



**QUEEN'S
UNIVERSITY
BELFAST**

Additive multiple predator effects of two specialist paradiaptomid copepods towards larval mosquitoes

Cuthbert, R. N., Dalu, T., Wasserman, R. J., Weyl, O. L. F., Froneman, P. W., Callaghan, A., & Dick, J. T. A. (2019). Additive multiple predator effects of two specialist paradiaptomid copepods towards larval mosquitoes. *Limnologia*, 79, [125727]. <https://doi.org/10.1016/j.limno.2019.125727>

Published in:
Limnologia

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights

Copyright 2019 Elsevier.

This manuscript is distributed under a Creative Commons Attribution-NonCommercial-NoDerivs License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

1 *Research Article*

2 **Additive multiple predator effects of two specialist paradiaptomid**
3 **copepods towards larval mosquitoes**

4

5 Ross N. Cuthbert ^{1,2,3,*}, Tatenda Dalu ^{4,5}, Ryan J. Wasserman ^{6,5}, Olaf L. F. Weyl ², P.

6 William Froneman ⁷, Amanda Callaghan ³, Jaimie T. A. Dick ¹

7

8 ¹*Institute for Global Food Security, School of Biological Sciences, Queen's University*

9 *Belfast, Belfast BT9 5DL, Northern Ireland*

10 ²*DST/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African*

11 *Institute for Aquatic Biodiversity, Makhanda 6140, South Africa*

12 ³*Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading,*

13 *Harborne Building, Reading RG6 6AS, England*

14 ⁴*Department of Ecology and Resource Management, University of Venda, Thohoyandou*

15 *0950, South Africa*

16 ⁵*South African Institute for Aquatic Biodiversity, Makhanda 6140, South Africa*

17 ⁶*Department of Biological Sciences and Biotechnology, Botswana International University of*

18 *Science and Technology, Palapye, Botswana*

19 ⁷*Department of Zoology and Entomology, Rhodes University, Makhanda 6140, South Africa*

20

21 ***Corresponding author:** email, rossnoelcuthbert@gmail.com, tel: +44 28 4272 8230

22 **Abstract**

23 Interactions between multiple predators can profoundly affect prey risk, with implications for
24 prey population stability and ecosystem functioning. In austral temporary wetlands, arid-area
25 adapted specialist copepods are key predators for much of the hydroperiod. Limited
26 autoecological information relating to species interactions negates understandings of trophic
27 dynamics in these systems. In the present study, we examine multiple predator effects of two
28 key predatory paradiaptomid copepods which often coexist in austral temporary wetlands,
29 *Lovenula raynerae* Suárez-Morales et al., 2015 and *Paradiaptomus lamellatus* Sars, 1985.
30 Predation rates towards larval mosquito prey across different water depths are quantified.
31 Using a comparative functional response approach, individual *L. raynerae* exhibited
32 significantly higher feeding rates than *P. lamellatus*, characterised by higher attack rates,
33 shorter handling times and higher maximum feeding rates. Increasing water depth tended to
34 negatively affect prey consumption, particularly for *L. raynerae*. Interspecific multiple
35 predator consumption combined additively, and thus prey risks were well-predicted from
36 consumption rates by individual paradiaptomid copepod species, irrespective of water depth.
37 Our results suggest that increasing copepod predator diversity in austral temporary wetland
38 ecosystems additively heightens prey risk across different water volumes, and may help to
39 regulate disease vector mosquito populations. Therefore, the numerical extent, phenology and
40 diversity of predator hatching events during the hydroperiod may substantially mediate
41 interaction strengths in these ecosystems.

42

43 **Keywords**

44 zooplankton; temporary wetland; predator-predator interaction; larval mosquitoes; water
45 depth; functional response

46 **1. Introduction**

47 Predator-prey interaction strengths are pervasive in their influence on community
48 composition within ecosystems (Paine, 1980; Sih et al., 1985; Wasserman et al., 2013).
49 However, predator species rarely exist in isolation, with an array of trophic and non-trophic
50 interactions known to embed predators and their prey in complex ecological networks
51 (Thompson, 2005; Sentis et al., 2017; Veselý et al., 2017, 2019). For instance, predators do
52 not only interact with their prey, but may also interact with one another as they compete for
53 shared resources (Polis et al., 1989; Johnson et al., 2009; Wasserman et al., 2016a; Sentis and
54 Boukal, 2018). These interactions are recognised as important components that contribute to
55 outcomes of predator-prey interactions by mediating levels of prey risk (Sih et al., 1998;
56 Bolker et al., 2003; Griffen, 2006). In turn, such processes may impart stability to prey
57 populations if, for example, predation risk is reduced. Therefore, quantifications of multiple
58 predator effects are important for robust food web models (McCoy et al., 2012; Sentis and
59 Boukal, 2018), and for understanding the implications of predator extirpation implications
60 within ecosystems. Furthermore, such interactions may have ecological and even economic
61 implications, for instance in agroecosystems pest species regulation by multiple natural
62 enemies (Losey and Denno, 1998), or the regulation of pathogen and parasite vectors such as
63 mosquitoes (Cuthbert et al., 2019b).

64 Multiple predator effects can manifest in a number of ways in a prey risk context, and there
65 are many examples where predator impacts on prey do not combine additively (Beddington,
66 1975; Soluk, 1993; Sih et al., 1998; Barrios-O'Neill et al., 2014). In this context, predation
67 risk can either be greater (prey risk enhancement) or lesser (prey risk reduction) than that
68 expected based on additivity among predators (Griffen, 2006). A functional response
69 approach is often used to quantify the density-dependence of interaction strengths through
70 examination of the relationship between resource availability and resource consumption

71 (Holling, 1959). This density-dependence can, theoretically, have profound implications for
72 the stability of lower trophic groups within ecosystems, with hyperbolic Type II functional
73 responses regarded as destabilising for prey populations whilst, conversely, sigmoid Type III
74 functional responses may allow for stability of prey through low-density refuge effects
75 (Hassell, 1978). Despite the importance of density-mediated effects for predator-prey
76 dynamics, relatively few studies have examined multiple predator effects using a functional
77 response approach (Soluk, 1993; Losey and Denno, 1998; Lampropoulos et al., 2013;
78 Barrios-O'Neill et al., 2014; Wasserman et al., 2016a; Sentis et al., 2017; Veselý et al., 2017,
79 2019; Mofu et al., 2019). Furthermore, the limited work which has been done has produced
80 equivocal results, with examples of synergism, additivity and antagonism displayed among
81 predators. This demonstrates the complexity and system-specificity of predator-predator
82 exchanges that impedes broad-scale extrapolations across taxonomic groups and habitat
83 types.

