louis encephalitis, Rift Valley

64 fever virus and lymphatic filariasis (Turell, 2012; Manimegalai and Sukanya, 2014). This

65 mosquito breeds rapidly in artificial, urban environments, enabling a high potential for human

66 contact (Yee, 2008). Furthermore, the species is characterised as being invasive, exhibiting

67 the potential to negatively impact and replace native species (Juliano, 2010). Climate change

68 may additionally bolster the potential of *C. quinquefasciatus* to invade novel ecosystems

69 through range expansion (Samy et al. 2016). Cyclopoid copepods have had marked success in

70   the biological control of *Aedes aegypti* mosquito larvae (e.g. Kay and Nam, 2005), and

71   predatory impacts have been demonstrated towards *Culex* spp. (e.g. Calliari et al. 2003;

72   Tranchida et al. 2009). Furthermore, the global distribution, high fecundity, environmental

73   hardiness, voraciousness and ease of mass-production of copepods enable high levels of

74   potential impact on target populations, whilst negating the need for potentially ecologically

75   harmful species introductions (Marten and Reid, 2007). Yet, while interspecific variations in

76   control efficiencies between cyclopoids have been demonstrated (Marten, 1989), as well as

77   demographic variations across differing species strains (Marten, 1990b), many candidate

78   copepod species are yet to be examined in the context of mosquito control.

79   Functional responses (FRs), which discern the *per capta* intake rate of a resource as a

80   function of its availability (Holling, 1959; 1966), exhibit great utility in the quantification of

81   interspecific differences in consumer-resource interactions (Dick et al. 2017). The search,

82   capture and handling components of FRs can be highly informative in the contexts of

83   biological control (O'Neil, 1990; Van Driesche and Bellows, 2011) and invasive species

84   (Dick et al. 2014). Indeed, high impact invasive species are strongly associated with higher

85   maximum feeding rates driven by low handling times (Dick et al. 2017), and this associates

86   strongly with measurable changes in affected populations (i.e. ecological impact). Functional

87   response form and magnitude can also be significant with respect to prey population stability;

88   a Type II FR is deemed to have potentially population destabilising effects due to high levels

89   of proportional predation at low prey densities, whilst a Type III FR may impart stability to

90   prey by providing refugia at low prey densities (Holling, 1959). Moreover, coupling such *per*

91   *capita* effects with corresponding numerical responses at the consumer population level can

92   bolster the robustness of predictions of consumer impacts on resources (Dick et al. 2017).

93   These approaches thus yield high potential in the comparison of efficacies of potential

94   biocontrol agents. Further, context-dependencies, such as habitat complexity, can modify the

95   nature of interactions within predator-prey systems and can be quantified using a FR

96   approach (e.g. Barrios-O'Neill et al. 2015; South et al.2017). Habitat complexity may enable

97   physical refuge for prey, directly affecting interaction strengths, and, accordingly, the

98   structuring of ecological communities (e.g. Alexander et al. 2012). These interactions are

99   often specific to predator-prey pairings, with habitat complexity having been found to also

100   heighten predation (e.g. Alexander et al. 2015).

101   Besides direct consumptive effects, trait-mediated indirect interactions (TMIIs),

102   including behavioural responses of target organisms to predators, can exert strong effects on

103   predator-prey dynamics (e.g. Alexander et al. 2013), and can be as strong or stronger in their

104   population effects (Peacor and Werner 2001; Trussell et al. 2004; 2008). Behavioural

105   responses of mosquitoes to predator cues can be marked, yet also species-specific (e.g.

106   Vonesh and Blaustein, 2010; Zuhurah and Lester, 2011). Thus, considering oviposition

107   selectivity among potential habitats by mosquitoes is of integral importance to biological

108   control, particularly for species which invest all of their eggs in one environment at one time,

109   such as *C. quinquefasciatus* (Wachira et al. 2010). Broadly, natural selection favours

110   individuals that are able to actively evade predation through avoidance of oviposition habitats

111   containing predators (Sih, 1986; Blaustein and Chase, 2007). Water colouration, serving as a

112   proxy for nutritional load, has also been demonstrated to significantly attract oviposition by

113   *Culex* mosquitoes (Ortiz Perea and Callaghan, 2017). The effects of the presence of copepods

114   on oviposition of mosquitoes has been seldom considered, however, Torres-Estrada et al.

115   (2001) found that *A. aegypti* preferentially oviposited in water treated with the copepod

116   *Mesocyclops longisetus* due to the emission of attractive compounds. This finding is an

117   oddity in the context of the general avoidance by mosquitoes of other predators (Vonesh and

118   Blaustein, 2010).

119   Here, we quantify the FRs of three locally-abundant and widespread cyclopoid

120 copepods, *Macrocyclops albidus* (Jurine 1820), *Macrocyclops fuscus* (Jurine, 1820) and

121 *Megacyclops viridis* (Jurine, 1820) towards larvae of the mosquito *C. quinquefasciatus*. We

122 then combine maximum feeding rate and attack rate estimations generated from the FRs with

123 reproductive effort data from Maier (1994) to compare the Relative Control Potential (RCP;

124 Cuthbert et al. 2018) among the three species. Benthic habitat complexity is integrated in a

125 separate experiment to assess potential context-dependencies relevant to the efficacy of

126 copepods in their predation of *C. quinquefasciatus*. Furthermore, we utilise pairwise choice

127 tests to assess the ovipositional responses of *C. quinquefasciatus* to the predators in several

128 bioassays consisting of visual and chemical cues from predatory copepods, and examine

129 whether the integration of pond dye reduces avoidance behaviour by the mosquito, hence

130 enhancing biocontrol efforts. Thus, we seek to decipher the most effective biocontrol agent

131 using the RCP metric, and consider the effects of habitat complexity and target prey

132 behavioural responses on agent efficacies.

