



# *Muddy waters: efficacious predation of container-breeding mosquitoes by a newly-described calanoid copepod across differential water clarities*

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1 **Muddy waters: efficacious predation of container-breeding mosquitoes by**  
2 **a newly-described calanoid copepod across differential water clarities**

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## 22 **Abstract**

23 Mosquito-borne diseases induce unrivalled morbidity and mortality in human  
24 populations. In recent times, greater urbanisation has facilitated vector population expansion,  
25 particularly of those which proliferate in container-style habitats. To combat increased  
26 associated disease risk, we urgently require innovative and efficacious control mechanisms to  
27 be identified and implemented. Predatory biological control of vectorially-important  
28 mosquitoes can be effective. Despite their high prevalence in freshwater ecosystems,  
29 predatory calanoid copepods have yet to be examined comprehensively for mosquito control.  
30 Moreover, environmental context-dependencies can cause substantial variations in natural  
31 enemy impacts on target populations. Accordingly, improved understanding of the effects of  
32 context-dependencies upon predatory biocontrol is needed. Here, we use functional responses  
33 (FRs) to examine the predatory impact of a newly-described species of calanoid copepod,  
34 *Lovenula raynerae*, upon larval *Culex pipiens* prey across variations in prey supply and water  
35 clarity. Using outdoor field trials, we assess the viability of *L. raynerae* in reducing mosquito  
36 survival in container-style habitats. *Lovenula raynerae* displayed destabilising Type II FRs  
37 towards larval mosquito prey across all water clarities tested, with overall predation rates  
38 remaining largely unaffected across all clarity treatments. In the outdoor experiment, *L.*  
39 *raynerae* applications resulted in substantial reductions in larval *C. pipiens* populations, with  
40 close to total eradication achieved following the experimental period under higher predator  
41 densities. These results demonstrate that environmental context such as water clarity may  
42 have little effect on vector control by calanoid copepods, which suggests a predatory reliance  
43 on hydromechanical signalling. Further, for the first time, we demonstrate the applicability of  
44 calanoid copepods to artificial container-style habitats where mosquitoes proliferate.  
45 Therefore, our results indicate that further examination into the applicability of this species  
46 group to aid vector biocontrol strategies is warranted.

47 **Keywords**

48 biological control; predator-prey; functional response; turbidity; *Culex pipiens*; *Lovenula*  
49 *raynerae*

50

51 **1. Introduction**

52 The effective control of mosquito-borne diseases and their vectors is of substantial  
53 public health importance (Mehlhorn, 2012; Beneli and Mehlhorn, 2016; WHO, 2017).  
54 Currently, a variety of chemical, physical, genetic and biological approaches are used to  
55 control mosquitoes (see Becker et al. 2010). However, many population management  
56 approaches are associated with drawbacks which impede their sustainability (e.g.  
57 Baldacchino et al. 2015). For instance, commonly-used insecticidal chemicals have caused  
58 environmental pollution, and emergent effects of insecticide resistance have presented major  
59 challenges to mosquito control strategies (e.g. Scholte et al. 2004; Ranson et al. 2016; Main  
60 et al. 2018). Mosquitoes which exploit artificial container-style habitats are of particular  
61 public health importance due to an association with urban areas and thus high potential for  
62 contact with human populations, wherein urban ‘heat islands’ can result in higher disease  
63 vector mosquito abundances (Townroe and Callaghan, 2014). Indeed, exploitation of human  
64 environments has facilitated invasive mosquito species to radically extend their geographic  
65 range (e.g. Lambrechts et al. 2010).

66 Biological control (hereafter biocontrol) provides a relatively environmentally-  
67 friendly and economical option in vector control (Rodríguez-Pérez et al. 2012). Natural  
68 enemies can efficaciously suppress vectorially-important mosquito populations (Marten,  
69 1990; Marten and Reid, 2007; Baldacchino et al. 2017; Cuthbert et al. 2018a, b; but see  
70 Thomas, 2018), and have successfully induced community-wide disease extirpations (Kay

71 and Nam, 2005; Nam et al. 2012). However, many candidate biocontrol agents remain  
72 entirely unexplored, or underexploited in the context of container-style aquatic habitats where  
73 vectorially-efficient mosquitoes can proliferate *en masse* (e.g. Townroe and Callaghan,  
74 2014). Biological control of larval mosquito populations by deliberate application of  
75 predatory copepod species has proven to be highly efficacious (reviewed by Marten and Reid,  
76 2007). Presently, however, only those present within the cyclopoid order have been examined  
77 and utilised for control. Yet, copepods represent a vastly extensive group of crustaceans,  
78 comprising a broad range of orders adapted to both ephemeral and perennial hydrologic  
79 ecosystems (Dussart and Defaye, 2001). Despite previous erroneous categorisation as  
80 herbivorous, considered unable to prey upon mosquito larvae (Marten and Reid, 2007),  
81 predatory calanoid copepods exist and can exert profound trophic impacts in aquatic  
82 environments (Wasserman et al. 2016a; Dalu et al. 2016a; Cuthbert et al. 2018d). Moreover,  
83 certain calanoid copepod species can be atypically large in size (e.g. Suárez-Morales et al.  
84 2015), and can therefore handle larval mosquito stages throughout their ontogeny (Cuthbert  
85 et al. 2018d). This contrasts to cyclopoid copepods which impart a size-refuge to larger prey  
86 (Marten and Reid 2007). Therefore, examining the efficacy of calanoid copepods towards  
87 container-breeding mosquitoes across ranging environmental contexts is of pertinence for the  
88 applied biocontrol of mosquito-borne disease vectors.