84 Predation has a marked influence on zooplankton communities in aquatic ecosystems (Lynch,
85 1979; Neill, 1990; Wasserman et al., 2013). In temporary aquatic ecosystems, predation
86 pressure is transient owing to successive wet and dry periods coupled with temporal
87 variability in colonisation dynamics (Wasserman et al., 2016b, 2018). In the early-mid stages
88 of hydroperiod, internal recruitment of zooplankters from resting egg banks drives
89 community composition and biotic interactions (Brendonck and De Meester, 2003; O'Neill
90 and Thorp, 2014; Cuthbert et al., 2019c). Higher-order predators, such as hexapods, are
91 externally recruited later (O'Neill and Thorp, 2014; Wasserman et al., 2016b). Temporary
92 ponds can, accordingly, contain substantive and diverse abundances of zooplankton shortly
93 after wet phase initiation (O'Neill and Thorp, 2014), and some hatching copepods are
94 predatory (Wasserman et al., 2016b; Cuthbert et al., 2018a). Given that more than one
95 predatory copepod species often emerges from dormant eggs in these ecosystems (Day et al.,

96 2001; Wasserman et al., 2016b), predator-predator exchanges may have implications for
97 colonist prey. In the present study, we examined emergent multiple predator effects among
98 key pioneer zooplankters which inhabit austral temporary aquatic ecosystems.

99 Two pioneering paradiaptomid copepod species numerically dominate temporary wetland
100 ecosystems in the early stages of hydroperiod in the Eastern Cape province of South Africa,
101 *Lovenula raynerae* Suárez-Morales, Wasserman and Dalu, 2015 (Suárez-Morales et al.,
102 2015) and *Paradiaptomus lamellatus* Sars, 1985. Both species belong to Paradiaptomidae, a
103 temporary-water adapted African subfamily (Day et al., 2001). These copepod species are
104 atypically large (4 - 5 mm) and predatory, and their simultaneous occurrence in high
105 abundances drives high potential for interspecific predator-predator exchanges. Furthermore,
106 the two species appear to occupy distinct areas of the water column, with *L. raynerae* an
107 active forager in the open water, whilst *P. lamellatus* is an ambush hyperbenthic predator
108 (Cuthbert, pers. obs.). Given such spatial differences in habitat use, alongside inherent
109 variations in water volumes in temporary wetlands over the hydroperiod, it is possible that
110 differences in water depth further mediate interactions between these predatory species. For
111 example, in shallower systems, predator-predator encounters may be intensified due to spatial
112 overlaps in habitat use between predator species (see also Schmitz, 2007). In turn, this may
113 exacerbate or dampen emergent multiple predator effects. Furthermore, search area alone is
114 known to modulate predator-prey interactions, and this can be readily represented in
115 laboratory studies through systematic alterations to experimental arena size or volume
116 (Uiterwaal and DeLong, 2016).

117 In the present study, we quantify emergent multiple predator effects between *L. raynerae* and
118 *P. lamellatus* towards common larval mosquito prey, which also externally colonise
119 temporary pond environments during wet phases. We employ a comparative functional
120 response approach to elucidate density-dependence of multiple predator interactions and

121 examine whether differences in water depth further alter the nature of these interactions. As
122 relatively little information is available on the trophic ecology of austral temporary wetlands,
123 the present study provides valuable insights on predator-prey relationships in these food
124 webs, including for the regulation of pest and vector species such as mosquitoes.

125

126

127 **2. Materials and methods**

128

129 *2.1. Animal collection and maintenance*

130 Gravid adult females of *Lovenula raynerae* (mean length \pm 1 SE: 5.02 ± 0.07 mm) and
131 *Paradiaptomus lamellatus* (4.00 ± 0.07 mm) were collected from a temporary pond near the
132 town of Peddie in the Eastern Cape Province of South Africa ($33^{\circ}15'23.1''S$ $27^{\circ}02'48.1''E$)
133 by pulling a 64 μ m mesh zooplankton net through the water column. Copepods were
134 transported in source water to a controlled environment (CE) room in the Department of
135 Zoology and Entomology, Rhodes University, Makhanda and housed in separate 5 L aquaria
136 containing 100 μ m mesh-strained source water according to species. The CE room was
137 maintained at 21 ± 1 °C and under a 14:10 light and dark photoperiod regime. Copepods were
138 starved for 24 h prior to experimentation. The prey, second instar larval *Culex pipiens*
139 complex mosquitoes were cultured from egg rafts collected from container-style habitats on
140 the University campus, and reared to the desired size class (2.71 ± 0.11 mm) in the same CE
141 room on a diet of rabbit food pellets (Agricol, Port Elizabeth).

142

143 *2.2. Experimental design*

144 Consumption (i.e. numbers of prey killed or eaten) by individuals or interspecific pairs of the
145 two predatory copepods (i.e. *L. raynerae*; *P. lamellatus*; *L. raynerae* + *P. lamellatus*) was
146 quantified towards five densities of the larval mosquito prey (2; 4; 8; 16; 32) and under two
147 water depth (i.e. volume) treatments (40 mL; 80 mL) in a factorial design. The associated
148 water depths were 1.6 and 3.3 cm at 40 mL and 80 mL volumes, respectively. In the CE
149 room, mosquito prey were introduced under allotted densities into glass aquaria (5.6 cm dia.)
150 containing the allocated water depth in a randomised array. Prey were allowed to settle for 2
151 h, after which a pre-designated copepod predator treatment was introduced. Predators were
152 allowed to feed for 4 h, following which they were removed and remaining live prey
153 enumerated to quantify those killed. Three replicates were conducted per experimental group,
154 with one set of predator-free controls established under each prey density and depth
155 treatment. Whilst these two species have been suggested to engage in predator-prey
156 interactions, with *L. raynerae* feeding on *P. lamellatus* (Dalu et al., 2017), we did not observe
157 this behaviour in the present study and thus predator depletion and replacement was not a
158 factor.