133 **2. Methods**

134 *2.1. Experimental organisms*

135   The predators, *Macrocyclops albidus*, *M. fuscus* and *Megacyclops viridis*, were

136 collected from Glastry Clay Pit Ponds, Northern Ireland (54°29'18.5"N; 5°28'19.9"W) during

137 January 2017 using a polypropylene dipper, transported to Queen's Marine Laboratory,

138 Portaferry in source water, and maintained at 25 ± 2 °C under a 16:8 light:dark regime.

139 Cultures were initiated separately for each species by placing a single ovigerous adult female

140 into 250 mL dechlorinated tap water, with the protists *Paramecium caudatum* and

141 *Chilomonas paramecium* supplied *ad libitum*. Emerging nauplii were transferred into larger

142   10 L tanks and fed on the same protist diet, while autoclaved wheat seeds were used to

143   sustain the protists.

144   The prey, *Culex quinquefasciatus*, were obtained from a laboratory colony maintained

145   at the same conditions as the predators. *Culex quinquefasciatus* were originally collected in

146   Cyprus in 2005 by Dr A. Callaghan and had been reared in laboratory conditions at the

147   University of Reading since then. Adults were maintained in 32.5 cm^3 cages (Bugdorm,

148   Watkins and Doncaster, Leominster, England). Defibrinated horse blood (TCS Biosciences,

149   Buckingham, England) was fed to adults three times per week using an artificial membrane

150   feeding system (Hemotek Ltd., Accrington, England). Cotton pads soaked in 10% sucrose

151   solution were provided for other sustenance. A black oviposition cup containing 200 mL tap

152   water was kept in each cage, with egg rafts removed three times per week and placed into 3 L

153   larval bowls containing tap water and ground guinea pig pellets (Pets at Home,

154   Newtownabbey, Northern Ireland) provided *ad libitum* until mosquito pupation.

155   *2.2. Experiment 1: Functional responses (FRs) and Relative Control Potential (RCP)*

156   Adult, non-ovigerous female *M. albidus* (1.6 – 1.8 mm body length), *M. fuscus* (1.9 –

157   2.1 mm body length) and *M. viridis* (2.0 – 2.3 mm body length) were selected for

158   experiments to standardise predators as cyclopoids are sexually dimorphic (Laybourn-Parry

159   et al. 1988). Predators were starved for 24 hours to standardise hunger levels. Prey, first instar

160   *C. quinquefasciatus* (1.1- 1.3 mm), were provided to copepods at six densities (2, 4, 8, 15, 30,

161   60, $n = 3$ per density) in 20 mL arenas of 42 mm diameter, and allowed to settle for three

162   hours prior to the addition of individual predators. Predators were allowed to feed for 24

163   hours, after which they were removed and remaining live prey counted. Controls consisted of

164   three replicates at each prey density without a predator. We integrated reproductive effort

165   data derived from Maier (1994) with maximum feeding rates and attack rates to calculate

166   RCP among the three predators (see below).

2.3. Experiment 2: Habitat complexity

168   Adult female *M. albidus* (1.6 – 1.8 mm body length) were selected for experiments

169   and starved for 24 hours to standardise hunger levels. Prey, first instar *C. quinquefasciatus*

170   (1.1- 1.3 mm), were provided at the same densities ($n = 3$ per experimental group) and in the

171   same arenas as Experiment 1, and also allowed the same acclimation. However, arenas

172   exhibited either 'low' or 'high' complexity, with 'low' complexity treatment arenas being

173   vacant and 'high' complexity arenas containing five polypropylene caps (7 mm diameter, 10

174   mm height), arranged in a uniform array (Figure 1) and positioned using non-toxic mounting

175   putty. As in Experiment 1, predators were allowed to feed for 24 hours before being removed

176   and remaining live prey counted. Controls consisted of three replicates at each prey density

177   and complexity level without predators.

2.4. Experiment 3: Ovipositional responses

179   We assessed ovipositional preferences of *C. quinquefasciatus* with pairwise choice

180   tests. Recently emerged female *C. quinquefasciatus* were blood fed for the first time over a

181   24 hour period. Following this, each experimental replicate consisted of cages (32.5 cm^3)

182   containing 20 blood-fed females and a 10% sucrose solution, refilled *ad libitum*. Mosquitoes

183   were maintained in these cages for 144 hours in bioassay 1, and 168 hours in following

184   bioassays, at 25 °C ± 2 °C and in a 16:8 light:dark regime. In all bioassays, control and

185   treatment cups were established 48 hours before being added to cages in 80 ml arenas of 6.5

186   cm diameter using water from a continuously aerated source. Adult female *M. albidus* were

187   used to produce all bioassays and were fed with mosquito larvae *ad libitum* prior to use. In

188   bioassay 1, three copepods were physically present in the treatment cups when added to the

