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1 Biological control agent selection under environmental change using  
2 functional responses, abundances and fecundities; the Relative  
3 Control Potential (RCP) metric

4  
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21 **ABSTRACT**

22 We currently lack the capacity to rapidly and reliably predict the efficacy of biological  
23 control agents due to inadequate consistency in derivations of functional and numerical  
24 responses and potential effects of context-dependencies. Here, we propose and apply a novel  
25 metric, Relative Control Potential (RCP), which combines the functional response (FR, *per*  
26 *capita* effect) with proxies for the numerical response (NR, agent population response) to  
27 compare agent efficacies, where  $RCP = FR \times \text{abundance}$  (or other proxies e.g. fecundity).  
28 The RCP metric is a comparative ratio between potential biocontrol agents, where values  $> 1$   
29 indicate higher relative control efficacy. Further, RCP can compare the efficacy of agents  
30 under environmental contexts, such as temperature change. We thus derived the RCP for two  
31 predatory cyclopoid copepods, *Macrocyclus albidus* (Cyclopoida: Cyclopidae) and  
32 *Megacyclus viridis* (Cyclopoida: Cyclopidae), towards larvae of the mosquito *Culex pipiens*  
33 (Diptera: Culicidae) under temperatures representative of current and future climate. Both  
34 copepods exhibited potentially population destabilising Type II FRs, with increasing  
35 temperatures inducing greater magnitude FRs through increased attack rates and decreased  
36 handling times. Attack rates by *M. albidus* were higher than *M. viridis*, yet handling times  
37 and maximum feeding rates were similar between the species across all temperatures. The  
38 inclusion of abundance data drives an elevated RCP of *M. albidus* and the integration of  
39 fecundity drives greater RCP of *M. albidus* at peak temperatures.  $Q_{10}$  values are indicative of  
40 increased feeding activity by both copepods with temperature increases, however relative  
41 feeding level increases of *M. viridis* slowed towards the peak temperature. We present RCP  
42 calculations and biplots that represent the comparative efficacies of the two biological control  
43 agents across temperatures. The Relative Control Potential (RCP) metric thus provides a new  
44 tool for practitioners to better assess the potential efficacy of biocontrol agents before their  
45 integration into management approaches for pests, vectors and invasive species.

46 Keywords: Functional response; Numerical response; Relative Control Potential; *Culex*  
47 *pipiens*; *Macrocyclus albidus*; *Megacyclus viridis*

48

## 49 **1. Introduction**

50 Biological control has been applied to manage pests, vectors and invasive species in a  
51 variety of ecological systems (O’Neil, 1990; Marten and Reid, 2007; Van Driesche and  
52 Bellows, 2011; Calvo et al. 2016). However, attempts to reveal agent efficacy through the  
53 coupling of functional and numerical responses (FRs, NRs) are limited in practice, reducing  
54 our predictive capacity for population-level effects (but see Heisswolf et al. 2009; Costa et al.  
55 2017). Further, natural systems are characterised by a number of abiotic and biotic context-  
56 dependencies that can alter species interaction strengths, including structural complexity  
57 (Barrios-O’Neill et al. 2015), temperature (Wasserman et al. 2016; South et al. 2017),  
58 dissolved oxygen (Lavery et al. 2015), parasitism (Bunke et al. 2015; Lavery et al. 2017a)  
59 and multiple/higher predators (Alexander et al. 2013; Barrios-O’Neill et al. 2014). Thus,  
60 rapid and reliable FR and NR derivations under context-dependencies are critical for the  
61 future of biocontrol strategies.

62 Effects associated with environmental change can be particularly profound in  
63 modulating natural systems. Indeed, climate change, coupled with urbanisation, is stimulating  
64 an unprecedented change in the population dynamics and status of mosquito vectors and their  
65 transmission of disease (Townroe and Callaghan, 2014; Medlock and Leach, 2015; Siraj et al.  
66 2017), with mosquito invasions increasing with the transportation of goods and humans (e.g.  
67 Yee, 2016; Medlock et al. 2017). The *Culex pipiens* (Diptera: Culicidae; Linnaeus, 1758)  
68 complex is widespread globally and act as one of the primary vectors of West Nile virus in  
69 the USA and continental Europe (Hubalek and Halouzka, 1999; Fonseca et al. 2004).

70 Freshwater cyclopoid copepods exhibit marked potential for the biological control of  
 71 mosquitoes (Marten and Reid, 2007; Baldacchino et al. 2017), and have been operationalised  
 72 in large-scale field applications (Kay and Nam, 2005). However, we require rapid assessment  
 73 of the relative biocontrol potential of such agents under changing climatic conditions. In this  
 74 study, we therefore present and apply a new metric, based on FRs and NRs, to compare the  
 75 efficacy of two native and widely-distributed copepods, *Macrocyclops albidus* (Cyclopoida:  
 76 Cyclopidae; Jurine, 1820) and *Megacyclops viridis* (Cyclopoida: Cyclopidae; Jurine, 1820)  
 77 under current and predicted temperature regimes.

