

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

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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

The use of predatory biological control agents can form an effective component in the 22 23 management of vectors of parasitic diseases and arboviruses. However, we require predictive methods to assess the efficacies of potential biocontrol agents under relevant environmental 24 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 larvae of the mosquito *Culex quinquefasciatus*. The effects of habitat complexity on such 28 predatory impacts were examined, as well as ovipositional responses of C. quinquefasciatus 29 to copepod cues in pairwise choice tests. All three copepod species demonstrated a 30 31 population destabilising Type II FR. M. albidus demonstrated the shortest handling time and highest maximum feeding rate, whilst M. fuscus exhibited the highest attack rate. The 32 integration of reproductive effort estimations in the new RCP metric identifies M. albidus as 33 34 a very promising biocontrol agent. Habitat complexity did not impact the FR form or maximum feeding rate of *M. albidus*, indicating that potentially population destabilising 35 effects are robust to habitat variations; however, attack rates of *M. albidus* were reduced in 36 the presence of such complexity. C. quinquefasciatus avoided ovipositing where M. albidus 37 was physically present, however it did not avoid chemical cues alone. C. quinquefasciatus 38 39 continued to avoid *M. albidus* during oviposition when both the treatment and control water were dyed; however, when an undyed, predator-free control was paired with dyed, predator-40 treated water, positive selectivity towards the treatment water was stimulated. We thus 41 42 demonstrate the marked predatory potential of cyclopoid copepods, utilising our new RCP metric, and advocate their feasibility in biological control programmes targeting container-43 style habitats. We also show that behavioural responses of target organisms and 44 45 environmental context should be considered to maximise agent efficacy.

Keywords: Functional response; Numerical response; Relative Control Potential; Copepod;
Mosquito; Oviposition

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#### 49 **1. Introduction**

Mosquitoes continue to endanger public health globally through the transmission of 50 vector-borne diseases, which account for hundreds of thousands of deaths annually (World 51 Health Organisation, 2017). Transmission rates of arboviruses and the ecology of their 52 53 vectors are shifting rapidly under environmental change (Medlock and Leach, 2015; Benelli and Mehlhorn, 2016), and at different spatiotemporal scales (Townroe and Callaghan, 2014; 54 Cunze et al. 2016). Furthermore, incursions and proliferations of highly invasive mosquito 55 species are occurring as a direct result of anthropogenic activity (e.g. Yee, 2016; Medlock et 56 al. 2017), with implications for public health and persistence of native species (Juliano, 2010; 57 Schäffner et al. 2013). Thus, the development of effective strategies to assist or augment the 58 control of vectors of mosquito-borne disease is critical. These strategies in turn rely on rapid 59 and reliable assessments of the efficacy of biocontrol agents under relevant environmental 60 61 contexts.

Culex quinquefasciatus Say, 1823 is a member of the C. pipiens complex, which are 62 63 vectors of diseases such as West Nile virus, Sandbis virus, St Louis encephalitis, Rift Valley fever virus and lymphatic filariasis (Turell, 2012; Manimegalai and Sukanya, 2014). This 64 mosquito breeds rapidly in artificial, urban environments, enabling a high potential for human 65 66 contact (Yee, 2008). Furthermore, the species is characterised as being invasive, exhibiting the potential to negatively impact and replace native species (Juliano, 2010). Climate change 67 may additionally bolster the potential of C. quinquefasciatus to invade novel ecosystems 68 through range expansion (Samy et al. 2016). Cyclopoid copepods have had marked success in 69

the biological control of Aedes aegypti mosquito larvae (e.g. Kay and Nam, 2005), and 70 predatory impacts have been demonstrated towards *Culex* spp. (e.g. Calliari et al. 2003; 71 72 Tranchida et al. 2009). Furthermore, the global distribution, high fecundity, environmental hardiness, voraciousness and ease of mass-production of copepods enable high levels of 73 potential impact on target populations, whilst negating the need for potentially ecologically 74 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 interspecific differences in consumer-resource interactions (Dick et al. 2017). The search, 81 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 (Dick et al. 2014). Indeed, high impact invasive species are strongly associated with higher 84 maximum feeding rates driven by low handling times (Dick et al. 2017), and this associates 85 strongly with measurable changes in affected populations (i.e. ecological impact). Functional 86 response form and magnitude can also be significant with respect to prey population stability; 87 a Type II FR is deemed to have potentially population destabilising effects due to high levels 88 of proportional predation at low prey densities, whilst a Type III FR may impart stability to 89 prey by providing refugia at low prey densities (Holling, 1959). Moreover, coupling such per 90 91 capita effects with corresponding numerical responses at the consumer population level can bolster the robustness of predictions of consumer impacts on resources (Dick et al. 2017). 92 These approaches thus yield high potential in the comparison of efficacies of potential 93 94 biocontrol agents. Further, context-dependencies, such as habitat complexity, can modify the

nature of interactions within predator-prey systems and can be quantified using a FR
approach (e.g. Barrios-O'Neill et al. 2015; South et al.2017). Habitat complexity may enable
physical refuge for prey, directly affecting interaction strengths, and, accordingly, the
structuring of ecological communities (e.g. Alexander et al. 2012). These interactions are
often specific to predator-prey pairings, with habitat complexity having been found to also
heighten predation (e.g. Alexander et al. 2015).