89 Environmental context-dependencies can cause substantial variations in natural  
90 enemy impacts on target populations (e.g. Cuthbert et al. 2018a), both as a result of biotic  
91 (e.g. Alexander et al. 2013; Barrios-O'Neill et al. 2014; Wasserman et al. 2016c) and abiotic  
92 (e.g. Wasserman et al. 2016b; Cuthbert et al. 2018a, b) factors, and is thus highly relevant to  
93 biocontrol agent selection. However, the implications of these context-dependencies on the  
94 efficacy of biocontrol agents often remain poorly understood. This, in turn, reduces the  
95 capacity of practitioners to fully understand and quantify biocontrol agent impacts. As

96 vectorially-efficient mosquito species are adapted to breed in a highly variable range of  
97 aquatic habitats (see Becker et al. 2010), understanding the implications of environmental  
98 context is integral to robust quantifications of biocontrol agent impacts on target mosquito  
99 species. Further, finding biocontrol agents that are also robust to environmental variability  
100 would be desirable. In particular, water clarity is highly variable in hydrological  
101 environments, and variations in water clarity can affect food webs through alterations of  
102 predation efficacy by visual predators (e.g. van De Meutter et al. 2005; Lunt and Smee,  
103 2015), manipulations of microhabitat structures and temperature regimes (e.g. Meysman et al.  
104 2006; Paaijmans et al. 2017), and by directly impacting filter feeders (e.g. Rellstab and Spak,  
105 2007), including larvae of many mosquito species. In addition, disease vector mosquitoes  
106 have been shown to be attracted to low-clarity habitats due to perceived higher nutritional loads  
107 or greater depth (Ortiz-Perea and Callaghan, 2017; Cuthbert et al. 2018b), with concurrent  
108 implications for mosquito abundances (e.g. Medlock and Vaux, 2014). Therefore, identifying  
109 biocontrol agents to target disease vector mosquitoes which are not impacted by turbid  
110 environments is crucial for successful field applications in diverse aquatic habitats (see  
111 Cuthbert et al. 2018c).

112         Functional responses (FRs), i.e. the *per capita* consumption rates of consumers with  
113 changes to resource densities (Solomon, 1949; Holling, 1959; Juliano, 2001), have been  
114 applied extensively to quantify the resource regulation potential of consumers (e.g. Abrams,  
115 1990; Dick et al. 2014), and can be applied to concurrently test environmental context-  
116 dependencies of consumer impact (e.g. South et al. 2017; Cuthbert et al. 2018a, b). Given that  
117 density- and context- dependencies of *per capita* impact may affect the viability of biocontrol  
118 agents in regulating target organisms (O’Neil, 1990; Van Driesche and Bellows, 1996;  
119 Cuthbert et al. 2018a), and the regulatory efficacy of many agents is yet to be explored, here,  
120 we examine the predatory potential of *Lovenula raynerae* Suárez-Morales, Wasserman and

121 Dalu 2015, a recently described and remarkably large (4 – 5 mm) calanoid copepod species,  
122 towards larvae of the disease vector complex *Culex pipiens* in container-style environments.  
123 *Lovenula raynerae* is a predatory ephemeral pond specialist species which hatches from  
124 dormant eggs within sediment during the early stages of hydroperiod (Suárez-Morales et al.  
125 2015; Wasserman et al. 2016a). Such ephemeral aquatic systems are highly varied with  
126 respect to their water clarity, particularly as a result of bioturbation which can heavily impact  
127 ecosystem functioning (e.g. Waterkeyn et al. 2016). Although high predatory impacts of *L.*  
128 *raynerae* have recently been described upon larval mosquitoes across their ontogeny  
129 (Cuthbert et al. 2018d), further research is required to elucidate additional context-  
130 dependencies of their impact, alongside assessments of their use in container-style habitats  
131 which foster disease vector mosquitoes (Townroe and Callaghan 2014). Therefore, the  
132 present study examines the FRs of *L. raynerae* towards larvae of the mosquito *C. pipiens*  
133 across a water clarity gradient, and also assesses the predation potential of the copepod in  
134 outdoor artificial container-style habitats under varying modes of predator and prey density.

## 135 **2. Materials and methods**

### 136 *2.1. Animal collection and rearing*

137 Adult male and female *L. raynerae* (4 – 5 mm) were collected from an ephemeral  
138 pond in the Eastern Cape, South Africa (33° 10' 04.1" S 27° 16' 10.6" E) by towing a 64 µm  
139 zooplankton net through the upper water column. Copepods were transported in source water  
140 to a controlled environment (CE) room at Rhodes University, Grahamstown (25 °C ± 1 °C;  
141 14:10 light:dark) and housed in 25 L aquaria containing strained (200 µm) water from the  
142 collection site prior to the experiments. *Culex pipiens* complex larvae originated from egg  
143 rafts collected from artificial container-style aquatic habitats on the Rhodes University



144 campus, and were reared to the desired size class on a diet of crushed rabbit food pellets  
145 (Agricol, Port Elizabeth).