78 Functional responses (FRs) quantify consumption under differing resource densities,  
 79 describing the key components of search, capture and handling time. Three broad FR types  
 80 have been described: the linear Type I, hyperbolic Type II and sigmoidal Type III (Solomon,  
 81 1949; Holling, 1959). Functional response form and magnitude are both powerful predictors  
 82 of the impacts of consumers on resource populations across taxonomic and trophic groups  
 83 (Dick et al. 2014; 2017). However, as FRs only assess *per capita* impacts, incorporation of  
 84 the Numerical Response (NR), that is the consumer population response, is also required to  
 85 discern the Total Response (TR) of consumers, whereby:

$$86 \qquad \qquad \qquad \text{TR} = \text{FR} \times \text{NR} \qquad \qquad \qquad (1)$$

88 In comparison to FRs, NRs are inherently more nebulous and difficult to quantify (Dick et al.  
 89 2017). Thus, simple consumer abundance (AB) has recently been proposed as a proxy for the  
 90 numerical response, giving the ‘Impact Potential’ (IP) metric, which has proved robust in  
 91 predicting ecological impact in the context of invasion biology (Dick et al. 2017; Laverly et  
 92 al. 2017b):

$$93 \qquad \qquad \qquad \text{IP} = \text{FR} \times \text{AB}$$

94 (2)

95 where FR is the maximum feeding rate (reciprocal of handling time) and AB is a measure of  
 96 consumer field abundance. In the context of biocontrol, we can express this as ‘Control  
 97 Potential’, CP:

$$98 \qquad \qquad \qquad CP = FR \times AB$$

99 (3)

100 where FR is the maximum feeding rate as above. However, in addition, we propose that  
 101 attack rate be used as a second measure of FR. The attack rate parameter describes the slope  
 102 of the FR curve at low prey densities and high attack rates can thus be particularly  
 103 destabilising to prey populations. CP as an absolute measure is, however, rather meaningless,  
 104 and needs a comparator. Thus, where two or more biocontrol agents require assessment as to  
 105 their relative potential efficacies, CP can become ‘Relative Control Potential’ (RCP):

$$106 \qquad \qquad \qquad RCP = \left( \frac{FR \text{ agent A}}{FR \text{ agent B}} \right) \times \left( \frac{AB \text{ agent A}}{AB \text{ agent B}} \right)$$

107 (4)

108 where RCP = 1, we predict no difference between biocontrol agents; for RCP < 1, we predict  
 109 agent A to have lesser efficacy than agent B; whereas when RCP > 1, agent A is predicted to  
 110 have greater efficacy than agent B. Further, increasing values above 1 indicate increasing  
 111 relative efficacy of agent A compared to agent B.

112 Furthermore, we propose the use of fecundity as a second proxy for NR, which  
 113 enables the incorporation into RCP of how quickly biocontrol agents can proliferate. Error  
 114 can also be incorporated into the RCP metric depending on data availability, using a  
 115 probability density function (pdf) to generate confidence intervals (CIs) and probabilities that  
 116 RCP > 1 or > 10 (see Dick et al. 2017). Moreover, as environmental gradients such as

117 temperature can have profound impacts on consumer-resource interactions (Englund et al.  
118 2011; Rall et al. 2012), RCP can be used to compare the efficacy of each biocontrol agent  
119 across environmental gradients. Here, we apply the RCP metric (Eqn. 4) to compare the  
120 biological control potentials of the copepods *M. albidus* and *M. viridis* towards the mosquito  
121 complex *C. pipiens* over a temperature gradient reflective of current and future UK climate  
122 change scenarios. We also apply the  $Q_{10}$  coefficient to further illustrate feeding activity  
123 responses of the two agents across temperatures (Bennett, 1990).

124

## 125 **2. Materials and methods**

### 126 *2.1. Animal collection and rearing*

127 *M. albidus* and *M. viridis* were collected at Glastry Clay Pit Ponds, Northern Ireland  
128 (54°29'18.5"N; 5°28'19.9"W) in January 2017 and kept in Queen's Marine Laboratory,  
129 Portaferry, N. Ireland, at  $25 \pm 2$  °C under a 16:8 light:dark regime and 50 – 60% relative  
130 humidity, since these conditions stimulated proliferation. Cultures were initiated using  
131 ovigerous females, placed individually into 250 mL cups with dechlorinated tap water and  
132 fed *ad libitum* with *Chilomonas paramecium* and *Paramecium caudatum* to obtain nauplii.  
133 Starter cultures of these protozoans were available commercially (Sciento, Manchester,  
134 England) and cultured under the same laboratory conditions in 2 L glass beakers using  
135 autoclaved wheat seeds, with *C. paramecium* providing nourishment for nauplii and early  
136 copepodids and *P. caudatum* for late copepodids and adults. Adult copepods were identified  
137 by Maria Holyńska, Museum and Institute of Zoology, Warsaw, Poland. Copepods were  
138 mass-reared in 10 L tanks and fed *ad libitum* on the protozoan diet. At maturity, copepods  
139 were maintained at  $12 \pm 2$  °C under a 12:12 light and dark regime and acclimatised for 7 d

140 prior to experimentation in 5 L holding arenas of 22 cm diameter fed *ad libitum* on the  
141 protozoan prey.