189   cages (n = 7 per experimental group). In bioassay 2, three copepods were removed from the

190   treatment cups immediately before being added to cages (n = 13 per experimental group). In

191   bioassay three, ten copepods were ground up using a pestle and mortar and added to

192   treatment cups (n = 7 per experimental group). In bioassays 4 and 5, three copepods were

193   added to arenas containing black liquid pond dye (0.3 g L^{-1}, Dyofix, Leeds, United Kingdom,

194   n = 7 per experimental group). Controls lacked predators, and those in bioassays 1-3 and 5

195   consisted of dechlorinated tap water, whilst controls in bioassay 4 consisted of dyed

196   dechlorinated tap water. Paired cups were added to opposing corners within cages in a

197   randomised design and mosquitoes given 48 hours to oviposit. Following this, cups were

198   removed and the numbers of egg rafts counted.

199   *2.5. Statistical analyses*

200   Statistical analyses were undertaken in R v3.4.2. (R Core Team, 2017). In

201   Experiments 1 and 2, logistic regression was used to infer FR types with respect to

202   proportions of prey eaten as functions of prey density. Categorically, a Type II FR is

203   indicated by a significantly negative first order term. Rogers' random predator equation was

204   fitted to the data as prey were not replaced as they were consumed (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 constant,

208   h is the handling time and T is the total experimental period. The Lambert W function was

209   used to aid model fitting (Bolker, 2008). Estimates of attack rate, handling time and

210   maximum feeding rate (1/h) were non-parametrically bootstrapped (n = 30) to provide means

211   and standard errors (SEs). In Experiment 1, we calculated RCP (see Cuthbert et al. 2018)

212   using maximum feeding and attack rates with reproductive effort data (clutch weight

213 produced per female body weight per day) from Maier (1994) as a proxy for the numerical

214 response:

215 $$RCP = \left(\frac{FR \text{ agent A}}{FR \text{ agent B}}\right) \times \left(\frac{RE \text{ agent A}}{RE \text{ agent B}}\right)$$

216   (2)

217 where FR represents maximum feeding or attack rates, and RE is reproductive effort. Where

218 RCP = 1, no difference between biocontrol agents is predicted; where RCP > 1, agent A has a

219 greater control potential than agent B, whilst where RCP < 1, agent A has a lesser control

220 potential than agent B (see Dick et al. 2017; Cuthbert et al. 2018). Uncertainty around RCP

221 values, in the form of confidence intervals (CIs) and p-values, were calculated as per Dick et

222 al. (2017). RCP 'biplots' were used to visually illustrate the results (e.g. see Cuthbert et al.

223 2018).

224   In Experiment 3, we used Shapiro-Wilk and Bartlett tests to assess normality and

225 homoscedasticity for each treatment pair, respectively. Where data were normal and

226 homoscedastic, we employed paired t-tests for analysis of oviposition data. Wilcoxon tests

227 were used where data did not meet the assumptions of a parametric test. We calculated

228 oviposition activity index (OAI) values (Kramer and Mulla, 1979), which enable data to be

229 standardised as proportions for graphical visualisation:

230 $$OAI = \frac{(NT - NC)}{(NT + NC)}$$

231   (3)

232 where the oviposition activity index (OAI) is a function of the number of egg rafts laid in

233 treated water (NT) relative to controls (NC). The OAI ranges from -1 to 1, with 0 indicating

234 no preference between options. Positive values indicate that greater preference is

235 demonstrated for the treatment rather than control cups, whilst negative values show

236 preference for controls.

3. Results

237

238 *3.1. Experiment 1: Functional responses (FRs) and Relative Control Potential (RCP)*

239   Prey survival in controls exceeded 99% and thus most prey deaths were attributed to

240 cyclopoid copepod predation. Type II FRs were found for each predator (Table 1; Figure 2).

241 *M. albidus* demonstrated the shortest handling times and, inversely, highest maximum

242 feeding rates, followed by *M. viridis*, which exhibited shorter handling times and higher

243 maximum feeding rates than *M. fuscus* (Table 2; Figure 3a, b). Attack rates varied among the

244 predators; *M. fuscus* exhibited greater attack rates than *M. albidus*, which in turn exhibited

245 greater attack rates than *M. viridis* (Table 2; Figure 3c).

246   Relative Control Potential (RCP) calculations and probabilities are illustrated in Table

247 2. When maximum feeding rates are used in the RCP metric (Figure 4a), *M. albidus* exhibits

248 greater efficacy in controlling *C. quinquefasciatus* than both *M. fuscus* (RCP = 2.73) and *M.*

249 *viridis* (RCP = 1.33). *M. viridis* exhibits a greater efficacy compared to *M. fuscus* (RCP =

250 2.13). With attack rates incorporated into the RCP metric (Figure 4b), *M. albidus*

251 demonstrates similar efficacy predictions to *M. fuscus* (RCP = 0.96), and greater efficacy than

252 *M. viridis* (RCP = 1.42). *M. viridis* exhibits a lower efficacy than *M. fuscus* here (RCP =

253 0.71). These calculations are bolstered graphically in Figure 4 using RCP biplots, with *M.*

254 *albidus* showing greatest efficacy overall when maximum feeding rates are integrated, and

255 similarities in efficacies projected between these predators when attack rates are applied.

