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1 SHORT COMMUNICATION

2 **Calanoid copepods: an overlooked tool in the control of disease vector mosquitoes**

3 ROSS N. CUTHBERT^{1,2,3*}, TATENDA DALU^{4,5}, RYAN J. WASSERMAN^{5,6},

4 AMANDA CALLAGHAN³, OLAF L.F. WEYL² and JAIMIE T.A. DICK¹

5 ¹*Institute for Global Food Security, School of Biological Sciences, Medical Biology Centre, Queen's*
6 *University Belfast, Belfast BT9 7BL, Northern Ireland*

7 ²*DST/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute for*
8 *Aquatic Biodiversity (SAIAB), Grahamstown 6140, South Africa*

9 ³*Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading, Harborne*
10 *Building, Reading RG6 6AS, England*

11 ⁴*Ecology and Resource Management, University of Venda, Thohoyandou, Limpopo, South Africa*

12 ⁵*South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown 6140, South Africa*

13 ⁶*Department of Biological Sciences and Biotechnology, Botswana International University of Science*
14 *and Technology, Palapye, Botswana*

15 *Corresponding author: Ross N. Cuthbert (rcuthbert03@qub.ac.uk)

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21 **Abstract**

22 Biological control can assist in the management of disease vector mosquitoes. However, we urgently
23 require the identification of novel and effective agents to aid population management strategies.
24 Previously, predatory biocontrol of disease vector mosquito species has focused extensively on
25 cyclopoid copepods, but prey size refuge effects have been identified as a hindrance to their predatory
26 efficacy. Calanoid copepods have yet to be examined in the context of mosquito control, despite their
27 high prevalence, diversity and distribution. Here, we apply functional responses (FRs; resource use as a
28 function of resource density) to examine predation efficiencies of a recently described ephemeral pond
29 specialist species, the freshwater calanoid copepod *Lovenula raynerae* Suárez-Morales, Wasserman &
30 Dalu 2015, using different size classes of larvae of the disease vector complex *Culex pipiens* as prey.
31 *Lovenula raynerae* effectively consumed *C. pipiens* larvae across their ontogeny. A potentially
32 population destabilising Type II FR was exhibited towards both early and late instar mosquitoes,
33 indicative of a lack of prey refuge across ontogenetic stages. Attack rates were greatest and handling
34 times lowest for early instar larvae compared to late instar larvae. These traits contrast to other copepods
35 commonly applied in biocontrol, which are only able to handle early instars, and in much smaller
36 numbers. We thus advocate that calanoid copepods can exert particularly marked predatory impact on
37 lower trophic groups, and that their use in disease vector mosquito control strategies should be further
38 explored.

39 **Keywords:**

40 Biological control; functional response; copepod; mosquito; disease vector; *Lovenula raynerae*

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44 **Introduction**

45 Predation is a key biotic process underpinning structuring of populations and communities within
46 ecosystems (Solomon, 1949; Murdoch, 1969; Carpenter *et al.* 1985), and can be an effective means of
47 vector control (e.g. Marten & Reid, 2007). In their most basic form, interactions between consumers
48 and prey can be quantified by the functional response (FR; resource use as a function of resource
49 density), and the derivation of FRs has been integral to the development of predator-prey theory
50 (Holling, 1959). Three broad forms of FR have been frequently observed: linear Type I; hyperbolic Type
51 II; sigmoidal Type III (Murdoch & Oaten, 1975). Within the context of predatory biocontrol, Type II
52 FRs are desirable given that these predator-prey relationships are, theoretically, associated with localised
53 prey extinction (Dick *et al.* 2014).

54 Biological control of mosquito-borne disease vectors has often integrated copepods as predatory agents
55 (Marten & Reid, 2007). While the efficacy and viability of copepods in the biocontrol of disease vectors
56 has been frequently explored (e.g. Cuthbert *et al.* 2018a, b), this work has all focused on cyclopoid
57 copepods, ignoring other copepod groups (Marten & Reid, 2007). Freshwater calanoid copepods have
58 remained unexamined in mosquito biocontrol (but see Cuthbert *et al.* 2018d), labelled as a herbivorous
59 group that is unable to handle mosquito prey (e.g. Marten & Reid, 2007). Predatory calanoid copepods
60 do, however, exist and can be relatively large in size (Suárez-Morales *et al.* 2015; Wasserman *et al.*
61 2015), making them biocontrol candidates. Further, cyclopoid copepods have been shown to be unable
62 to effectively handle late instar larval mosquito prey (Marten & Reid, 2007), and predators often display
63 reduced capture efficacy towards resources which are relatively large or small (Vonesh & Bolker, 2005).
64 Thus, identifying agents that can handle larval mosquito prey throughout ontogenetic variation is
65 important for reducing size refuge effects.