142 *Culex pipiens* were obtained from a laboratory colony established at the University of  
143 Reading, England several years prior, originating from field-collected mosquitoes at the  
144 Pirbright Institute, Surrey, England. The colony was sustained under the same conditions as  
145 the copepod cultures, at  $25 \pm 2$  °C in  $32.5 \times 32.5 \times 32.5$  cm cages (Bugdorm, Watkins and  
146 Doncaster, Leominster, England) and fed three times per week with defibrinated horse blood  
147 (TCS Biosciences, Buckingham, England) using a Hemotek® blood-feeding system  
148 (Hemotek Ltd., Accrington, England) and additionally provided with cotton pads soaked in a  
149 10% sucrose solution. Cages contained black cups filled with 200 mL dechlorinated tap water  
150 for oviposition. Egg rafts were extracted three times per week and placed into larval bowls  
151 containing 3 L dechlorinated tap water, and fed *ad libitum* with ground guinea pig pellets  
152 (Pets at Home, Newtownabbey, Northern Ireland) until pupation.

## 153 2.2. Experimental procedure

154 Non-ovigerous adult female *M. albidus* and *M. viridis* (1.6 – 1.8 mm and 2.0 – 2.3  
155 mm body length excluding caudal setae, respectively) were selected for experiments. We  
156 selected non-ovigerous females to standardise predators as cyclopoids are sexually dimorphic  
157 (Laybourn-Parry et al. 1988) and to eliminate cannibalism of hatching juveniles (Toscano et  
158 al. 2016). First instar *C. pipiens* larvae (1.1 – 1.3 mm) were used as prey. Functional response  
159 experiments were undertaken in transparent polypropylene cups (42 mm dia.) containing 20  
160 mL dechlorinated tap water from a continuously aerated source in a 12:12 light and dark  
161 regime over 24 h at temperatures representing reasonable current autumn/winter (12 °C),  
162 spring/summer (16 °C) and future spring/summer (20 °C) conditions in the UK ( $\pm 0.1$  °C;  
163 Clifton NEIB water baths; Hulme et al. 2002; Hammond and Pryce, 2007). Dissolved oxygen



164 was monitored using a YSI model 550A meter (Letchworth, England) to ensure levels  
 165 remained above 80% saturation. Both predators and prey were acclimatised to the two  
 166 elevated temperatures over a two hour period prior to experiments; temperatures were  
 167 increased every 30 minutes by either 1 °C or 2 °C (i.e. to 16 °C or 20 °C). Following the  
 168 acclimatisation period, we added single adult females of either *M. albidus* or *M. viridis* to  
 169 containers with prey densities of 2, 4, 8, 15, 30 and 60 ( $n = 4$  per experimental group).  
 170 Controls consisted of four replicates at each prey density and temperature in the absence of  
 171 predators. Predators were individually starved for 24 h in containers of the same volume and  
 172 diameter as the experimental arenas before being transferred to containers holding the  
 173 corresponding prey density. Predators were removed from experimental arenas after 24  
 174 hours, with the numbers of prey alive counted to derive the numbers killed in each replicate.

### 175 *2.3. Data manipulation and statistical analyses*

176 Statistical analyses were undertaken in R v3.3.1. (R Core Team, 2016). Logistic  
 177 regression of proportion of prey killed as a function of prey density was used to infer FR  
 178 types; Type II FRs are characterised by a significant negative first-order term and Type III  
 179 FRs by a significant positive first order term followed by a significant negative second order  
 180 term. To account for prey depletion, we fitted Rogers' random predator equation for  
 181 conditions without prey replacement (Trexler et al. 1988; Juliano, 2001):

$$182 \quad N_e = N_0(1 - \exp(a(N_e h - T)))$$

183 (5)

184 where  $N_e$  is the number of prey eaten,  $N_0$  is the initial density of prey,  $a$  is the attack rate,  $h$  is  
 185 the handling time and  $T$  is the total experimental period. The Lambert W function was  
 186 applied due to the implicit nature of the random predator equation (Bolker, 2008). Attack  
 187 rates and maximum feeding rates ' $1/h$ ' were non-parametrically bootstrapped ( $n = 30$ ) to

188 facilitate modelling of FR parameters with respect to ‘predator’ and ‘temperature’ factors and  
 189 their interactions. Bootstrapped parameters were analysed using generalised linear models  
 190 (GLMs) assuming a quasi-Poisson distribution. *F*-tests were used in a step-deletion process to  
 191 compare deviance between models (Crawley, 2007). We applied Tukey’s HSD method to  
 192 infer specific pairwise differences using the ‘multcomp’ package in R (Hothorn et al. 2008).