256 *3.2. Experiment 2: Habitat complexity*

257   Prey survival in controls was 100%, and thus all prey deaths in treatment groups were

258 attributed to predation by cyclopoid copepods. Type II FRs were observed under both levels

259   of habitat complexity (Table 3; Figure 5). Handling times and maximum feeding rates were

260   similar between both levels of habitat complexity (Figure 6a, b). Attack rates were higher in

261   low compared to high complexity treatments (Figure 6c).

3.3. Experiment 3: Ovipositional responses

263   In bioassay 1, significantly fewer egg rafts were laid in cups containing copepods in

264   comparison to controls ($W = 45$, $p = 0.01$). However, in bioassays 2 and 3, no significant

265   differences in oviposition levels were detected between the chemical treatments with

266   predatory cyclopoids removed ($t = 1.41$, $df = 12$, $p = 0.18$), or with ground cyclopoids ($W =$

267   29.5, $p = 0.55$). In bioassay 4, significantly fewer egg rafts were oviposited in dyed water

268   containing predatory copepods compared to dyed water alone ($t = 5.35$, $df = 6$, $p = 0.002$). In

269   bioassay 5, significantly more egg rafts were laid in cups treated with both dye and copepods

270   compared to undyed, predator-free controls ($t = 5.60$, $df = 6$, $p = 0.001$). Figure 7 illustrates

271   OAI scores for each of the five treatments graphically.

4. Discussion

273   The development and application of control strategies to combat mosquito-borne

274   disease is of immense public health importance (Benelli and Mehlhorn, 2016), particularly in

275   light of changes to population dynamics being driven by globalisation, urbanisation and

276   climate change (Townroe and Callaghan, 2014; Medlock and Leach, 2015), as well as

277   emergent context-dependencies (e.g. Fischer et al. 2013). Biological control of mosquitoes

278   using predatory agents can be an effective tool to assist or augment control strategies

279   targeting important disease vector species (e.g. Nam et al. 2012). In this context, the coupled

280   use of functional and numerical responses (or their proxies) provides a robust comparative

281   framework to decipher and project the potential efficacy in controlling target organisms by

282   agents (see Dick et al. 2017; Cuthbert et al. 2018). Consumer-resource interactions are,

283   however, subject to a range of context-dependencies which may modulate interaction

284   strengths, such as habitat complexity (Barrios-O'Neill et al. 2015; Alexander et al. 2015),

285   temperature (Wasserman et al. 2016; Cuthbert et al. 2018) and parasitism (Laverty et al.

286   2017a). Such effects may reduce or nullify the potential of agents in the field, and thus are

287   critical to consider during biocontrol agent evaluations. Furthermore, elucidating behavioural

288   responses, such as those relating to ovipositional choice, of target organisms is imperative to

289   derive a holistic view of agent efficacy, particularly in ecological systems where organisms

290   are not confined to a single habitat patch, such as is often the case with container-breeding

291   mosquitoes in urban and peri-urban environments (Silberbush and Blaustein, 2011; Townroe

292   and Callaghan, 2014).

293   Here, we applied functional responses (FRs) to assess the potential predatory impact

294   of three native cyclopoid copepods towards *C. quinquefasciatus*, a container-breeding

295   mosquito and capable vector of diseases such as West Nile virus and lymphatic filariasis

296   (Turell, 2012; Manimegalai and Sukanya, 2014). Further, we examined the impact of habitat

297   complexity on interaction strengths in this predator-prey system and discerned the

298   ovipositional responses of *C. quinquefasciatus* to predatory cues. The focal predators, *M.*

299   *albidus*, *M. fuscus* and *M. viridis* all exhibited potentially population destabilising Type II

300   FRs towards first instar *C. quiquefasciatus*. This characteristic encompasses high levels of

301   killing at low prey densities, limiting conditions for prey refugia (Holling, 1959) and,

302   concurrently, avoids the compensatory effect of reduced competitive interactions in surviving

303   mosquito prey (Juliano, 2007). This destabilising FR may thus reduce the likelihood of size

304   refuge attainment in prey, given that cyclopoid copepods only effectively consume early

305   instar mosquito larvae (Marten and Reid, 2007). *Macrocyclops albidus* exhibited the highest

306   magnitude FR of the predators, characterised by comparatively low handling times and thus

307   high maximum feeding rates. *M. fuscus* demonstrated the greatest attack rates, indicating

308   strong *per capita* efficacies at lower prey densities. *M. albidus* has already shown promise

309   during previous trials for the control of arbovirus vectors (e.g. Marten, 1990a; Veronesi et al.

310   2015), however the predatory potential of the congeneric *M. fuscus* had yet to be examined

311   prior to this study. The use of RCP illustrates that *M. albidus* exhibits the highest efficacy for

312   the control of *C. quinquefasciatus* when maximum feeding rates are integrated, particularly

313   given the comparatively low reproductive effort of *M. fuscus* (Maier, 1994). When attack

314   rates are used in the RCP metric, similarities between *M. albidus* and *M. fuscus* are deduced.

