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Short Communication

The effect of the alternative prey, *Paramecium caudatum* (Peniculida: Parameciidae), on the predation of *Culex pipiens* (Diptera: Culicidae) by the copepods *Macrocyclops albidus* and *Megacyclops viridis* (Cyclopoida: Cyclopidae)

Ross N. Cuthbert[1,2,3], Amanda Callaghan[2], Jaimie T.A. Dick[1]

[1]Institute for Global Food Security, School of Biological Sciences, Medical Biology Centre, Queen's University Belfast, Belfast BT9 7BL, Northern Ireland, [2]Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading, Harborne Building, Whiteknights, Reading RG6 6AS, England, [3]Corresponding author, email: rcuthbert03@qub.ac.uk

Abstract

Biological control can be an effective tool to combat public health risks associated with mosquito-borne disease. However, target impacts of biological control agents may be reduced by biotic contexts such as the presence of alternative prey. In turn, this can impede our ability to realistically assess biocontrol agent efficacy. Here, we examine the effects of alternative ciliate prey on the predation potential of two cyclopoid copepods, *Macrocyclops albidus* Jurine (Cyclopoida: Cyclopidae) and *Megacyclops viridis* Jurine (Cyclopoida: Cyclopidae), towards larvae of the West Nile virus vector mosquito *Culex pipiens* Linnaeus (Diptera: Culicidae). Using functional responses (FRs; resource use under different resource densities), we demonstrate that both copepods exhibit potentially destabilising Type II FRs towards mosquito prey. However, where the alternative prey was present, we observed species-specific modulations to FR form and magnitude. For *M. albidus*, FRs remained Type II where ciliate prey were present, however, maximum feeding rates on mosquito larvae were reduced. Conversely, for *M. viridis*, FRs moved towards more stabilising Type III, whilst maximum feeding rates on mosquito larvae were not significantly reduced. Whilst both species of cyclopoid copepod were able to effectively target and consume larval mosquitoes in the presence of alternative prey, we demonstrate that overall efficacies may be reduced in aquatic habitats which contain multiple prey types. We thus advocate that biotic contexts such as prey selectivity should be integrated into predatory biocontrol agent examinations for mosquitoes which vector pathogens and parasites, to more holistically assess their efficacy.

Key words:

mosquito-borne disease, functional response, *Culex pipiens*, prey choice, cyclopoid

Introduction

Public health endangerment from mosquito-borne diseases has never been more apparent (Fernandes et al. 2018). To this end, a variety of vector control approaches have been developed and implemented, with varying levels of success (Becker et al. 2010). Biological control could present new tools to combat mosquito-borne disease in future (Thomas, 2018), with potential to reduce mosquito-borne disease circulation at large scales (Kay and Nam, 2005). Predatory cyclopoid copepods have proven especially efficacious in the biocontrol of mosquitoes which vector pathogens and parasites (Marten and Reid, 2007; Cuthbert et al. 2018a). Indeed, copepod efficacy has proven particularly high in artificial, human-mediated aquatic environments (Townroe and Callaghan, 2014; Veronesi et al. 2015). Although predation efficiencies of copepods have proven robust to abiotic environmental contexts such as habitat complxity (Cuthbert et al. 2018b) and turbidity (Cuthbert et al. 2018c), quantifications of biotic effects associated with alternative prey on their predatory impacts are lacking.

Within ecosystems, the presence of alternative prey can drive patterns of coexistence and thus impart stability to populations through frequency-dependent prey selection processes which alleviate predation pressures towards specific prey through switching (Murdoch, 1969; Cuthbert et al. 2018d). Accordingly, alternative prey may reduce biocontrol agent efficacies towards target organisms. Functional responses (FRs) have been used widely to quantify consumer-resource interactions under differing resource densities (Holling 1959), with FR form and magnitude offering insights into consumer impacts (e.g. biocontrol agents) towards target organisms (Dick et al. 2014). Type II FRs are characterized by a decelerating intake rate, where consumption rates are high at low prey densities. On the other hand, Type III FRs are sigmoidal, with low proportional intake at low prey densities. A Type II FR can therefore be population destabilising due to high proportional consumption at low prey densities, whilst

