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Original Article

Additive multiple predator effects can reduce mosquito populations

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Abstract

1. Multiple predator interactions may profoundly alter ecological community dynamics and can complicate predictions of simpler pairwise predator/prey interaction strengths. In particular, multiple predator effects may lessen or enhance prey risk, with implications for community-level stability. Such emergent effects may modulate natural enemy efficacy towards target organisms.

2. In the present study, we use a functional response approach to quantify emergent multiple predator effects among natural enemies towards the disease vector mosquito complex, *Culex pipiens.* We quantify conspecific multiple predator-predator interactions of the cyclopoid copepod *Macrocyclops albidus* (intermediate predator) by comparing multiple predator consumption simulations, based on individual consumption rates, with multiple predator consumption rates experimentally observed. Further, we examine the influence of the presence of a predator at a higher trophic level, *Chaoborus flavicans*, on copepod group predation.

3. Both predators displayed Type II functional responses, with *C. flavicans* consuming significantly more prey than *M. albidus* individually. Overall consumption levels of mosquitoes increased with greater predator density and richness. We did not detect antagonistic or synergyistic emergent multiple predator effects between conspecifics of *M. albidus*, and the higher level predator did not reduce effects of the intermediate predator. Accordingly, evidence for additive multiple predator interactions was found.

4. The lack of predator-predator interference between cyclopoid copepods and larval chaoborid midges provides strong support for their combined application in mosquito biocontrol. We propose increased examination of multiple predator effects in assessments of

natural enemy efficacies to better understand overall predatory effects within communities and utilities in vector control.

Keywords

biological control; mosquito-borne disease; functional response; multiple predator effects; cyclopoid; chaoborid

Introduction

Ecological communities typically comprise multiple consumers (e.g., predators) sharing common resources (Sih et al., 1998; Barrios O'Neill et al., 2014; Wasserman et al., 2016). Whilst community dynamics are often inferred on the basis of simple pairwise interaction strengths (e.g., the effects of a predator on a prey population) (Schmitz, 2007, Tang et al., 2014; Vázquez et al., 2015; Cuthbert et al., 2018d), biotic factors such as ontogenic shifts (Bofill and Yee, 2019) and multiple predator-predator interactions (Sih et al., 1998; Bolker et al., 2003; Wasserman et al., 2016) can also have profound implications for the outcomes of predator-prey interactions. Utilisation of shared resources may often lead to competition among predator species, and it is well established that these interactions may not combine additively in the context of prey risk (Soluk, 1993; Sih et al., 1998; Barrios-O'Neill et al., 2014). This can lead to erroneous inferences based on pairwise interaction strengths at the community-level. Additionally, the presence of predators at a higher trophic level may alter the efficacy of intermediate consumers through both density-mediation (i.e., direct predation) and trait-mediation (i.e., non-consumptive effects) (Peacor and Werner, 2001; Werner and Peaor, 2003). There is increasing evidence for the primary importance of trait-mediation as a driver of trophic cascades within ecosystems (Gabowski, 2004; Schmitz et al., 2004; Trussell et al., 2004). In particular, the impacts of aquatic predator cues in ecosystems may be profound due to their immediacy and influence at the population-level, and simuntaneously across multiple life history stages (Peacor and Werner, 2001; Trussell et al., 2004, 2008; Alexander et al., 2013).

Multiple predator effects can manifest in three ways: (1) additively, (2) synergystically or (3) antagonistically. Whilst synergistic multiple predator effects result in enhancement of prey risk to predation (Soluk and Collins, 1998; Losey and Denno, 1998; Sih et al., 1998), antagonistic effects result in prey risk reductions (Sih et al., 1998; Vance-Chalcraft and Soluk, 2005), both of which have implications that differ from additive multiple predator effects inferred from pairwise predator-prey interaction strengths.

Functional reponses quantify the relationship between resource (e.g., prey) density and consumption rate (Holling, 1959), and have been applied extensively by ecologists as a means of quantifying the density-dependence of *per capita* interaction strengths across multiple fields (Abrams, 1982, Dick et al., 2014; Cuthbert et al., 2018d). Both functional response form or Type (i.e., Types I, II, III), and their magnitude (e.g., curve asymptote), are powerful indicators of the strength of interactions between trophic groups and, at least theoretically, can aid inferences of population-level stability under different environmental contexts (Murdoch and Oaten, 1975; Dick et al., 2014). Centrally, the attack rate (i.e., initial curve slope) and handling time (i.e., curve asymptote, inversely maximum feeding rate) of consumers can be used to quantatively compare ecological impacts under different resouce densities and context-dependencies (e.g., Cuthbert et al., 2018b). However, the application of functional responses to derivations of multiple predator effects has remained scarce and produced equivocal results across different experimental systems (Soluk, 1993; Losey and Denno, 1998; Barrios-O’Neill et al., 2014; Wasserman et al., 2016).

