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

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ABSTRACT

The use of predatory biological control agents can form an effective component in the management of vectors of parasitic diseases and arboviruses. However, we require predictive methods to assess the efficacies of potential biocontrol agents under relevant environmental contexts. Here, we applied functional responses (FRs) and reproductive effort as a proxy of numerical responses (NRs) to compare the Relative Control Potential (RCP) of three cyclopoid copepods, *Macrocyclops albidus*, *M. fuscus* and *Megacyclops viridis* towards larvae of the mosquito *Culex quinquefasciatus*. The effects of habitat complexity on such predatory impacts were examined, as well as ovipositional responses of *C. quinquefasciatus* to copepod cues in pairwise choice tests. All three copepod species demonstrated a 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 integration of reproductive effort estimations in the new RCP metric identifies *M. albidus* as 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 effects are robust to habitat variations; however, attack rates of *M. albidus* were reduced in the presence of such complexity. *C. quinquefasciatus* avoided ovipositing where *M. albidus* was physically present, however it did not avoid chemical cues alone. *C. quinquefasciatus* 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-treated water, positive selectivity towards the treatment water was stimulated. We thus demonstrate the marked predatory potential of cyclopoid copepods, utilising our new RCP metric, and advocate their feasibility in biological control programmes targeting container-style habitats. We also show that behavioural responses of target organisms and environmental context should be considered to maximise agent efficacy.
Keywords: Functional response; Numerical response; Relative Control Potential; Copepod; Mosquito; Oviposition

1. Introduction

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

* Culex quinquefasciatus* Say, 1823 is a member of the *C. pipiens* complex, which are 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 mosquito breeds rapidly in artificial, urban environments, enabling a high potential for human 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 may additionally bolster the potential of *C. quinquefasciatus* to invade novel ecosystems through range expansion (Samy et al. 2016). Cyclopoid copepods have had marked success in...
the biological control of *Aedes aegypti* mosquito larvae (e.g. Kay and Nam, 2005), and predatory impacts have been demonstrated towards *Culex* spp. (e.g. Calliari et al. 2003; Tranchida et al. 2009). Furthermore, the global distribution, high fecundity, environmental hardiness, voraciousness and ease of mass-production of copepods enable high levels of potential impact on target populations, whilst negating the need for potentially ecologically harmful species introductions (Marten and Reid, 2007). Yet, while interspecific variations in control efficiencies between cyclopoids have been demonstrated (Marten, 1989), as well as demographic variations across differing species strains (Marten, 1990b), many candidate copepod species are yet to be examined in the context of mosquito control.

Functional responses (FRs), which discern the *per capta* intake rate of a resource as a 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, capture and handling components of FRs can be highly informative in the contexts of biological control (O’Neil, 1990; Van Driessche and Bellows, 2011) and invasive species (Dick et al. 2014). Indeed, high impact invasive species are strongly associated with higher maximum feeding rates driven by low handling times (Dick et al. 2017), and this associates strongly with measurable changes in affected populations (i.e. ecological impact). Functional response form and magnitude can also be significant with respect to prey population stability; a Type II FR is deemed to have potentially population destabilising effects due to high levels of proportional predation at low prey densities, whilst a Type III FR may impart stability to prey by providing refugia at low prey densities (Holling, 1959). Moreover, coupling such *per 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). These approaches thus yield high potential in the comparison of efficacies of potential 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).

