

*The effect of the alternative prey,  
Paramecium caudatum (Peniculida:  
Parameciidae), on the predation of Culex  
pipiens (Diptera: Culicidae) by the  
copepods Macrocyclus albidus and  
Megacyclus viridis (Cyclopoida:  
Cyclopidae)*

Article

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1 Short Communication

2 **The effect of the alternative prey, *Paramecium caudatum***  
3 **(Peniculida: Parameciidae), on the predation of *Culex pipiens***  
4 **(Diptera: Culicidae) by the copepods *Macrocyclus albidus* and**  
5 ***Megacyclus viridis* (Cyclopoida: Cyclopidae)**

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21 **Abstract**

22 Biological control can be an effective tool to combat public health risks associated with  
23 mosquito-borne disease. However, target impacts of biological control agents may be reduced  
24 by biotic contexts such as the presence of alternative prey. In turn, this can impede our ability  
25 to realistically assess biocontrol agent efficacy. Here, we examine the effects of alternative  
26 ciliate prey on the predation potential of two cyclopoid copepods, *Macrocyclops albidus*  
27 Jurine (Cyclopoida: Cyclopidae) and *Megacyclops viridis* Jurine (Cyclopoida: Cyclopidae),  
28 towards larvae of the West Nile virus vector mosquito *Culex pipiens* Linnaeus (Diptera:  
29 Culicidae). Using functional responses (FRs; resource use under different resource densities),  
30 we demonstrate that both copepods exhibit potentially destabilising Type II FRs towards  
31 mosquito prey. However, where the alternative prey was present, we observed species-  
32 specific modulations to FR form and magnitude. For *M. albidus*, FRs remained Type II where  
33 ciliate prey were present, however, maximum feeding rates on mosquito larvae were reduced.  
34 Conversely, for *M. viridis*, FRs moved towards more stabilising Type III, whilst maximum  
35 feeding rates on mosquito larvae were not significantly reduced. Whilst both species of  
36 cyclopoid copepod were able to effectively target and consume larval mosquitoes in the  
37 presence of alternative prey, we demonstrate that overall efficacies may be reduced in aquatic  
38 habitats which contain multiple prey types. We thus advocate that biotic contexts such as  
39 prey selectivity should be integrated into predatory biocontrol agent examinations for  
40 mosquitoes which vector pathogens and parasites, to more holistically assess their efficacy.

41 **Key words:**

42 mosquito-borne disease, functional response, *Culex pipiens*, prey choice, cyclopoid

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## 45 **Introduction**

46 Public health endangerment from mosquito-borne diseases has never been more apparent  
47 (Fernandes et al. 2018). To this end, a variety of vector control approaches have been  
48 developed and implemented, with varying levels of success (Becker et al. 2010). Biological  
49 control could present new tools to combat mosquito-borne disease in future (Thomas, 2018),  
50 with potential to reduce mosquito-borne disease circulation at large scales (Kay and Nam,  
51 2005). Predatory cyclopoid copepods have proven especially efficacious in the biocontrol of  
52 mosquitoes which vector pathogens and parasites (Marten and Reid, 2007; Cuthbert et al.  
53 2018a). Indeed, copepod efficacy has proven particularly high in artificial, human-mediated  
54 aquatic environments (Townroe and Callaghan, 2014; Veronesi et al. 2015). Although  
55 predation efficiencies of copepods have proven robust to abiotic environmental contexts such  
56 as habitat complexity (Cuthbert et al. 2018b) and turbidity (Cuthbert et al. 2018c),  
57 quantifications of biotic effects associated with alternative prey on their predatory impacts are  
58 lacking.

59 Within ecosystems, the presence of alternative prey can drive patterns of coexistence and thus  
60 impart stability to populations through frequency-dependent prey selection processes which  
61 alleviate predation pressures towards specific prey through switching (Murdoch, 1969;  
62 Cuthbert et al. 2018d). Accordingly, alternative prey may reduce biocontrol agent efficacies  
63 towards target organisms. Functional responses (FRs) have been used widely to quantify  
64 consumer-resource interactions under differing resource densities (Holling 1959), with FR  
65 form and magnitude offering insights into consumer impacts (e.g. biocontrol agents) towards  
66 target organisms (Dick et al. 2014). Type II FRs are characterized by a decelerating intake  
67 rate, where consumption rates are high at low prey densities. On the other hand, Type III FRs  
68 are sigmoidal, with low proportional intake at low prey densities. A Type II FR can therefore  
69 be population destabilising due to high proportional consumption at low prey densities, whilst