101 Besides direct consumptive effects, trait-mediated indirect interactions (TMIIs), including behavioural responses of target organisms to predators, can exert strong effects on 102 predator-prey dynamics (e.g. Alexander et al. 2013), and can be as strong or stronger in their 103 population effects (Peacor and Werner 2001; Trussell et al. 2004; 2008). Behavioural 104 105 responses of mosquitoes to predator cues can be marked, yet also species-specific (e.g. Vonesh and Blaustein, 2010; Zuhurah and Lester, 2011). Thus, considering oviposition 106 107 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, such as C. quinquefasciatus (Wachira et al. 2010). Broadly, natural selection favours 109 individuals that are able to actively evade predation through avoidance of oviposition habitats 110 containing predators (Sih, 1986; Blaustein and Chase, 2007). Water colouration, serving as a 111 proxy for nutritional load, has also been demonstrated to significantly attract oviposition by 112 Culex mosquitoes (Ortiz Perea and Callaghan, 2017). The effects of the presence of copepods 113 on oviposition of mosquitoes has been seldom considered, however, Torres-Estrada et al. 114 (2001) found that A. aegypti preferentially oviposited in water treated with the copepod 115 116 Mesocyclops longisetus due to the emission of attractive compounds. This finding is an oddity in the context of the general avoidance by mosquitoes of other predators (Vonesh and 117 Blaustein, 2010). 118

Here, we quantify the FRs of three locally-abundant and widespread cyclopoid 119 copepods, Macrocyclops albidus (Jurine 1820), Macrocyclops fuscus (Jurine, 1820) and 120 121 Megacyclops viridis (Jurine, 1820) towards larvae of the mosquito C. quinquefasciatus. We then combine maximum feeding rate and attack rate estimations generated from the FRs with 122 reproductive effort data from Maier (1994) to compare the Relative Control Potential (RCP; 123 Cuthbert et al. 2018) among the three species. Benthic habitat complexity is integrated in a 124 125 separate experiment to assess potential context-dependencies relevant to the efficacy of copepods in their predation of C. quinquefasciatus. Furthermore, we utilise pairwise choice 126 127 tests to assess the ovipositional responses of C. quinquefasciatus to the predators in several bioassays consisting of visual and chemical cues from predatory copepods, and examine 128 whether the integration of pond dye reduces avoidance behaviour by the mosquito, hence 129 enhancing biocontrol efforts. Thus, we seek to decipher the most effective biocontrol agent 130 using the RCP metric, and consider the effects of habitat complexity and target prey 131 behavioural responses on agent efficacies. 132

#### 133 **2. Methods**

#### 134 2.1. Experimental organisms

135The predators, Macrocylops albidus, M. fuscus and Megacyclops viridis, were136collected from Glastry Clay Pit Ponds, Northern Ireland ( $54^{\circ}29'18.5$ "N;  $5^{\circ}28'19.9$ "W) during137January 2017 using a polypropylene dipper, transported to Queen's Marine Laboratory,138Portaferry in source water, and maintained at  $25 \pm 2$  °C under a 16:8 light:dark regime.139Cultures were initiated separately for each species by placing a single ovigerous adult female140into 250 mL dechlorinated tap water, with the protists Paramecium caudatum and141Chilomonas 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 to143 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 <sup>3</sup> 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 <i>M. viridis</i> $(2.0 - 2.3 \text{ mm body length})$ 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

data derived from Maier (1994) with maximum feeding rates and attack rates to calculateRCP among the three predators (see below).

167 2.3. Experiment 2: Habitat complexity

Adult female *M. albidus* (1.6 - 1.8 mm body length) were selected for experiments 168 and starved for 24 hours to standardise hunger levels. Prey, first instar C. quinquefasciatus 169 (1.1-1.3 mm), were provided at the same densities (n = 3 per experimental group) and in the 170 same arenas as Experiment 1, and also allowed the same acclimation. However, arenas 171 172 exhibited either 'low' or 'high' complexity, with 'low' complexity treatment arenas being vacant and 'high' complexity arenas containing five polypropylene caps (7 mm diameter, 10 173 174 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 and remaining live prey counted. Controls consisted of three replicates at each prey density 176 and complexity level without predators. 177

178 2.4. Experiment 3: Ovipositional responses

We assessed ovipositional preferences of *C. quinquefasciatus* with pairwise choice 179 tests. Recently emerged female C. quinquefasciatus were blood fed for the first time over a 180 24 hour period. Following this, each experimental replicate consisted of cages (32.5 cm<sup>3</sup>) 181 containing 20 blood-fed females and a 10% sucrose solution, refilled ad libitum. Mosquitoes 182 were maintained in these cages for 144 hours in bioassay 1, and 168 hours in following 183 bioassays, at 25 °C  $\pm$  2 °C and in a 16:8 light:dark regime. In all bioassays, control and 184 185 treatment cups were established 48 hours before being added to cages in 80 ml arenas of 6.5 cm diameter using water from a continuously aerated source. Adult female M. albidus were 186 187 used to produce all bioassays and were fed with mosquito larvae ad libitum prior to use. In bioassay 1, three copepods were physically present in the treatment cups when added to the 188

cages (n = 7 per experimental group). In bioassay 2, three copepods were removed from the 189 treatment cups immediately before being added to cages (n = 13 per experimental group). In 190 191 bioassay three, ten copepods were ground up using a pestle and mortar and added to treatment cups (n = 7 per experimental group). In bioassays 4 and 5, three copepods were 192 added to arenas containing black liquid pond dye (0.3 g L<sup>-1</sup>, Dyofix, Leeds, United Kingdom, 193 n = 7 per experimental group). Controls lacked predators, and those in bioassays 1-3 and 5 194 195 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 removed and the numbers of egg rafts counted. 198

#### 199 2.5. Statistical analyses

Statistical analyses were undertaken in R v3.4.2. (R Core Team, 2017). In Experiments 1 and 2, logistic regression was used to infer FR types with respect to proportions of prey eaten as functions of prey density. Categorically, a Type II FR is indicated by a significantly negative first order term. Rogers' random predator equation was fitted to the data as prey were not replaced as they were consumed (Juliano, 2001):

