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Alternative prey impedes the efficacy of a natural enemy of mosquitoes

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Abstract

Adaptive foraging behaviour in the presence of multiple prey types may mediate stability to predator-prey relationships. For biological control agents, the presence of alternative prey may thus reduce ecological impacts towards target organisms, presenting a key challenge to the derivation of agent efficacies. Quantifications of non-target effects are especially important for generalist biocontrol agents in their regulation of pests, vectors and invasive species. We examined the predatory impact of the notonectid *Anisops debilis* towards larvae of the vector mosquito complex *Culex pipiens* in the presence of varying densities of alternative daphniid prey. Experimentally, we quantified functional responses of *A. debilis* towards target mosquito prey under different background daphniid compositions, and also tested for prey switching propensities by the notonectid predator. Increasing background densities of daphniids significantly reduced the predatory impact of notonectids on mosquitoes, characterised by reductions in attack rates, lengthening of handling times and lessening of maximum feeding rates. Furthermore, notonectids displayed a significant selective preference in favour of daphniid prey over mosquitoes, irrespective of prey proportions in aquatic environments. Accordingly, notonectids did not display a prey switching pattern. We thus demonstrate that the presence of alternative prey can dampen predatory impacts of notonectids towards mosquitoes, as compared to more simplistic pairwise systems with singular prey choice. Accordingly, the effects of stabilising mechanisms, such as adaptive foraging, should be further integrated in biocontrol agent assessments.

Key words

functional response; prey switching; prey preference; trophic interaction strength; *Anisops*; *Culex*

Introduction

Predatory interactions can profoundly influence population- and community-level stabilities (Paine, 1980; Sih et al., 1985; Dick et al., 2017). Within ecosystems, predators can drive cascading effects across trophic levels through both density- and trait-mediated interactions with their prey (Connell, 1972; Paine, 1980; Werner and Peacor, 2003). However, experimental and theoretical inferences of community dynamics are frequently grounded in interaction strengths between simplified consumer-resource pairings, which ignore additional environmental complexities (Bolker et al., 2003; Werner and Peacor, 2003; Schmitz, 2007). Rather, a community module approach, comprising three or more interacting species, provides a powerful tool to examine the effect of predation on community composition (Holt, 1997; Paterson et al., 2015). In particular, predators in the wild are often presented with multiple prey types, which may act as a stabilising mechanism within food webs (Polis et al., 1989). Indeed, adaptive feeding behaviours have been identified as key stabilising components which reduce prey risk, alongside factors such as habitat complexity, antipredator behaviour, omnivory and cannibalism (Kratina et al., 2012).

Predator-prey interaction strengths have been harnessed and exploited for the biological control of target species, such as mosquitoes (e.g. Kay and Nam, 2005; Cuthbert et al., 2018a). However, many natural enemies are generalists which consume a wide variety of prey species (Simberloff and Stiling, 1996). Accordingly, the proliferation of generalist natural enemies following release is likely to directly impact on non-target organisms through inadvertent predatory interactions or competition, thus potentially reducing levels of ecological impact on target prey. Moreover, biological control agents which adversely affect non-target communities through these processes may, paradoxically, become economically- and environmentally-damaging in themselves (Louda et al., 1997). Quantifications of

70 predatory interaction strengths which consider both target and non-target biota are, therefore,
71 integral to robust biological control agent assessments at the community-level.

72 The functional response has been applied extensively in quantifications of biological control
73 agent *per capita* impacts (Holling, 1959; Lester et al., 1999; Cuthbert et al., 2018a). Whilst
74 experimental functional response systems often comprise simplistic paired predator-prey
75 model species (see Lester and Harmsen, 2002), the presence of alternative prey may decrease
76 the functional response magnitude due to predator switching or satiation (see later), or drive
77 categorical changes to functional response form (Murdoch, 1969; Murdoch and Oaten, 1975).
78 In particular, propensities of predators to switch between prey may drive transitions to type
79 III functional responses empirically. Such changes are thought to impart more stability to
80 populations by remediating destabilising type II responses, which are characterised by a lack
81 of low-density prey refuge (Hassell, 1978; Cuthbert et al., 2019a).