## 146 2.2. Experimental protocols

147 We conducted two experiments to discern the efficacy of the calanoid copepod *L.*  
148 *raynerae* in mosquito control. In experiment 1, in the CE room, we quantified the effect of a  
149 water clarity gradient on the predatory impact of *L. raynerae* towards larval mosquito prey.  
150 Adult male *L. raynerae* were starved for 48 h prior to experimentation. Here, males were  
151 selected for experimentation to provide standardisation of predator type, given the various  
152 reproductive stages of female copepods that may influence predation rates. Functional  
153 responses of copepods were constrained under three water clarity treatments, conducive with  
154 the variability observed in ephemeral systems (Cuthbert, pers. obs.). Water clarity was  
155 defined as 0%, 50% and 100% against a predefined scale using a water clarity tube  
156 (GroundTruth, Leonard) by diluting turbid water to the prescribed clarity, with each treatment  
157 continuously aerated and filtered (200 µm) prior to use. *Culex pipiens* ( $3.3 \pm 0.2$  mm) larvae  
158 were established at five prey densities (2, 4, 8, 16, 32;  $n = 4$  per density) in 80 mL arenas of  
159 5.6 cm diameter containing the appropriate clarity treatment. Once predators were added,  
160 they were allowed to feed undisturbed for 6 h, after which they were removed and remaining  
161 prey counted to derive those killed. Controls consisted of three replicates at each density and  
162 clarity treatment without predators.

163 In experiment 2, we ascertained the efficacy of *L. raynerae* in outdoor artificial  
164 container-style habitats at regulating *C. pipiens* populations. This was done in a partially  
165 shaded outdoor location within the Rhodes University campus, similar to the environments  
166 from which *C. pipiens* rafts were collected. *Culex pipiens* larvae ( $1.89 \text{ mm} \pm 0.08$ ) were  
167 added at two densities (50, 100), to 2 L arenas of 13.5 cm diameter, each containing 1.5 L

168 filtered (200 µm) aerated water from the copepod collection site and 0.3 g of crushed rabbit  
169 food pellets. Then, three predator densities were added (0, 4, 8) in a fully randomised array.  
170 We maintained copepod sex ratios of 3:1 male:female across predator treatments (i.e. 0:0,  
171 3:1, 6:2) to minimise cannibalism (Lavens and Sorgeloos, 1996). After 72 h, the predators  
172 were removed and remaining live mosquito prey counted to derive the number eaten. We  
173 conducted at least three replicates per experimental group. Water temperatures within arenas  
174 were found to be within the 16 – 20 °C range across the duration of the experiment.

### 175 2.3. Statistical analyses

176 All statistical analyses were undertaken in R v3.4.2. (R Core Team, 2017). In  
177 experiment 1, generalised linear models (GLMs) assuming a Poisson error distribution were  
178 used to examine the effects of ‘water clarity’ and ‘prey density’ on raw prey consumption.  
179 All FR analyses were undertaken within the ‘frair’ package in R (Pritchard et al. 2017).  
180 Logistic regression considering the proportion of prey consumed as a function of the ‘prey  
181 density’ factor was used to infer FR types. Here, a Type II FR is determined categorically by  
182 a significantly negative first order term, and a Type III FR by a significantly positive first  
183 order term followed by a significantly negative second order term. We fit Rogers’ random  
184 predator equation to account for non-replacement of prey during the experiment (Trexler et  
185 al. 1998; Juliano, 2001):

$$186 \quad N_e = N_0(1 - \exp(a(N_e h - T)))$$

187 Eqn. 1.

188

189 where  $N_e$  is the number of prey eaten,  $N_0$  is the initial density of prey,  $a$  is the attack constant,  
190  $h$  is the handling time and  $T$  is the total experimental period. We applied the Lambert W  
191 function to fit the random predator equation (Bolker, 2008). The difference (delta) method

192 (see Juliano, 2001) was employed to compare FR attack rates and handling times between  
193 treatments with respect to the ‘water clarity’ factor. We applied Bonferroni corrections to  
194 account for multiplicity of comparisons (i.e.  $\alpha = 0.017$ ). Furthermore, we employed a non-  
195 parametric bootstrapping procedure ( $n = 2000$ ) to generate 95% confidence intervals around  
196 the FR curves (see Pritchard et al. 2017).

197 In experiment 2, GLMs assuming a quasibinomial error distribution, as residuals were  
198 found to be over-dispersed relative to degrees of freedom, were used to model mortality rates  
199 with respect to the ‘predator density’ and ‘prey density’ factors. Here, we used Tukey’s  
200 comparisons *via* the ‘multcomp’ package in R (Hothorn et al. 2008). In all cases, non-  
201 significant terms and interactions were removed stepwise to obtain models with maximal  
202 parsimony (as per Crawley, 2007).

### 203 **3. Results**

204 In experiment 1, survival in control groups was 100% and so experimental deaths of  
205 larval mosquitoes were attributed to predation by copepods, which were also observed eating  
206 the larvae. Overall consumption was not significantly affected by water clarity ( $\chi^2 = 1.76$ ,  $df$   
207  $= 2$ ,  $p = 0.42$ ) but increased significantly with higher prey densities ( $\chi^2 = 80.45$ ,  $df = 4$ ,  $p <$   
208  $0.001$ ). The consumptive effect of ‘prey density’ was not dependent on the water clarity as  
209 the ‘water clarity  $\times$  prey density’ effect was not significant ( $\chi^2 = 7.46$ ,  $df = 8$ ,  $p = 0.49$ ). Type  
210 II FRs were detected in all water clarity treatments (Table 1; Figure 1). Functional response  
211 parameters (attack rate,  $a$ ; handling time,  $h$ ) did not differ significantly between any water  
212 clarity treatment pairs (Table 1; Figure 1;  $a$ , low – medium,  $z = 0.64$ ,  $p = 0.52$ ;  $a$ , medium –  
213 high,  $z = 0.25$ ,  $p = 0.80$ ;  $a$ , low – high,  $z = 0.87$ ,  $p = 0.39$ ;  $h$ , low – medium,  $z = 0.55$ ,  $p =$   
214  $0.58$ ;  $h$ , medium – high,  $z = 0.79$ ,  $p = 0.43$ ;  $h$ , low – high,  $z = 0.32$ ,  $p = 0.75$ ).