66 Calanoid copepods form part of zooplankton assemblages which dominate ephemeral aquatic
67 ecosystems, facilitated through *in situ* hatching of dormant eggs (Dalu *et al.* 2017). These atypical

68 ecosystems are understudied given their impermanency and spatial heterogeneity, with interaction
69 strengths between predators and their prey poorly constrained (though see Wasserman *et al.* 2015).
70 Thus, examining the predatory potential of calanoids towards basal mosquito prey at differing
71 ontogenetic stages would be informative towards interaction strength quantifications within ephemeral
72 systems where mosquitoes often breed, further to the potential role of calanoid copepods in biocontrol.
73 Accordingly, in this study we examine, using FRs, the predatory potential of *Lovenula raynerae* Suárez-
74 Morales, Wasserman & Dalu 2015, a recently described and remarkably large freshwater calanoid
75 (Suárez-Morales *et al.* 2015), towards different sized larvae of the vectorially-important *Culex pipiens*
76 mosquito complex in order to constrain biocontrol efficacy and potential size refuge effects.

77 **Materials and Methods**

78 Adult *L. raynerae* were collected from an ephemeral pond in Bathurst, Eastern Cape, South Africa
79 (33°29'21.4"S 26°49'48.4"E) using a 200 µm mesh net and transported in source water to a controlled
80 environment room at Rhodes University, Grahamstown. Copepods were acclimated at 25 ± 1 °C and
81 under a 12:12 light:dark regime for 7 days, being fed on a standard diet of crushed fish flakes for the
82 first 5 days (Aqua Plus, Grahamstown) and starved for the last 2 days in continuously aerated 25 L tanks
83 containing dechlorinated tapwater. The focal prey, larvae of the *C. pipiens* complex, were cultured using
84 egg rafts collected from artificial containers within the Rhodes University campus, and reared to the
85 desired size class in the same laboratory using a diet of crushed rabbit pellets (Agricol, Port Elizabeth),
86 supplied *ad libitum*. Non-gravid adult female copepods (5.1 ± 0.1 mm) were selected for
87 experimentation and provided either early (1.4 ± 0.1 mm) or late (4.4 ± 0.2 mm) instar mosquito prey at
88 six densities (2, 4, 8, 16, 32, 64; $n = 4$ per treatment group) in arenas of 5.6 cm diameter containing 80
89 mL dechlorinated tapwater from a continuously aerated source. Prey were allowed to settle for two
90 hours before the addition of predators. Once predators were added they were allowed to feed

91 undisturbed for 5 hours, after which they were removed and remaining live prey counted. Controls
 92 consisted of a replicate at each density and prey size class without a predator.

93 All statistical analyses were conducted using ‘R’. Overall prey consumption with respect to ‘prey size’
 94 and ‘prey density’ factors and their interactions was analysed using generalised linear models (GLMs)
 95 assuming a Poisson error distribution. We removed non-significant terms and interactions stepwise to
 96 obtain the minimum adequate model using likelihood ratio tests. We used the package ‘frair’ (Pritchard
 97 *et al.* 2017) for FR analyses. Here, our approach to FR analysis is phenomenological as opposed to
 98 mechanistic, and thus our results are comparative across standardised experimental conditions (see
 99 Jeschke *et al.* 2002; Dick *et al.* 2014). Logistic regression of proportional prey consumption as a
 100 function of prey density was used to derive FR types (*frair::frair_test*), wherein, categorically, a Type II
 101 FR is identified by a significantly negative first order term, whilst a Type III FR is ascribed from a
 102 significantly negative second order term following a positive first order term. As prey supplies were not
 103 replenished, the Rogers’ random predator equation was used to fit FRs (Juliano, 2001; *frair::frair_fit*):

$$104 \quad N_e = N_0(1 - \exp(a(N_e h - T)))$$

105 Eqn. 1.

106 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
 107 handling time and T is the total experimental period. We used a non-parametric bootstrap procedure ($n =$
 108 2000; *frair::frair_boot*) to generate FR confidence intervals, enabling their consideration in population
 109 terms. We subsequently used the difference method (see Juliano, 2001) to undertake pairwise
 110 comparisons of FRs with respect to the attack rate and handling time parameters.