159

160 2.3. Statistical analyses

161 Prey consumption (i.e. numbers of prey killed) was examined with respect to *predator* (3
162 levels), *depth* (2 levels) and *density* (5 levels), and their two- and three-way interactions, in a
163 Poisson generalised linear model with log link. Model averaging *via* a second-order
164 derivation of Akaike's information criterion (AICc) was used to select predictors which
165 minimised information loss (Burnham and Anderson, 2002). The initial tested model
166 included all fixed effects and their interaction terms. Model comparisons were made using
167 ΔAICc between the best model and model *i*. Models with $\Delta\text{AICc} \leq 2$ were considered

168 interchangeable (Burnham and Anderson, 2002). The relative variable importance (RVI) of
169 predictors was also calculated based on the sum of AICc model weights, whereby predictors
170 with RVI close to 1 are considered to yield high importance (Bartoń, 2016). Analysis of
171 deviance was used to discern effect sizes and significance levels of predictors in the top
172 model(s) (Fox and Weisberg, 2011), and Tukey comparisons were used *post-hoc* based on
173 estimated marginal means (Lenth, 2018).

174 Functional response analyses were undertaken phenomenologically (see Alexander et al.,
175 2012). Logistic regression was used to distinguish between functional response types (i.e.
176 type II or type III) among *predator* and *depth* treatments. Here, a significantly negative first-
177 order term indicates a type II functional response, whilst a significantly positive first-order
178 and significantly negative second-order term indicates a type III functional response (Trexler
179 et al., 1988; Juliano, 2001). Owing to the non-replacement of prey during the experiment,
180 Rogers' random predator equation was employed (Rogers, 1972):

$$181 \quad N_e = N_0(1 - \exp(a(N_e h - T))) \quad (1)$$

183 where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is
184 the handling time and T is the total experimental period. The Lambert W function was
185 implemented due to the recursive nature of the Random predator equation (Bolker, 2008).
186 Given that we did not empirically measure these parameter estimates, attack rates and
187 handling times were not considered mechanistically, but were instead used for comparative
188 purposes among the factorial experimental treatment groups (see Jeschke et al., 2002). Where
189 evidence for a given functional response type (i.e. type II or type III; see above) was
190 equivocal, a generalised form of the random predator equation with scaling exponent q was
191 fit (see Real, 1977; Vucic-Pestic et al., 2010), and suitability of each model compared based

192 on $\Delta AICc$. The use of the scaling exponent allows functional responses to be quantified in a
193 continuous manner between types II and III.

194 To quantify emergent multiple predator effects, we followed McCoy et al. (2012) and Sentis
195 and Boukal (2018) and employed a population dynamic approach. This method has been
196 shown to be more robust than other commonly-used approaches for simulating multiple
197 predator effects in additive experimental designs (Sentis and Boukal, 2018). We generated
198 predictions of consumption in paired copepod groups based on the a and h estimates from the
199 single predator treatments for both species and water depths:

$$\frac{dN}{dt} = - \sum_{i=1}^n f_i(N)P_i$$

201 (2)

202 where N is the prey population density, P_i ($i = 1, 2, \dots, n$) are the population densities of
203 predators i and $f_i(N)$ is the functional response of predator i (i.e. Eqn. 1). These predictions
204 were subsequently compared to actual experimental observations of paired predator
205 consumption to quantify multiple predator effects. Predictions were made under all
206 experimental prey densities, for the entire experimental duration. Global sensitivity analysis
207 was used to generate 95 % confidence intervals based on the a and h estimates, and their
208 standard errors and covariance-variance matrices, from the single predator treatments. A
209 Latin hypercube sampling algorithm was then used to generate parameter sets over time
210 (Soetaert and Petzoldt, 2010). For both predicted and observed functional responses, 95 %
211 confidence intervals were generated from 2.5 % and 97.5 % prey survival quantiles. Lower
212 confidence interval limits were capped at 0. Functional responses were then compared across
213 prey densities on the basis of confidence interval overlaps. Statistical analyses were
214 performed using R v3.5.1 (R Core Development Team, 2018).

215

216

217 **3. Results**

218 Two models with $\Delta\text{AICc} \leq 2$ were identified, and neither contained interaction terms. The
219 *predator* and *density* terms were retained in both top models (both RVI = 1.00), whilst *depth*
220 was present in one (RVI = 0.62). All interaction terms were relatively unimportant (all RVI \leq
221 0.06) and thus removed from the final model of prey mortality from consumption. Prey
222 consumption differed significantly according to the *predator* treatment ($\chi^2 = 109.30$, $\text{df} = 2$, p
223 < 0.001) (Fig. 1). *Paradiaptomus lamellatus* killed significantly fewer prey than *L. raynerae*
224 or paired predator treatments (both $p < 0.001$). Differences between *L. raynerae* and paired
225 predator groups were not statistically clear ($p = 0.94$). Numbers of prey killed increased
226 significantly with *density* ($\chi^2 = 152.15$, $\text{df} = 2$, $p < 0.001$). Whilst increasing *depth* tended to
227 reduce consumptive prey mortality (Fig. 1), the effect was not statistically clear ($\chi^2 = 3.18$, df
228 $= 1$, $p = 0.07$).

229 Evidence for type II functional responses was displayed under all predator treatments (Table
230 1). Whilst single *P. lamellatus* did not display a significantly negative first-order term, the
231 random predator equation provided a better fit than in the non-fixed presence of scaling
232 exponent q under both water depths (both $\Delta\text{AICc} > 2$). Attack rates were inclined to be
233 higher, handling times lower and thus maximum feeding rates greater for *L. raynerae*
234 compared to *P. lamellatus* at the shallower water depth (Table 1). Increasing water depths
235 generally lengthened handling times of *L. raynerae*, yet reduced those of *P. lamellatus*,
236 resulting in more similar maximum feeding rates at the higher depth between predator species
237 (Table 1). In turn, maximum feeding rates were similar under paired multiple predator
238 treatments between water depths.