Besides direct consumptive effects, trait-mediated indirect interactions (TMIIs), including behavioural responses of target organisms to predators, can exert strong effects on predator-prey dynamics (e.g. Alexander et al. 2013), and can be as strong or stronger in their population effects (Peacor and Werner 2001; Trussell et al. 2004; 2008). Behavioural 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 selectivity among potential habitats by mosquitoes is of integral importance to biological 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 individuals that are able to actively evade predation through avoidance of oviposition habitats containing predators (Sih, 1986; Blaustein and Chase, 2007). Water colouration, serving as a proxy for nutritional load, has also been demonstrated to significantly attract oviposition by Culex mosquitoes (Ortiz Perea and Callaghan, 2017). The effects of the presence of copepods on oviposition of mosquitoes has been seldom considered, however, Torres-Estrada et al. (2001) found that A. aegypti preferentially oviposited in water treated with the copepod 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 Blaustein, 2010).
Here, we quantify the FRs of three locally-abundant and widespread cyclopoid copepods, *Macrocyclops albidus* (Jurine 1820), *Macrocyclops fuscus* (Jurine, 1820) and *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 reproductive effort data from Maier (1994) to compare the Relative Control Potential (RCP; Cuthbert et al. 2018) among the three species. Benthic habitat complexity is integrated in a separate experiment to assess potential context-dependencies relevant to the efficacy of copepods in their predation of *C. quinquefasciatus*. Furthermore, we utilise pairwise choice 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 whether the integration of pond dye reduces avoidance behaviour by the mosquito, hence enhancing biocontrol efforts. Thus, we seek to decipher the most effective biocontrol agent using the RCP metric, and consider the effects of habitat complexity and target prey behavioural responses on agent efficacies.

### 2. Methods

#### 2.1. Experimental organisms

The predators, *Macrocyclops albidus*, *M. fuscus* and *Megacyclops viridis*, were collected from Glastry Clay Pit Ponds, Northern Ireland (54°29′18.5″N; 5°28′19.9″W) during January 2017 using a polypropylene dipper, transported to Queen’s Marine Laboratory, Portaferry in source water, and maintained at 25 ± 2 °C under a 16:8 light:dark regime. Cultures were initiated separately for each species by placing a single ovigerous adult female into 250 mL dechlorinated tap water, with the protists *Paramecium caudatum* and *Chilomonas paramecium* supplied *ad libitum*. Emerging nauplii were transferred into larger...
10 L tanks and fed on the same protist diet, while autoclaved wheat seeds were used to sustain the protists.

The prey, *Culex quinquefasciatus*, were obtained from a laboratory colony maintained at the same conditions as the predators. *Culex quinquefasciatus* were originally collected in Cyprus in 2005 by Dr A. Callaghan and had been reared in laboratory conditions at the University of Reading since then. Adults were maintained in 32.5 cm³ cages (Bugdorm, Watkins and Doncaster, Leominster, England). Defibrinated horse blood (TCS Biosciences, Buckingham, England) was fed to adults three times per week using an artificial membrane feeding system (Hemotek Ltd., Accrington, England). Cotton pads soaked in 10% sucrose solution were provided for other sustenance. A black oviposition cup containing 200 mL tap water was kept in each cage, with egg rafts removed three times per week and placed into 3 L larval bowls containing tap water and ground guinea pig pellets (Pets at Home, Newtownabbey, Northern Ireland) provided *ad libitum* until mosquito pupation.

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

Adult, non-ovigerous female *M. albidus* (1.6 – 1.8 mm body length), *M. fuscus* (1.9 – 2.1 mm body length) and *M. viridis* (2.0 – 2.3 mm body length) were selected for experiments to standardise predators as cyclopoids are sexually dimorphic (Laybourn-Parry et al. 1988). Predators were starved for 24 hours to standardise hunger levels. Prey, first instar *C. quinquefasciatus* (1.1- 1.3 mm), were provided to copepods at six densities (2, 4, 8, 15, 30, 60, *n* = 3 per density) in 20 mL arenas of 42 mm diameter, and allowed to settle for three hours prior to the addition of individual predators. Predators were allowed to feed for 24 hours, after which they were removed and remaining live prey counted. Controls consisted of 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 calculate RCP among the three predators (see below).

2.3. Experiment 2: Habitat complexity

Adult female *M. albicus* (1.6 – 1.8 mm body length) were selected for experiments and starved for 24 hours to standardise hunger levels. Prey, first instar *C. quinquefasciatus* (1.1- 1.3 mm), were provided at the same densities (n = 3 per experimental group) and in the same arenas as Experiment 1, and also allowed the same acclimation. However, arenas 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 mm height), arranged in a uniform array (Figure 1) and positioned using non-toxic mounting 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 and complexity level without predators.