82 Patterns of prey switching (i.e. frequency-dependent predation) and preferences by predators
83 may facilitate coexistence among taxa, in turn potentially stabilising diverse communities
84 (Murdoch, 1969). Switching propensities are considered to be driven by differential
85 profitability and abundance among prey types (McCann et al., 2005). Characteristically,
86 through avoidance of rare prey and disproportionate utilisation of abundant prey, patterns of
87 density-dependent refuge may emerge that mitigate population extirpations within
88 communities (Cuthbert et al., 2018b). This, in turn, may allow for population recovery of rare
89 prey types. Biological control agents which exhibit a propensity to switch between prey types
90 may, therefore, facilitate low density refugia for target prey, thus inadvertently enabling their
91 persistence within communities. Conversely, agents which do not switch from target prey
92 (i.e. demonstrate frequency-independent predation) are desirable, owing to efficient
93 consumption of target prey even when they are rare in environments. Accordingly, studies
94 which explicitly quantify the influence of alternative prey types on ecological impacts

towards target organisms are urgently required in biological control and other ecological contexts.

Mosquitoes are important vectors of pathogens and parasites which cause disease in humans and wildlife, with infectious disease risks being increasingly exacerbated by ongoing climate change (Lafferty, 2009; Ryan et al., 2019). According to the World Health Organisation (WHO), malaria alone accounted for 435,000 human deaths in 2018. Biological and environmental control measures can assist or augment control strategies targeting vector mosquitoes, without imposing environmental risks associated with the use of chemical insecticides (Cameron and Lorenz, 2013). Many predatory arthropods have been identified as effective natural enemies of mosquitoes (e.g. Shaalan and Canyon, 2009), and have been shown capable of eliminating disease risks at community-scales through reductions of mosquito populations (Kay and Nam, 2005). However, there is currently a lack of understanding of how mosquito prey risk is altered within food webs where they coexist and compete with other organisms (but see Chesson, 1989; Fischer et al., 2013; Kumar et al., 2008; Cuthbert et al., 2019a).

Notonectids are important generalist predators in freshwater aquatic ecosystems colonised by mosquitoes (Blaustein, 1998; Dalu et al., 2016; Wasserman et al., 2018). Daphniids have also been identified as an important notonectid prey species, in both natural and artificial ephemeral systems, and are often pioneering crustaceans given their presence within resting egg banks (Brendonck and De Meester, 2003; Wasserman et al., 2016a). Given the capacity of daphniid and mosquito prey to rapidly colonise temporary aquatic habitats, there is high potential for these species to overlap and compete for resources (Stav et al. 2005). Further, both are nektonic prey types which are preferred by notonectid predators (Klecka and Boukal, 2012). However, although predatory impacts of notonectids have been shown to be high towards mosquitoes in pairwise laboratory experiments (e.g. Cuthbert et al. 2019b), the

presence of daphniids as alternative prey could impede such impacts. In the present study, we thus use a comparative functional response and prey switching approach to quantify the ecological impacts of the pelagic notonectid *Anisops debilis* Gersaeker 1873 (Hemiptera: Notonectidae) towards larvae of the vector mosquito complex *Culex pipiens* (Diptera: Culicidae), in the presence of alternative *Daphnia pulex* (Cladocera: Daphniidae) group prey at varying densities.

Materials and methods

Animal collection and husbandry

Adult *A. debilis* (7.45 ± 0.17 mm), were collected using a 1000 μ m mesh scoop net from the water column of an impounded stream in Makhanda, Eastern Cape, South Africa ($33^{\circ}19'00.4''\text{S}$ $26^{\circ}31'21.0''\text{E}$). Notonectids were transported in source water to a controlled environment (CE) room in the Department of Zoology and Entomology, Rhodes University, Makhanda, maintained at $25 (\pm 1^{\circ}\text{C})$ and under a 14:10 light and dark photoperiod regime. In the CE room, notonectids were concurrently acclimated and starved for 48 hours prior to experimentation in filtered (100 μ m mesh size) source water. Larval *C. pipiens* complex prey from egg rafts collected from aquatic container-style habitats on the university campus were reared to the desired size class in the CE room on a diet of crushed guinea pig food pellets (Agricol, Port Elizabeth). The alternative prey, *D. pulex* were collected by hauling a 64 μ m mesh zooplankton net through the water column of small reservoir in Makhanda ($33^{\circ}18'33.9''\text{S}$ $26^{\circ}30'03.2''\text{E}$), transported in source water and housed in the CE room until experimental use.