239 At the shallow water depth (Fig. 2a), functional response confidence intervals tended to
240 diverge at low-intermediate prey densities, suggesting significant differences in feeding rates
241 in favour of *L. raynerae*. Multiple predator functional responses were well-predicted from
242 simulations based on individual consumption (Fig. 2b), and thus prey risk was additive here
243 as predator density increased. At the greater depth, functional responses were more similar
244 between predators (Fig. 2c), with confidence intervals overlapping at intermediate-high prey
245 densities. In turn, simulations of multiple predator functional responses were not significantly
246 different to those observed experimentally under the greater depth (Fig. 2d). Thus, additive
247 multiple predator effects between the copepod predators were observed across both levels of
248 water depth. Nevertheless, predictions of multiple predator functional responses tended to be
249 more similar to those observed under the greater water depth.

250

251

252 **4. Discussion**

253 The two key arid-area adapted specialist predatory copepods, *L. raynerae* and *P. lamellatus*,
254 displayed additive multiple predator effects irrespective of water depth in the present study.
255 As such, larval mosquito prey risk was predictable from simulations based on individual
256 predator functional response parameters. Nevertheless, significant differences in predatory
257 impact were exhibited between predators, with *L. raynerae* overall significantly more
258 voracious than *P. lamellatus*. This finding corroborates with a previous comparative
259 functional response study between the two copepod species individually towards cladoceran
260 prey (Wasserman et al., 2016a), and stable isotope analysis whereby *P. lamellatus* was
261 suggested to be a less predaceous than *L. raynerae* (Dalu et al., 2017). These consumptive
262 traits were generally consistent irrespective of water depth, however *P. lamellatus* tended to

263 be less affected by variations in water depth than *L. raynerae*. Although these species are
264 considered pioneers which hatch relatively early in the hydroperiod, they often persist to the
265 end of a pond's wet phase (Dalu et al., 2016). Therefore, our results are applicable to various
266 hydroperiod stages, with additive multiple predator effects driving increases in prey risk
267 synonymous with predator abundances in these systems.

268 The feeding magnitude of gravid female *L. raynerae* in the present study was shown to be
269 significantly higher than gravid *P. lamellatus*, irrespective of prey density and water depth.
270 Gravid female paradiaptomid copepods may be more voracious than males or non-gravid
271 females, influencing population-level interaction strengths (Cuthbert et al., 2019c).
272 Interspecific differences in predatory efficiencies between *L. raynerae* and *P. lamellatus*
273 appear to persist across sex demographics, as Wasserman et al. (2016b) demonstrated greater
274 functional response magnitudes of adult male *L. raynerae* over *P. lamallatus*. This
275 consumptive difference suggests that *L. raynerae* contributed most substantially to observed
276 predation rates in multiple predator groups. Furthermore, the lack of significant influence of
277 water depth (i.e. search area) on interaction strengths in the present study suggests that high
278 predation rates may be sustained throughout different pond water volumes during the
279 hydroperiod. This corroborates with the time-stresses associated with maturation and
280 reproduction during short hydroperiods. Nevertheless, increasing depth tended to relate
281 negatively to consumption overall, and this effect may become more pronounced under
282 greater experimental depths than tested here. Further, although the extrapolation of laboratory
283 results to empirical systems should be viewed with caution, comparative functional response
284 studies such as the present can provide useful phenomenological insights as to the effects of
285 environmental heterogeneity on interaction strengths (Jeschke et al., 2002; Dick et al., 2014),
286 and particularly in austral temporary wetland ecosystems where little autoecological
287 information currently exists.

288 Functional response form was unchanged across predator groups in the present study, with
289 Type II models providing the best fit to the data. The functional response of *P. lamellatus*
290 was, however, more equivocal in comparison to *L. raynerae*, with asymptotic declines in
291 consumption rates less marked. Wasserman et al. (2016b) found similarly equivocal results
292 between these species; however, in the present study, the categorical model minimised
293 information loss as compared to generalised forms of the functional response (see Pritchard et
294 al., 2017). This is also reflected in the considerable differences in attack rates between the
295 two species, with *L. raynerae* exhibiting a substantially higher attack coefficient. This may be
296 driven by higher encounter rates with prey, owing to the foraging behaviour of *L. raynerae* in
297 the open water. As attack rates correspond to the initial slope of functional response curves
298 (see Cuthbert et al., 2019d), high attack rates can be particularly destabilising to prey
299 populations at low densities. Accordingly, differences in predator hatching phenology from
300 egg banks in temporary wetlands may promote prey stability if community composition
301 favours high abundances of *P. lamellatus* over *L. raynerae*. Little is currently known about
302 environmental drivers that influence hatching success in austral temporary systems (but see
303 Mabidi et al., 2018), with many temporary-water inhabitants employing temporal bet hedging
304 strategies (Brendonck et al., 1998).