215 In experiment 2, overall, the presence of *L. raynerae* resulted in significant larval  
216 mosquito reductions given that the ‘predator density’ factor significantly affected mortality  
217 rates ( $F_{2, 17} = 72.59, p < 0.001$ ; Figure 1). Greater mortality rates were found between all  
218 incremental predator density increases (0 – 4,  $z = 6.69, p < 0.001$ ; 4 – 8:  $z = 4.35, p < 0.001$ ; 0  
219 – 8,  $z = 9.90, p < 0.001$ ). Significantly greater mortality rates of larval mosquito prey were  
220 demonstrated under the lower prey density treatments overall ( $F_{1, 16} = 10.23, p = 0.006$ ;  
221 Figure 2). There was no ‘predator density × prey density’ interaction ( $F_{2, 14} = 1.37, p = 0.29$ ),  
222 and so the efficacy of *L. raynerae* at different densities was robust to treatment variations  
223 associated with prey density.

#### 224 **4. Discussion**

225 Here, for the first time, we demonstrate high *per capita* predation potential of an  
226 ephemeral pond specialist calanoid copepod, *L. raynerae*, towards vector mosquito prey  
227 irrespective of water clarity regime. Equally, in outdoor trials, we show that this species can  
228 substantially reduce larval mosquito abundances in container-style habitats which frequently  
229 foster vectorially-efficient mosquito species (Townroe and Callaghan, 2014). Copepods are  
230 highly efficacious predatory biocontrol agents for disease vectoring mosquitoes (Marten,  
231 1984; Marten and Reid, 2007; Cuthbert et al 2018a, b). Although biocontrol examinations  
232 have hitherto focused on cyclopid copepods, other groups of copepods are also predatory  
233 and thus may be of value in biocontrol strategies (Wasserman et al. 2016a; Cuthbert et al.  
234 2018d).

235 Functional responses of the calanoid copepod *L. raynerae* were not significantly  
236 affected by variations in water clarity, either in terms of form or magnitude. In a predation  
237 context, both FR form and magnitude are powerful predictors of the interaction strengths  
238 between predators and prey (Dick et al. 2014), and may be combined with predator

239 population responses to holistically assess ecological impact (Dick et al. 2017; Cuthbert et al.  
240 2018a, b). Three broad forms of FR have been defined (Hassell, 1978): the linear Type I,  
241 hyperbolic Type II and sigmoidal Type III. Whilst Type I FRs are mechanistically restricted  
242 to filter feeders (Jeschke et al. 2004), Type II FRs are conducive to high ecological impact as  
243 a result of high predation pressures at low prey densities (Dick et al. 2014). Accordingly,  
244 Type II FRs are particularly desirable in biocontrol contexts as they trend towards target prey  
245 eradications (Cuthbert et al. 2018a). However, Type III FRs are regarded as more stabilising,  
246 wherein there is a provisioning of low-density refugia for prey, driven, empirically, by  
247 processes such as prey switching (Hassell, 1978; Cuthbert et al. 2018e).

248         The present study observed Type II FRs regardless of water clarity regime. Therefore,  
249 *L. raynerae* is effectively able to locate, capture and handle prey at low densities, even in  
250 highly turbid conditions. These results are pertinent as ephemeral aquatic ecosystems, both  
251 natural and artificial, are highly varied with respect to their water clarity regime, for instance  
252 due to detritus inputs or bioturbation (Cuthbert, pers. obs.). Indeed, bioturbation associated  
253 with biocontrol agents can also affect the viability of multiple management interventions in  
254 aquatic habitats which target larval mosquitoes (e.g. Fry-O'Brien and Mulla, 1996).  
255 Mosquitoes often exhibit predator avoidance behaviours when ovipositing (see Vonesh and  
256 Blaustein, 2010), and water clarity can offset this avoidance behaviour under certain  
257 conditions (Cuthbert et al. 2018b). For this reason, identifying predatory agents which are  
258 unaffected by water clarity variations is imperative for effective biocontrol applications. Our  
259 results suggest a reliance on hydromechanical cues by this predatory calanoid copepod when  
260 detecting and capturing mosquito prey, as opposed to visual cues, which may account for the  
261 lack of overall consumptive variation between water clarity treatments. Indeed, these results  
262 corroborate with those demonstrating a lack of reliance on visual signals in cyclopoid

263 copepods when detecting prey across diurnal and water clarity regime shifts (e.g. Hwang and  
264 Strickler, 2001; Cuthbert et al. 2018c).

265           Attack rates of *L. raynerae* were relatively unaffected by variations in water clarity,  
266 and even trended towards being higher under lower water clarities. Attack rates correspond to  
267 the initial slope steepness in FR curves, and thus high attack rates can be particularly  
268 destabilising to prey populations at low prey densities. On the other hand, handling times  
269 reflect the asymptote in FR curves, and can be reciprocated to infer maximum feeding rates  
270 of predators (Dick et al. 2014; Cuthbert et al. 2018a). Here, although larval mosquitoes can  
271 exhibit high responsiveness to predatory cues (e.g. Zuharah and Lester 2011), it is feasible  
272 that low water clarity increases vulnerability to predation in larval mosquito prey, in turn  
273 enhancing the capture efficiency by predators at low prey densities. Furthermore, handling  
274 times were not significantly different across the water clarity gradient in the present study,  
275 and so maximum feeding rates were similar between treatments. Yet, handling times trended  
276 towards being lowest, and thus maximum feeding rates highest, at intermediate water  
277 clarities. Hence, we present strong and sustained destabilising predatory impacts of the  
278 calanoid copepod *L. raynerae* towards varying prey supplies of larval *C. pipiens* irrespective  
279 of this environmental context. Importantly, although FR examinations here only considered  
280 males, female *L. raynerae* are also voracious consumers of larval mosquitoes, with the  
281 potential to kill over 5 first instar larvae per hour and the ability to handle late instar prey  
282 (Cuthbert et al. 2018d). This intake rate is considerably higher than cyclopid copepods  
283 which are often used in biological control, and which also impart a size refuge to late instar  
284 mosquito prey (Marten and Reid, 2007).