70 Type III FRs may impart stability to prey populations through the provision of low density  
71 prey refugia (Holling 1959; Alexander et al. 2012). Thus, in a biocontrol context, agents  
72 which demonstrate a Type II FR irrespective of environmental contexts are desirable.  
73 Cyclopoid copepods have been shown to exhibit potentially population destabilising Type II  
74 FRs towards larval mosquito prey (Cuthbert et al. 2018a, b). However, effects of alternative  
75 prey, which often coexist with larval mosquitoes, on copepod-mosquito interaction strengths  
76 have not been comprehensively assessed. In aquatic habitats, ciliate protists are ubiquitous in  
77 stagnant waters where mosquitoes breed (Duguma et al. 2017). Thus, since alternative prey  
78 may reduce predatory impact and hence natural enemy efficacy towards mosquitoes, here we  
79 examine the effects of a common alternative ciliate prey on the biocontrol efficiency of  
80 cyclopoid copepods towards larvae of the vectorially-important mosquito *Culex pipiens*  
81 Linnaeus (Diptera: Culicidae).

## 82 **Materials and Methods**

83 The focal predators, *Macrocyclops albidus* Jurine (Cyclopoida: Cyclopidae) and *Megacyclops*  
84 *viridis* Jurine (Cyclopoida: Cyclopidae) were collected from Glastry Clay Pit ponds, Northern  
85 Ireland (54°29'18.5"N; 5°28'19.9"W) and transported in water from the collection site to  
86 Queen's Marine Laboratory, Portaferry. We initiated separate copepod cultures for each  
87 species from single gravid origerous females. Cultures were maintained at 25±2 °C, 50-60%  
88 RH and under a 16:8 light:dark regime. *Paramecium caudatum* and *Chilomonas paramecium*  
89 were supplied *ad libitum* to the copepods for culture proliferation, with adult copepod  
90 samples from each culture dissected and identified to species. Whilst *P. caudatum* is a food  
91 source for adults and copepodids, *C. paramecium* provides nurishment for nauplii stages. The  
92 prey, *Cx. pipiens* were obtained from a colony maintained in the same laboratory. This  
93 colony originated from eggs supplied in 2017 by The Pirbright Institute (Woking, England).  
94 Adult females were kept in 32.5 cm<sup>3</sup> cages (Bugdorm, Watkins and Doncaster, Leominster,

95 England) and fed regularly using defibrinated horse blood *via* a membrane feeding system  
96 (Hemotek Ltd, Accrington, England). Sucrose-soaked cotton pads were additionally supplied  
97 for sustenance in each cage. Eggs rafts were removed regularly and larvae reared in 3 L larval  
98 bowls with crushed guinea pig food pellets provided *ad libitum* (Pets at Home,  
99 Newtownabbey, Northern Ireland) until pupation.

100 To quantify the effects of alternative prey on the FR of both copepod species, we employed a  
101 factorial  $2 \times 2 \times 6$  experimental design with respect to ‘predator species’ (*M. albidus*/*M.*  
102 *viridis*), ‘alternative prey’ (present/absent) and ‘prey supply’ (prey densities of either 2, 4, 6,  
103 8, 15 or 30). *Culex pipiens* first instar larvae (1.1 – 1.3 mm) were supplied to adult female *M.*  
104 *albidus* (1.6 – 1.8 mm) or *M. viridis* (2 – 2.3 mm) over a 6 hour experimental period ( $n = 5$   
105 per experimental treatment). Experiments were undertaken in polypropylene arenas of 42 mm  
106 diameter containing 20 ml of dechlorinated tap water from an aerated source. For the  
107 alternative prey treatment, we added 3 ml of *P. caudatum* culture (approx. 150 ciliates ml<sup>-1</sup>  
108 before addition to 17 ml dechlorinated tap water) to experimental arenas. This concentration  
109 aligns with densities used in other studies (e.g. Reiss and Schmid-Araya 2011). We starved  
110 non-ovigerous adult female copepods individually for 24 hours before the experiment to  
111 standardise hunger levels. To eliminate dissolved oxygen variability among treatments, we  
112 bubbled oxygen into the *P. caudatum* culture for 2 minutes prior to its dissemination. Prey  
113 were allowed to settle for 2 hours before the experiment was initiated through the addition of  
114 predators. After 6 hours during the photoperiod, the predators were removed and remaining  
115 live prey counted. Controls were five replicates at each prey density and alternative prey  
116 treatment in the absence of a predator.

117 All statistical analyses were undertaken in R (R Core Team, 2018). We compared overall  
118 prey consumption (numbers eaten) with respect to the ‘predator species, ‘alternative prey’  
119 and ‘prey supply’ factors using a generalized linear model (GLM) assuming a Poisson

120 distribution and log link as counts were not overdispersed. Non-significant factors and  
121 interactions were removed to generate the most parsimonious model through a step-deletion  
122 process using  $\chi^2$  for model selection *via* analysis of deviance (Crawley, 2007).