305 Copepods are present in most aquatic systems and often provide an important link between
306 trophic groups (i.e. from primary producers to predators) (Naganuma, 1996; Calbet and Saiz,
307 2005). In previous studies, copepods have been shown to exhibit all three classic functional
308 response forms (Paffenhöfer and Stearns, 1988; Hooff and Bollens, 2004; Jeschke et al.,
309 2004; Cuthbert et al., 2019a). While many freshwater copepods exhibit Type I functional
310 responses, this linear feeding response is mechanistically restricted to filter feeders, with
311 predatory copepods such as those in the present study subject to prey handling restraints
312 which cause asymptotic consumption declines with increasing prey densities (Jeschke et al.,

313 2002, 2004). Prey handling restraints were generally more pronounced in *P. lamellatus* as
314 compared to *L. raynerae* in the present study, with the latter species displaying substantially
315 higher maximum feeding rates. However, difference in feeding magnitude tended to be less
316 pronounced at the higher water depth between species, suggesting that *L. raynerae* is more
317 substantially impacted by changes in water depth, and thus search area, over the hydroperiod.
318 As *L. raynerae* is an active forager through the entire water column, alterations to water
319 volume likely drive differences in encounter rates for this species. By contrast, *P. lamellatus*,
320 as a hyperbenthic feeder, may be less affected by changes in water depth. The spatial
321 partitioning of these two key predatory copepods within aquatic habitats may limit the
322 potential for interspecific multiple predator effects which alter prey risk (see Schmitz, 2007),
323 with multiple predators shown to impact prey independently in the present study. The
324 additive multiple predator interaction found here corroborates with other studies on
325 freshwater crustaceans, where predator feeding rates also appear to combine independently
326 under certain conditions (Barrios-O'Neill et al., 2014). Accordingly, in temporary wetland
327 ecosystems, greater predator diversity likely does not result in significant alterations to prey
328 risk. Furthermore, given the potential importance of paradiaptomid copepods for the
329 regulation of vectorially-efficient mosquito species (see Cuthbert et al., 2018a), additive
330 multiple predator effects may further assist with the natural regulation of pests and disease.
331 As the coexistence of *L. raynerae* and *P. lamellatus* is common in austral temporary ponds in
332 their native range, our results contribute important information on the trophic dynamics of
333 these systems where little autoecological information currently exists. Predator diversity is
334 thus likely an important component that heightens interaction strengths in these aquatic
335 habitats.

336

337

338 **Acknowledgements**

339 This study was funded by the Department for the Economy, Northern Ireland. We extend
340 gratitude to Rhodes University for the provision of laboratory facilities. We acknowledge use
341 of infrastructure and equipment provided by the South African Institute for Aquatic
342 Biodiversity (SAIAB) Research Platform and the funding channelled through the National
343 Research Foundation – SAIAB Institutional Support system. This study was partly funded by
344 the National Research Foundation – South African Research Chairs Initiative of the
345 Department of Science and Technology (Inland Fisheries and Freshwater Ecology, Grant No.
346 110507). TD is supported by the National Research Foundation Thuthuka Grant (No.
347 117700).

348

349

350 **References**

- 351 Alexander, M.E., Dick, J.T.A., O'Connor, N.E., Haddaway, N.R., Fansworth, K.D., 2012.
352 Functional responses of the intertidal amphipod *Echinogammarus marinus*: effects of
353 prey supply, model selection and habitat complexity. *Mar. Ecol. Prog. Ser.* 468, 191–
354 202.
- 355 Barrios-O'Neill, D., Dick, J.T.A., Emmerson, M.C., Ricciardi, A., MacIsaac, H.J., Alexander,
356 M.E., Bovy, H.C., 2014. Fortune favours the bold: a higher predator reduces the
357 impact of a native but not an invasive intermediate predator. *J. Anim. Ecol.* 83, 693–
358 701.
- 359 Bartoń, K., 2016. MuMIn: Multi-model inference. R package

- 360 Beddington, J.R., 1975. Mutual interference between parasites or predators and its effect on
361 searching efficiency. *J. Anim. Ecol.* 44, 331–340.
- 362 Bolker, B.M., 2008. *emdbook: Ecological models and data in R*, R Package. Princeton
363 University Press, Princeton.
- 364 Bolker, B.M., Holyoak, M., Křivan, V., Rowe, L., Schmitz, O., 2003. Connecting theoretical
365 and empirical studies of trait-mediated interactions. *Ecology* 84, 1101–1114.
- 366 Brendonck, L., De Meester, L., 2003. Egg banks in freshwater zooplankton: evolutionary and
367 ecological archives in the sediment. *Hydrobiologia* 491, 65–84.
- 368 Brendonck, L., Riddoch, B.J., Van de Weghe, V., Van Dooren, T., 1998. The maintenance of
369 egg banks in very short lived pools – a case study with anostracans (Branchiopoda).
370 *Arch. Hydrobiol.* 52, 141–161.
- 371 Burnham, K.P., Anderson, D.R., 2002. *Model selection and multimodel inference. A*
372 *practical information-theoretic approach*. Springer, New York.
- 373 Calbet, A., Saiz, E., 2005. The ciliate-copepod link in marine ecosystems. *Aquat. Microb.*
374 *Ecol.* 38, 157–167.
- 375 Cuthbert, R.N., Callaghan, A., Dick, J.T.A., 2019a. The effect of the alternative prey,
376 *Paramecium caudatum* (Peniculida: Parameciidae), on the predation of *Culex pipiens*
377 (Diptera: Culicidae) by the copepods *Macrocyclus albidus* and *Megacyclus viridis*
378 (Cyclopoida: Cyclopidae). *J. Med. Entomol.* 56, 276–279.
- 379 Cuthbert, R.N., Callaghan, A., Sentis, A., Dalal, A., Dick, J.T.A., 2019b. Additive multiple
380 predator effects can reduce mosquito populations. *Ecol. Entomol.* in press.

381 Cuthbert, R.N., Dalu, T., Wasserman, R.J., Callaghan, A., Weyl, O.L.F., Dick, J.T.A., 2018a.
382 Calanoid copepods: an overlooked tool in the control of disease vector mosquitoes. *J.*
383 *Med. Entomol.* 25, 1656–1658.

384 Cuthbert, R.N., Dalu, T., Wasserman, R.J., Weyl, O.L.F., Callaghan, A., Froneman, P.W.,
385 Dick, J.T.A., 2019c. Sex-skewed trophic impacts in temporary wetlands. *Freshwater*
386 *Biol.* 64, 359–366.

387 Cuthbert, R.N., Dickey, J.W.E., Coughlan, N.E., Joyce, P.W.S., Dick, J.T.A., 2019d. The
388 Functional Response Ratio (FRR): advancing comparative metrics for predicting the
389 ecological impacts of invasive alien species. *Biol. Invasions* in press.