Experimental protocols

We conducted two experiments to quantify the effects of alternative daphniid prey on notonectid predatory impacts towards larval mosquitoes. First, we quantified functional responses of *A. debilis* towards larval mosquito prey in the presence of varying background densities of daphniids. In the CE room, five densities (2, 4, 8, 16 or 32) of third instar larval *C. pipiens* (4.12 ± 0.19 mm) were introduced separately into 100 mL glass jars (5.6 cm dia.) containing filtered source water. Within each larval mosquito density level, four densities (0, 10, 20 or 40) of *D. pulex* (1.75 ± 0.05 mm) were introduced. All experimental groups were fully crossed and replicated five times. Following one hour of prey settling, individual *A. debilis* were introduced into experimental arenas containing both prey types and allowed to feed for two hours, after which predators were removed and remaining live prey of both types counted. Controls consisted of a replicate of mosquito/daphniid prey in the absence of predators under all crossed density treatment groups.

Second, we examined frequency-dependent predation (prey switching) by notonectids towards both prey types. As before, predators were acclimated and starved, with second-third instar larval *C. pipiens* (3.30 ± 0.10 mm) and *D. pulex* (1.84 ± 0.06 mm) introduced simultaneously into 100 mL experimental arenas. Thirty prey were introduced into each arena, under five different ratios (*C. pipiens*:*D. pulex*: 5:25, 10:20, 15:15, 20:10, 25:5), with 3 replicates per experimental group. After settling, *A. debilis* were introduced individually and allowed to feed for thirty minutes, after which predators were removed and remaining live prey counted. Controls consisted of a replicate of each prey ratio in the absence of predators. Pilot studies were used to inform appropriate feeding times for the two experiments, which are analysed separately.

Statistical analyses

All statistical analyses were performed in R v3.5.1 (R Core Development Team, 2018). In the functional response experiment, generalised linear models were used to examine counts of overall mosquito mortality with respect to the alternative prey treatment density (4 levels) and mosquito density (5 levels), and their interaction. A negative binomial family with log link was implemented owing to residual overdispersion (residual deviance > degrees of freedom). An information theoretic approach was used to select models which minimised information loss *via* second-order Akaike's information criterion (AICc) and model averaging, with the relative variable importance (RVI) of terms additionally discerned (Burnham and Anderson, 2002; Bartoń, 2015). Models with $\Delta\text{AICc} < 2$ were considered interchangeable (Burnham and Anderson, 2002). In the top model, the significance of factors was inferred using analysis of deviance. Type III sums of squares were implemented in the presence of an interaction term and type II sums of squares were applied where an interaction was not present in the top model (see Langsrud, 2003). Where applicable, least square means estimates were used for pairwise comparisons of significant terms, with Tukey adjustments of p values. A critical α of 0.05 was used in all analyses.

Logistic regression considering the proportion of mosquito prey killed as a function of initial density (continuous predictor) was used to discern functional response types. Here, a significantly negative first order term is indicative of a type II functional response and a significantly positive first order term followed by a significantly negative second order term indicates a type III functional response (Juliano, 2001). As prey were not replaced during the experiment, we fit Rogers' random predator equation (Rogers, 1972; Trexler et al., 1988; Juliano, 2001):

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

(1)

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is the handling time and T is the total experimental period. The *Lambert W* function was implemented due to the recursive nature of the Random predator equation (Bolker, 2008). Non-parametric bootstrapping ($n = 1999$) was used, based on starting parameter estimates, to generate 95 % confidence intervals of the attack rate and handling time parameters. Functional response curves, and these parameters, were then compared on the basis of confidence interval overlaps across prey densities.