285           The study also highlights that the *L. raynerae* predation efficiency observed under  
286 controlled laboratory conditions also persists in outdoor environments exposed to natural  
287 conditions, where vector mosquitoes proliferate. In outdoor experiments within container-

288 style habitats, *L. raynerae* induced substantial mortality rates in larval mosquito populations.  
289 As vectorially-important mosquitoes increasingly proliferate in such artificial, container-style  
290 habitats (Townroe and Callaghan, 2014), exploring the suitability for biocontrol agents over  
291 longer-term experiments in these environments is critical for empirical derivations of their  
292 efficacy. Indeed, these aquatic habitats can often be minute and ephemeral in nature, negating  
293 the use of larger larval mosquito antagonists, such as fish (see Azevedo-Santos et al. 2016).  
294 Our results demonstrate that, over the experimental period, higher densities of *L. raynerae*  
295 exhibited higher predation capacities relative to lower densities towards all prey supplies.  
296 Thus, multiple *L. raynerae* conspecifics may be additive in their consumption of *C. pipiens*  
297 larvae. Moreover, as *L. raynerae* is an ephemeral pond specialist and is capable of producing  
298 dormant, drought-resistant eggs, applications of this species to ephemeral aquatic habitats  
299 which foster mosquitoes may enable predator hatching *in situ* prior to, or simultaneous with,  
300 colonisation by mosquitoes. Although our results are theoretically promising in this respect,  
301 further research is required to test the efficacy of single applications of dormant eggs of *L.*  
302 *raynerae* over recurrent hydroperiods, and thus over longer experimental times overall.  
303 Furthermore, examinations of prey preferences and cannibalism in *L. raynerae* towards  
304 juveniles would be of value in further discerning factors that may impede their applied  
305 efficacy in biocontrol. However, it has been proposed that the most efficacious copepod  
306 species in biocontrol are able to curtail overpopulation and growth stunting *via* cannibalism  
307 of juveniles when the population becomes too high relative to the food supply (Marten and  
308 Reid, 2007).

309 In conclusion, our results suggest that calanoids and other copepod groups warrant  
310 further consideration as biocontrol agents of disease vector mosquitoes. In particular,  
311 ephemeral pond specialist species, such as *L. raynerae*, may be particularly promising  
312 candidates, as they are often particularly large, develop rapidly, exhibit dormancy, and have

313 adapted to occupy relatively high trophic levels (Dalu et al. 2016a). Further, their especially  
314 large size may make *L. raynerae* less vulnerable to higher-order predation as compared to  
315 physically smaller copepod species. Generally, biotic interactions within ephemeral aquatic  
316 ecosystems are often poorly studied due to spatial and temporal heterogeneity (Dalu et al.  
317 2016b), and thus these systems hold much potential for biocontrol agent exploration, as  
318 demonstrated in the present study. Our results show strong and destabilising predatory  
319 impacts of *L. raynerae* towards larvae of *C. pipiens* across a water clarity regime, which may  
320 enable consistently high impacts upon target populations under differing environmental  
321 conditions. Furthermore, we show efficacious predatory potential of this species in artificial  
322 container-style habitats in outdoor environments. Future research should test additional  
323 environmental contexts as to their effects on biotic interaction strengths between biocontrol  
324 agents and target organisms, and further explore the sustained potential of calanoid copepods  
325 over longer hydroperiods within container-style habitats which harbour disease vector  
326 mosquitoes.

## 327 **References**

328           Abrams, P.A., 1990. The effects of adaptive behaviour on the type-2 functional  
329 response. *Ecology* 71, 877–885.

330           Alexander, M.E., Dick, J.T.A., O'Connor, N.E., 2013. Trait-mediated indirect  
331 interactions in a marine intertidal system as quantified by functional responses. *Oikos* 122,  
332 1521–1531.

333           Azevedo-Santos, V.M., Vitule, J.R.S., Pelicice, F.M., García-Berthou, E., Simberloff,  
334 D, 2017. Non-native fish to control *Aedes* mosquitoes: A controversial, harmful tool.  
335 *BioScience* 67, 84–90.



336 Baldacchino, F., Caputo, B., Chandre, F., Drago, A., della Torre, A., Montarsi, F.,  
337 Rizzoli, A. 2015. Control methods against invasive *Aedes* mosquitoes in Europe: a review.  
338 Pest Manag. Sci. 71, 1471–1485.

339 Baldacchino, F., Bruno, M.C., Visentin, P., Blondel, K., Arnolid, D., Hauffe, H.C.,  
340 Rizzoli, A., 2017. Predation efficiency of copepods against the new invasive mosquito  
341 species *Aedes koreicus* (Diptera: Culicidae) in Italy. Eur. Zool. J. 84, 43–48.

342 Barrios-O'Neill, D., Dick, J.T.A., Emmerson, M.C., Ricciardi, A., MacIsaac, H.J.,  
343 Alexander, M.E., Bovy, H.C., 2014. Fortune favours the bold: a higher predator reduces the  
344 impact of a native but not an invasive intermediate predator. J. Anim. Ecol. 83, 693–701.