2.4. Experiment 3: Ovipositional responses

We assessed ovipositional preferences of *C. quinquefasciatus* with pairwise choice tests. Recently emerged female *C. quinquefasciatus* were blood fed for the first time over a 24 hour period. Following this, each experimental replicate consisted of cages (32.5 cm$^3$) containing 20 blood-fed females and a 10% sucrose solution, refilled *ad libitum*. Mosquitoes were maintained in these cages for 144 hours in bioassay 1, and 168 hours in following bioassays, at 25 °C ± 2 °C and in a 16:8 light:dark regime. In all bioassays, control and 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. albicus* were 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
cages \((n = 7\) per experimental group). In bioassay 2, three copepods were removed from the treatment cups immediately before being added to cages \((n = 13\) per experimental group). In 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 added to arenas containing black liquid pond dye \((0.3\ \text{g L}^{-1},\ \text{Dyofix, Leeds, United Kingdom,}\ n = 7\) per experimental group). Controls lacked predators, and those in bioassays 1-3 and 5 consisted of dechlorinated tap water, whilst controls in bioassay 4 consisted of dyed dechlorinated tap water. Paired cups were added to opposing corners within cages in a randomised design and mosquitoes given 48 hours to oviposit. Following this, cups were removed and the numbers of egg rafts counted.

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):

\[ N_e = N_0(1 - \exp(a(N_e h - T))) \]

\[(1)\]

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 numerical response:

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

(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:

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

(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 show preference for controls.

3. Results

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 2. When maximum feeding rates are used in the RCP metric (Figure 4a), *M. albidus* exhibits greater efficacy in controlling *C. quinquefasciatus* than both *M. fuscus* (RCP = 2.73) and *M. viridis* (RCP = 1.33). *M. viridis* exhibits a greater efficacy compared to *M. fuscus* (RCP = 2.13). With attack rates incorporated into the RCP metric (Figure 4b), *M. albidus* demonstrates similar efficacy predictions to *M. fuscus* (RCP = 0.96), and greater efficacy than *M. viridis* (RCP = 1.42). *M. viridis* exhibits a lower efficacy than *M. fuscus* here (RCP = 0.71). These calculations are bolstered graphically in Figure 4 using RCP biplots, with *M. albidus* showing greatest efficacy overall when maximum feeding rates are integrated, and similarities in efficacies projected between these predators when attack rates are applied.

3.2. Experiment 2: Habitat complexity

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

3.3. Experiment 3: Ovipositional responses

In bioassay 1, significantly fewer egg rafts were laid in cups containing copepods in comparison to controls ($W = 45, p = 0.01$). However, in bioassays 2 and 3, no significant differences in oviposition levels were detected between the chemical treatments with 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 dyed water containing predatory copepods compared to dyed water alone ($t = 5.35, df = 6, p = 0.002$). In 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 OAI scores for each of the five treatments graphically.

4. Discussion

The development and application of control strategies to combat mosquito-borne disease is of immense public health importance (Benelli and Mehlhorn, 2016), particularly in light of changes to population dynamics being driven by globalisation, urbanisation and 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 using predatory agents can be an effective tool to assist or augment control strategies targeting important disease vector species (e.g. Nam et al. 2012). In this context, the coupled use of functional and numerical responses (or their proxies) provides a robust comparative framework to decipher and project the potential efficacy in controlling target organisms by agents (see Dick et al. 2017; Cuthbert et al. 2018). Consumer-resource interactions are,
however, subject to a range of context-dependencies which may modulate interaction strengths, such as habitat complexity (Barrios-O’Neill et al. 2015; Alexander et al. 2015), 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 critical to consider during biocontrol agent evaluations. Furthermore, elucidating behavioural responses, such as those relating to ovipositional choice, of target organisms is imperative to derive a holistic view of agent efficacy, particularly in ecological systems where organisms are not confined to a single habitat patch, such as is often the case with container-breeding mosquitoes in urban and peri-urban environments (Silberbush and Blaustein, 2011; Townroe and Callaghan, 2014).