For the prey switching experiment, generalised linear mixed effects models, assuming a Poisson error distribution with log link, were used to analyse counts of prey mortality with respect to prey type (2 levels) and prey proportion (5 levels), and their interaction. A random effects structure was applied to account for repeated measures of both prey types within each experimental replicate. A model averaging approach was followed to select the model which minimised information loss (see before). Analysis of deviance was used to infer significance, as before. Manly's selectivity indices, modified by Chesson (1983) and assuming non-replacement of prey, were used to quantify preferences towards mosquito prey (Manly, 1974; Chesson, 1983):

$$\alpha_i = (\ln((n_{i0} - r_i)/n_{i0})) / \sum_{j=1}^m (\ln((n_{j0} - r_j)/n_{j0})) \quad (2)$$

where α_i is Manly's selectivity index for prey type i , n_{i0} is the number of prey type i available at the start of the experiment, r_i is the number of prey type i consumed, m the number of prey types, n_{j0} the number of prey type j available at the start of the experiment and r_j is the number of prey type j consumed. In a two-prey system, α_i ranges from 0 to 1, with 1 indicating complete preference, 0 indicating complete avoidance and 0.5 indicating neutral selectivity. If all of a given prey type were consumed, Eqn. 2 was modified by adding one

individual to the corresponding prey supply (see Klecka and Boukal, 2012). This assumes that one individual survived, and therefore the index estimate is slightly conservative. To remove extremes prior to analysis, we transformed indices:

$$a_t = (\alpha_i(n - 1) + 0.5)/n \quad (3)$$

where α_t is the transformed output and n is the sample size. Beta regression was used to analyse the strength of preference indices towards larval mosquito prey across proportional availability (5 levels) (Cribari-Neto and Zeileis, 2010). A nested likelihood ratio test against the null model (0 predictors) was used to infer significance (Zeileis and Hothorn, 2002), with least square means estimates used *post-hoc* (see before).

Results

Control survival of both prey types exceeded 98 % in each experiment, and thus experimental mortality was not adjusted for background mortality. In the functional response experiment, the top model included alternative prey density (RVI = 1.00) and mosquito density (RVI = 1.00) as predictors of mosquito mortality. The ‘alternative prey density × mosquito density’ interaction term was not included ($\Delta AICc = 22.17$; $RVI < 0.01$), and thus differences in predation driven by alternative prey were consistent across mosquito densities. Alternative prey had a significant effect on mosquito mortality overall ($\chi^2 = 38.99$, $df = 3$, $p < 0.001$) (Fig. 1), with significantly fewer mosquitoes consumed under alternative prey densities of 20 and 40 compared to where alternative prey were absent (both $p \leq 0.001$). Differences in mosquito mortality rates with 10 daphniids compared to in the absence of alternative prey were not statistically clear ($p = 0.07$), yet tended to be reduced with daphniids. Mosquito mortality was also significantly reduced with 40 alternative daphniid prey as compared to 10

($p = 0.002$), but not 20 ($p = 0.09$) daphniids; mortality rates between 10 and 20 alternative prey were more similar ($p = 0.57$). Significantly more mosquito prey were consumed under higher density availabilities ($\chi^2 = 76.52$, $df = 4$, $p < 0.001$).

Type II functional responses were evidenced across all alternative prey treatments, owing to significantly negative first order terms (Table 1; Fig. 2). The maximum alternative prey treatment (40) was an exception to this (Table 1); however, the type II model was shown to minimise information loss as compared to either type III or flexible models *via* AICc (type II: 66.27; type III: 68.87; flexible: 67.68) (see Pritchard et al. 2017). Attack rates towards mosquitoes tended to decrease under increasing quantities of alternative prey, whilst handling times generally lengthened (Table 1). Accordingly, mosquito maximum feeding rates were substantially reduced in all instances in the presence of alternative daphniid prey. Functional responses towards mosquitoes by notonectids in the absence of alternative prey were of significantly higher magnitude than all alternative prey treatments under high mosquito densities. Conversely, magnitudes among different densities of alternative prey were more similar (Fig. 2).