111 **Results and Discussion**

112 No prey deaths occurred in controls and so mortality was deemed a result of predation by copepods, which
 113 was directly observed towards both prey sizes. Overall predation was significantly greater towards early

114 instar prey compared to later instar prey ($\chi^2 = 79.39$, $df = 1$, $p < 0.001$) and increased significantly with
115 increasing prey supplied ($\chi^2 = 200.03$, $df = 5$, $p < 0.001$). No significant ‘prey size \times prey supply’
116 interaction was detected ($\chi^2 = 4.06$, $df = 5$, $p = 0.54$). Type II FRs were detected for both prey sizes (Table
117 1; Fig. 1), and 95% confidence intervals did not overlap across any prey supplies. Attack rates were
118 significantly higher towards early instar prey ($z = 3.65$, $p < 0.001$), evidenced by the steeper initial gradient
119 in the FR curve. Handling times were significantly reduced for smaller prey items ($z = 3.15$, $p = 0.002$),
120 with a greater maximum feeding rate reached here.

121 The application of FRs is relevant within the predatory biocontrol context, providing a mainstay to the
122 derivation of predator-prey interactions (Murdoch & Oaten, 1975). Here, we demonstrate that the calanoid
123 copepod *L. raynerae* can handle mosquito larvae throughout their ontogeny, with a potentially population-
124 destabilising Type II FR exhibited towards both early and late instar mosquito prey. These results are
125 promising when compared to similar studies on cyclopoid copepod predation efficiency (e.g. Cuthbert *et*
126 *al.* 2018a, b, c). Indeed, when prey was not limited, *L. raynerae* individuals consumed several multitudes
127 more culicid larvae than all cyclopoids investigated (Marten & Reid, 2007). In addition, unlike *L. raynerae*
128 predation as highlighted in this study, studies on cyclopoids have shown that late-stage mosquito larvae
129 experience refuge given the relatively large size of these prey (Marten & Reid, 2007).

130 *Lovenula raynerae* attack rates were significantly greater, whilst handling times significantly lower and,
131 inversely, maximum feeding rates higher, for small prey compared to large prey. However, the calanoid
132 copepod examined here may foster localised extinctions of mosquito populations under certain conditions
133 across their ontogeny. This is due to high levels of consumption at low resource densities, in light of the
134 Type II FR form exhibited for each prey size (Murdoch & Oaten, 1975). Indeed, these results corroborate
135 with those of Wasserman *et al.* (2015) whereby a destabilising Type II FR towards daphniids was exhibited
136 by *L. raynerae*. Though *L. raynerae* can handle particularly large quantities of early instar prey, predation
137 on late instars was marked relative to the predator size. As such, the strength of these biotic interactions

138 may drive profound impacts upon mosquito prey in aquatic systems, particularly as their predation has
139 proven robust to environmental variations (Cuthbert et al. 2018d). However, as context-dependencies e.g.
140 temperature (Cuthbert et al. 2018a) and alternative prey (Cuthbert et al. 2018e) can influence predator-
141 prey interaction strengths, examinations of additional environmental effects on predation rates of such
142 calanoid copepods towards target prey are urgently required.

143 In summary, we demonstrate that, contrary to suggestions that freshwater calanoids offer little value for
144 biocontrol (Marten & Reid, 2007), *L. raynerae* offers much potential. The species is a voracious,
145 carnivorous copepod, able to consume much higher numbers of mosquito prey than cyclopoid copepods
146 which are frequently considered in vector control strategies. Our findings suggest high and destabilising
147 predatory pressures from *L. raynerae* towards mosquito prey, with the potential to drive eradications.
148 Moreover, as *L. raynerae* is an ephemeral pond specialist species and lays desiccation-resistant eggs,
149 applications of their dormant eggs to transient aquatic habitats which foster mosquitoes may be efficacious
150 for vector control strategies. Thus, we propose further investigation into the predatory role of calanoid
151 copepods, an extremely diverse and widespread crustacean group, in the structuring of populations and
152 communities within aquatic ecosystems, alongside examinations of their efficacy as part of vector control
153 strategies.

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159 which was revised by all authors.