Type III FRs may impart stability to prey populations through the provision of low density prey refugia (Holling 1959; Alexander et al. 2012). Thus, in a biocontrol contex, agents which demonstrate a Type II FR irrespective of environmental contexts are desirable. Cyclopoid copepods have been shown to exhibit potentially population destabilising Type II FRs towards larval mosquito prey (Cuthbert et al. 2018a, b). However, effects of alternative prey, which often coexist with larval mosquitoes, on copepod-mosquito interaction strengths have not been comprehensively assessed. In aquatic habitats, ciliate protists are ubiquitous in stagnant waters where mosquitoes breed (Duguma et al. 2017). Thus, since alternative prey may reduce predatory impact and hence natural enemy efficacy towards mosquitoes, here we examine the effects of a common alternative ciliate prey on the biocontrol efficiency of cyclopoid copepods towards larvae of the vectorially-important mosquito *Culex pipiens* Linnaeus (Diptera: Culicidae).

Materials and Methods

The focal predators, *Macocyclops albidus* Jurine (Cyclopoida: Cyclopidae) and *Megacyclops viridis* Jurine (Cyclopoida: Cyclopidae) were collected from Glastry Clay Pit ponds, Northern Ireland (54°29'18.5"N; 5°28'19.9"W) and transported in water from the collection site to Queen’s Marine Laboratory, Portaferry. We initiated separate copepod cultures for each species from single gravid origerous females. Cultures were maintained at 25±2 ^{o}C, 50-60% RH and under a 16:8 light:dark regime. *Paramecium caudatum* and *Chilomonas paramecium* were supplied *ad libitum* to the copepods for culture proliferation, with adult copepod samples from each culture dissected and identified to species. Whlst *P. caudatum* is a food source for adults and copepodids, *C. paramecium* provides nurishment for nauplii stages. The prey, *Cx. pipiens* were obtained from a colony maintained in the same laboratory. This colony originated from eggs supplied in 2017 by The Pirbright Institute (Woking, England). Adult females were kept in 32.5 cm^3 cages (Bugdorm, Watkins and Doncaster, Leominster, England) and fed regularly using defibrinated horse blood *via* a membrane feeding system (Hemotek Ltd, Accrington, England). Sucrose-soaked cotton pads were additionally supplied for sustenance in each cage. Eggs rafts were removed regularly and larvae reared in 3 L larval bowls with crushed guinea pig food pellets provided *ad libitum* (Pets at Home, Newtownabbey, Northern Ireland) until pupation.

To quantify the effects of alternative prey on the FR of both copepod species, we employed a factorial 2 × 2 × 6 experimental design with respect to 'predator species' (*M. albidus*/*M. viridis*), 'alternative prey' (present/absent) and 'prey supply' (prey densities of either 2, 4, 6, 8, 15 or 30). *Culex pipiens* first instar larvae (1.1 – 1.3 mm) were supplied to adult female *M. albidus* (1.6 – 1.8 mm) or *M. viridis* (2 – 2.3 mm) over a 6 hour experimental period (*n* = 5 per experimental treatment). Experiments were undertaken in polypropylene arenas of 42 mm diameter containing 20 ml of dechlorinated tap water from an aerated source. For the alternative prey treatment, we added 3 ml of *P. caudatum* culture (approx. 150 ciliates ml^{-1} before addition to 17 ml dechlorinated tap water) to experimental arenas. This concentration aligns with densities used in other studies (e.g. Reiss and Schmid-Araya 2011). We starved non-ovigerous adult female copepods individually for 24 hours before the experiment to standardise hunger levels. To eliminate dissolved oxygen variability among treatments, we bubbled oxygen into the *P. caudatum* culture for 2 minutes prior to its dissemination. Prey were allowed to settle for 2 hours before the experiment was initiated through the addition of predators. After 6 hours during the photoperiod, the predators were removed and remaining live prey counted. Controls were five replicates at each prey density and alternative prey treatment in the absence of a predator.