In the switching experiment, prey type alone was retained in the top model (RVI = 1.00); prey proportion (RVI = 0.16; Δ AICc = 3.28) and the ‘prey type $\times$ prey proportion’ interaction (RVI < 0.01; Δ AICc = 13.14) were relatively unimportant. Accordingly, significantly higher numbers of daphniid prey were consumed than mosquitoes when presented simultaneously, irrespective of proportional availability ($\chi^2 = 28.18$, $df = 1$, $p < 0.001$) (Fig. 3). Notonectids did not display a prey switching propensity between prey types (Fig. 3), instead consuming significantly higher numbers of daphniid prey than expected overall. Nevertheless, preference indices towards mosquito prey were significantly affected by proportional mosquito availabilities ($\chi^2 = 22.73$, $df = 4$, $p < 0.001$), reflecting complete avoidance of mosquito prey at certain low-intermediate proportions (Fig. 3).

263

264 **Discussion**

265 Predatory impacts of the notonectid *A. debilis* towards vector mosquito prey were
266 significantly reduced by the presence of alternative daphniid prey during the present study.
267 As daphniid background densities increased, the functional response curve magnitude of *A.*
268 *debilis* fell concurrently, with attack rates decreasing and handling times lengthening.
269 Furthermore, *A. debilis* did not display a prey switching propensity away from daphniid prey
270 types. That is, irrespective of their proportional availability within the environment, positive
271 selection in favour of daphniids over mosquitoes was displayed. Indeed, complete avoidance
272 (i.e. zero mortality) of mosquito prey was evidenced under certain proportions (i.e. 0.17, 0.5).
273 Accordingly, the efficacy of notonectids as natural enemies towards mosquito prey may be
274 impeded by alternative prey, particularly where cladocerans are available in elevated
275 abundances.

276 Functional response magnitudes towards mosquito prey reduced incrementally with
277 alternative daphniid prey density increases. This is further reflected by consistent reductions
278 in raw prey consumption of mosquitoes as the alternative prey became more abundant. Attack
279 rates correspond to the scaling coefficient of functional responses, and describe the initial
280 slope of functional response curves (Hassell and May, 1973; Jeschke et al., 2002). Therefore,
281 the reductions in attack rates as alternative prey increased align with reduced predatory
282 impact under low mosquito prey densities, thus potentially increasing low-density refuge
283 effects for mosquito prey. However, although the presence of alternative prey has been
284 proposed to stabilise communities and facilitate transition from type II to type III functional
285 responses (Steele, 1972; Murdoch and Oaten, 1975), the present study did not find evidence
286 for this, with *A. debilis* displaying a type II functional response irrespective of alternative

prey density. Thus, predatory impacts at low mosquito prey densities may be sustained. Nevertheless, under the highest alternative prey density, the functional response type became somewhat equivocal.

Handling times increased concurrently with greater alternative prey densities in the present study. Inversely, the handling time corresponds to the maximum feeding rate of the functional response. Accordingly, the functional response maximum feeding rate towards mosquito prey reduced significantly as background densities of daphniids increased. Conversely, Chesson (1989) found alternative daphniid prey to have a less marked effect on mosquito prey consumption in *Notonecta hoffmani*, with only a slight change to attack rates and handling times. This may relate to general behavioural differences between notonectid subfamilies. Although Anisopinae (e.g. *Anisops* spp.), unlike all other diving insects, are able to attain near-neutral buoyancy, Notonectinae (e.g. *Notonecta* spp.) are positively buoyant and rely on clinging to habitat structures to maintain depth (Matthews and Seymour, 2008). Therefore, their greater co-occurrence in the water column may have driven more profound reductions in predatory impacts by *A. debilis*, as compared to *Notonecta* spp. reported in previous studies in simple habitats.