123 We determined FR types using logistic regression of the proportion of prey killed as a  
124 function of prey density. A significantly negative first order term is indicative of a Type II  
125 FR, whilst a significantly positive first order term followed by a significantly negative second  
126 order term indicates a Type III FR (Juliano, 2001). We additionally used locally weighted  
127 scatterplot smoothing (LOWESS; 9/10 smoothing factor) to further examine proportional  
128 consumption across varying prey densities. We then fit flexible functional response models  
129 using the *frair* package in R (Pritchard et al. 2017), which can integrate a scaling exponent  
130 ( $q$ ) if the results of logistic regression are equivocal (Real, 1977; Wasserman et al. 2016):

$$131 \quad N_e = N_0 (1 - \exp(bN_0^q(hN_e - T)))$$

132 Eqn. 1

133 where  $N_e$  is the number of prey eaten,  $N_0$  is the initial prey density,  $b$  is the search coefficient,  
134  $h$  is the handling time,  $q$  is the scaling exponent and  $T$  is the total time available. Where a  
135 categorical Type II functional response is evidenced, the scaling exponent  $q$  may be fixed at  
136 0, whilst responses are increasingly sigmoidal where  $q > 0$ . We then non-parametrically  
137 bootstrapped ( $n = 2000$ ) initial  $b$  and  $h$  parameter estimates to construct 95% confidence  
138 intervals (CIs) around FR curves. This enabled results to be considered at the population-  
139 level, with differences subsequently inferred on the basis of FR overlaps across prey  
140 densities.

## 141 **Results and Discussion**



142 Prey survival in controls was 99.9% overall, and thus we assumed that prey deaths in  
143 treatments resulted from predation, which was also evidenced through partially consumed  
144 remains of larval mosquitoes. Overall, significantly more prey items were consumed by *M.*  
145 *viridis* than *M. albidus* ( $\chi^2 = 3.95$ ,  $df = 1$ ,  $P = 0.047$ ), whilst predation was significantly  
146 reduced in the presence of alternative prey for both species ( $\chi^2 = 8.40$ ,  $df = 1$ ,  $P = 0.004$ ). The  
147 alternative prey effect was consistent between predators as there were no interactions  
148 between the ‘predator species’ and ‘alternative prey’ factors ( $\chi^2 = 0.73$ ,  $df = 1$ ,  $P = 0.39$ ).  
149 Further, overall consumption was significantly greater as more prey were supplied ( $\chi^2 =$   
150  $114.86$ ,  $df = 5$ ,  $P < 0.001$ ). No further significant interactions were found for the ‘predator  $\times$   
151 prey supply’ ( $\chi^2 = 1.47$ ,  $df = 5$ ,  $P = 0.92$ ), ‘alternative prey  $\times$  prey supply’ ( $\chi^2 = 2.75$ ,  $df = 5$ ,  $P$   
152  $= 0.74$ ), or ‘alternative prey  $\times$  predator  $\times$  prey supply’ terms in the model ( $\chi^2 = 2.59$ ,  $df = 5$ ,  $P$   
153  $= 0.76$ ).

154 Both *M. albidus* and *M. viridis* exhibited Type II FRs when no alternative prey were available  
155 (Table 1; Figure 1a,b). *Macrocyclus albidus* also displayed a categorical Type II FR in the  
156 presence of alternative prey (Figure 1a). However, in the presence of alternative prey, the FR  
157 form of *M. viridis* was equivocal between Type II and Type III (Figure 1b). Thus, for this  
158 treatment, a scaling exponent was integrated, where the FR was deemed to be moving  
159 towards being a sigmoidal Type III ( $q = 0.83$ ; Table 1).

160 The search coefficient  $b$  trended towards being lower under the presence of alternative prey,  
161 whilst handling time  $h$  tended to be higher, and thus maximum feeding rate  $1/h$  lower (Table  
162 1; Figure 2a, b). Shaded FR CIs overlapped only at low prey densities in the case of *M.*  
163 *albidus* (Figure 2a), and thus maximum feeding rates were significantly greater in the absence  
164 of alternative prey for this species. On the other hand, in the case of *M. viridis*, FR CIs  
165 overlapped at all except the lowest prey densities (Figure 2b). This reflects the sigmoidal FR  
166 form and low search coefficient  $b$  in the presence of alternative prey for *M. viridis*. Where

167 there were no alternative prey, FR CIs of the two copepod species converged across all prey  
168 supplies, and thus FRs were not significantly different within this treatment. However, where  
169 there were alternative prey present, at intermediate densities the FR CIs of *M. albidus* were  
170 significantly lower in comparison to *M. viridis* (Figure 2).