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

where  $N_e$  is the number of prey eaten,  $N_0$  is the initial density of prey, *a* is the attack constant, *h* is the handling time and *T* is the total experimental period. The Lambert W function was used to aid model fitting (Bolker, 2008). Estimates of attack rate, handling time and maximum feeding rate (1/*h*) were non-parametrically bootstrapped (n = 30) to provide means and standard errors (SEs). In Experiment 1, we calculated RCP (see Cuthbert et al. 2018) using maximum feeding and attack rates with reproductive effort data (clutch weight produced per female body weight per day) from Maier (1994) as a proxy for the numericalresponse:

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)

where FR represents maximum feeding or attack rates, and RE is reproductive effort. Where RCP = 1, no difference between biocontrol agents is predicted; where RCP > 1, agent A has a greater control potential than agent B, whilst where RCP < 1, agent A has a lesser control potential than agent B (see Dick et al. 2017; Cuthbert et al. 2018). Uncertainty around RCP values, in the form of confidence intervals (CIs) and *p*-values, were calculated as per Dick et al. (2017). RCP 'biplots' were used to visually illustrate the results (e.g. see Cuthbert et al. 2018).

In Experiment 3, we used Shapiro-Wilk and Bartlett tests to assess normality and homoscedasticity for each treatment pair, respectively. Where data were normal and homoscedastic, we employed paired *t*-tests for analysis of oviposition data. Wilcoxon tests were used where data did not meet the assumptions of a parametric test. We calculated oviposition activity index (OAI) values (Kramer and Mulla, 1979), which enable data to be standardised as proportions for graphical visualisation:

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

231 (3)

where the oviposition activity index (OAI) is a function of the number of egg rafts laid in
treated water (NT) relative to controls (NC). The OAI ranges from -1 to 1, with 0 indicating
no preference between options. Positive values indicate that greater preference is

demonstrated for the treatment rather than control cups, whilst negative values showpreference for controls.

#### 237 **3. Results**

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

Prey survival in controls exceeded 99% and thus most prey deaths were attributed to
cyclopoid copepod predation. Type II FRs were found for each predator (Table 1; Figure 2). *M. albidus* demonstrated the shortest handling times and, inversely, highest maximum
feeding rates, followed by *M. viridis*, which exhibited shorter handling times and higher
maximum feeding rates than *M. fuscus* (Table 2; Figure 3a, b). Attack rates varied among the
predators; *M. fuscus* exhibited greater attack rates than *M. albidus*, which in turn exhibited
greater attack rates than *M. viridis* (Table 2; Figure 3c).

Relative Control Potential (RCP) calculations and probabilities are illustrated in Table 246 2. When maximum feeding rates are used in the RCP metric (Figure 4a), *M. albidus* exhibits 247 greater efficacy in controlling C. quinquefasciatus than both M. fuscus (RCP = 2.73) and M. 248 viridis (RCP = 1.33). M. viridis exhibits a greater efficacy compared to M. fuscus (RCP = 249 2.13). With attack rates incorporated into the RCP metric (Figure 4b), M. albidus 250 demonstrates similar efficacy predictions to *M. fuscus* (RCP = 0.96), and greater efficacy than 251 252 *M. viridis* (RCP = 1.42). *M. viridis* exhibits a lower efficacy than *M. fuscus* here (RCP =  $\frac{1}{2}$ ) 0.71). These calculations are bolstered graphically in Figure 4 using RCP biplots, with M. 253 albidus showing greatest efficacy overall when maximum feeding rates are integrated, and 254 255 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 were258 attributed to predation by cyclopoid copepods. Type II FRs were observed under both levels

of habitat complexity (Table 3; Figure 5). Handling times and maximum feeding rates were
similar between both levels of habitat complexity (Figure 6a, b). Attack rates were higher in
low compared to high complexity treatments (Figure 6c).

262 3.3. Experiment 3: Ovipositional responses

In bioassay 1, significantly fewer egg rafts were laid in cups containing copepods in 263 comparison to controls (W = 45, p = 0.01). However, in bioassays 2 and 3, no significant 264 differences in oviposition levels were detected between the chemical treatments with 265 266 predatory cyclopoids removed (t = 1.41, df = 12, p = 0.18), or with ground cyclopoids (W =29.5, p = 0.55). In bioassay 4, significantly fewer egg rafts were oviposited in dved water 267 containing predatory copepods compared to dyed water alone (t = 5.35, df = 6, p = 0.002). In 268 269 bioassay 5, significantly more egg rafts were laid in cups treated with both dye and copepods compared to undyed, predator-free controls (t = 5.60, df = 6, p = 0.001). Figure 7 illustrates 270 OAI scores for each of the five treatments graphically. 271

#### 272 **4. Discussion**

The development and application of control strategies to combat mosquito-borne 273 disease is of immense public health importance (Benelli and Mehlhorn, 2016), particularly in 274 light of changes to population dynamics being driven by globalisation, urbanisation and 275 276 climate change (Townroe and Callaghan, 2014; Medlock and Leach, 2015), as well as emergent context-dependencies (e.g. Fischer et al. 2013). Biological control of mosquitoes 277 using predatory agents can be an effective tool to assist or augment control strategies 278 targeting important disease vector species (e.g. Nam et al. 2012). In this context, the coupled 279 use of functional and numerical responses (or their proxies) provides a robust comparative 280 framework to decipher and project the potential efficacy in controlling target organisms by 281 agents (see Dick et al. 2017; Cuthbert et al. 2018). Consumer-resource interactions are, 282

however, subject to a range of context-dependencies which may modulate interaction 283 strengths, such as habitat complexity (Barrios-O'Neill et al. 2015; Alexander et al. 2015), 284 285 temperature (Wasserman et al. 2016; Cuthbert et al. 2018) and parasitism (Laverty et al. 2017a). Such effects may reduce or nullify the potential of agents in the field, and thus are 286 critical to consider during biocontrol agent evaluations. Furthermore, elucidating behavioural 287 288 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 and Callaghan, 2014). 292