390 Dalu, T., Wasserman, R.J., Vink, T.J.F., Weyl, O.L.F., 2017. Sex and species specific
391 isotopic niche specialisation increases with trophic complexity: evidence from an
392 temporary pond ecosystem. *Sci. Rep.* 7, 43229.

393 Dalu, T., Weyl, O.L.F., Froneman, P.W., Wasserman, R.J., 2016. Trophic interactions in an
394 Austral temperate temporary pond inferred using stable isotope analysis.
395 *Hydrobiologia* 768, 81–94.

396 Day, J.A., De Moor, I.J., Stewart, B.A., Louw, A.E., 2001. Guides to the freshwater
397 invertebrates of Southern Africa. Vol 3: Crustacea II. Ostracoda, Copepoda and
398 Branchiura. Report No. TT148/01. Water Research Commission, Pretoria.

399 Dick, J.T.A., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J., Robinson, T.B.,
400 Kumschick, S., Weyl, O.L.F., Dunn, A.M., Hatcher, M.J., Paterson, R.A., Farnsworth,
401 K.D., Richardson, D.M., 2014. Advancing impact prediction and hypothesis testing in
402 invasion ecology using a comparative functional response approach. *Biol. Invasions*
403 16, 735–753.

- 404 Fox, J., Weisberg, S., 2011. An R companion to applied regression. Sage, Thousand Oaks.
- 405 Griffen, B.D., 2006. Detecting emergent effects of multiple predator species. *Oecologia* 148,
406 702–709.
- 407 Hassell, M.P., 1978. The dynamics of arthropod predator-prey systems. Princeton University
408 Press, Princeton.
- 409 Holling, C.S., 1959. Some characteristics of simple types of predation and parasitism. *Can.*
410 *Entomol.* 91, 385–398.
- 411 Hooff, R.C., Bollens, S.M., 2004. Functional response and potential predatory impact of
412 *Tortanus dextrilobatus*, a carnivorous copepod recently introduced to the San
413 Francisco Estuary. *Mar. Ecol. Progr. Ser.* 277, 167–179.
- 414 Jeschke, J.M., Kopp, M., Tollrian, R., 2002. Predator functional responses: discriminating
415 between handling and digesting prey. *Ecol. Monogr.* 72, 95–112.
- 416 Jeschke, J.M., Kopp, M., Tollrian, R., 2004. Consumer-food systems: why type I functional
417 responses are exclusive to filter feeders. *Biol. Rev.* 79, 337-349.
- 418 Johnson, P.T.J., Olden, J.D., Solomon, C.T., Vander Zanden, M.J., 2009. Interactions among
419 invaders: community and ecosystem effects of multiple invasive species in an
420 experimental aquatic system. *Oecologia* 159, 161–170.
- 421 Juliano, S.A., 2001. Non-linear curve fitting: predation and functional response curves, In:
422 Scheiner, S.M., Gurevitch, J. (Eds.), *Design and analysis of ecological experiments.*
423 Oxford University Press, Oxford, pp. 178–196.
- 424 Lampropoulos, P.D., Perdikis, D.C., Fantinou, A.A., 2013. Are multiple predator effects
425 directed by prey availability? *Basic Appl. Ecol.* 14, 605–613.

426 Lenth, R., 2018. emmeans: estimated marginal means, aka least-squares means. R package.

427 Losey, J.E., Denno, R.F., 1998. Positive predator – predator interactions: enhanced predation
428 rates and synergistic suppression of aphid populations. *Ecology* 79, 2143–2152.

429 Lynch, M., 1979. Predation, competition, and zooplankton community structure: an
430 experimental study. *Limnol. Oceanogr.* 24, 253–272.

431 Mabidi, A., Bird, M. S., Perissinotto, R. 2018. Increasing salinity drastically reduces hatching
432 success of crustaceans from depression wetlands of the semi-arid Eastern Cape Karoo
433 region, South Africa. *Sci Rep.*, 8, 5983.

434 McCoy, M.W., Stier, A.C., Osenberg, C.W., 2012. Emergent effects of multiple predators on
435 prey survival: the importance of depletion and the functional response. *Ecol. Lett.* 15,
436 1449–1456.

437 Mofu, L., South, J., Dalu, T., Wasserman, R.J., Woodford, D.J., Weyl, O.L.F., 2019.
438 Interspecific differences in invader and native fish functional responses illustrate
439 neutral effects on prey but superior invader competitive ability. *Freshwater Biol.* in
440 press.

441 Naganuma, T., 1996. Calanoid copepods: Linking lower-higher trophic levels by linking
442 lower-higher Reynolds numbers. *Mar. Ecol. Prog. Ser.* 136, 311–313.

443 Neill, W.E., 1990. Induced vertical migration in copepods as a defence against invertebrate
444 predation. *Nature* 345, 524-526.

445 O’Neill, B.J., Thorp, J.H., 2014. Untangling food-web structure in an temporary ecosystem.
446 *Freshwater Biol.* 59, 1462–1473.

447 Paffenhöfer, G.-A., Stearns, D.E., 1988. Why is *Acartia tonsa* (Copepoda: Calanoida)
448 restricted to nearshore environments? *Mar. Ecol. Progr. Ser.* 42, 33–38.

449 Paine, R.T., 1980. Food webs: linkage, interaction strength and community infrastructure. *J.*
450 *Anim. Ecol.* 49, 667–685.

451 Polis, G.A., Myers, C.A., Holt, R.D., 1989. The ecology and evolution of intraguild
452 predation: potential competitors that eat each other. *Annu. Rev. Ecol. Syst.* 20, 297–
453 330.

454 Pritchard, D.W., Paterson, R.A., Bovy, H.C., Barrios-O’Neill, D., 2017. Frair: an R package
455 for fitting and comparing consumer functional responses. *Methods Ecol. Evol.* 8,
456 1528–1534.

457 R Core Development Team., 2018. R: a language and environment for statistical computing.
458 Vienna.

459 Real, L.A., 1977. The kinetics of functional response. *Am. Nat.* 111, 289–299.

460 Rogers, D., 1972. Random search and insect population models. *J. Anim. Ecol.* 41, 369–383.

461 Schmitz, O.J., 2007. Predator diversity and trophic interactions. *Ecology* 88, 2415–2426.

462 Sentis, A., Boukal, D.S., 2018. On the use of functional responses to quantify emergent
463 multiple predator effects. *Sci. Rep.* 8, 11787.