193 Benthic survey data for *M. albidus* and *M. viridis* (as *Acanthocyclops viridis*) derived  
 194 from Tinson and Laybourn-Parry (1986) were used to calculate RCP based on maximum  
 195 field abundances using pooled bootstrapped mean maximum feeding and attack rates across  
 196 all three temperatures. To calculate RCP using fecundity, we used results from Laybourn-  
 197 Parry et al. (1988) to discern the proportion of total consumed energy devoted to reproduction  
 198 across corresponding temperatures for the two copepods:

$$199 \quad \text{Fecundity} = \left( \frac{P_r}{C} \right) \times 100$$

200 (6)

201 where  $P_r$  is the quantity of energy expended through the production of eggs and  $C$  is the total  
 202 energy consumed at a given temperature (Table 1). Reproductive energy proportions at 16 °C  
 203 were supplemented with those available for 15 °C. We generated ‘RCP biplots’ to present the  
 204 RCP (see Laverty et al. 2017b) of the two predators using both the abundance (AB) and  
 205 fecundity (FE) proxies for the numerical response.

206 We additionally calculated  $Q_{10}$  values to further quantify the effects of increased  
 207 temperature on feeding rates and compare how these varied between predatory cyclopoids:

$$208 \quad Q_{10} = \left( \frac{FR_2}{FR_1} \right)^{\left( \frac{10}{T_2 - T_1} \right)}$$

209 (7)

210 where  $Q_{10}$  is a coefficient without units,  $FR_1$  is the maximum feeding rate at temperature  $T_1$   
 211 and  $FR_2$  is the maximum feeding rate at temperature  $T_2$ . The  $Q_{10}$  coefficient assesses how  
 212 temperature increases of 10 °C affect the rate of biological processes (Bennett, 1990); values  
 213 of 1 – 1.5 are associated with a thermal plateau and values of 2 – 4 indicate substantive  
 214 increases in activity as temperature increases (Huey, 1982; Bennett, 1990).

215

### 216 3. Results

217 Prey survival in control treatments was a minimum of 98.5% across temperatures, and  
 218 thus experimental deaths were attributed to predation by copepods, which was also directly  
 219 observed. Type II FRs were found in all predator and temperature combinations, as indicated  
 220 by significantly negative first order terms (Table 2; Figure 1). Overall, attack rates (initial FR  
 221 slopes; see Figure 1) for *M. albidus* were significantly higher than for *M. viridis* ( $F_{1, 178} =$   
 222  $7.25, p = 0.01$ ) and increased significantly with temperature ( $F_{2, 176} = 74.41, p < 0.001$ ). There  
 223 were significant increases in attack rates between 12 °C and 16 °C ( $z = 5.61, p < 0.001$ ), and  
 224 12 °C and 20 °C ( $z = 6.75, p < 0.001$ ), but not between 16 °C and 20 °C ( $z = 1.20, p = 0.45$ ).  
 225 There was a significant ‘predator × temperature’ interaction ( $F_{2, 174} = 3.09, p = 0.05$ ),  
 226 reflecting significantly greater attack rates by *M. albidus* only at 12 °C ( $z = 3.42, p = 0.01$ ;  
 227 Table 2; Figure 1). Overall, maximum feeding rates (asymptotes of FR curves; see Figure 1)  
 228 did not differ significantly between the two predators ( $F_{1, 178} = 2.88, p = 0.09$ ), but increased  
 229 significantly with temperature ( $F_{2, 176} = 110.29, p < 0.001$ ; Figure 1). There were significant  
 230 increases in maximum feeding rates between all temperature levels (12 °C – 16 °C,  $z = 4.23,$   
 231  $p < 0.001$ ; 16 °C – 20 °C,  $z = 4.79, p < 0.001$ ; 12 °C – 20 °C,  $z = 8.81, p < 0.001$ ). There was a  
 232 significant ‘predator × temperature’ interaction ( $F_{2, 174} = 3.46, p = 0.03$ ), reflecting a non-  
 233 significant difference between maximum feeding rates of *M. albidus* at 20 °C and *M. viridis*

234 at 16 °C ( $z = 2.48, p = 0.13$ ), compared to a significantly lower maximum feeding rate of *M.*  
235 *albidus* at 16 °C in comparison to *M. viridis* at 20 °C ( $z = 6.24, p < 0.001$ ).

236 The RCP calculations integrating field abundances with maximum feeding and attack  
237 rates are presented in Table 3. *M. albidus* displays much higher abundance than *M. viridis*,  
238 driving greater RCP using both FR parameters (Figure 2a and 2b). There is a general increase  
239 in the proportion of total consumed energy devoted to reproduction as temperature increases  
240 (Table 1). Anomalous to this is the response to warming of *M. viridis* at 20 °C, with fecundity  
241 here falling markedly (Table 1). There are relatively similar levels of RCP for the two species  
242 illustrated at both 12 °C and 16 °C, followed by a substantial decrease in efficacy of *M.*  
243 *viridis* at 20 °C (Table 4; Figures 2c and 2d). Indeed, the certainty of the RCP using fecundity  
244 strengthens at peak temperatures (Table 4). Under both NR proxies, differential efficacies  
245 between predators were more pronounced using the attack rate parameter (Figure 2). The  $Q_{10}$   
246 coefficients for the two predators across the temperature gradient indicate that between 12 °C  
247 and 20 °C both the feeding rates of *M. albidus* and *M. viridis* were highly responsive to  
248 temperature increases. There was a marked difference between the predators in the  
249 incremental drivers of this response, with *M. viridis* exhibiting a rapid increase between 12 °C  
250 and 16 °C which slowed between 16 °C and 20 °C. Conversely, *M. albidus* was consistent in  
251 its feeding response to increased temperatures (Table 5).