293 Here, we applied functional responses (FRs) to assess the potential predatory impact of three native cyclopoid copepods towards C. quinquefasciatus, a container-breeding 294 mosquito and capable vector of diseases such as West Nile virus and lymphatic filariasis 295 296 (Turell, 2012; Manimegalai and Sukanya, 2014). Further, we examined the impact of habitat complexity on interaction strengths in this predator-prey system and discerned the 297 ovipositional responses of C. quinquefasciatus to predatory cues. The focal predators, M. 298 albidus, M. fuscus and M. viridis all exhibited potentially population destabilising Type II 299 FRs towards first instar C. quiquefasciatus. This characteristic encompasses high levels of 300 301 killing at low prey densities, limiting conditions for prey refugia (Holling, 1959) and, concurrently, avoids the compensatory effect of reduced competitive interactions in surviving 302 mosquito prey (Juliano, 2007). This destabilising FR may thus reduce the likelihood of size 303 304 refuge attainment in prey, given that cyclopoid copepods only effectively consume early instar mosquito larvae (Marten and Reid, 2007). Macrocyclops albidus exhibited the highest 305 magnitude FR of the predators, characterised by comparatively low handling times and thus 306 307 high maximum feeding rates. *M. fuscus* demonstrated the greatest attack rates, indicating

strong per capita efficacies at lower prey densities. M. albidus has already shown promise 308 during previous trials for the control of arbovirus vectors (e.g. Marten, 1990a; Veronesi et al. 309 310 2015), however the predatory potential of the congeneric *M. fuscus* had yet to be examined prior to this study. The use of RCP illustrates that *M. albidus* exhibits the highest efficacy for 311 the control of *C. quinquefasciatus* when maximum feeding rates are integrated, particularly 312 given the comparatively low reproductive effort of *M. fuscus* (Maier, 1994). When attack 313 314 rates are used in the RCP metric, similarities between *M. albidus* and *M. fuscus* are deduced. However, in a meta-analysis of the impacts of invasive species, the maximum feeding rate 315 316 combined with numerical response proxies gave 100% association with degree of ecological impact. That is, the 'offtake rate' of prey, sometimes known as the 'total response', was the 317 best predictor of consumer impact on resources (Dick et al. 2017; Laverty et al. 2017b). Also, 318 the attack rate in experiments where prey are not replaced following consumption may be 319 artificially constrained (Alexander et al. 2012). Thus, we propose that, while attack rates may 320 321 give insight into predation at low prey densities, it is the maximum feeding rate combined with numerical response proxies that gives best RCP predictions. 322

Habitat complexity was found to modulate interactions between *M. albidus* and *C.* 323 quinquefasciatus through a reduction in attack rates where complexity was present. However, 324 the Type II FR exhibited was robust to variations in habitat complexity, and thus population 325 326 destabilising effects towards disease vectors such as C. quinquefasciatus may be sustained under such conditions. Indeed, habitat structure has previously been demonstrated to affect 327 survivability of mosquito prey to predatory copepods (e.g. Rey et al. 2004), however, density-328 329 dependent effects (i.e. FRs) had yet to be considered. Our results contrast to other studies whereby variations to benthic habitat structure fundamentally change the nature or magnitude 330 of the observed FR (e.g. Alexander et al. 2012; but see Alexander et al. 2015). Furthermore, 331 332 handling times and thus maximum feeding rates were largely unaffected by habitat

complexity, and hence feeding magnitudes of *M. albidus* are maintained under these contexts.
These traits favour the application of *M. albidus* as a biocontrol agent given the dynamic
nature of container-style habitats within which *C. quinquefasciatus* colonises (Bohart and
Washino, 1978; Meyer and Durso, 1993).

Predator avoidance by culicids during oviposition is common (see Vonesh and 337 338 Blaustein, 2010), yet interspecific variations with respect to coexistence patterns are 339 commonplace (e.g. van Dam and Walton, 2008). Furthermore, interacting environmental factors and density-dependent effects may further modulate oviposition responses (Silberbush 340 and Blaustein, 2011; Silberbush et al. 2014). Pairwise choice tests have been deemed more 341 powerful and advocated over multiple-choice comparisons when examining responses to 342 343 predatory cues (Silberbush and Blaustein, 2011). Avoidance behaviours have been deemed stronger for mosquito species which oviposit directly onto waterbodies, such as *Culex* spp. 344 (Vonesh and Blaustein, 2010). Our results are indicative of predator avoidance behaviour of 345 346 C. quinquefasciatus towards M. albidus when the copepod is physically present. Yet, these effects were dependent upon the nature of the aquatic environment. Generally, this contrasts 347 with limited results which demonstrate positive selection by A. aegypti for sites treated with 348 cyclopoid copepods (Torres-Estrada et al. 2001). We found no evidence for avoidance 349 behaviour in the presence of chemical cues of *M. albidus*. Mosquitoes within the *C. pipiens* 350 complex have been shown to prefer black dyed water when ovipositing, likely as a result of 351 greater perceived depth or a higher nutritional load for their progeny (Ortiz Perea and 352 Callaghan, 2017). Here, when both predator and control treatments were dyed, C. 353 354 quinquefasciatus continued to avoid M. albidus in pairwise tests. However, when the control treatment was undyed and predator-free, C. quinquefasciatus demonstrated clear preference 355 for dyed, predator-treated water. These results indicate interactive complexity, whereby the 356 357 preference towards dyed habitats overrides the avoidance stimulus relating to physical

predator presence. Thus, the use of dye in synergy with predator inoculations may fostermore effective biocontrol potential in certain environments.