Here, we applied functional responses (FRs) to assess the potential predatory impact of three native cyclopoid copepods towards *C. quinquefasciatus*, a container-breeding mosquito and capable vector of diseases such as West Nile virus and lymphatic filariasis (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 ovipositional responses of *C. quinquefasciatus* to predatory cues. The focal predators, *M. albidus*, *M. fuscus* and *M. viridis* all exhibited potentially population destabilising Type II FRs towards first instar *C. quinquefasciatus*. This characteristic encompasses high levels of killing at low prey densities, limiting conditions for prey refugia (Holling, 1959) and, concurrently, avoids the compensatory effect of reduced competitive interactions in surviving mosquito prey (Juliano, 2007). This destabilising FR may thus reduce the likelihood of size refuge attainment in prey, given that cyclopoid copepods only effectively consume early instar mosquito larvae (Marten and Reid, 2007). *Macrocylops albidus* exhibited the highest magnitude FR of the predators, characterised by comparatively low handling times and thus 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 during previous trials for the control of arbovirus vectors (e.g. Marten, 1990a; Veronesi et al. 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 the control of *C. quinquefasciatus* when maximum feeding rates are integrated, particularly given the comparatively low reproductive effort of *M. fuscus* (Maier, 1994). When attack 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 combined with numerical response proxies gave 100% association with degree of ecological impact. That is, the ‘oftake rate’ of prey, sometimes known as the ‘total response’, was the best predictor of consumer impact on resources (Dick et al. 2017; Laverty et al. 2017b). Also, the attack rate in experiments where prey are not replaced following consumption may be artificially constrained (Alexander et al. 2012). Thus, we propose that, while attack rates may give insight into predation at low prey densities, it is the maximum feeding rate combined with numerical response proxies that gives best RCP predictions.

Habitat complexity was found to modulate interactions between *M. albidus* and *C. quinquefasciatus* through a reduction in attack rates where complexity was present. However, the Type II FR exhibited was robust to variations in habitat complexity, and thus population destabilising effects towards disease vectors such as *C. quinquefasciatus* may be sustained under such conditions. Indeed, habitat structure has previously been demonstrated to affect survivability of mosquito prey to predatory copepods (e.g. Rey et al. 2004), however, density-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 of the observed FR (e.g. Alexander et al. 2012; but see Alexander et al. 2015). Furthermore, 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 Blaustein, 2010), yet interspecific variations with respect to coexistence patterns are commonplace (e.g. van Dam and Walton, 2008). Furthermore, interacting environmental factors and density-dependent effects may further modulate oviposition responses (Silberbush and Blaustein, 2011; Silberbush et al. 2014). Pairwise choice tests have been deemed more powerful and advocated over multiple-choice comparisons when examining responses to predatory cues (Silberbush and Blaustein, 2011). Avoidance behaviours have been deemed stronger for mosquito species which oviposit directly onto waterbodies, such as *Culex* spp. (Vonesh and Blaustein, 2010). Our results are indicative of predator avoidance behaviour of *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 with limited results which demonstrate positive selection by *A. aegypti* for sites treated with cyclopoid copepods (Torres-Estrada et al. 2001). We found no evidence for avoidance behaviour in the presence of chemical cues of *M. albidus*. Mosquitoes within the *C. pipiens* complex have been shown to prefer black dyed water when ovipositing, likely as a result of greater perceived depth or a higher nutritional load for their progeny (Ortiz Perea and Callaghan, 2017). Here, when both predator and control treatments were dyed, *C. quinquefasciatus* continued to avoid *M. albidus* in pairwise tests. However, when the control treatment was undyed and predator-free, *C. quinquefasciatus* demonstrated clear preference for dyed, predator-treated water. These results indicate interactive complexity, whereby the preference towards dyed habitats overrides the avoidance stimulus relating to physical
predator presence. Thus, the use of dye in synergy with predator inoculations may foster more effective biocontrol potential in certain environments.

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 forms conducive to population destabilising effects. We illustrate that this overarching FR form is robust to interspecific variations and habitat complexities, however, variations in underlying FR parameters are apparent among cyclopoid species and habitat complexities. The integration of reproductive effort estimations allows the better resolution of differential RCP between the species, favouring the use of *M. albidus* over other comparators overall. These results corroborate generally with the success of cyclopoid copepods in mosquito 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 which has been demonstrated (e.g. Marten, 1990a; Veronesi et al. 2015). Understanding the non-consumptive effects of predators is central to biocontrol applications and broader concerns relating to predator-prey interactions (Preisser and Bolnick, 2008; Vonesh and Blaustein, 2010). Here, we demonstrate context-dependent avoidance behaviour of *C. quinquefasciatus* in response to copepod predators, yet highlight that the synergistic use of dye may bolster the efficacy of biocontrol releases due to favoured oviposition in these conditions. Additional field-based research to elucidate the effects of dye on community structure and predatory impact is thus warranted.