315   However, in a meta-analysis of the impacts of invasive species, the maximum feeding rate

316   combined with numerical response proxies gave 100% association with degree of ecological

317   impact. That is, the 'offtake rate' of prey, sometimes known as the 'total response', was the

318   best predictor of consumer impact on resources (Dick et al. 2017; Laverty et al. 2017b). Also,

319   the attack rate in experiments where prey are not replaced following consumption may be

320   artificially constrained (Alexander et al. 2012). Thus, we propose that, while attack rates may

321   give insight into predation at low prey densities, it is the maximum feeding rate combined

322   with numerical response proxies that gives best RCP predictions.

323   Habitat complexity was found to modulate interactions between *M. albidus* and *C.*

324   *quinquefasciatus* through a reduction in attack rates where complexity was present. However,

325   the Type II FR exhibited was robust to variations in habitat complexity, and thus population

326   destabilising effects towards disease vectors such as *C. quinquefasciatus* may be sustained

327   under such conditions. Indeed, habitat structure has previously been demonstrated to affect

328   survivability of mosquito prey to predatory copepods (e.g. Rey et al. 2004), however, density-

329   dependent effects (i.e. FRs) had yet to be considered. Our results contrast to other studies

330   whereby variations to benthic habitat structure fundamentally change the nature or magnitude

331   of the observed FR (e.g. Alexander et al. 2012; but see Alexander et al. 2015). Furthermore,

332   handling times and thus maximum feeding rates were largely unaffected by habitat

333   complexity, and hence feeding magnitudes of *M. albidus* are maintained under these contexts.

334   These traits favour the application of *M. albidus* as a biocontrol agent given the dynamic

335   nature of container-style habitats within which *C. quinquefasciatus* colonises (Bohart and

336   Washino, 1978; Meyer and Durso, 1993).

337   Predator avoidance by culicids during oviposition is common (see Vonesh and

338   Blaustein, 2010), yet interspecific variations with respect to coexistence patterns are

339   commonplace (e.g. van Dam and Walton, 2008). Furthermore, interacting environmental

340   factors and density-dependent effects may further modulate oviposition responses (Silberbush

341   and Blaustein, 2011; Silberbush et al. 2014). Pairwise choice tests have been deemed more

342   powerful and advocated over multiple-choice comparisons when examining responses to

343   predatory cues (Silberbush and Blaustein, 2011). Avoidance behaviours have been deemed

344   stronger for mosquito species which oviposit directly onto waterbodies, such as *Culex* spp.

345   (Vonesh and Blaustein, 2010). Our results are indicative of predator avoidance behaviour of

346   *C. quinquefasciatus* towards *M. albidus* when the copepod is physically present. Yet, these

347   effects were dependent upon the nature of the aquatic environment. Generally, this contrasts

348   with limited results which demonstrate positive selection by *A. aegypti* for sites treated with

349   cyclopoid copepods (Torres-Estrada et al. 2001). We found no evidence for avoidance

350   behaviour in the presence of chemical cues of *M. albidus*. Mosquitoes within the *C. pipiens*

351   complex have been shown to prefer black dyed water when ovipositing, likely as a result of

352   greater perceived depth or a higher nutritional load for their progeny (Ortiz Perea and

353   Callaghan, 2017). Here, when both predator and control treatments were dyed, *C.*

354   *quinquefasciatus* continued to avoid *M. albidus* in pairwise tests. However, when the control

355   treatment was undyed and predator-free, *C. quinquefasciatus* demonstrated clear preference

356   for dyed, predator-treated water. These results indicate interactive complexity, whereby the

357   preference towards dyed habitats overrides the avoidance stimulus relating to physical

358   predator presence. Thus, the use of dye in synergy with predator inoculations may foster

359   more effective biocontrol potential in certain environments.

360   In conclusion, the use of FR demonstrates strong predatory impact and potential for

361   the application of cyclopoid copepods in biocontrol strategies, particularly in light of Type II

362   forms conducive to population destabilising effects. We illustrate that this overarching FR

363   form is robust to interspecific variations and habitat complexities, however, variations in

364   underlying FR parameters are apparent among cyclopoid species and habitat complexities.

365   The integration of reproductive effort estimations allows the better resolution of differential

366   RCP between the species, favouring the use of *M. albidus* over other comparators overall.

367   These results corroborate generally with the success of cyclopoid copepods in mosquito

368   control during field trials, whereby large-scale eradication has been achieved (Kay and Nam,

369   2005; Nam et al. 2012), and the particular interest in the use of *M. albidus* in field-based trials

370   which has been demonstrated (e.g. Marten, 1990a; Veronesi et al. 2015). Understanding the

371   non-consumptive effects of predators is central to biocontrol applications and broader

372   concerns relating to predator-prey interactions (Preisser and Bolnick, 2008; Vonesh and

373   Blaustein, 2010). Here, we demonstrate context-dependent avoidance behaviour of *C.*

374   *quinquefasciatus* in response to copepod predators, yet highlight that the synergistic use of

375   dye may bolster the efficacy of biocontrol releases due to favoured oviposition in these

376   conditions. Additional field-based research to elucidate the effects of dye on community

377   structure and predatory impact is thus warranted.

378

382   Warsaw, Poland, for assistance with copepod identification. We also are grateful to two

383   anonymous referees for helpful comments.