All statistical analyses were undertaken in R (R Core Team, 2018). We compared overall prey consumption (numbers eaten) with respect to the 'predator species, 'alternative prey' and 'prey supply' factors using a generalized linear model (GLM) assuming a Poisson distribution and log link as counts were not overdispersed. Non-significant factors and interactions were removed to generate the most parsimonious model through a step-deletion process using χ^2 for model selection *via* analysis of deviance (Crawley, 2007).

We determined FR types using logistic regression of the proportion of prey killed as a function of prey density. A significantly negative first order term is indicative of a Type II FR, whilst a significantly positive first order term followed by a significantly negative second order term indicates a Type III FR (Juliano, 2001). We additionally used locally weighted scatterplot smoothing (LOWESS; 9/10 smoothing factor) to further examine proportional consumption across varying prey densities. We then fit flexible functional response models using the *frair* package in R (Pritchard et al. 2017), which can integrate a scaling exponent (q) if the results of logistic regression are equivocal (Real, 1977; Wasserman et al. 2016):

$$N_e = N_0\,(1 - \exp(bN_0^q(hN_e - T))) \qquad \text{Eqn. 1}$$

where N_e is the number of prey eaten, N_0 is the initial prey density, b is the search coefficient, h is the handling time, q is the scaling exponent and T is the total time available. Where a categorical Type II functional response is evidenced, the scaling exponent q may be fixed at 0, whilst responses are increasingly sigmoidal where $q > 0$. We then non-parametrically bootstrapped (n = 2000) initial b and h parameter estimates to construct 95% confidence intervals (CIs) around FR curves. This enabled results to be considered at the population-level, with differences subsequently inferred on the basis of FR overlaps across prey densities.

Results and Discussion

Prey survival in controls was 99.9% overall, and thus we assumed that prey deaths in treatments resulted from predation, which was also evidenced through partially consumed remains of larval mosquitoes. Overall, significantly more prey items were consumed by *M. viridis* than *M. albidus* ($\chi^2 = 3.95$, $df = 1$, $P = 0.047$), whilst predation was significantly reduced in the presence of alternative prey for both species ($\chi^2 = 8.40$, $df = 1$, $P = 0.004$). The alternative prey effect was consistent between predators as there were no interactions between the 'predator species' and 'alternative prey' factors ($\chi^2 = 0.73$, $df = 1$, $P = 0.39$). Further, overall consumption was significantly greater as more prey were supplied ($\chi^2 = 114.86$, $df = 5$, $P < 0.001$). No further significant interactions were found for the 'predator × prey supply' ($\chi^2 = 1.47$, $df = 5$, $P = 0.92$), 'alternative prey × prey supply' ($\chi^2 = 2.75$, $df = 5$, $P = 0.74$), or 'alternative prey × predator × prey supply' terms in the model ($\chi^2 = 2.59$, $df = 5$, $P = 0.76$).

Both *M. albidus* and *M. viridis* exhibited Type II FRs when no alternative prey were available (Table 1; Figure 1a,b). *Macrocyclops albidus* also displayed a categorical Type II FR in the presence of alternative prey (Figure 1a). However, in the presence of alternative prey, the FR form of *M. viridis* was equivocal between Type II and Type III (Figure 1b). Thus, for this treatment, a scaling exponent was integrated, where the FR was deemed to be moving towards being a sigmoidal Type III ($q = 0.83$; Table 1).

The search coefficient b trended towards being lower under the presence of alternative prey, whilst handling time h tended to be higher, and thus maximum feeding rate $1/h$ lower (Table 1; Figure 2a, b). Shaded FR CIs overlapped only at low prey densities in the case of *M. albidus* (Figure 2a), and thus maximum feeding rates were significantly greater in the absence of alternative prey for this species. On the other hand, in the case of *M. viridis*, FR CIs overlapped at all except the lowest prey densities (Figure 2b). This reflects the sigmoidal FR form and low search coefficient b in the presence of alternative prey for *M. viridis*. Where there were no alternative prey, FR CIs of the two copepod species converged across all prey supplies, and thus FRs were not significantly different within this treatment. However, where there were alternative prey present, at intermediate densities the FR CIs of *M. albidus* were significantly lower in comparison to *M. viridis* (Figure 2).