464 Sentis, A., Gémard, C., Jaugeon, B., Boukal, D.S., 2017. Predator diversity and
465 environmental change modify the strengths of trophic and nontrophic interactions.
466 *Glob. Change Biol.* 23, 2629–2640.

467 Sih, A., Crowley, P., McPeck, M., Petranka, J., Strohmeier, K., 1985. Predation, competition
468 and prey communities: a review of field experiments. *Annu. Rev. Ecol. Syst.* 16, 269–
469 311.

470 Sih, A., Englund, G., Wooster, D., 1998. Emergent impacts of multiple predators on prey.
471 Trends Ecol. Evol. 13, 350–355.

472 Soetaert, K., Petzoldt, T., 2010. Inverse modelling, sensitivity and Monte Carlo analysis in R
473 using package FME. J. Stat. Softw. 33, 1–28.

474 Soluk, D.A., 1993. Multiple predator effects: predicting combined functional response of
475 stream fish and invertebrate predators. Ecology 74, 219–255.

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

479 Thompson, J.N., 2005. Coevolution: the geographic mosaic of coevolutionary arms races.
480 Curr. Biol. 15, 992–994.

481 Trexler, J.C., McCullough, C.E., Travis, J., 1988. How can the functional response best be
482 determined? Oecologia 76, 206–214.

483 Uiterwaal, S.F., DeLong, J.P., 2018. Multiple factors, including arena size, shape the
484 functional responses of ladybird beetles. J. Appl. Ecol. 55, 2429–2438.

485 Veselý, L., Boukal, D.S., Buřič, M., Kuklina, I., Fořt, M., Yazicioglu, B., Prechal, M., Kozák,
486 P., Kouba, A., Sentis, A., 2019. Temperature and prey density jointly influence
487 trophic and non-trophic interactions in multiple predator communities. Freshwater
488 Biol. in press.

489 Veselý, L., Boukal, D. S., Buřič, M., Kozák, P., Kouba, A., Sentis, A., 2017. Effects of prey
490 density, temperature and predator diversity on nonconsumptive predator-driven
491 mortality in a freshwater food web. Sci. Rep. 7, 18075.

492 Vucic-Pestic, O., Rall, B. C., Kalinkat, G., Brose, U., 2010. Allometric functional response
493 model: body masses constrain interaction strengths. *J. Anim. Ecol.* 79, 249–256.

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

497 Wasserman, R.J., Alexander, M.E., Dalu, T., Ellender, B.R., Kaiser, H., Weyl, O.L.F., 2016b.
498 Using functional responses to quantify interaction effects among predators. *Funct.*
499 *Ecol.* 30, 1988–1998.

500 Wasserman, R.J., Noyon, M., Avery, T.S., Froneman, P.W., 2013. Trophic level stability-
501 inducing effects of predaceous early juvenile fish in an estuarine mesocosm study.
502 *PLOS One* 8, e61019.

503 Wasserman, R.J., Weston, M., Weyl, O.L.F., Froneman, P.W., Welch, R.J., Vink, T.J., Dalu,
504 T., 2018. Sacrificial males: the potential role of copulation and predation in
505 contributing to copepod sex-skewed ratios. *Oikos* 127, 970–980.

506

507

508

509

510

511

512

513 **Table 1.** Functional response parameter estimates across predator treatments and between
 514 water depths. First-order terms are estimated from logistic regression of prey mortality rates
 515 from consumption as a function of density, whilst attack rate and handling time estimates are
 516 discerned from the random predator equation.

Predator(s)	Depth	First-order term, p	Attack rate, p	Handling time, h	Maximum feeding rate, $1/h$
<i>L. raynerae</i>	40	-0.08, < 0.001	4.52, 0.005	0.07, < 0.001	13.86
<i>P. lamellatus</i>	40	-0.03, 0.14	0.37, 0.05	0.16, 0.10	9.66
<i>L. raynerae</i> + <i>P. lamellatus</i>	40	-0.07, < 0.001	4.38, 0.004	0.08, < 0.001	13.32
<i>L. raynerae</i>	80	-0.08, < 0.001	5.38, 0.06	0.11, < 0.001	8.95
<i>P. lamellatus</i>	80	-0.01, 0.50	0.18, 0.04	0.09, 0.49	10.93
<i>L. raynerae</i> + <i>P. lamellatus</i>	80	-0.06, < 0.001	2.44, < 0.001	0.07, < 0.001	14.37