384

385   **References**

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648 **Tables**

649 Table 1

Predator	First order term	p	h	p	a	p
M. albidus	-0.03	< 0.001	0.03	< 0.001	2.11	< 0.001
M. fuscus	-0.05	< 0.001	0.04	< 0.001	3.85	< 0.001
M. viridis	-0.02	< 0.001	0.04	< 0.001	1.36	< 0.001

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661   Table 2

Agent A, agent B comparison	FR (± SD)	Parameter	Reproductive effort	RCP	CIs (60%), $p_{RIP} > 1$ (%)
M. albidus, M. fuscus	35.72 (± 7.15), 24.06 (± 5.41)	1/*h*	0.35, 0.20	2.73	2.03 – 3.36, 99.94***
M. albidus, M. viridis	35.72 (± 7.15), 26.30 (± 4.82)	1/*h*	0.35, 0.37	1.33	1.02 – 1.61, 82.13
M. viridis, M. fuscus	26.30 (± 4.82), 24.06 (± 5.41)	1/*h*	0.37, 0.20	2.13	1.60 – 2.60, 99.35**
M. albidus, M. fuscus	2.04 (± 0.36), 4.03 (± 1.20)	*a*	0.35, 0.20	0.96	0.68 – 1.21, 39.10
M. albidus, M. viridis	2.04 (± 0.36), 1.42 (± 0.29)	*a*	0.35, 0.37	1.42	1.09 – 1.71, 87.82
M. viridis, M. fuscus	1.42 (± 0.29), 4.03 (± 1.20)	*a*	0.37, 0.20	0.71	0.49 – 0.90, 12.63

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665   Table 3

Habitat complexity	First order term	*p*	*h*	*p*	*a*	*p*
Low	-0.03	< 0.001	0.04	< 0.001	1.68	< 0.001
High	-0.02	< 0.001	0.04	< 0.001	0.90	< 0.001

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680 **Captions**

681 Table 1. First order terms resulting from logistic regression (proportion of prey killed versus

682 prey density) for each predator species in Experiment 1, alongside functional response

683 parameter estimates (h, handling time; a, attack rate) resulting from Rogers' random predator

684 equation.

685 Table 2. Relative Control Potential (RCP; means) calculations between species using

686 bootstrapped ($n = 30$ per parameter) maximum feeding rates ($1/h$) and attack rates (a) with

687 reproductive effort data (Maier, 1994). Uncertainties are reflected through CIs. Asterisks

688 denote significant levels of certainty that the RCP score is greater than 1 (* > 95%, ** > 99%,

689 *** > 99.9%).

690 Table 3. First order terms resulting from logistic regression (proportion of prey killed versus

691 prey density) for each level of habitat complexity in Experiment 2, alongside functional

692 response parameter estimates (h, handling time; a, attack rate) resulting from Rogers' random

693 predator equation.

694 Figure 1. Illustration of low and high habitat complexity treatment arenas used in Experiment

695 2.

696 Figure 2. Functional responses of *M. albidus* (a), *M. fuscus* (b) and *M. viridis* (c) towards *C.*

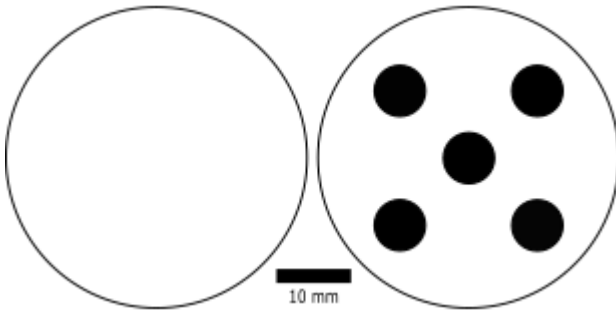697 *quinquefasciatus* prey over a 24 hour experimental period. Means are ± SE ($n = 3$ per

698 density).

699 Figure 3. Bootstrapped functional response parameters (handling time, a; maximum feeding

700 rate, b; attack rate, c) of *M. albidus*, *M. fuscus* and *M. viridis* towards *C. quinquefasciatus*.

701 Means are ± SE ($n = 30$ per parameter).

702   Figure 4. RCP biplot integrating mean bootstrapped maximum feeding rate (a) and attack rate

703   (b) and reproductive effort (Maier, 1994) for each predator. Increasing efficacy is projected

704   from bottom left to top right. Means are ± SE (n = 30 per FR parameter).

705   Figure 5. Functional responses of *M. albidus* towards *C. quinquefasciatus* prey under low and

706   high habitat complexity over a 24 hour experimental period. Means are ± SE (n = 3 per

707   density).

708   Figure 6. Bootstrapped functional response parameters (handling time, a; maximum feeding

709   rate, b; attack rate, c) of *M. albidus* towards *C. quinquefasciatus* under low and high habitat

710   complexity. Means are ± SE (n = 30 per parameter).

711   Figure 7. Oviposition activity index values (OAI, ± SE) shown by *C. quinquefasciatus*

712   resulting from pairwise tests with predator-free controls and treatments cups inoculated with:

713   (a) three physically-present *M. albidus* (n = 7); (b) the cues emitted by three removed *M.*

714   *albidus* (n = 13); (c) ten ground *M. albidus* (n = 7); (d) three physically-present *M. albidus* in

715   dyed water with a dyed control (n = 7); (e) three physically present *M. albidus* in dyed water

716   with an undyed control (n = 7). Controls in bioassays (a) – (c) comprised undyed arenas. OAI

717   values of 0 indicate no preference between predator-control treatments, with values closer to -

718   1 indicating an increasing preference for controls (i.e. predator avoidance), and those closer

719   to 1 indicating increasing selection for predator-treated water. Labels above indicate

720   significance levels resulting from statistical tests using raw oviposition data (p: * < 0.05, ** <

721   0.01, *** < 0.001, NS ≥ 0.05).