160 **References**

- 161 Carpenter, S.R., Kitchell, J.F. & Hodgson, J.R. (1985) Cascading trophic interactions and lake
162 productivity. *BioScience*, 3, 634–639.
- 163 Cuthbert, R.N., Dick, J.T.A., Callaghan, A. & Dickey, J.W.E. (2018a) Biological control agent selection
164 under environmental change using functional responses, abundances and fecundities; the
165 Relative Control Potential (RCP) metric. *Biological Control*, 121, 50–57.
- 166 Cuthbert, R.N., Dick, J.T.A., Callaghan, A. (2018b) Interspecific variation, habitat complexity and
167 ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control.
168 *Biological Control*, 121, 80–87.
- 169 Cuthbert, R.N., Dick, J.T.A., Callaghan, A. (2018c) Dye another day: the predatory impact of cyclopoid
170 copepods on larval mosquito *Culex pipiens* is unaffected by dyed environments. *Journal of*
171 *Vector Ecology*, in press.
- 172 Cuthbert, R.N., Dalu, T., Wasserman, R.J., Coughlan, N.E., Callaghan, A., Weyl, O.L.F., Dick, J.T.A.
173 (2018d) Muddy waters: efficacious predation of container-breeding mosquitoes by a newly-
174 described calanoid copepod across differential water clarities. Under review.
- 175 Cuthbert, R.N., Dickey, J.W.E., McMorro, C., Laverty, C., Dick, J.T.A. (2018e) Resistance is futile:
176 lack of predator switching and a preference for native prey predict the success of an invasive
177 prey species. *Royal Society Open Science*, in press.
- 178 Dalu, T., Wasserman, R.J., Vink, T.J.F., Weyl, O.L.F. (2017) Sex and species specific isotopic niche
179 specialisation increases with trophic complexity: evidence from an ephemeral pond ecosystem.
180 *Scientific Reports*, 7, 43229.
- 181 Dick, J.T.A., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J., Robinson, T.B. *et al.* (2014)
182 Advancing impact prediction and hypothesis testing in invasion ecology using a comparative
183 functional response approach. *Biological Invasions*, 16, 735–753.

- 184
185 Holling, C.S. (1959) Some characteristics of simple types of predation and parasitism. *The Canadian*
186 *Entomologist*, 91, 385–398.
- 187 Jeschke J.M., Kopp, M., Tollrian R. (2002) Predator functional responses: discriminating between
188 handling and digesting prey. *Ecological Monographs*, 72, 95–112.
- 189 Juliano, S.A. (2001) Nonlinear curve fitting: predation and functional response curves. In: Scheiner,
190 S.M. & Gurevitch, J. (Eds.) *Design and analysis of ecological experiments*. Oxford University
191 Press, UK, pp. 178–196.
- 192 Marten, G.G. & Reid, J.W. (2007) Cyclopid copepods. *Journal of the American Mosquito Control*
193 *Association*, 23, 65–92.
- 194 Murdoch, W.W. & Oaten, A (1975) Predation and population stability. *Advances in Ecological Research*,
195 9, 1–131.
- 196 Murdoch, W.W. (1969) Switching in general predators: experiments on predator specificity and stability
197 of prey populations. *Ecological Monographs*, 39, 335–354.
- 198 Pritchard, D.W., Paterson, R., Bovy, H.C. & Barrios-O’Neill, D. (2017) Frair: an R package for fitting
199 and comparing consumer functional responses. *Methods in Ecology and Evolution*, 8, 1528–
200 1534.
- 201 Solomon, M.E. (1949) The natural control of animal populations. *Journal of Animal Ecology*, 18, 1–35.
- 202 Suárez-Morales, E., Wasserman, R.J. & Dalu, T. (2015) A new species of *Lovenula* Schmeil (Copepoda,
203 Calanoida, Diaptomidae) from East Cape Province, South Africa. *Crustaceana*, 88, 324–342.
- 204 Vonesh, J.R. & Bolker, B.M. (2005) Compensatory larval responses shift trade-offs associated with
205 predator-induced hatching plasticity. *Ecology*, 86, 1580–1591.

206 Wasserman, R.J., Alexander, M.E., Barrios-O'Neill, D., Weyl, O.L.F. & Dalu, T. (2015) Using functional
207 responses to assess predator hatching phenology implications for pioneering prey in arid temporary
208 pools. *Journal of Plankton Research*, 38, 154–158.

