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

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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 *Macrocyclops albidus* and**

5   ***Megacyclops 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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Introduction

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

Within ecosystems, the presence of alternative prey can drive patterns of coexistence and thus impart stability to populations through frequency-dependent prey selection processes which alleviate predation pressures towards specific prey through switching (Murdoch, 1969; Cuthbert et al. 2018d). Accordingly, alternative prey may reduce biocontrol agent efficacies towards target organisms. Functional responses (FRs) have been used widely to quantify consumer-resource interactions under differing resource densities (Holling 1959), with FR form and magnitude offering insights into consumer impacts (e.g. biocontrol agents) towards target organisms (Dick et al. 2014). Type II FRs are characterized by a decelerating intake rate, where consumption rates are high at low prey densities. On the other hand, Type III FRs are sigmoidal, with low proportional intake at low prey densities. A Type II FR can therefore 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 contex, 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, *Macocyclops 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. Whlst *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^3 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^{-1}

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 χ^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
$$N_e = N_0 \left(1 - \exp(bN_0^q(hN_e - T))\right)$$

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). *Macrocyclops 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 aplications, 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.

	Macrocyclops albidus		Megacyclops viridis	
(a) Alterative prey	No	Yes	No	Yes
(b) Logistic regression				
Type II response				
First term	-0.04	-0.03	-0.03	-0.02
P value	0.001	0.03	0.006	0.11
Type III response				
First term	-0.07	-0.10	-0.04	0.02
P value	0.35	0.25	0.54	0.78
Second term	0.0007	0.002	0.0003	-0.001
P value	0.71	0.43	0.88	0.58
(c) Parameter estimates				
b	0.63	0.35	0.62	0.11
h	0.13	0.18	0.08	0.16
q	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 *Macrocyclops albidus* (a) and *Megacyclops 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 ±SE ($n = 5$ per experimental group).

303   Figure 2. Functional responses ($n = 5$ per experimental group) of predatory copepods

304   *Macrocyclops albidus* (a) and *Megacyclops 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).