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

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ABSTRACT

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

1. Introduction

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

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facilitated invasive mosquito species to radically extend their geographic range (e.g., Lambrechts et al., 2010).

Biological control (hereafter biocontrol) provides a relatively environmentally-friendly and economical option in vector control (Rodríguez-Pérez et al., 2012). Natural enemies can efficaciously suppress vectorially-important mosquito populations (Marten, 1990; Marten and Reid, 2007; Baldacchino et al., 2017; Cuthbert et al., 2018a,b; but see Thomas, 2018), and have successfully induced community-wide disease extirpations (Kay and Nam, 2005; Nam et al., 2012). However, many candidate biocontrol agents remain entirely unexplored, or underexploited in the context of container-style aquatic habitats where vectorially-efficient mosquitoes can proliferate *en masse* (e.g., Townroe and Callaghan, 2014). Biological control of larval mosquito populations by deliberate application of predatory copepod species has proven to be highly efficacious (reviewed by Marten and Reid, 2007). Presently, however, only cyclopoids have been examined and utilised for control. Yet, copepods represent a vastly extensive group of crustaceans, comprising a broad range of orders adapted to both ephemeral and perennial hydrologic ecosystems (Dussart and Defaye, 2001). Despite previous erroneous categorisation as herbivorous, considered unable to prey upon mosquito larvae (Marten and Reid, 2007), predatory calanoid copepods exist and can exert profound trophic impacts in aquatic environments (Wasserman et al., 2016a; Dalu et al., 2016a; Cuthbert et al., 2018d). Moreover, certain calanoid copepod species can be atypically large in size (e.g., Suárez-Morales et al., 2015), and can therefore handle larval mosquito stages throughout their ontogeny (Cuthbert et al., 2018d). This contrasts to cyclopoid copepods which impart a size-refuge to larger prey (Marten and Reid, 2007). Therefore, examining the efficacy of calanoid copepods towards container-breeding mosquitoes across ranging environmental contexts is of pertinence for the applied biocontrol of mosquito-borne disease vectors.

Environmental context-dependencies can cause substantial variations in ecological impacts on target populations (e.g., Cuthbert et al., 2018a), both as a result of biotic (e.g., Alexander et al., 2013; Barrios-O'Neill et al., 2014; Wasserman et al., 2016c) and abiotic (e.g., Wasserman et al., 2016b; Cuthbert et al., 2018a,b) factors, and are thus highly relevant to biocontrol agent selection. However, the implications of these context-dependencies on the efficacy of biocontrol agents often remain poorly understood. This, in turn, reduces the capacity of practitioners to fully understand and quantify biocontrol agent impacts. As vectorially-efficient mosquito species are adapted to breed in a highly variable range of aquatic habitats (see Becker et al., 2010), understanding the implications of environmental context is integral to robust quantifications of biocontrol agent impacts on target mosquito species. Further, finding biocontrol agents that are also robust to environmental variability would be desirable. In particular, water clarity is highly variable in hydrological environments, and variations in water clarity can affect food webs though alterations of predation efficacy by visual predators (e.g., Van de Meutter et al., 2004; Lunt and Smee, 2015), manipulations of microhabitat structures and temperature regimes (e.g., Meysman et al., 2006; Paaijmans et al., 2017), and by directly impacting filter feeders (e.g., Rellstab and Spaak, 2007), including larvae of many mosquito species. In addition, disease vector mosquitoes have been shown be attracted to low-clarity habitats due to perceived higher nutritional loads or greater depth (Ortiz-Perea and Callaghan, 2017; Cuthbert et al., 2018b), with concurrent implications for mosquito abundances (e.g., Medlock and Vaux, 2014). Therefore, identifying biocontrol agents to target disease vector mosquitoes which are not impacted by turbid environments is crucial for successful field applications in diverse aquatic habitats (see Cuthbert et al., 2018c).

Functional responses (FRs), i.e., the *per capita* consumption rates of consumers with changes to resource densities (Solomon, 1949; Holling, 1959; Juliano, 2001), have been applied extensively to quantify the resource regulation potential of consumers (e.g., Abrams, 1990; Dick et al., 2014), and can be applied to concurrently test environmental context-dependencies of consumer impact (e.g., South and Dick, 2017; Cuthbert et al., 2018a,b). Given that density- and context-dependencies of *per capita* impact may affect the viability of biocontrol agents in regulating target organisms (O'Neil, 1990; Van Driesche and Bellows, 2011; Cuthbert et al., 2018a), and the regulatory efficacy of many agents is yet to be explored, here, we examine the predatory potential of *Lovenula raynerae* Suárez-Morales, Wasserman and Dalu 2015, a recently described and remarkably large (4–5 mm) calanoid copepod species, towards larvae of the disease vector complex *Culex pipiens* in container-style environments. *Lovenula raynerae* is a predatory ephemeral pond specialist species which hatches from dormant eggs within sediment during the early stages of hydroperiod (Suárez-Morales et al., 2015; Wasserman et al., 2016a). Such ephemeral aquatic systems are highly varied with respect to their water clarity, particularly as a result of bioturbation which can heavily impact ecosystem functioning (e.g., Waterkeyn et al., 2016). Although high predatory impacts of *L. raynerae* have recently been described upon larval mosquitoes across their ontogeny (Cuthbert et al., 2018d), further research is required to elucidate additional context-dependencies of their impact, alongside assessments of their use in container-style habitats which foster disease vector mosquitoes (Townroe and Callaghan 2014). Therefore, the present study examines the FRs of *L. raynerae* towards larvae of the mosquito *C. pipiens* across a water clarity gradient, and also assesses the predation potential of the copepod in outdoor artificial container-style habitats under varying modes of predator and prey density.

2. Materials and methods

2.1. Animal collection and rearing

Adult male and female *L. raynerae* (4–5 mm) were collected from an ephemeral pond in the Eastern Cape, South Africa (33°10′04.1″S 27°16′10.6″E) by towing a 64 µm zooplankton net through the upper water column. Copepods were transported in source water to a controlled environment (CE) room at Rhodes University, Grahamstown (25 °C ± 1 °C; 14:10 light:dark) and housed in 25 L aquaria containing strained (200 µm) water from the collection site prior to the experiments. *Culex pipiens* complex larvae originated from egg rafts collected from artificial container-style aquatic habitats on the Rhodes University campus, and were reared to the desired size class on a diet of crushed rabbit food pellets (Agricol, Port Eizabeth).

2.2. Experimental protocols

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

In experiment 2, we ascertained the efficacy of *L. raynerae* in outdoor artificial container-style habitats at regulating *C. pipiens*