252

#### 253 **4. Discussion**

254 Biological control of pests, disease vectors and invasive species can be effective (e.g.  
255 Hajek, 2007; Nam et al. 2012; Veronesi et al. 2015), but efforts to predict the efficacy of  
256 natural enemies are limited when *per capita* effects (e.g. Functional Responses; FRs) are  
257 solely considered (Lester and Harmsen, 2002; Fernández-arhex and Corley, 2003). The

258 complementary agent population response (e.g. Numerical Response; NR) is, however,  
259 somewhat nebulous and more difficult to derive, with proxies for the NR required to allow  
260 rapid assessment of the overall impact of a consumer (i.e. Total Response; TR; Dick et al.  
261 2017). Recent developments that combine functional and numerical responses (or their  
262 proxies) into the comparative Relative Impact Potential metric (RIP; Dick et al. 2017) yield  
263 high explanatory and predictive power for the ecological impacts of invasive species. Indeed,  
264 this RIP approach was 100% successful at identifying high impact invasive species, and the  
265 metric correlated tightly with degree of ecological impact (i.e. reduction of native species  
266 populations). Hence, the present RCP metric is promising to assess the efficacy of biocontrol  
267 agents, which are chosen on the same basis regarding impact on populations of target species  
268 (see Dick et al. 2017). Further value in the application of RCP surrounds the integration of  
269 context-dependencies associated with environmental change, which can strongly affect  
270 interactions between consumers and their resources (e.g. oxygen availability: Lavery et al.  
271 2015; habitat complexity: Barrios-O'Neill et al. 2015). Thus, we present the new Relative  
272 Control Potential (RCP) metric that uses *per capita* and consumer population responses to  
273 compare efficacy among biocontrol agents and can allow predictions of changes in such  
274 efficacies under context-dependencies.

275         The risk of mosquito-borne disease at continental scales has reached unprecedented  
276 levels in recent decades (Medlock and Leach, 2015). Arboviruses such as Zika, West Nile,  
277 dengue and chikungunya present enormous public health concerns, with disease dynamics  
278 shifting rapidly under environmental change (Benelli and Mehlhorn, 2016; Siraj et al. 2017).  
279 Agricultural systems will additionally be impacted (Chevalier, 2013). This risk necessitates  
280 the formation of techniques to assess and compare the potential efficacies of biological  
281 control agents. Here, RCP revealed *M. albidus* as a more effective agent than *M. viridis*.  
282 Temperature was shown to mediate changes to the FR parameters of *M. albidus* and *M.*

283 *viridis*, driving higher magnitude FRs through increasing attack rates and decreasing handling  
284 times. The temperature-dependence of attack rates reported here contrasts to suggestions that  
285 this FR parameter is temperature-independent (Rall et al. 2012; Dell et al. 2014). We show  
286 that both predators exhibit high maximum feeding rates that exceed 30 of the West Nile virus  
287 vector *C. pipiens* per day at 20 °C. Critically, the Type II FRs found are indicative of a  
288 capacity to destabilise prey populations due to high proportional consumption at low prey  
289 densities (Long and Whitefleet-Smith, 2013). *M. albidus* and *M. viridis* show strong  
290 similarities in their *per capita* consumption, although the attack rates of *M. albidus* were  
291 significantly greater overall, illustrated by steeper gradients in the FR curves at low densities.  
292 As a result, *M. albidus* may be more effective in controlling *C. pipiens* populations.

293         We demonstrate that integrating field abundances with RCP reveals far stronger  
294 control efficacies of *M. albidus* compared to *M. viridis*. The utility of abundance estimates  
295 lies in the projection of how many conspecifics may engage in the predator-prey (or other  
296 consumer-resource) interaction and it is thus a useful NR proxy. On the other hand,  
297 incorporating measures of fecundity estimates how rapidly biological control agents can  
298 reproduce when introduced. Using fecundity, we demonstrate temperature-dependencies of  
299 control efficacy. Large fecundity variabilities are illustrated in the RCP biplots, with the  
300 reproductive allocation of *M. viridis* declining rapidly at 20 °C, whilst that of *M. albidus*  
301 continues to rise. The reduction in fecundity shown by *M. viridis* at 20 °C is concurrent with a  
302 slowing feeding activity response discerned through  $Q_{10}$  analysis, whereas *M. albidus*  
303 displays a consistent incremental increase in feeding with warming. Overall, *M. albidus*  
304 displays greater potential for the control of West Nile virus vector *C. pipiens* than *M. viridis*,  
305 particularly under climate change projections where our certainty for differential efficacy  
306 increases (e.g. Hulme et al. 2002). This differential efficacy is more pronounced when the  
307 attack rate parameter is considered. An increased metabolic demand could enable *M. albidus*

308 to sustain efficiency in the consumption of *C. pipiens*, which proliferate more rapidly at  
309 higher temperatures, particularly in domestic and peri-domestic habitats in urbanised  
310 environments (Townroe and Callaghan, 2014).