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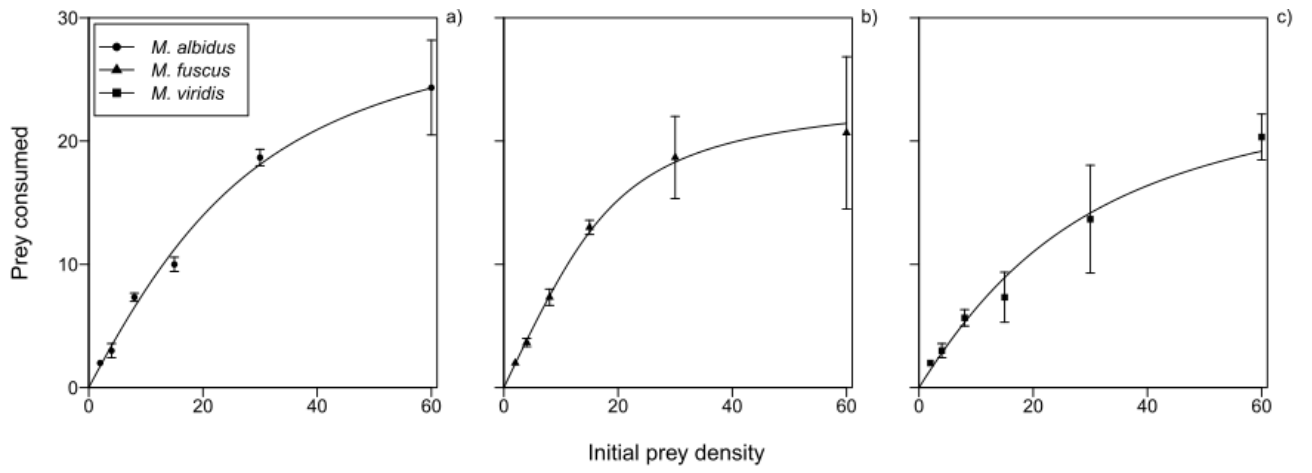
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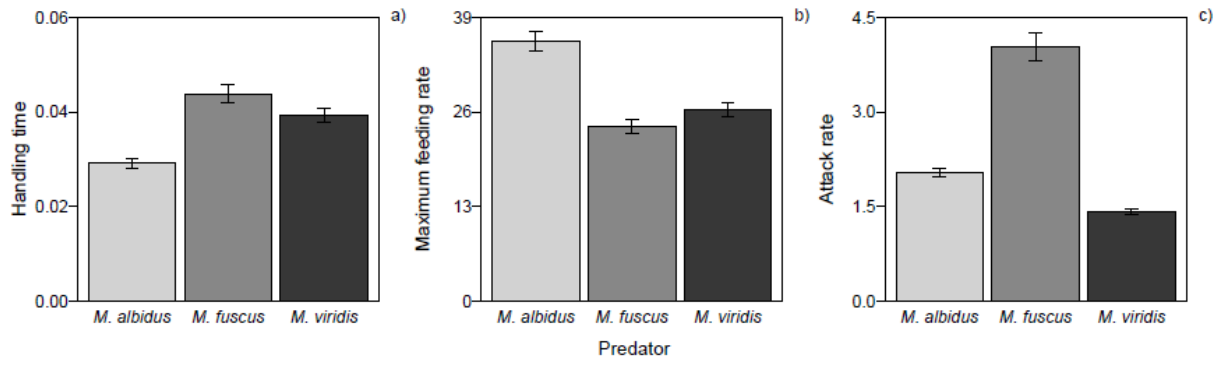
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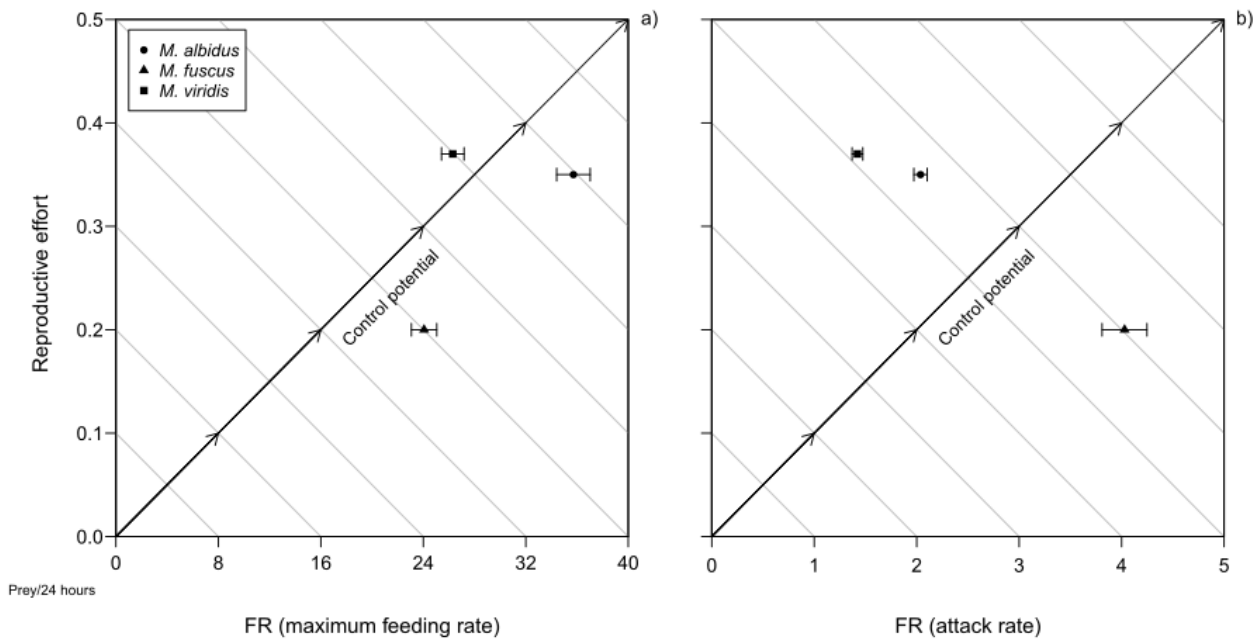