345 Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M.B., Dahl, C., Kaiser, A.,  
346 2010. Mosquitoes and their control. Springer-Verlag, Berlin/Heidelberg.

347 Benelli, G., Mehlhorn, H. 2016. Declining malaria, rising of dengue and Zika virus:  
348 insights for mosquito vector control. Parasitol. Res. 115, 1747–1754.

349 Bolker, B.M., 2008. Ecological Models and Data in R. Princeton University Press,  
350 Princeton.

351 Crawley, M.J., 2007. The R book. John Wiley & Sons, Chichester.

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

355 Cuthbert, R.N., Dick, J.T.A., Callaghan, A., 2018b. Interspecific variation, habitat  
356 complexity and ovipositional responses modulate the efficacy of cyclopid copepods in  
357 disease vector control. Biol. Control 121, 80–87.

358 Cuthbert, R.N., Callaghan, A., Dick, J.T.A., 2018c. Dye another day: the predatory  
359 impact of cyclopoid copepods on larval mosquito *Culex pipiens* is unaffected by dyed  
360 environments. *J. Vector Ecol.* In press.

361 Cuthbert, R.N., Dalu, T., Wasserman, R.J., Callaghan, A., Weyl, O.L.F., Dick, J.T.A.,  
362 2018d. Calanoid copepods: an overlooked tool in the control of disease vector mosquitoes. *J.*  
363 *Med. Entomol.* In press.

364 Cuthbert, R.N., Dickey, J.W.E., McMorrow, C., Lavery, C., Dick, J.T.A., 2018e.  
365 Resistance is futile: lack of predator switching and a preference for native prey predict the  
366 success of an invasive prey species. *Royal Soc. Open Sci.* In press.

367 Dalu, T., Weyl, O.L.F., Froneman, P.W., Wasserman, P.W., 2016a. Trophic  
368 interactions in an austral temperate ephemeral pond inferred using stable isotope analysis.  
369 *Hydrobiologia* 768, 81–94.

370 Dalu, T., Wasserman, R.J., Dalu, M.T.B., 2016b. Agricultural intensification and  
371 drought frequency increases may have landscape-level consequences for ephemeral  
372 ecosystems. *Glob. Change Biol.* 23, 983–985.

373 Dick, J.T.A., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J.,  
374 Robinson, T.B., Kumschick, S., Weyl, O.L.F., Dunn, A.M., Hatcher, M.J., Paterson, R.A.,  
375 Farnsworth, K.D., Richardson, D.M., 2014. Advancing impact prediction and hypothesis  
376 testing in invasion ecology using a comparative functional response approach. *Biol. Invasions*  
377 16, 735–753.

378 Dick, J.T.A., Lavery, C., Lennon, J.J., Barrios-O'Neill, D., Mensink, P.J., Britton, R.,  
379 Médoc, V., Boets, P., Alexander, M.E., Taylor, N.G., Dunn, A.M., Hatcher, M.J., Rosewarne,  
380 P.J., Crookes, S., MacIsaac, H.J., Xu, M., Ricciardi, A., Wasserman, R.J., Ellender, B.R.,  
381 Weyl, O.L.F., Lucy, F.E., Banks, P.B., Dodd, J.A., MacNeil, C., Penk, M.R., Aldridge, D.C.,

382 Caffrey, J.M., 2017. Invader Relative Impact Potential: a new metric to understand and  
383 predict the ecological impacts of existing, emerging and future invasive alien species. J.  
384 Appl. Ecol. 54, 1259–1267.

385 Dussart, B.H., Defaye, D., 2001. Introduction to the Copepoda. Guides to the  
386 Identification of the Microinvertebrates of the Continental Waters of the World 16. Backhuys  
387 Publishers, Leiden.

388 Fry-O'Brien, L.L., Mulla, M.S., 1996. Effect of tadpole shrimp, *Triops longicaudatus*,  
389 (Notostraca: Triopsidae), on the efficacy of the microbial control agent *Bacillus thuringiensis*  
390 var. *israelensis* in experimental microcosms. J. Am. Mosq. Contr. Assoc. 12, 33–38.

391 Hassell, M.P., 1978. The dynamics of arthropod predator-prey systems. Princeton  
392 University Press, Princeton.

393 Holling, C.S., 1959. Some characteristics of simple types of predation and parasitism.  
394 Can. Entomol. 91, 385–398.

395 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general  
396 parametric models. Biom. J. 50, 346–363.

397 Hwang, J.S., Strickler, R., 2001. Can copepods differentiate prey from predator  
398 hydromechanically? Zool. Stud. 40, 1–7

399 Jeschke, J.M., Kopp, M., Tollrian, R., 2004. Consumer-food systems: why type I  
400 functional responses are exclusive to filter feeders. Biol. Rev. 79, 337–349.

401 Juliano, S.A., 2001. Non-linear curve fitting: predation and functional response  
402 curves. In: Scheiner, S.M., Gurevitch, J. (Eds.), Design and Analysis of Ecological  
403 Experiments. Oxford University Press, Oxford, pp. 178–196.

404 Kay, B.H., Nam, V.S., 2005. New strategy against *Aedes aegypti* in Vietnam. *Lancet*  
405 365, 613–617.

406 Lambrechts, L., Scott, T.W., Gubler, D.J., 2010. Consequences of the expanding  
407 global distribution of *Aedes albopictus* for Dengue virus transmission. *PLoS Negl. Trop. Dis.*  
408 4, 645.

409 Lavens, P., Sorgeloos, P., 1996. Manual on the production and use of live food for  
410 aquaculture (No. 361). Food and Agriculture Organization (FAO), Rome.