311 In this study, temperature had a profound effect on predator-prey interactions. It has  
312 been suggested that interaction strengths of ambush predators such as the benthic copepods  
313 examined here are temperature independent (Awasthi et al. 2012; Novich et al. 2014). Thus,  
314 marked thermal dependencies may result, rather, from individual prey foraging responses to  
315 temperature change, wherein motility and velocity may increase with warming. These  
316 interactions make predator-prey systems highly specific to both the species (Englund et al.  
317 2011) and environment (Broitman et al. 2011), with optimal foraging patterns of ectothermic  
318 prey potentially peaking at intermediate temperatures (Englund et al. 2011; Kalinoski and  
319 DeLong 2016). Further, for endotherms, such interactions are often dependent on the specific  
320 feeding strategy (Dell et al. 2014). In this study, feeding rates of candidate biocontrol agents  
321 peaked at the highest temperature, however the rate of increase slowed between 16 °C and 20  
322 °C, as compared to between 12 °C and 16 °C; this trend was particularly marked for *M.*  
323 *viridis*. Notably, these temperatures are well within the thermal tolerances of common  
324 cyclopoid copepods (Marten and Reid, 2007). Temperature additionally has a substantial  
325 influence on the development of the focal prey, *C. pipiens*, driving significant reductions in  
326 development times under conditions of warming (Loetti et al. 2011; Ruybal et al. 2016), and  
327 necessitating increased foraging intensity. Yet, increases in *C. pipiens* mortality due to  
328 drivers outside of predation are also evident as temperatures rise due to thermal stressors  
329 (Ruybal et al. 2016). *M. albidus* has been proved particularly effective against the invasive  
330 arbovirus vector *A. albopictus* following field trials (Marten, 1990; Veronesi et al. 2015).  
331 Previous research has suggested that copepods are more efficient consumers of *Aedes* spp.  
332 than *Culex* spp. as a result of morphological variations between the genera (Marten and Reid,

333 2007). However, laboratory trials have shown similar levels of overall predation by *M.*  
334 *albidus* towards both *A. albopictus* and *C. pipiens* (Veronesi et al. 2015), as well as by  
335 *Mesocyclops annulatus* (Micieli et al. 2002). Further field trials are required to elucidate  
336 whether their efficiencies towards *C. pipiens* translate empirically, particularly as it is the  
337 major West Nile virus vector in the USA and Europe (Hubalek and Halouzka, 1999; Fonseca  
338 et al. 2004) and part of one of the most widespread mosquito complexes in the world  
339 (Harbach, 2012).

340 In conclusion, this is the first study to develop and apply the new RCP metric to  
341 biological control agent selection. We demonstrate that the integration of abundance and  
342 fecundity estimations can provide a means to differentiate between biocontrol agents that  
343 display similar *per capita* efficacies across temperature gradients. We additionally illustrate  
344 the value in the use of maximum feeding and attack rates for instructing agent selection.  
345 Adaptations of this metric have been applied successfully in the context of invasion biology  
346 to explain and predict the ecological impact of invasive species (Dick et al. 2017; Lavery et  
347 al. 2017b), and similar fundamental principles enable its application to the selection of  
348 biocontrol agents. Overall, temperature increases will induce greater *per capita* predation  
349 pressure by predatory copepods towards *C. pipiens* and likely other mosquito species.  
350 Copepod applications to waterbodies can form an integral part of mosquito control efforts  
351 (Baldacchino et al. 2015), with large-scale field trials having proved successful (e.g. Kay and  
352 Nam, 2005), particularly given their ability to thrive in both natural and artificial waterbodies  
353 (Marten and Reid, 2007). Importantly, copepods can be augmented synergistically using  
354 existing control methods, such as the use of bacterial *Bacillus thuringiensis* var. *israelensis*  
355 (*Bti*; Kosiyachinda et al. 2003). Nanoparticles have additionally been found to heighten  
356 predation (Murugan et al. 2015). The straightforward derivation of the RCP metric, and its  
357 visual representation in biplots, will allow comparisons of biological control agents across



358 many ecological systems, and could increase cost-effectiveness of natural enemies in the  
359 long-term. Further proxies for the numerical response, such as biocontrol agent longevity or  
360 biomass, can be integrated into the RCP metric as per the requirements of the assessed system  
361 or biocontrol approach, increasing the robustness and flexibility of the method. Moreover, the  
362 additional integration of a qualifier to account for target organism responses under matched  
363 environmental change scenarios could bolster the power of the RCP metric in reliably  
364 selecting biocontrol agents, and this is worth further consideration in future research.

365

### 366 **Authors' contributions**

367 Relative Control Potential (RCP) concept and framework: RNC, JTAD and JWED. Design  
368 and execution of experimental trials: RNC and JTAD. Fitting functional response models and  
369 undertaking RCP calculations: RNC. First draft of the manuscript: RNC. Manuscript  
370 revisions: all authors.

