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

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Abstract

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

Keywords

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

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

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

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

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

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

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

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

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

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

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

predation (Wasserman et al. 2014). It is plausible that the necessity of high resource acquisition for reproduction, compounded by time pressures associated with characteristically short hydroperiods in arid environments, may negate anti-predator responses of *L. raynerae*. Alternatively, these copepods may respond to conspecific alarm cues rather than predator cues; however, this was not tested in the study. Furthermore, the effects of predator cues on vertical migration strategies in copepods, alongside other anti-predator mechanisms, requires further investigation in heterogenous aquatic environments. Nevertheless, the lack of motility response to predation, coupled with consistently higher activity levels of males, may further increase the susceptibility of male copepods to predation by invertebrates such a notonectids, potentially further accentuating the development of female-skewed ratios which have been shown to arise in wild *L. raynerae* populations in ephemeral ponds (Wasserman et al. 2018). Thus, these traits may further contribute to the development of sex-skewed demographics 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 + SE (*n*=12 per experimental group).

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