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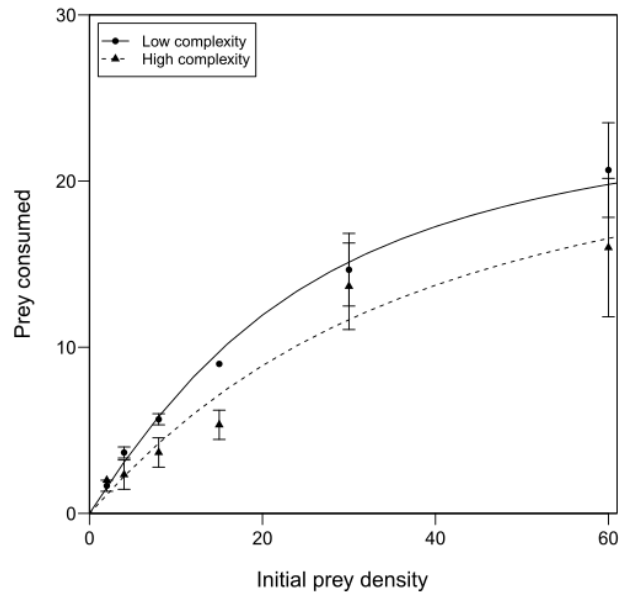
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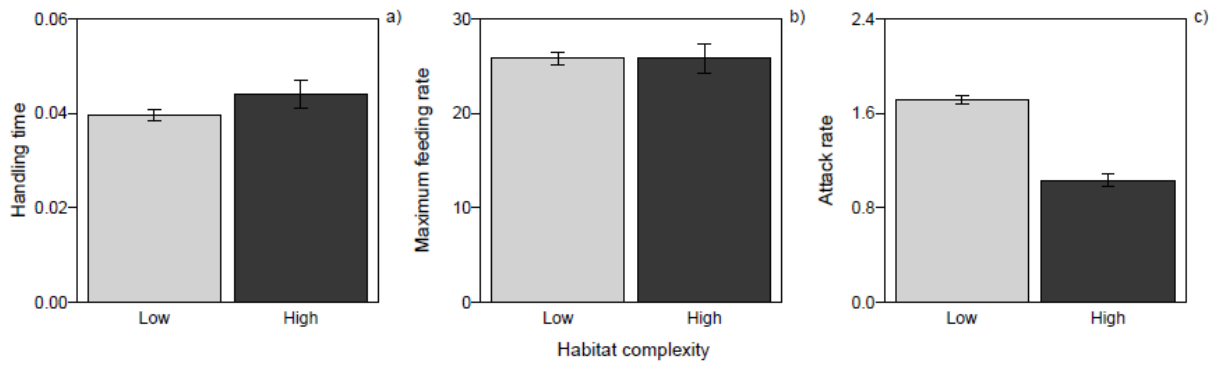795

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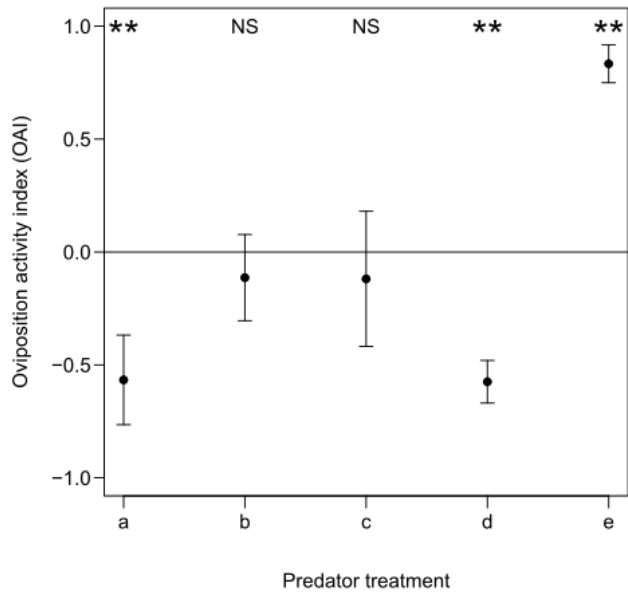801

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