Prey switching has been proposed as a central, stabilising mechanism within ecological communities (Murdoch and Oaten, 1975). Experimental applications of prey switching enable quantifications of ecological impacts towards specific prey under different environmental availabilities. In the biocontrol and invasion sciences, species which do not exhibit a prey switching propensity are likely to exert greater ecological impacts on target species, with lower impacts on alternative prey (Cuthbert et al. 2018b). In the present study, *A. debilis* did not display a prey switching propensity between mosquito and daphniid prey. Daphniid prey were positively selected over mosquitoes under all environmental availabilities and, therefore, predatory selection by notonectids was relatively frequency-independent. The

continuous movement of cladocerans in the water column compared to the relatively static surface position exhibited by *Culex* mosquitoes likely explains this preference (Scott and Murdoch, 1983). Such inherent differences in prey movement and behaviour likely drive higher detectability of daphniid prey, with notonectids reliant on both visual and tactile mechanisms to capture prey (Peckarsky, 1984; Diéguez and Gilbert, 2003; Gergs et al., 2010). Moreover, previous studies have shown consistently high *per capita* ecological impacts of notonectids towards daphniid prey, with an ability to capture these prey across different aquatic environmental contexts (Wasserman et al., 2016b).

Notonectids have been demonstrated to be key predator species which often occupy top trophic positions in temporary aquatic habitats (Dalu et al., 2016), and have the capacity to shape the structuring of communities and eliminate pelagic species (Blaustein, 1998). Aerial dispersal during their adult life history stages enables efficient colonisation of transient aquatic habitat patches, where vector mosquitoes may proliferate. Our results demonstrate that, in communities fostering multiple alternative prey types, risk reductions may be imparted to target mosquito prey in biocontrol programs that utilise such predators. Increasing densities of alternative prey resulted in consistent decreases in predatory impact, and clear preferential selection for alternative prey was exhibited across proportional availabilities. Our results corroborate with Chesson (1989) and Fischer et al. (2013), where different notonectid species were found to selectively prefer cladocerans over larval mosquitoes. Given the coexisting prey types in aquatic food webs, increasing diversity of lower trophic groups likely reduces the efficiency of notonectids in regulating vector mosquito populations. Contrastingly, cladoceran populations may be more severely impacted. However, studies incorporating a greater range of prey types would elucidate these findings further. The potential for notonectids to drive temporal localised extirpation of planktonic crustaceans within temporary wetland systems has recently been highlighted (Wasserman et

al. 2018). The production of dormant eggs by crustaceans under adverse conditions may facilitate population resurgence following dry periods (Stross and Hill, 1965; Wasserman et al., 2016a).

Whilst caution should be exercised when relating laboratory experiment results to complex real-world systems, classical ecological concepts such as functional responses, prey preferences and prey switching offer great utility in comparative derivations of ecological impacts relevant for the assessment of biocontrol agents under context-dependencies. Future research should thus continue to experimentally integrate mechanisms which may stabilise pairwise predator-prey relationships, such as prey switching, omnivory, cannibalism and habitat complexity, in order to better quantify the influence of external factors on biocontrol agent impacts towards target organisms.

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Table 1. First order terms and functional response parameter estimates from *Anisops debilis* feeding on larval *Culex pipiens* prey in the presence of different daphniid alternative prey densities.

Alternative prey	First order term, p	Attack rate (a), p	Handling time (h), p	Maximum feeding rate ($1/h$)
0	-0.03, 0.005	0.73, < 0.001	0.06, 0.004	17.99
10	-0.06, < 0.001	0.72, 0.001	0.18, < 0.001	5.70
20	-0.03, 0.05	0.36, 0.01	0.17, 0.03	6.05
40	-0.03, 0.13	0.21, 0.11	0.38, 0.10	2.64

Fig. 1. Boxplot showing larval *Culex pipiens* consumption by *Anisops debilis* across all mosquito prey densities in the presence of different densities of alternative daphniid prey. The horizontal bar displays the median, the box gives the interquartile ranges and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range.

Fig. 2. Functional responses of *Anisops debilis* towards larval *Culex pipiens* prey in the presence of different background densities of alternative daphniid prey. Shaded areas represent 95 % confidence intervals.

Fig. 3. Proportional predation patterns by *Anisops debilis* between larval *Culex pipiens* and daphniid prey. The solid line represents expected consumption values under equal selective preference and the dashed sigmoid line represents a hypothetical prey switching pattern. Means are $\pm$ SE.