360 In conclusion, the use of FR demonstrates strong predatory impact and potential for the application of cyclopoid copepods in biocontrol strategies, particularly in light of Type II 361 forms conducive to population destabilising effects. We illustrate that this overarching FR 362 363 form is robust to interspecific variations and habitat complexities, however, variations in 364 underlying FR parameters are apparent among cyclopoid species and habitat complexities. The integration of reproductive effort estimations allows the better resolution of differential 365 RCP between the species, favouring the use of *M. albidus* over other comparators overall. 366 These results corroborate generally with the success of cyclopoid copepods in mosquito 367 368 control during field trials, whereby large-scale eradication has been achieved (Kay and Nam, 2005; Nam et al. 2012), and the particular interest in the use of *M. albidus* in field-based trials 369 370 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 concerns relating to predator-prey interactions (Preisser and Bolnick, 2008; Vonesh and 372 Blaustein, 2010). Here, we demonstrate context-dependent avoidance behaviour of C. 373 quinquefasciatus in response to copepod predators, yet highlight that the synergistic use of 374 dye may bolster the efficacy of biocontrol releases due to favoured oviposition in these 375 376 conditions. Additional field-based research to elucidate the effects of dye on community structure and predatory impact is thus warranted. 377

378

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384	
385	References
386	Alexander, M.E., Dick, J.T.A., O'Connor, N.E., Haddaway, N.R., Farnsworth, K.D.,
387	2012. Functional responses of the intertidal amphipod Echinogammarus marinus: effects of
388	prey supply, model selection and habitat complexity. Mar. Ecol. Prog. Ser. 468, 191-202.
389	http://dx.doi.org/10.3354/meps09978.
390	
391	Alexander, M.E., Dick, J.T.A., O'Connor, N.E., 2013. Trait-mediated indirect
392	interactions in a marine intertidal system as quantified by functional responses. Oikos
393	122(11), 1521–1531. <u>http://dx.doi.org/10.1111/j.1600-0706.2013.00472.x</u> .
394	
395	Alexander, M.E., Kaiser, H., Weyl, O.L.F., Dick, J.T.A., 2015. Habitat simplification
396	increases the impact of a freshwater invasive fish. Environ. Biol. Fish. 98(2), 477-486.
397	http://dx.doi.org/10.1007/s10641-014-0278-z.
398	
399	Benelli, G., Mehlhorn, H., 2016. Declining malaria, rising of dengue and Zika virus:
400	insights for mosquito vector control. Parasitol. Res. 115(5), 1747-1754.
401	http://dx.doi.org/10.1007/s00436-016-4971-z.
402	
403	Barrios-O'Neill, D., Dick, J.T.A., Emmerson, M.C., Ricciardi, A., MacIsaac, H.J.,
404	2015. Predator-free space, functional responses and biological invasions. Funct. Ecol. 29(3),
405	377–384. <u>http://dx.doi.org/10.1111/1365-2435.12347</u> .

407	Bohart, R.M., Washino, R.K., 1978. Mosquitoes of California. University of
408	California Press, Berkeley.
409	
410	Bolker, B.M., 2008. Ecological Models and Data in R. Princeton University Press,
411	Princeton.
412	
413	Blaustein, L., Chase, J.M., 2007. Interactions between mosquito larvae and species
414	that share the same trophic level. Annu. Rev. Entomol. 52, 489–507.
415	https://doi.org/10.1146/annurev.ento.52.110405.091431.
416	
417	Calliari, D., Sanz, K., Martínez, M., Cervetto, G., Gómez, M., Basso, C., 2003.
418	Comparison of the predation rate of freshwater cyclopoid copepod species on larvae of the
419	mosquito Culex pipiens. Med. Vet. Entomol. 17, 339-342.
420	
421	Cunze, S., Koch, L.K., Kochmann, J., Klimpel, S., 2016. Aedes albopictus and Aedes
422	japonicus - two invasive mosquito species with different temperature niches in Europe.
423	Parasit. Vectors 9(1), 573. <u>http://dx.doi.org/10.1186/s13071-016-1853-2</u> .
424	
425	Cuthbert, R.N., Dick, J.T.A., Callaghan, A., Dickey, J.W.E., 2018. Biological control
426	agent selection under environmental change using functional responses, abundances and
427	fecundities; the Relative Control Potential (RCP) metric. Biol. Control 121, 50-57.
428	https://doi.org/10.1016/j.biocontrol.2018.02.008.
429	
430	Dick, J.T.A., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J.,

