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SHORT COMMUNICATION

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

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Abstract

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

Keywords:

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

Introduction

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

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

Calanoid copepods form part of zooplankton assemblages which dominate ephemeral aquatic ecosystems, facilitated through *in situ* hatching of dormant eggs (Dalu *et al*. 2017). These atypical ecosystems are understudied given their impermanency and spatial heterogeneity, with interaction strengths between predators and their prey poorly constrained (though see Wasserman *et al*. 2015). Thus, examining the predatory potential of calanoids towards basal mosquito prey at differing ontogenetic stages would be informative towards interaction strength quantifications within ephemeral systems where mosquitoes often breed, further to the potential role of calanoid copepods in biocontrol. Accordingly, in this study we examine, using FRs, the predatory potential of *Lovenula raynerae* Suárez-Morales, Wasserman & Dalu 2015, a recently described and remarkably large freshwater calanoid (Suárez-Morales *et al*. 2015), towards different sized larvae of the vectorially-important *Culex pipiens* mosquito complex in order to constrain biocontrol efficacy and potential size refuge effects.

Materials and Methods

Adult *L. raynerae* were collected from an ephemeral pond in Bathurst, Eastern Cape, South Africa (33°29'21.4"S 26°49'48.4"E) using a 200 µm mesh net and transported in source water to a controlled environment room at Rhodes University, Grahamstown. Copepods were acclimated at 25 ±1 °C and under a 12:12 light:dark regime for 7 days, being fed on a standard diet of crushed fish flakes for the first 5 days (Aqua Plus, Grahamstown) and starved for the last 2 days in continuously aerated 25 L tanks containing dechlorinated tapwater. The focal prey, larvae of the *C. pipiens* complex, were cultured using egg rafts collected from artificial containers within the Rhodes University campus, and reared to the desired size class in the same laboratory using a diet of crushed rabbit pellets (Agricol, Port Elizabeth), supplied *ad libitum*. Non-gravid adult female copepods (5.1 ± 0.1 mm) were selected for experimentation and provided either early (1.4 ± 0.1 mm) or late (4.4 ± 0.2 mm) instar mosquito prey at six densities (2, 4, 8, 16, 32, 64; $n = 4$ per treatment group) in arenas of 5.6 cm diameter containing 80 mL dechlorinated tapwater from a continuously aerated source. Prey were allowed to settle for two hours before the addition of predators. Once predators were added they were allowed to feed undisturbed for 5 hours, after which they were removed and remaining live prey counted. Controls consisted of a replicate at each density and prey size class without a predator.

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

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

Eqn. 1.

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

Results and Discussion

No prey deaths occurred in controls and so mortality was deemed a result of predation by copepods, which was directly observed towards both prey sizes. Overall predation was significantly greater towards early instar prey compared to later instar prey ($\chi^2 = 79.39$, $df = 1$, $p < 0.001$) and increased significantly with increasing prey supplied ($\chi^2 = 200.03$, $df = 5$, $p < 0.001$). No significant 'prey size × prey supply' interaction was detected ($\chi^2 = 4.06$, $df = 5$, $p = 0.54$). 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 may drive profound impacts upon mosquito prey in aquatic systems, particularly as their predation has proven robust to environmental variations (Cuthbert et al. 2018d). However, as context-dependencies e.g. temperature (Cuthbert *et al*., 2018a) and alternative prey (Cuthbert *et al*., 2018e) can influence predator-prey interaction strengths, examinations of additional environmental effects on predation rates of such calanoid copepods towards target prey are urgently required.

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

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References

Carpenter, S.R., Kitchell, J.F. & Hodgson, J.R. (1985) Cascading trophic interactions and lake productivity. *BioScience*, 3, 634–639.

Cuthbert, R.N., Dick, J.T.A., Callaghan, A. & Dickey, J.W.E. (2018a) Biological control agent selection under environmental change using functional responses, abundances and fecundities; the Relative Control Potential (RCP) metric. *Biological Control*, 121, 50–57.

Cuthbert, R.N., Dick, J.T.A., Callaghan, A. (2018b) Interspecific variation, habitat complexity and ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control. *Biological Control*, 121, 80–87.

Cuthbert, R.N., Dick, J.T.A., Callaghan, A. (2018c) Dye another day: the predatory impact of cyclopoid copepods on larval mosquito *Culex pipiens* is unaffected by dyed environments. *Journal of Vector Ecology*, in press.

Cuthbert, R.N., Dalu, T., Wasserman, R.J., Coughlan, N.E., Callaghan, A., Weyl, O.L.F., Dick, J.T.A. (2018d) Muddy waters: efficacious predation of container-breeding mosquitoes by a newly-described calanoid copepod across differential water clarities. Under review.