371

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377

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564 **Tables**

## 565 Table 1

Species	Temperature (°C)	$C$ (mJ)	$P_r$ (mJ)	Fecundity (%)
<i>M. albidus</i>	12	31210	2355	7.55
<i>M. albidus</i>	16	26150	2907	11.12
<i>M. albidus</i>	20	29150	3691	12.66
<i>M. viridis</i>	12	34433	2851	8.28
<i>M. viridis</i>	16	25311	3020	11.93
<i>M. viridis</i>	20	24960	1671	6.70

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575 Table 2

Species	Temperature (°C)	1 <sup>st</sup> order term	<i>p</i>	Attack rate ( <i>a</i> )	<i>p</i>	Handling time ( <i>h</i> )	<i>p</i>
<i>M. albidus</i>	12	-0.02	< 0.001	1.28	< 0.001	0.07	< 0.001
<i>M. albidus</i>	16	-0.03	< 0.001	1.92	< 0.001	0.04	< 0.001
<i>M. albidus</i>	20	-0.04	< 0.001	2.37	< 0.001	0.04	< 0.001
<i>M. viridis</i>	12	-0.03	< 0.001	0.95	< 0.001	0.07	< 0.001
<i>M. viridis</i>	16	-0.03	< 0.001	1.90	< 0.001	0.04	< 0.001
<i>M. viridis</i>	20	-0.04	< 0.001	2.19	< 0.001	0.03	< 0.001

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586 Table 3

Agent A, agent B comparison	Mean FR parameter ( $\pm$ SD)	Mean abundance (ind. $m^{-2} \pm$ SD)	RCP	CIs $p_{RCP} > 1$ (%)
<i>M. albidus</i> , <i>M. viridis</i>	1/h: 22.80 ( $\pm$ 8.37), 24.41 ( $\pm$ 10.55)	6727 ( $\pm$ 1018.23), 562 ( $\pm$ 288.50)	16.77	6.79 – 23.78 99.97***
<i>M. albidus</i> , <i>M. viridis</i>	a: 1.98 ( $\pm$ 0.72), 1.77 ( $\pm$ 0.67)	6727 ( $\pm$ 1018.23), 562 ( $\pm$ 288.50)	19.34	8.18 – 27.35 99.99***

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600 Table 4

Agent A, agent B comparison	Temperature (°C)	Mean FR parameter ( $\pm$ SD)	Fecundity (%)	RCP	CI $p_{RCP} > 1$ (%)
<i>M. albidus</i> , <i>M. viridis</i>	12	1/h: 15.88 ( $\pm$ 4.36), 13.99 ( $\pm$ 2.67)	7.55, 8.28	1.07	0.77 – 1.34 51.93
<i>M. albidus</i> , <i>M. viridis</i>	16	1/h: 22.12 ( $\pm$ 7.64), 25.98 ( $\pm$ 9.68)	11.12, 11.93	0.90	0.53 – 1.21 32.58
<i>M. albidus</i> , <i>M. viridis</i>	20	1/h: 30.42 ( $\pm$ 5.24), 33.25 ( $\pm$ 6.73)	12.66, 6.70	1.80	1.39 – 2.17 98.21*
<i>M. albidus</i> , <i>M. viridis</i>	12	$\alpha$ : 1.43 ( $\pm$ 0.52), 1.05 ( $\pm$ 0.34)	7.55, 8.28	1.37	0.82 – 1.83 66.71
<i>M. albidus</i> , <i>M. viridis</i>	16	$\alpha$ : 2.17 ( $\pm$ 0.51), 2.02 ( $\pm$ 0.39)	11.12, 11.93	1.04	0.77 – 1.28 49.04
<i>M. albidus</i> , <i>M. viridis</i>	20	$\alpha$ : 2.34 ( $\pm$ 0.75), 2.24 ( $\pm$ 0.54)	12.66, 6.70	2.09	1.39 – 2.69 95.34*

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606 Table 5

Species	Temperature (°C)	Mean max. feeding rate (1/h)	$Q_{10}$ value
<i>M. albidus</i>	12 – 20	15.88 – 30.42	2.25
<i>M. albidus</i>	12 – 16	15.88 - 22.12	2.29
<i>M. albidus</i>	16 – 20	22.12 – 30.42	2.22
<i>M. viridis</i>	12 – 20	13.99 – 33.25	2.95
<i>M. viridis</i>	12 – 16	13.99 – 25.98	4.70
<i>M. viridis</i>	16 – 20	25.98 – 33.25	1.85

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## 622 Captions

623 Table 1. Fecundity (%) calculations, entailing proportion of total consumed energy ( $C$ )  
 624 devoted to production of eggs ( $P_r$ ) in adult female *M. albidus* and *M. viridis* across 12 °C, 16  
 625 °C and 20 °C. Data relating to energetics adapted from Laybourn-Parry et al. (1988;  $n = 5$ ;  
 626 Eqn. 6).

627 Table 2. Results of logistic regression to denote functional response type across all predator  
 628 and temperature treatments, alongside the starting attack rate ( $a$ ) and handling time ( $h$ )  
 629 parameter estimates and associated  $p$ -values generated using the Rogers' random predator  
 630 equation (Eqn. 5).