431 Robinson, T.B., Kumschick, S., Weyl, O.L.F., Dunn, A.M., Hatcher, M.J., Paterson, R.A.,

432	Farnsworth, K.D., Richardson, D.M., 2014. Advancing impact prediction and hypothesis
433	testing in invasion ecology using a comparative functional response approach. Biol. Invasions
434	16(4), 735–753. <u>http://dx.doi.org/10.1007/s10530-013-0550-8</u> .
435	
436	Dick, J.T.A., Laverty, C., Lennon, J.J., Barrios-O'Neill., D., Mensink, P.J., Britton,
437	R., Médoc, V., Boets, P., Alexander, M.E., Taylor, N.G., Dunn, A.M., Hatcher, M.J.,
438	Rosewarne, P.J., Crookes, S., MacIsaac, H.J., Xu, M., Ricciardi, A., Wasserman, R.J.,
439	Ellender, B.R., Weyl, O.L.F., Lucy, F.E., Banks, P.B., Dodd, J.A., MacNeil, C., Penk, M.R.,
440	Aldridge, D.C., Caffrey, J.M., 2017. Invader Relative Impact Potential: a new metric to
441	understand and predict the ecological impacts of existing, emerging and future invasive alien
442	species. J. Appl. Ecol. 54(4), 1259–1267. http://dx.doi.org/10.1111/1365-2664.12849.
443	
444	Fischer, S., Zanotti, G., Castro, A., Quiroga, L., Vargas, D.V., 2011. Effect of habitat
445	complexity on the predation of Buenoa fuscipennis (Heteroptera: Notonectidae) on mosquito
446	immature stages and alternative prey. J. Vector Ecol. 38(2), 215-223.
447	https://doi.org/10.1111/j.1948-7134.2013.12033.x.
448	
449	Holling, C.S., 1959. Some characteristics of simple types of predation and parasitism.
450	Can. Entomol. 91(7), 385–398. http://dx.doi.org/10.4039/Ent91385-7.
451	
452	Holling, C. S., 1966. The functional response of invertebrate predators to prey
453	density. Mem. Entomol. Soc. Can. 48: 1-86. https://doi.org/10.4039/entm9848fv.
454	
455	Juliano, S.A., 2001. Non-linear curve fitting: predation and functional response
456	curves, in: Scheiner, S.M., Gurevitch, J. (Eds.), Design and Analysis of Ecological

457	Experiments. Oxford University Press, Oxford, pp. 178–196.
458	http://dx.doi.org/10.4319/lo.1995.40.8.1533.
459	
460	Juliano, S.A., 2007. Population dynamics, in: T.G. Floore (Ed.). Biorational Control
461	of Mosquitoes. J. Am. Mosq. Contr. Assoc. 23 (Suppl. 2), pp. 93-109.
462	
463	Juliano, S.A., 2010. Coexistence, exclusion, or neutrality? A meta-analysis of
464	competition between Aedes albopictus and resident mosquitoes. Israel J. Ecol. Evol. 56(3-4),
465	325-351. https://doi.org/10.1560/ijee.55.3-4.325.
466	
467	Kay, B.H., Nam, V.S., 2005. New strategy against Aedes aegypti in Vietnam. Lancet
468	365(9459), 613–617. <u>http://dx.doi.org/10.1016/S0140-6736(05)17913-6</u> .
469	
470	Kramer, W.L., Mulla, M.I.R.S., 1979. Oviposition attractants and repellents of
471	mosquitoes: oviposition responses of <i>Culex</i> mosquitoes to organic infusions. Environ.
472	Entomol. 8(6), 1111–1117. https://doi.org/10.1093/ee/8.6.1111.
473	
474	Laverty, C., Brenner, D., McIlwaine, C., Lennon, J.J., Dick, J.T.A., Lucy, F.E.,
475	Christian, K.A., 2017a. Temperature rise and parasitic infection interact to increase the
476	impact of an invasive species. Int. J. Parasitol. 47(5), 291–296.
477	http://dx.doi.org/10.1016/j.ijpara.2016.12.004.
478	
479	Laverty, C., Green, K.D., Dick, J.T.A., Barrios-O'Neill, D., Mensink, P.J., Médoc, V.,
480	Spataro, T., Caffrey, J.M., Lucy, F.E., Boets, P., Britton, J.R., Pegg, J., Callagher, C.,

481	2017b. Assessing the ecological impacts of invasive species based on their functional
482	responses and abundances. Biol. Invasions 19(5), 1653–1665.
483	http://dx.doi.org/10.1007/s10530-017-1378-4.
484	
485	Laybourn-Parry, J., Abdullahi, B.A., Tinson, S.V., 1988. Temperature-dependent energy
486	partitioning in the benthic copepods Acanthocyclops viridis and Macrocyclops albidus. Can.
487	J. Zool. 66(12), 2709–2713. <u>https://doi.org/10.1139/z88-397</u> .
488	
100	Maine C. 1004 Datterna afflife bistore are seen and a structure branches of a second set of a
489	Maler, G., 1994. Patterns of life history among cyclopold copepods of central Europe.
490	Freshw. Biol. 31(1), 77-86. https://doi.org/10.1111/j.1365-2427.1994.tb00840.x.
491	
492	Manimegalai, K., Sukanya, S., 2014. Biology of the filarial vector, Culex
493	quinquefasciatus (Diptera:Culicidae). Int. J. Curr. Microbiol. Appl. Sci. 3(4), 718-24.
494	
495	Marten, G.G., 1989. A survey of cyclopoid copepods for control of Aedes albopictus
496	larvae. Bull. Soc. Vector Ecol. 14(2), 232–236.
497	
498	Marten, G.G., 1990a. Elimination of Aedes albopictus from tire piles by introducing
499	Macrocyclops albidus (Copepoda, Cyclopidae). J. Am. Mosq. Control Assoc. 6(4), 689-693.
500	
501	Marten G.G., 1990b. Issues in the development of cyclops for mosquito control.
502	Arbovirus Res. Aust. 5, 159–164.
503	
504	Marten, G.G., Reid, J.W., 2007. Cyclopoid copepods. J. Am. Mosq. Control Assoc.
505	23(2), 65–92. http://dx.doi.org/10.2987/8756-971X(2007)23%5B65:CC%5D2.0.CO;2.