171 Biotic contexts such as the presence of alternative prey can affect derivations of ecological  
172 impact between consumers and resources (Médoc et al. 2018), and is relevant in the  
173 biocontrol context as multiple prey items often occur simultaneously within ecosystems.  
174 Indeed, for generalist consumers, prey switching and prey preferences can have a marked  
175 influence on ecological impacts (Murdoch 1969; Bolnick et al. 2002; Cuthbert et al. 2018d).  
176 Such biotic contexts are neglected where experimental designs focus upon singular prey  
177 species, and thus impact quantifications and transfer of results to empirical applications are  
178 inherently restricted. Several species of cyclopoid copepods have proven effective in field  
179 applications targetting mosquitoes which vector pathogens and parasites (Marten and Reid  
180 2007). As cyclopoid copepods are known to feed on a range of organisms (Kumar et al.  
181 2008), understanding the effects of such alternative prey on their biocontrol efficacy is vital  
182 for mosquito control efficacy assessments.

183 The present study demonstrates that the presence of alternative prey can significantly  
184 influence the predation efficiency of cyclopoid copepods towards vectorially-important  
185 mosquitoes. Our results corroborate with Rey et al. (2004) and Kumar et al. (2008), where  
186 alternative prey significantly reduced mosquito consumption by cyclopoid copepods. For  
187 both species here, the presence of alternative prey reduced the overall strength of interactions  
188 with larval mosquito prey. Whilst the Type II FRs exhibited by both copepods here in the  
189 absence of alternative prey corroborate with other studies (Cuthbert et al. 2018a, b, c), we  
190 found that the presence of alternative prey can drive species-specific shifts in FR form and  
191 magnitude. For *M. albidus*, whilst the Type II FR was sustained in the presence of alternative

192 prey, maximum feeding rates were significantly reduced. On the other hand, for *M. viridis*,  
193 FR form shifted towards a sigmoidal Type III in the presence of alternative prey whilst  
194 maximum feeding rates were similar to where alternative prey were absent. Thus, whilst the  
195 Type II FR demonstrates that *M. albidus* may offer high and sustained predation levels at low  
196 prey densities, *M. viridis* may give refuge to mosquito prey at low prey densities due to an  
197 increasingly sigmoidal FR. Indeed, *M. albidus* has been regularly operationalized in  
198 biocontrol and has proven particularly efficacious in field applications, and thus our results  
199 corroborate with these field-based trends in light of sustained population destabilising Type II  
200 FRs (Marten and Reid, 2007). Our results suggest that cyclopoid copepods are able to still  
201 effectively reduce mosquito numbers in the presence of alternative prey. However, future  
202 examinations of biocontrol agents should seek to incorporate assessments as to the effects of  
203 multiple alternative prey on ecological impact prior to agent release.

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283 Table 1. Results of logistic regression for both Type II and Type III models, considering prey  
 284 killed as a function of prey density, and parameter estimates resulting from the flexible  
 285 functional response models.

	<i>Macrocyclus albidus</i>		<i>Megacyclus viridis</i>	
(a) Alternative prey	No	Yes	No	Yes
(b) Logistic regression				
Type II response				
First term	-0.04	-0.03	-0.03	-0.02
<i>P</i> value	0.001	0.03	0.006	0.11
Type III response				
First term	-0.07	-0.10	-0.04	0.02
<i>P</i> value	0.35	0.25	0.54	0.78
Second term	0.0007	0.002	0.0003	-0.001
<i>P</i> value	0.71	0.43	0.88	0.58
(c) Parameter estimates				
<i>b</i>	0.63	0.35	0.62	0.11
<i>h</i>	0.13	0.18	0.08	0.16
<i>q</i>	Fixed at 0	Fixed at 0	Fixed at 0	0.83

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298 Figure 1. Functional response forms determined from proportion of prey consumed under  
299 differing initial prey densities for *Macrocyclus albidus* (a) and *Megacyclus viridis* (b)  
300 preying on larvae of *Culex pipiens* without (solid lines; circles) and with (dashed lines;  
301 triangles) alternative prey (*Paramecium caudatum*). Relationships are presented using  
302 LOWESS lines with means  $\pm$ SE ( $n = 5$  per experimental group).

303 Figure 2. Functional responses ( $n = 5$  per experimental group) of predatory copepods  
304 *Macrocyclus albidus* (a) and *Megacyclus viridis* (b) towards first instar *Culex pipiens*,  
305 without (solid lines) and with (dashed lines) alternative prey (*Paramecium caudatum*) over 6  
306 hour experimental period. Shaded areas indicate bootstrapped ( $n = 2000$ ) 95% confidence  
307 intervals (CIs).