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225 **Table 1.** First order terms and significance levels resulting from logistic regression of the proportion of
 226 prey eaten as a function of prey density, and FR parameter estimates (attack rate, a ; handling time, h)
 227 across differing prey treatments with significance levels resulting from Rogers' random predator equation
 228 with bootstrapped ($n = 2000$) 95% confidence intervals (CIs). Functional response parameters are
 229 estimated per hour ($T = 5$).

Prey size	1 st order term, p	a , p	95% CIs (a)	h , p	95% CIs (h)
Small	-0.04, < 0.001	1.08, < 0.001	0.55–3.07	0.19, < 0.001	0.13–0.25
Large	-0.03, < 0.001	0.20, < 0.001	0.11–0.42	0.47, < 0.001	0.30–0.69

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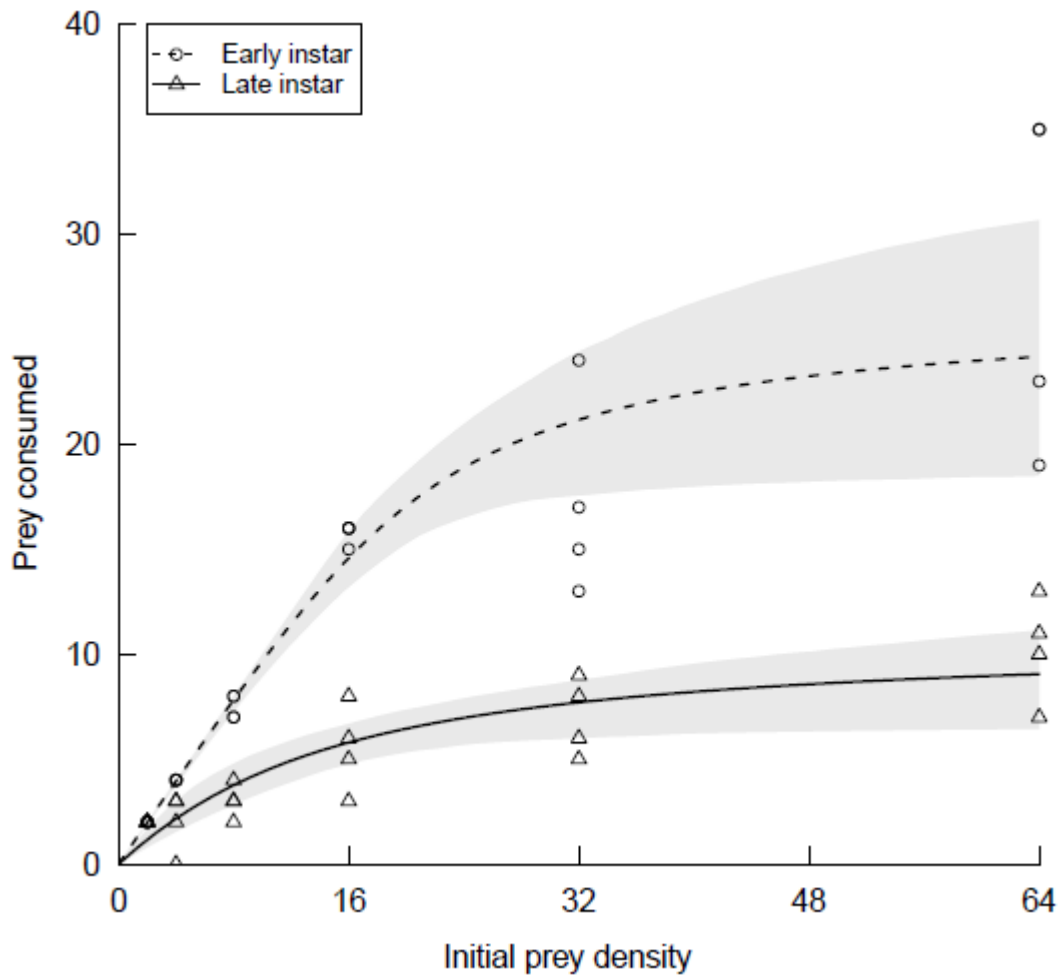
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245 **Fig. 1.** Functional responses of non-gravid adult female *Lovenula raynerae* towards early and late instar
 246 larval *Culex* prey over the total 5 hour experimental period. Shaded areas represent bootstrapped ($n =$
 247 2000) confidence intervals. Points are raw data ($n = 4$ per treatment group).