506	
507	Medlock, J.M., Leach, S.A., 2015. Effect of climate change on vector-borne disease
508	risk in the UK. Lancet Infect. Dis. 15(6), 721–730. <u>http://dx.doi.org/10.1016/S1473-</u>
509	<u>3099(15)70091-5</u> .
510	
511	Medlock, J.M., Vaux, A.G.C., Cull, B., Schäffner, F., Gillingham, E., Pfluger, V.,
512	Leach, S.A., 2017. Detection of the invasive mosquito species Aedes albopictus in southern
513	England. Lancet Infect. Dis. 17(2), 140. http://dx.doi.org/10.1016/S1473-3099(17)30024-5.
514	
515	Meyer, R.P., Durso, S.L. 1993. Identification of the mosquitoes of California. Mosq.
516	Vector Contr. Assoc. Cali., Sacramento, USA.
517	
518	Nam, V.S., Yen, N.T., Duc, H.M., Tu, T.C., Thang, V.T., Le, N.H., Le Loan, L.,
519	Huong, V.T.Q., Khanh, L.H.K., Trang, H.T.T., Lam, L.Z.Y., Kutcher, S.C., Aaskov, J.G.,
520	Jeffery, J.A.L., Ryan, P.A., Kay, B.H., 2012. Community-based control of Aedes aegypti by
521	using Mesocyclops in Southern Vietnam. Am. J. Trop. Med. Hyg. 86(5), 850-859.
522	http://dx.doi.org/10.4269/ajtmh.2012.11-0466.
523	
524	O'Neil, R.J., 1990. Functional response of arthropod predators and its role in the
525	biological control of insect pests in agricultural systems, in: Dunn, P.E., Baker, R.R. (Eds.)
526	New directions in biological control: alternatives for suppressing agricultural pests and
527	diseases. Alan R. Liss, Inc., New York, pp. 83–96.
528	
529	Ortiz Perea, N., Callaghan, A., 2017. Pond dyes are Culex mosquito oviposition
530	attractants. PeerJ 5, e3361. http://dx.doi.org/10.7717/peerj.3361.

531	
532	Peacor, S.D., Werner, E.E., 2001. The contribution of trait-mediated indirect effects to
533	the net effects of a predator. Proc. Natl. Acad. Sci. USA 98, 3904–3908.
534	https://doi.org/10.1073/pnas.071061998.
535	
536	Preisser, E.L., Bolnick, D.I., 2008. The many faces of fear: comparing the pathways
537	and impacts of nonconsumptive predator effects on prey populations. PLoS ONE 3(6), e2465.
538	https://doi.org/10.1371/journal.pone.0002465.
539	
540	R Core Team, 2017. R: A language and environment for statistical computing. R
541	Foundation for Statistical Computing, Vienna.
542	
543	Rey, J.R., O'Connell, S., Suárez, S., Menéndez, Z., Lounibos, L.P., Byer, G. 2004.
544	Laboratory and field studies of Macrocyclops albidus (Crustacea: Copepoda) for biological
545	control of mosquitoes in artificial containers in a subtropical environment. J. Vector Ecol. 29
546	(1), 124–134.
547	
548	Samy, A.M., Elaagip, A.H., Kenawy, M.A., Ayres, C.F.J., Townsend Peterson, A.,
549	Soliman, D.A., 2016. Climate change influences on the global potential distribution of the
550	mosquito Culex quinquefasciatus, vector of West Nile virus and lymphatic filariasis. PLOS
551	One 11(10), e0163863. https://doi.org/10.1371/journal.pone.0163863.
552	

553	Schäffner, F., Medlock, J.M., Van Bortel W., 2013. Public health significance of
554	invasive mosquitoes in Europe. Clin. Microbiol. Infect. 19(8), 685-692.
555	https://doi.org/10.1111/1469-0691.12189.
556	
557	Sih, A., 1986. Antipredator responses and the perception of danger by mosquito
558	larvae. Ecol. 67(2), 434. <u>https://doi.org/10.2307/1938587</u> .
559	
560	Silberbush, A., Blaustein, L., 2011. Mosquito females quantify risk of predation to
561	their progeny when selecting an oviposition site. Funct. Ecol. 25(5), 1091–1095.
562	https://doi.org/10.1111/j.1365-2435.2011.01873.x.
563	
564	Silberbush, A., Tsurim, I., Margalith, Y., Blaustein, L., 2014. Interactive effects of
565	salinity and a predator on mosquito oviposition and larval performance. Popul. Ecol. 175(2),
566	565–575. <u>https://doi.org/10.1007/s00442-014-2930-x</u> .
567	
568	South, J., Dick, J.T.A., McCard, M., Barrios-O'Neill, D., Anton, A., 2017. Predicting
569	predatory impact of juvenile invasive lionfish (Pterois volitans) on a crustacean prey using
570	functional response analysis: effects of temperature, habitat complexity and light regimes.
571	Environ. Biol. Fish. 100(10), 1155–1165. http://dx.doi.org/10.1007/s10641-017-0633-y.
572	
573	Townroe, S., Callaghan, A., 2014. British container breeding mosquitoes: The impact
574	of urbanisation and climate change on community composition and phenology. PLoS ONE,
575	9(4), e95325. <u>https://doi.org/10.1371/journal.pone.0095325</u> .
576	

