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1 SHORT COMMUNICATION

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

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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.

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Introduction

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

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

instar prey compared to later instar prey (χ^2 = 79.39, df = 1, p < 0.001) and increased significantly with

increasing prey supplied (χ^2 = 200.03, df = 5, p < 0.001). No significant 'prey size $\times$ prey supply'

interaction was detected (χ^2 = 4.06, df = 5, p = 0.54). Type II FRs were detected for both prey sizes (Table

1; Fig. 1), and 95% confidence intervals did not overlap across any prey supplies. Attack rates were

significantly higher towards early instar prey (z = 3.65, p < 0.001), evidenced by the steeper initial gradient

in the FR curve. Handling times were significantly reduced for smaller prey items (z = 3.15, p = 0.002),

with a greater maximum feeding rate reached here.

The application of FRs is relevant within the predatory biocontrol context, providing a mainstay to the

derivation of predator-prey interactions (Murdoch & Oaten, 1975). Here, we demonstrate that the calanoid

copepod *L. raynerae* can handle mosquito larvae throughout their ontogeny, with a potentially population-

destabilising Type II FR exhibited towards both early and late instar mosquito prey. These results are

promising when compared to similar studies on cyclopoid copepod predation efficiency (e.g. Cuthbert *et*

al. 2018a, b, c). Indeed, when prey was not limited, *L. raynerae* individuals consumed several multitudes

more culicid larvae than all cyclopoids investigated (Marten & Reid, 2007). In addition, unlike *L. raynerae*

predation as highlighted in this study, studies on cyclopoids have shown that late-stage mosquito larvae

experience refuge given the relatively large size of these prey (Marten & Reid, 2007).

Lovenula raynerae attack rates were significantly greater, whilst handling times significantly lower and,

inversely, maximum feeding rates higher, for small prey compared to large prey. However, the calanoid

copepod examined here may foster localised extinctions of mosquito populations under certain conditions

across their ontogeny. This is due to high levels of consumption at low resource densities, in light of the

Type II FR form exhibited for each prey size (Murdoch & Oaten, 1975). Indeed, these results corroborate

with those of Wasserman *et al*. (2015) whereby a destabilising Type II FR towards daphniids was exhibited

by *L. raynerae*. Though *L. raynerae* can handle particularly large quantities of early instar prey, predation

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.

154   **Acknowledgements**

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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 ($T = 5$).

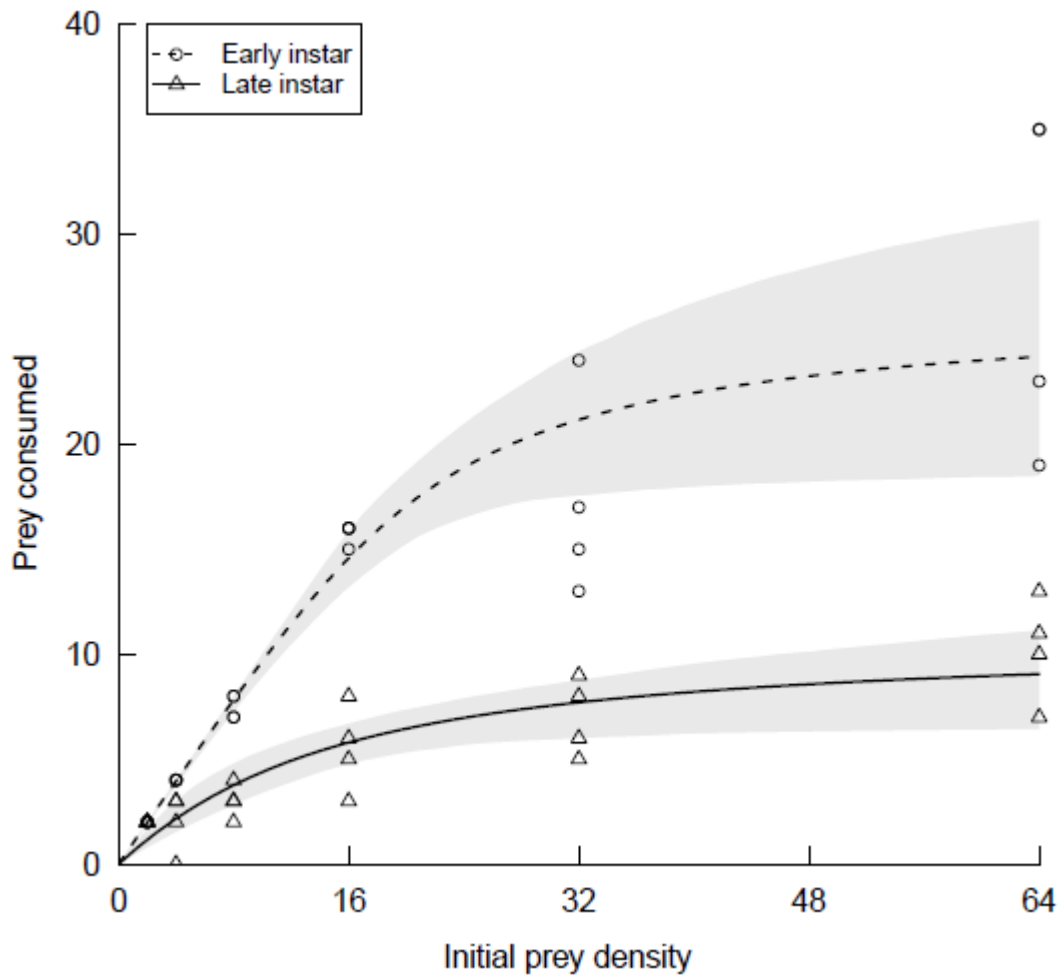| Prey size	1st order term, p	a, p	95% CIs (a)	h, p	95% CIs (h)
Small	-0.04, < 0.001	1.08, < 0.001	0.55–3.07	0.19, < 0.001	0.13–0.25
Large	-0.03, < 0.001	0.20, < 0.001	0.11–0.42	0.47, < 0.001	0.30–0.69

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Fig. 1. Functional responses of non-gravid adult female *Lovenula raynerae* towards early and late instar

larval *Culex* prey over the total 5 hour experimental period. Shaded areas represent bootstrapped (*n* =

2000) confidence intervals. Points are raw data (*n* = 4 per treatment group).