Biotic contexts such as the presence of alternative prey can affect derivations of ecological impact between consumers and resources (Médoc et al. 2018), and is relevant in the biocontrol context as multiple prey items often occur simultaneously within ecosystems. Indeed, for generalist consumers, prey switching and prey preferences can have a marked influence on ecological impacts (Murdoch 1969; Bolnick et al. 2002; Cuthbert et al . 2018d). Such biotic contexts are neglected where experimental designs focus upon singular prey species, and thus impact quantifications and transfer of results to empirical applications are inherently restricted. Several species of cyclopoid copepods have proven effective in field applications targetting mosquitoes which vector pathogens and parasites (Marten and Reid 2007). As cyclopoid copepods are known to feed on a range of organisms (Kumar et al. 2008), understanding the effects of such alternative prey on their biocontrol efficacy is vital for mosquito control efficacy assessments.

The present study demonstrates that the presence of alternative prey can significantly influence the predation efficiency of cyclopoid copepods towards vectorially-important mosquitoes. Our results corroborate with Rey et al. (2004) and Kumar et al. (2008), where alternative prey significantly reduced mosquito consumption by cyclopoid copepods. For both species here, the presence of alternative prey reduced the overall strength of interactions with larval mosquito prey. Whilst the Type II FRs exhibited by both copepods here in the absence of alternative prey corroborate with other studies (Cuthbert et al. 2018a, b, c), we found that the presence of alternative prey can drive species-specific shifts in FR form and magnitude. For *M. albidus*, whilst the Type II FR was sustained in the presence of alternative prey, maximum feeding rates were significantly reduced. On the other hand, for *M. viridis*, FR form shifted towards a sigmoidal Type III in the presence of alternative prey whilst maximum feeding rates were similar to where alternative prey were absent. Thus, whilst the Type II FR demonstrates that *M. albidus* may offer high and sustained predation levels at low prey densities, *M. viridis* may give refuge to mosquito prey at low prey densities due to an increasingly sigmoidal FR. Indeed, *M. albidus* has been regularly operationalized in biocontrol and has proven particularly efficacious in field aplications, and thus our results corroborate with these field-based trends in light of sustained population destabilising Type II FRs (Marten and Reid, 2007). Our results suggest that cyclopoid copepods are able to still effectively reduce mosquito numbers in the presence of alternative prey. However, future examinations of biocontrol agents should seek to incorporate assessments as to the effects of multiple alternative prey on ecological impact prior to agent release.

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Table 1. Results of logistic regression for both Type II and Type III models, considering prey killed as a function of prey density, and parameter estimates resulting from the flexible functional response models.

	Macrocyclops albidus		*Megacyclops viridis*	
(a) Alterative prey	No	Yes	No	Yes
(b) Logistic regression				
Type II response				
First term	-0.04	-0.03	-0.03	-0.02
P value	0.001	0.03	0.006	0.11
Type III response				
First term	-0.07	-0.10	-0.04	0.02
P value	0.35	0.25	0.54	0.78
Second term	0.0007	0.002	0.0003	-0.001
P value	0.71	0.43	0.88	0.58
(c) Parameter estimates				
b	0.63	0.35	0.62	0.11
h	0.13	0.18	0.08	0.16
q	Fixed at 0	Fixed at 0	Fixed at 0	0.83

Figure 1. Functional response forms determined from proportion of prey consumed under differing initial prey densities for *Macrocyclops albidus* (a) and *Megacyclops viridis* (b) preying on larvae of *Culex pipiens* without (solid lines; circles) and with (dashed lines; triangles) alternative prey (*Paramecium caudatum*). Relationships are presented using LOWESS lines with means ±SE (*n* = 5 per experimental group).

Figure 2. Functional responses (*n* = 5 per experimental group) of predatory copepods *Macrocyclops albidus* (a) and *Megacyclops viridis* (b) towards first instar *Culex pipiens*, without (solid lines) and with (dashed lines) alternative prey (*Paramecium caudatum*) over 6 hour experimental period. Shaded areas indicate bootstrapped (*n* = 2000) 95% confidence intervals (CIs).