577	Torres-Estrada, J., Rodriguez, M.H., Cruz-Lopez, L., Arredondo- Jimenez, J.I., 2001.
578	Selective oviposition by Aedes aegypti (Diptera: Culicidae) in response to Mesocyclops
579	longisetus (Copepoda: Cyclopoida) under laboratory and field conditions. J. Med. Entomol.
580	38(2), 188–192. <u>https://doi.org/10.1603/0022-2585-38.2.188</u> .
581	
582	Tranchida, M.C., Micieli, M.V., Maciá, A., García, J.J., 2009. Native Argentinean
583	cyclopoids (Crustacea: Copepoda) as predators of Aedes aegypti and Culex pipiens (Diptera:
584	Culicidae) mosquitoes. Rev. Biol. Trop. 57(4), 1059–1068.
585	
586	Trussell, G.C., Ewanchuk, P.J., Bertness, M.D., Silliman, B.R., 2004. Trophic
587	cascades in rocky shore tide pools: distinguishing lethal and nonlethal effects. Oecologia
588	139(3), 427–432. https://doi.org/10.1007/s00442-004-1512-8.
589	
590	Trussell, G.C., Ewanchuk, P.J., Matassa, C.M., 2008. Resource identity modifies the
591	influence of predation risk on ecosystem function. Ecol. 89(10), 2798–2807.
592	https://doi.org/10.1890/08-0250.1.
593	
594	Turell, M.J., 2012. Members of the Culex pipiens complex as vectors of viruses. J.
595	Am. Mosq. Control Assoc. 28(4), 123-6. https://doi.org/10.2987/8756-971x-28.4.123.
596	
597	Van Dam, A.R., Walton, W.E., 2008. The effect of predatory fish exudates on the
598	ovipostional behaviour of three mosquito species: Culex quinquefasciatus, Aedes aegypti and
599	Culex tarsalis. Med. Vet. Entomol. 22(4), 399-404. https://doi.org/10.1111/j.1365-
600	<u>2915.2008.00764.x</u> .

602	Van Driesche, R., Bellows, T.S., 2011. Biological Control. Springer, Berlin.
603	https://doi.org/10.1007/978-1-4613-1157-7.
604	
605	Veronesi, R., Carrieri, M., Maccagnani, B., Maini, S., Bellini, R., 2015. Macrocyclops
606	albidus (Copepoda: Cyclopidae) for the biocontrol of Aedes albopictus and Culex pipiens in
607	Italy. J. Am. Mosq. Control Assoc. 31(1), 32–43. https://doi.org/10.2987/13-6381.1.
608	
609	Vonesh, J.R., Blaustein, L., 2010. Implications of predator-induced shifts in mosquito
610	oviposition site selection for vector control: a meta-analysis. Israel J. Ecol. Evol. 56(3-4),
611	263-279. https://doi.org/10.1560/ijee.56.3-4.263.
612	
613	Wachira, S.W., Ndung'u, M., Njagi, P.G., Hassanali, A., 2010. Comparative
614	responses of ovipositing Anopheles gambiae and Culex quinquefasciatus females to the
615	presence of <i>Culex</i> egg rafts and larvae. Med. Vet. Entomol. 24(4), 369–374.
616	
617	Wasserman, R.J., Alexander, M.E., Weyl, O.L.F., Barrios-O'Neill, D., Froneman,
618	P.W., Dalu, T., 2016. Emergent effects of habitat complexity and temperature on predator-
619	prey interactions. Ecosphere, 7(2), e01239. http://dx.doi.org/10.1002/ecs2.1239.
620	
621	World Health Organisation, 2017. Vector-borne diseases fact sheet.
622	http://www.who.int/mediacentre/factsheets/fs387/en/ (accessed 01 December 2017).
623	
624	Yee, D.A., 2008. Tires as habitats for mosquitoes: A review of studies within the
625	eastern United States. J. Med. Entomol. 45(4), 581–593S. https://doi.org/10.1603/0022-
626	2585(2008)45[581:tahfma]2.0.co;2.

627	
628	Yee, D.A., 2016. Thirty years of Aedes albopictus (Diptera: Culicidae) in America:
629	An introduction to current perspectives and future challenges. J. Med. Entomol. 53(5), 989-
630	991. <u>https://doi.org/10.1093/jme/tjw063</u> .
631	
632	Zuharah, W.F., Lester, P.J., 2010. Can adults of the New Zealand mosquito Culex
633	pervigilans (Bergorth) detect the presence of a key predator in larval habitats? J. Vector Ecol.
634	35, 100–105. <u>https://doi.org/10.1111/j.1948-7134.2010.00065.x</u> .
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### 648 Tables

649 Table 1

Predator	First order	р	h	р	а	р
	term					
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

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

665	Table	3

	Habitat	First order	р	h	р	а	р
	complexity	term					
	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**

Table 1. First order terms resulting from logistic regression (proportion of prey killed versus
prey density) for each predator species in Experiment 1, alongside functional response
parameter estimates (*h*, handling time; *a*, attack rate) resulting from Rogers' random predator
equation.

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

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

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

denote significant levels of certainty that the RCP score is greater than 1 (\* > 95%, \*\* > 99%,
\*\*\* > 99.9%).

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

prey density) for each level of habitat complexity in Experiment 2, alongside functional
response parameter estimates (*h*, handling time; *a*, attack rate) resulting from Rogers' random
predator equation.

Figure 1. Illustration of low and high habitat complexity treatment arenas used in Experiment2.

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  $\pm$  SE (n = 3 per

698 density).

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

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

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

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

high habitat complexity over a 24 hour experimental period. Means are  $\pm$  SE (n = 3 per density).

Figure 6. Bootstrapped functional response parameters (handling time, a; maximum feeding rate, b; attack rate, c) of *M. albidus* towards *C. quinquefasciatus* under low and high habitat complexity. Means are  $\pm$  SE (n = 30 per parameter).

Figure 7. Oviposition activity index values (OAI,  $\pm$  SE) shown by *C. quinquefasciatus* 

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

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

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

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

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

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

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

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

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

721 0.01, \*\*\* < 0.001, NS  $\geq$  0.05).

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b)

2.4

38

C)



a)

30-

0.06