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### Table 1

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TABLES

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<td>&lt; 0.001</td>
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<td>&lt; 0.001</td>
<td>2.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><em>M. fuscus</em></td>
<td>-0.05</td>
<td>&lt; 0.001</td>
<td>0.04</td>
<td>&lt; 0.001</td>
<td>3.85</td>
<td>&lt; 0.001</td>
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<tr>
<td><em>M. viridis</em></td>
<td>-0.02</td>
<td>&lt; 0.001</td>
<td>0.04</td>
<td>&lt; 0.001</td>
<td>1.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Agent A, agent B comparison</td>
<td>FR (± SD)</td>
<td>Parameter</td>
<td>Reproductive effort</td>
<td>RCP</td>
<td>CI (60%), $p_{RIP} &gt; 1$ (%)</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
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</tr>
<tr>
<td><em>M. albidus, M. fuscus</em></td>
<td>35.72 (± 7.15), 24.06</td>
<td>1/h</td>
<td>0.35, 0.20</td>
<td>2.73</td>
<td>2.03 – 3.36, 99.94***</td>
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<tr>
<td><em>M. albidus, M. viridis</em></td>
<td>35.72 (± 4.82), 26.30</td>
<td>1/h</td>
<td>0.35, 0.37</td>
<td>1.33</td>
<td>1.02 – 1.61, 82.13</td>
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<tr>
<td><em>M. viridis, M. fuscus</em></td>
<td>26.30 (± 4.82), 24.06</td>
<td>1/h</td>
<td>0.37, 0.20</td>
<td>2.13</td>
<td>1.60 – 2.60, 99.35**</td>
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<tr>
<td><em>M. albidus, M. fuscus</em></td>
<td>2.04 (± 0.36), 4.03</td>
<td>a</td>
<td>0.35, 0.37</td>
<td>0.96</td>
<td>0.68 – 1.21, 39.10</td>
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</tr>
<tr>
<td><em>M. albidus, M. viridis</em></td>
<td>0.36, 4.03</td>
<td></td>
<td>0.20</td>
<td>0.68</td>
<td>0.68 – 1.21, 39.10</td>
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<tr>
<td><em>M. viridis, M. fuscus</em></td>
<td>0.36, 1.42</td>
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<td>0.37</td>
<td>1.42</td>
<td>1.09 – 1.71, 87.82</td>
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<tr>
<td><em>M. viridis, M. fuscus</em></td>
<td>1.42 (± 0.29), 4.03</td>
<td>a</td>
<td>0.37, 0.71</td>
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<td>0.49 – 0.90, 12.63</td>
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<tr>
<td>Habitat complexity</td>
<td>First order term</td>
<td>$p$</td>
<td>$h$</td>
<td>$p$</td>
<td>$a$</td>
<td>$p$</td>
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<tr>
<td>Low</td>
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<td>0.04</td>
<td>&lt; 0.001</td>
<td>0.90</td>
<td>&lt; 0.001</td>
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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%).

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 Experiment 2.

Figure 2. Functional responses of *M. albidus* (a), *M. fuscus* (b) and *M. viridis* (c) towards *C. quinquefasciatus* prey over a 24 hour experimental period. Means are ± SE ($n = 3$ per density).

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*. Means are ± SE ($n = 30$ per parameter).
Figure 4. RCP biplot integrating mean bootstrapped maximum feeding rate (a) and attack rate (b) and reproductive effort (Maier, 1994) for each predator. Increasing efficacy is projected from bottom left to top right. Means are ± SE (n = 30 per FR 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 ± 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 ± SE (n = 30 per parameter).

Figure 7. Oviposition activity index values (OAI, ± 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, ** < 0.01, *** < 0.001, NS ≥ 0.05).