517

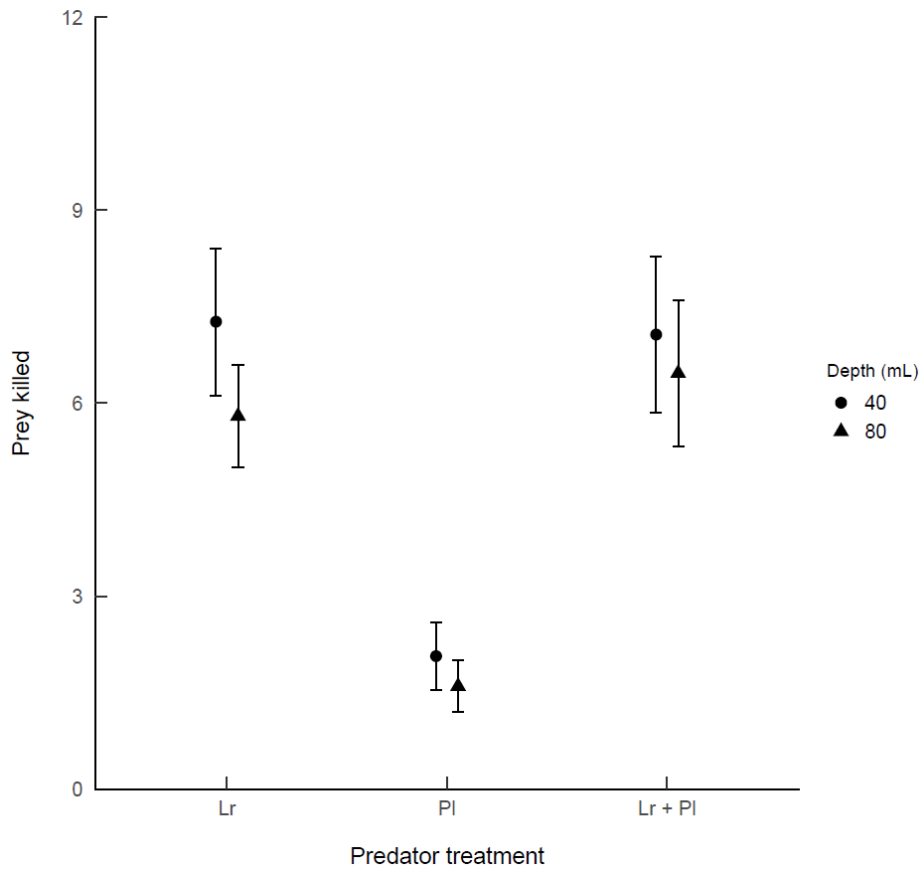
518

519

520

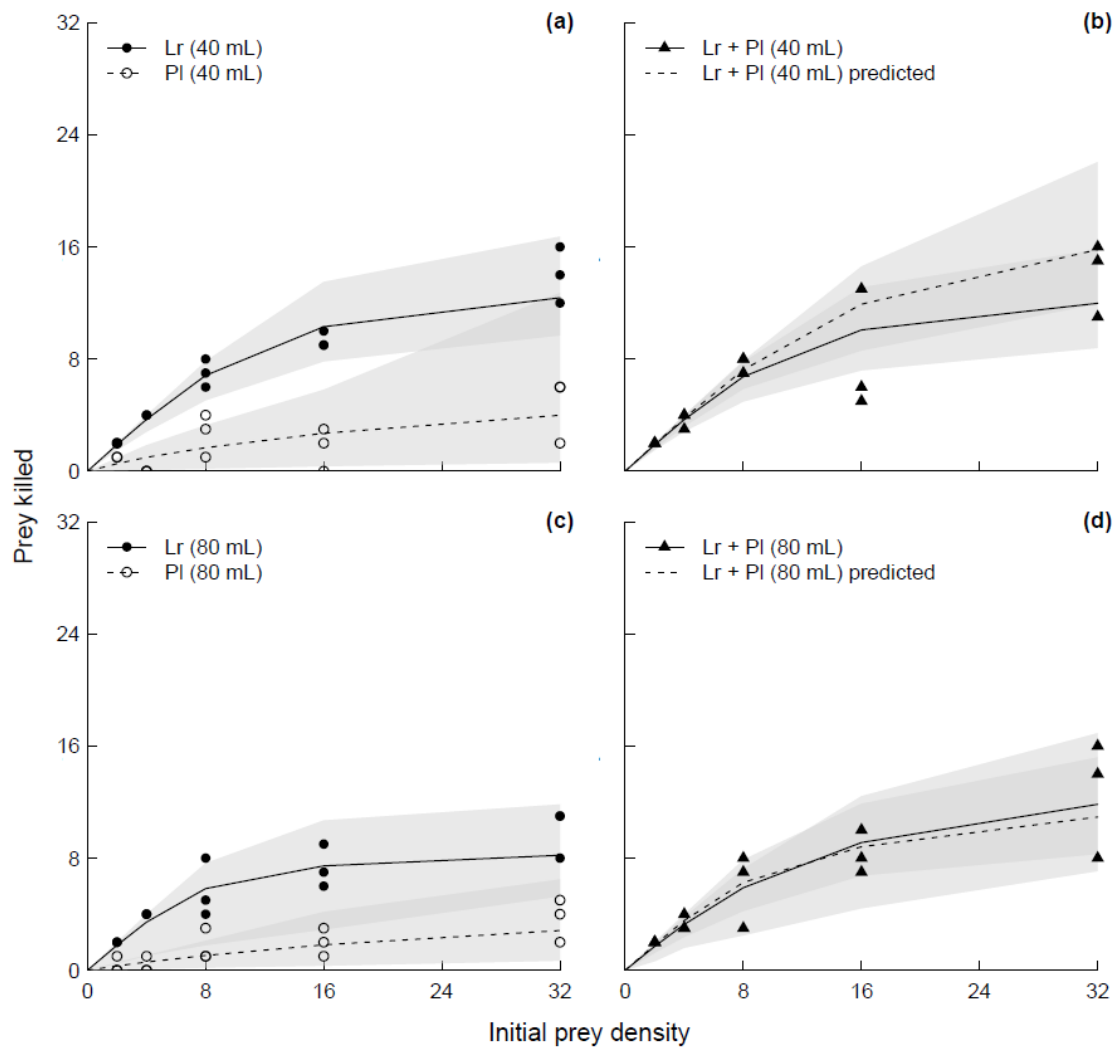
521

522



523

524 **Fig. 1.** Mosquito prey mortality from consumption under different predator treatments
 525 (*Lovenula raynerae*, Lr; *Paradiaptomus lamellatus*, Pl; *L. raynerae* + *P. lamellatus*, Lr + Pl)
 526 and between water depths. Means are ± 1 SE, pooled across all prey densities.



527

528 **Fig. 2.** Copepod functional responses considering mosquito prey mortality from consumption
 529 under different predator treatments (*Lovenula raynerae*, Lr; *Paradiaptomus lamellatus*, PI; *L.*
 530 *raynerae* + *P. lamellatus*, Lr + PI) and between water depths (40 mL: a, b; 80 mL: c, d). The
 531 left panels (a, c) display *per capita* feeding rates and the right panels total multiple predator
 532 feeding rates. Predictions of multiple predator feeding rates (b, d) are simulated based on
 533 individual consumption rates at corresponding depths (a, c) over the total experimental period
 534 (i.e. 4 h). Points are raw data and shaded areas are 95 % confidence intervals.