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1 *Short Communication*

2 **Quantifying reproductive state and predator effects on copepod motility in**  
3 **ephemeral ecosystems**

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21

22 **Abstract**

23 Ephemeral wetlands in arid environments are unique ecosystems with atypical trophic  
24 structuring, often dominated by invertebrate predation. Copepod behavioural traits and  
25 vulnerabilities to predation can vary substantially according to reproductive status. Gravid  
26 female copepods may be more vulnerable to predation due to reduced escape speeds or higher  
27 visibility for predators. Here, we quantify how reproductive status modulates horizontal  
28 motility rates of the predatory ephemeral pond specialist copepod *Lovenula raynerae*, and the  
29 responsiveness of the copepod to predator cues of the notonectid *Anisops debilis*. Males  
30 exhibited significantly higher motility rates than gravid female copepods, however chemical  
31 predator cues did not significantly influence activity rates in either sex. The lack of  
32 responsiveness to predator cues by specialist copepods in ephemeral wetlands may result  
33 from a lack of predation pressure in these systems, or due to time stress to reproduce during  
34 short hydroperiods. In turn, this could increase predation risk of copepods from externally-  
35 recruited top predators in ephemeral wetlands, and potentially contribute to the development  
36 of skewed sex ratios in favour of females.

37 **Keywords**

38 *Lovenula raynerae*; notonectid; hydroperiod; trait-mediated responses; temporary ponds; sex-  
39 skewed populations

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45 Ephemeral wetland ecosystems remain poorly studied due to high spatial and temporal  
46 heterogeneity, and function fundamentally differently from permanent waters (see Dalu et al.  
47 2017a). In arid environments, ephemeral aquatic ecosystems provide particularly important  
48 aquatic habitat patches for rare and endemic species (De Meester et al. 2005). Population  
49 demographics are known to vary profoundly over the hydroperiod in ephemeral wetlands  
50 (Wasserman et al. 2018; Cuthbert et al. 2019), and such variations can have marked  
51 implications for population success (Kiørboe 2006). In copepods, for example, predation  
52 pressure may not be equally shared by the sexes. Gravid female copepods may be more  
53 vulnerable to predation pressure as they are less agile and more conspicuous to predators  
54 (Maier 1995, Svensson 1997, Mahjoub et al. 2011). Behavioural examinations of copepods  
55 have, however, thus far focused on permanent aquatic ecosystems.

56 Ephemeral aquatic ecosystems exhibit atypical trophic structuring, where  
57 characteristically shallow food webs enable copepods to occupy high trophic levels (Dalu et  
58 al. 2017b). Predation risks are alleviated in these wetlands for some of the hydroperiod as  
59 many species are internally recruited (e.g. Wasserman et al. 2016), with higher order hexapod  
60 predation pressure (e.g. notonectids) arriving later (O'Neill and Thorp 2014, Wasserman et  
61 al. 2018). Generally, within aquatic systems, the prevalence of higher-order predatory cues  
62 profoundly affects invertebrate behaviour, for instance by reducing activity rates (Paterson et  
63 al. 2013; Alexander et al. 2013). Examinations of these trends are, however, lacking in  
64 ephemeral ecosystems where predation pressures are transient (Wasserman et al. 2018; but  
65 see Brendonck et al. 2002; De Roeck et al. 2005).

66 Here, we examine variabilities in motility rates according to reproductive status in the  
67 atypically large (4.0–5.0mm) and predatory ephemeral pond specialist copepod *Lovenula*  
68 *raynerae* Suárez-Morales, Wasserman, Dalu (Suárez-Morales et al. 2015) under the presence  
69 of different predator cue treatments. We were particularly interested in differences between

70 gravid and non-gravid copepods regarding invertebrate predation risk. Invertebrate predators,  
71 such as notonectids, utilise movement (visual) and hydromechanical cues for prey detection  
72 (e.g. Diéguez and Gilbert 2003). Responses of prey to predation threat may, therefore,  
73 involve a decrease in activity to avoid detection (passive avoidance), or an increase of  
74 activity for active avoidance. We hypothesised that males would exhibit active avoidance  
75 strategies, while gravid females would employ passive avoidance strategies, given the  
76 reduced mobility potential associated with the carrying of an egg-load of the latter.

77         Adult *L. raynerae* were sampled from an ephemeral wetland (33°10'04.1"S  
78 27°16'10.6"E) in the Eastern Cape, South Africa using a 64µm zooplankton net of 30cm  
79 diameter. Copepods were transported to a laboratory with a controlled environment (CE) at  
80 Rhodes University, Grahamstown, where adult males and females (4.5–5.0mm) were housed  
81 at 25±1°C in 25L tanks containing source pond water under a 14:10 light:dark photoperiod.  
82 Adults of the notonectid *Anisops debilis* Gersaeker (6.92±0.04mm) were collected by towing  
83 a kick net through an impounded stream (33°19'00.1"S 26°31'21.2"E) in Grahamstown and  
84 maintained in the same CE room. *Culex pipiens sensu lato* were collected from container-  
85 style habitats on the Rhodes University campus.