631 Table 3: Mean Relative Control Potential (RCP) using maximal abundances from Tinson and  
 632 Laybourn-Parry (1986;  $n = 8$ ) for *M. albidus* and *M. viridis*, alongside uncertainties reflected  
 633 through 60% confidence intervals (CIs) and probability that the RCP output exceeds 1 using  
 634 bootstrapped maximum feeding and attack rates. Asterisks denote significant levels of  
 635 certainty that the RCP score is greater than 1 (\* > 95%, \*\* > 99%, \*\*\* > 99.9%).

636 Table 4: Mean Relative Control Potential (RCP) using fecundities (%) across temperature  
 637 change for *M. albidus* and *M. viridis* alongside uncertainties reflected through 60%  
 638 confidence intervals (CIs) and probability that the RCP output exceeds 1 using bootstrapped  
 639 maximum feeding and attack rates. Asterisks denote significant levels of certainty that the  
 640 RCP score is greater than 1 (\* > 95%, \*\* > 99%, \*\*\* > 99.9%).

641 Table 5:  $Q_{10}$  coefficient (Eqn. 7) values associated with mean bootstrapped maximum feeding  
 642 rates for both predators between temperature gradients.

643 Figure 1. Functional responses of *M. albidus* (a, b, c) and *M. viridis* (d, e, f) towards first  
 644 instar *C. pipiens* larvae at 12 °C (a, d), 16 °C (b, e) and 20 °C (c, f) over the 24 hour  
 645 experimental period. Means are  $\pm$  SE at each prey density ( $n = 4$ ).

646 Figure 2. RCP biplots comparing *M. albidus* and *M. viridis* using abundance estimates (a, b;  $n$   
647 = 8) and fecundity calculations (c, d; Table 1), with FR parameters of maximum feeding (a,  
648 c) and attack rates (b, d). FR parameters in abundance biplots are pooled bootstrapped  
649 estimates across all temperatures ( $n = 90$ ); those in fecundity plots are temperature-specific  
650 estimates ( $n = 30$ ). Increasing CP is read from bottom left to top right. Abundance and FR  
651 parameter means are  $\pm$  SE.

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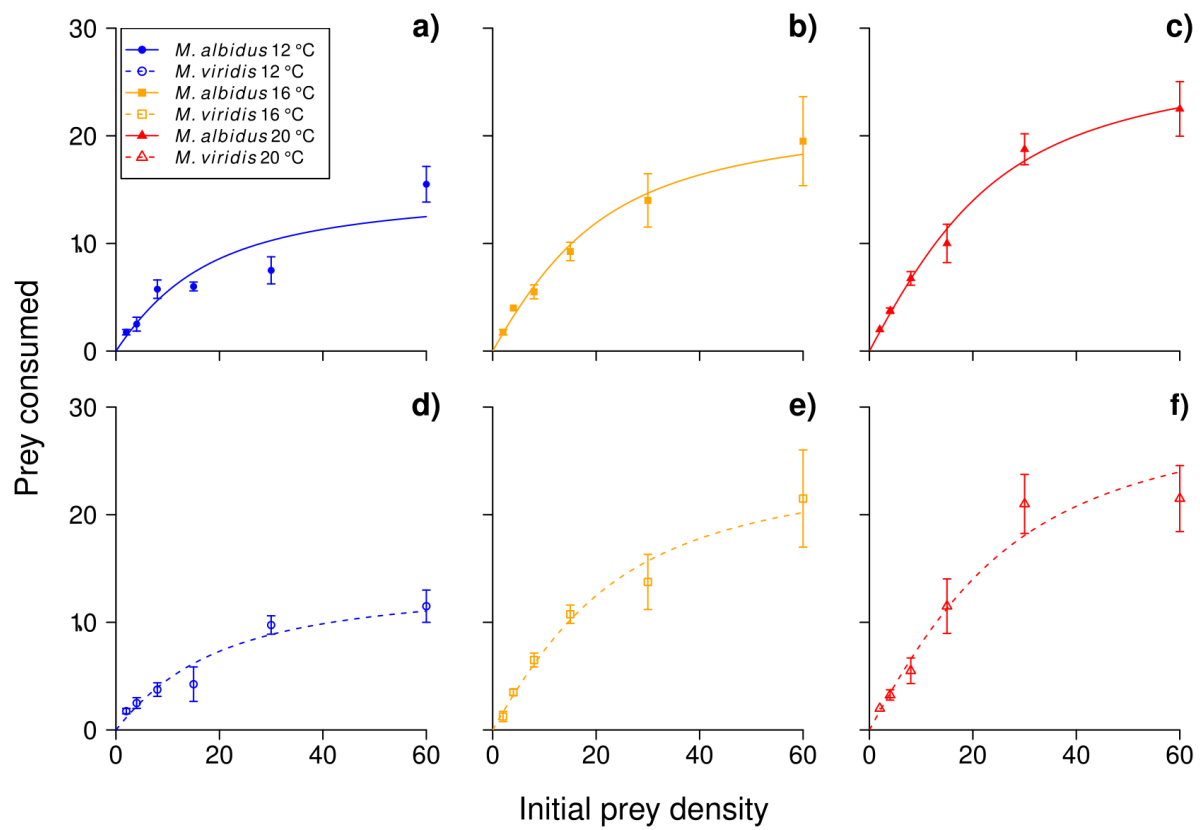
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