Cuthbert, R.N., Dickey, J.W.E., McMorrow, C., Laverty, C., Dick, J.T.A. (2018e) Lack of predator switching and a preference for native prey predict the success of an invasive prey species. *Royal Society Open Science*, in press.

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Dalu, T., Wasserman, R.J., Vink, T.J.F., Weyl, O.L.F. (2017) Sex and species specific isotopic niche specialisation increases with trophic complexity: evidence from an ephemeral pond ecosystem. *Scientific Repoirts*, 7, 43229.

Dick, J.T.A., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J., Robinson, T.B. *et al*. (2014) Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biological Invasions*, 16, 735–753.

Holling, C.S. (1959) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, 91, 385–398.

Jeschke J.M., Kopp, M., Tollrian R. (2002) Predator functional responses: discriminating between handling and digesting prey. *Ecological Monographs*, 72, 95–112.

Juliano, S.A. (2001) Nonlinear curve fitting: predation and functional response curves. In: Scheiner, S.M. & Gurevitch, J. (Eds.) *Design and analysis of ecological experiments*. Oxford University Press, UK, pp. 178–196.

Marten, G.G. & Reid, J.W. (2007) Cyclopoid copepods. *Journal of the American Mosquito Control Association*, 23, 65–92.

Murdoch, W.W. & Oaten, A (1975) Predation and population stability. *Advances in Ecological Research*, 9, 1–131.

Murdoch, W.W. (1969) Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monographs*, 39, 335–354.

Pritchard, D.W., Paterson, R., Bovy, H.C. & Barrios-O'Neill, D. (2017) Frair: an R package for fitting and comparing consumer functional responses. *Methods in Ecology and Evolution*, 8, 1528–1534.

Solomon, M.E. (1949) The natural control of animal populations. *Journal of Animal Ecology*, 18, 1–35.

Suárez-Morales, E., Wasserman, R.J. & Dalu, T. (2015) A new species of *Lovenula* Schmeil (Copepoda, Calanoida, Diaptomidae) from East Cape Province, South Africa. *Crustaceana*, 88, 324–342.

Vonesh, J.R. & Bolker, B.M. (2005) Compensatory larval responses shift trade-offs associated with predator-induced hatching plasticity. *Ecology*, 86, 1580–1591.

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

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

Prey size	1st order term,	*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

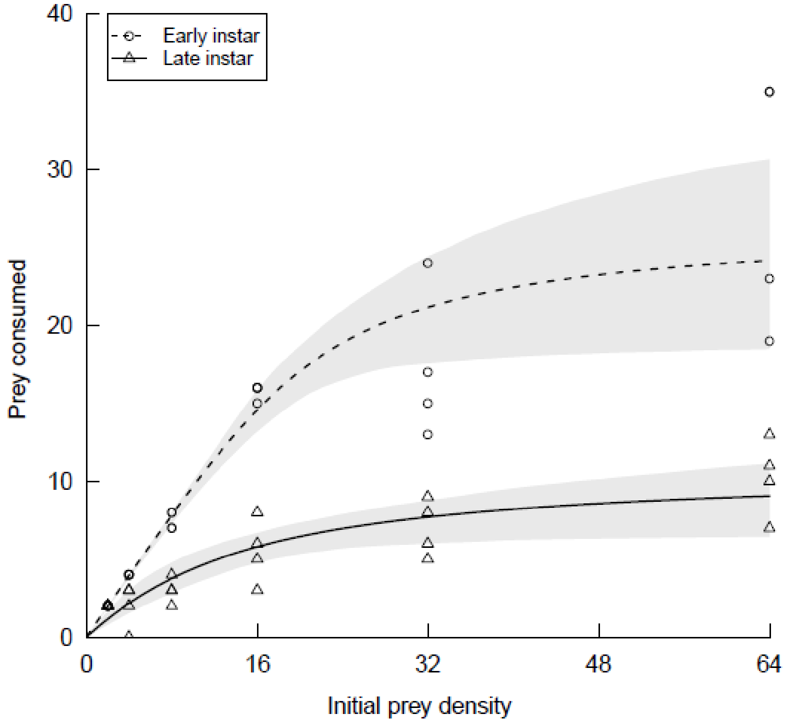

Fig. 1. Functional responses of non-gravid adult female *Lovenula raynerae* towards early and late instar larval *Culex* prey over the total 5 hour experimental period. Shaded areas represent bootstrapped (*n* = 2000) confidence intervals. Points are raw residuals (*n* = 4 per treatment group).