86         To derive the effects of sex and predator cues on copepod motility, we employed a  
87 2×2 experimental design with respect to sex (two levels) and predator cues (two levels). Only  
88 male and/or gravid female copepods were used for the study. Non-gravid females were,  
89 however, not employed as their reproductive (egg-production) state was difficult to determine  
90 and could have implications for behavioural performance. Adult male and gravid female *L.*  
91 *raynerae* were fed on a diet of *C. pipiens* larvae for 72h prior to use. To create chemical  
92 predator cues for experimental use, *A. debilis* were maintained in 2L aquaria of 16.5cm  
93 diameter at a stocking density of 7.5ind. L<sup>-1</sup> and fed *ad libitum* on a standard diet of *C.*  
94 *pipiens* larvae. After 48h of cue accumulation, water was extracted and strained through

95 200 $\mu$ m mesh for immediate use in the experiment. Control water was maintained in identical  
96 conditions, but in the absence of notonectid predators (i.e. 2L aquaria for 48h). Male and/or  
97 gravid female copepods were then added to jars of 5.6cm diameter, with a line marked across  
98 the middle of the bottom of the arena, containing 25mL of water of the allocated cue  
99 treatment, and allowed to settle for 20 min ( $n=12$  per experimental group). After this  
100 acclimation period, the number of line crosses was visually recorded over a 5min observation  
101 period. Lighting was positioned directly above arenas at an intensity of 930 lux.

102         Using the R environment, the effects of ‘sex’ and ‘predator cue’ on the number of line  
103 crosses exhibited were analysed using generalised linear models (GLMs) assuming a quasi-  
104 Poisson distribution as residuals were overdispersed. Non-significant terms and interactions  
105 were removed stepwise to maximise parsimony (Crawley, 2007).

106         Males exhibited an average of 22.54 ( $\pm$  SE:  $\pm$  1.55) line crosses, whilst gravid females  
107 exhibited 13.04 ( $\pm$  SE:  $\pm$  2.13). Overall, male *L. raynerae* exhibited significantly greater  
108 motility than gravid females ( $F_{(1,46)}=11.28$ ,  $p=0.002$ ; Fig. 1). The presence of predator cues  
109 had no effect on copepod motility ( $F_{(1,45)}=1.07$ ,  $p=0.31$ ). There was no significant ‘sex  $\times$   
110 predator cue’ interaction effect ( $F_{(1,44)}=0.52$ ,  $p=0.47$ ), indicating that the lack of predator cue  
111 effects on motility was consistent between copepod sexes.

112         The ephemeral pond-specialist copepod *L. raynerae* does not appear to use alternative  
113 anti-predation strategies, with respect to motility, based on reproductive state. Neither males  
114 nor females were responsive to the presence of predator cues from notonectids in terms of  
115 their horizontal motility levels. No significant increase or decrease in motility was detectable.  
116 Males were, however, more active than the gravid females irrespective of the predation cue  
117 treatment. A number of invertebrates have shown high responsiveness to predator cues (e.g.  
118 Paterson et al. 2013; Weiss et al. 2015) and even to conspecific alarm cues associated with

119 predation (Wasserman et al. 2014). It is plausible that the necessity of high resource  
120 acquisition for reproduction, compounded by time pressures associated with characteristically  
121 short hydroperiods in arid environments, may negate anti-predator responses of *L. raynerae*.  
122 Alternatively, these copepods may respond to conspecific alarm cues rather than predator  
123 cues; however, this was not tested in the study. Furthermore, the effects of predator cues on  
124 vertical migration strategies in copepods, alongside other anti-predator mechanisms, requires  
125 further investigation in heterogenous aquatic environments. Nevertheless, the lack of motility  
126 response to predation, coupled with consistently higher activity levels of males, may further  
127 increase the susceptibility of male copepods to predation by invertebrates such a notonectids,  
128 potentially further accentuating the development of female-skewed ratios which have been  
129 shown to arise in wild *L. raynerae* populations in ephemeral ponds (Wasserman et al. 2018).  
130 Thus, these traits may further contribute to the development of sex-skewed demographics  
131 within ephemeral ecosystems.

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207 Fig. 1. Effect of sex and notonectid cues on the motility (number of line crosses) of male and  
208 gravid female *Lovenula raynerae*. Means are  $\pm$  SE ( $n=12$  per experimental group).

209