411 Lunt, J., Smee, D.J., 2015. Turbidity interferes with foraging success of visual but not  
412 chemosensory predators. *PeerJ* 3, e1212.

413 Main, B.J., Everitt, A., Cornel, A.J., Hormozdiari, F., Lanzaro, G.C., 2018. Genetic  
414 variation associated with increased insecticide resistance in the malaria mosquito, *Anopheles*  
415 *coluzzii*. *Parasit. Vectors* 11, 225.

416 Marten, G.G., 1984. Impact of the copepod *Mesocyclops leuckarti pilosa* and the  
417 green alga *Kirchneriella irregularis* upon larval *Aedes albopictus* (Diptera: Culicidae). *Bull.*  
418 *Soc. Vector Ecol.* 9, 1–5.

419 Marten, G.G., 1990. Elimination of *Aedes albopictus* from tire piles by introducing  
420 *Macrocyclus albidus* (Copepoda, Cyclopidae). *J. Am. Mosq. Control Assoc.* 6, 689–693.

421 Marten, G.G., Reid, J.W., 2007. Cyclopoid copepods. *J. Am. Mosq. Control Assoc.*  
422 23, 65–92.

423 Medlock, J.M., Vaux, A.G.C., 2014. Colonization of a newly constructed urban  
424 wetland by mosquitoes in England: implications for nuisance and vector species. *J. Vector*  
425 *Ecol.* 39, 249–260.

426 Mehlnhorn, H., Al-Rasheid, K.A., Al-Quraishy, S., Abdel-Ghaffar, F. 2012. Research  
427 and increase of expertise in arachno-entomology are urgently needed. *Parasitol. Res.* 110,  
428 259–265.

429 Meysman, F.J.R., Middelburg, J.J., Heip, C.H.R., 2006. Bioturbation: a fresh look at  
430 Darwin's last idea. *Trends Ecol. Evol.* 21, 688–695.

431 Nam, V.S., Yen, N.T., Duc, H.M., Tu, T.C., Thang, V.T., Le, N.H., Le Loan, L.,  
432 Huong, V.T.Q., Khanh, L.H.K., Trang, H.T.T., Lam, L.Z.Y., Kutcher, S.C., Aaskov, J.G.,  
433 Jeffery, J.A.L., Ryan, P.A., Kay, B.H., 2012. Community-based control of *Aedes aegypti* by  
434 using *Mesocyclops* in Southern Vietnam. *Am. J. Trop. Med. Hyg.* 86, 850–859.

435 O'Neil, R.J., 1990. Functional response of arthropod predators and its role in the  
436 biological control of insect pests in agricultural systems. In: Dunn, P.E., Baker, R.R. (Eds.),  
437 *New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and*  
438 *Diseases.* Alan R Liss Inc, New York, pp. 83–96.

439 Ortiz-Perea, N., Callaghan, A., 2017. Pond dyes are *Culex* mosquito oviposition  
440 attractants. *PeerJ* 5, e3361.

441 Paaajmans, K., Takken, W., Githeko, A.K., Jacobs, A.F.G., 2017. The effect of water  
442 turbidity on the near-surface water temperature of larval habitats of the malaria mosquito  
443 *Anopheles gambiae*. *Int. J. Biomet.* 52, 747–753.

444 Pritchard, D.W., Paterson, R.A., Bovy, H.C., Barrios-O'Neill, D., 2017. Frair: an R  
445 package for fitting and comparing consumer functional responses. *Method. Ecol. Evol.* 8,  
446 1528–1534.

447 R Core Team, 2017. *R: A Language and Environment for Statistical Computing.* R  
448 Foundation for Statistical Computing, Vienna.

449 Ranson, H., Lissenden, N., 2016. Insecticide resistance in African *Anopheles*  
450 mosquitoes: a worsening situation that needs urgent action to maintain malaria control.  
451 Trends Parasitol. 32, 187–96.

452 Rellstab C., Spaak P., 2007. Starving with a full gut? Effect of suspended particles on  
453 the fitness of *Daphnia hyalina*. Hydrobiol. 594, 131–139.

454 Rodríguez-Pérez, M.A., Howard, A.F.V., Reyes-Villanueva, F., 2012. Biological  
455 Control of Dengue Vectors. In: Larramendy, M.L. and Soloneski, S. (Eds.) Integrated Pest  
456 Management and Pest Control – Current and Future Tactics. InTech. pp. 241–270.

457 Scholte, E.J., Bart, G., Knols, J., Samson, A., Takken, W., 2004. Entomopathogenic  
458 fungi for mosquito control: a review. J. Insect Sci. 4, 19.

459 Solomon, M.E., 1949. The natural control of animal populations. J. Anim. Ecol. 18,  
460 1–35.

461 South, J., Dick, J.T.A., 2017. Effects of acute and chronic temperature changes on the  
462 functional responses of the dogfish *Scyliorhinus canicula* (Linnaeus, 1758) towards  
463 amphipod prey *Echinogammarus marinus* (Leach, 1815). Environ. Biol. Fish. 100, 1251–  
464 1563.

465 Suárez-Morales, E., Wasserman, R.J., Dalu, T. 2015. A new species of *Lovenula*  
466 Schmeil (Copepoda, Diaptomidae) from the Eastern Cape province of South Africa.  
467 Crustaceana 88, 324–342.

468 Thomas, M.B. 2018. Biological control of human disease vectors: a perspective on  
469 challenges and opportunities. Biocontrol 63, 61–69.

470 Townroe, S., Callaghan, A., 2014. British container breeding mosquitoes: the impact  
471 of urbanisation and climate change on community composition and phenology. PLoS One 9,  
472 e95325.

473 Trexler, J.C., McCulloch, C.E., Travis, J., 1988. How can the functional response best  
474 be determined? Oecologia 76, 206–214.

475 Van de Meutter, F., Stoks, R., De Meester, L., 2004. Behavioural linkage of pelagic  
476 prey and littoral predators: microhabitat selection by *Daphnia* induced by damselfly larvae.  
477 Oikos 107, 265–272.

478 Van Driesche, R., Bellows, T.S., 2011. Biological Control. Springer, Berlin.

479 Vonesh, J.R., Blaustein, L., 2010. Predator-induced shifts in mosquito oviposition site  
480 selection: A meta-analysis and implications for vector control. Israel J. Ecol. Evol. 56, 123–  
481 139.

482 Wasserman, R.J., Alexander, M.E., Barrios-O’Neill, D., Weyl, O.L.F., Dalu, T.,  
483 2016a. Using functional responses to assess predator hatching phenology implications for  
484 pioneering prey in arid temporary pools. J. Plankt. Res. 38, 154–158.

485 Wasserman, R.J., Alexander, M.E., Weyl, O.J.F., Barrios-O’Neill, D., Froneman,  
486 P.W., Dalu, T., 2016b. Emergent effects of structural complexity and temperature on  
487 predator-prey interactions. Ecosphere 7, e01239.

488 Wasserman, R.J., Alexander, M.E., Dalu, T., Ellender, B.R., Kaiser, H., Weyl, O.L.F.,  
489 2016c. Using functional responses to quantify interaction effects among predators. Funct.  
490 Ecol. 30, 1988–1998.

491 Waterkeyn, A., Grillas, P., Brendonck, L., 2016. Experimental test of the ecosystem  
492 impacts of the keystone predator *Triops cancriformis* (Branchiopoda: Notostraca) in  
493 temporary ponds. *Freshwater Biol.* 61, 1392–1404.

494 World Health Organisation, 2017. Vector-borne diseases fact sheet.  
495 <http://www.who.int/mediacentre/factsheets/fs387/en/> (accessed 26 April 2018).

496 Zuharah, W.F., Lester, P.J., 2011. Are exotic invaders less susceptible to native  
497 predators? A test using native and exotic mosquito species in New Zealand. *Popul. Ecol.* 53,  
498 307–317.

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513 **Tables and Figure Captions**

514 Table 1. First order terms generated from logistic regression of proportional prey  
515 consumption as a function of prey density alongside parameter outputs from Rogers' random  
516 predator equation across water clarity regimes.

Water clarity	First order term, $p$	$a, p$	$h, p$
0%	-0.058620, < 0.001	1.572822, < 0.001	0.104547, < 0.001
50%	-0.044384, < 0.001	1.201939, < 0.001	0.087762, < 0.001
100%	-0.049398, < 0.001	1.082802, < 0.001	0.115452, < 0.001

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528 Figure 1. Functional responses of *Lovenula raynerae* towards larval *Culex pipiens* prey  
529 across a water clarity gradient (low, 0%; medium, 50%; high, 100%). Shaded areas represent  
530 bootstrapped ( $n = 2000$ ) confidence intervals.

531 Figure 2. Mortality rate ( $\pm$ SE) of larval *Culex pipiens* at different densities in outdoor trial  
532 resulting from the presence of predatory copepod *Lovenula raynerae* at three densities.

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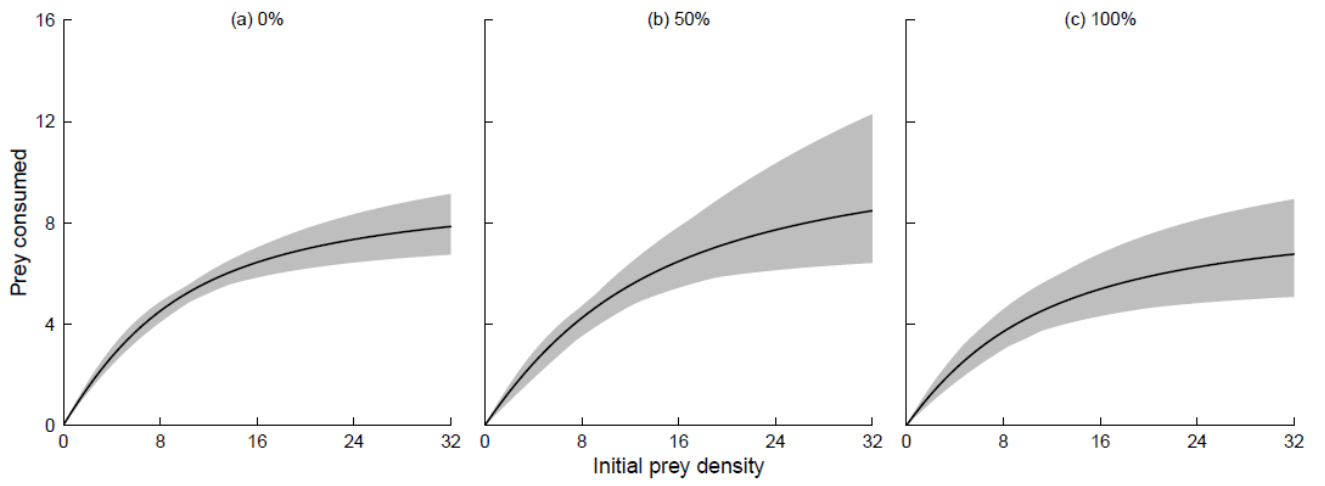
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