Functional responses have been a staple in quantifications of natural enemy efficacies towards target organisms (Solomon, 1949; O'Neill, 1990; Van Drieche and Bellows, 2011; Cuthbert et al., 2018c), but have again neglected multiple predator effects. Mosquitoes are exposed to a broad range of predators across their life history (e.g., copepods, dragonflies, spiders), and many important natural enemies have been identified across multiple habitat types (Medlock and Snow, 2008; Shaalan and Canyon, 2009). In a biological control context, synergism among predators is targetted as this may enhance effects upon target species groups. On the other hand, antagonistic predator-predator interactions could reduce predatory effects and alleviate target prey risk. In turn, this may heighten mosquito proliferations and worsen disease risk. Additive effects are also desirable as this indicates a lack of interference between predators. Copepods copepods (freshwater crustaceans) have been identified as particularly efficacious agents for the population-level suppression of mosquitoes (Marten and Reid, 2007; Cuthbert et al., 2018b). Their application has successfully eradicated pathogens vectored by medically important mosquitoes at community-level scales (Kay and Nam, 2005; Nam et al., 2012), and their predatory effects have been proven to be context-independent to variables such as water clarity and habitat complexity (Cuthbert et al., 2018b, c). Cyclopoids are a particularly diverse group with a worldwide distribution (Dussart and Defaye, 2001), and often dominate lentic freshwater habitats where medically important mosquitoes can breed. Furthermore, their ecological versatility facilitates application to minute or ephemeral container-style habitats where many other predators cannot persist (Marten and Reid, 2007).

Despite their importance for the natural regulation of mosquitoes, there has hitherto been a lack of consideration for the implications of con/heterospecific multiple predator interactions for the efficacy of copepods in mosquito biocontrol. Such multiple predator interactions have been shown to substantially affect predatory effects by other mosquito antagonists (e.g., Barry and Roberts, 2014; Brahma et al., 2015). Further, chaoborid larvae (flies with an aquatic larval stage) are important predators in many fishless aquatic ecosystems and have the capacity to influence zooplankton assemblages *via* predation (Yan et al., 1991; Arnott and Vanni, 1993; Nyberg, 1998). Although comparatively less studied than copepods in a vector control context, larval chaoborids have also been identified as effective predators of larval mosquitoes (Borkent, 1980; Cuthbert et al., 2019b), and have the capacity to colonise artificial container-style habitats where mosquitoes breed *via* aerial dispersal in their adult stage (Sunahara et al., 2002). Owing to their strong predatory capacity towards zooplankton such as cyclopoid copepods (Smyly, 1979), chaoborid-copepod interactions may result in prey risk reductions for mosquitoes, given that the two predator groups are known to co-occur and interact in freshwater habitats (e.g., Moore et al., 1994). Alternatively, synergystic or additive effects would indicate utility in their joint use. Therefore, the present study uses a functional response approach to quantify con- and heterospecific multiple predator effects within and between the cyclopoid copepod *Macrocyclops albidus* (Jurine, 1820) (Cyclopoida: Cyclopidae) and a predator at a higher trophic level, larvae of the chaoborid *Chaoborus flavicans* (Meigen, 1830) (Diptera: Chaoboridae), using larvae of the *Culex pipiens* (Diptera: Culicidae) mosquito complex as basal prey. Firstly, we quantify individual functional responses of the two predators, before secondly quantifying and comparing predicted multiple predator effects among conspecific copepod groups in the presence or absence of the predator at a higher trophic level. Based on previous findings, we hypothesise that: (1) *C. flavicans* will exhibit a higher consumption rate than *M. albidus*; (2) overall consumption rates will generally increase with greater predator density and richness; (3) the consumption rates by multiple conspecific copepods will combine independently in the absence of the higher predator, whilst negative interactions between chaoborids and copepods will reduce consumption rates for the prey.

Materials and methods

Animal collection and maintenance

The intermediate predator, *M. albidus*, was collected from Glastry Clay Pit ponds, Northern Ireland (54° 29' 18.5" N 5° 28' 19.9" W) using a polypropylene dipper. Copepods were transported in source water to a laboratory at Queen's Marine Laboratory (QML), and maintained in controlled laboratory conditions (25 ± 2 °C; 50 – 60 % relative humidity; 16:8 light:dark regime) to stimulate proliferation. Gravid females were isolated from samples and used to initiate pure cultures in accordance with the available literature (Suarez et al., 1992; Marten and Reid, 2007). After nauplii emerged (larval stage), originating females were dissected and identified to confirm the species. *Macrocyclops albidus* cultures were then initiated in 10 litre tanks and fed *ad libitum* with *Paramecium caudatum* and *Chilomonas paramecium* (Sciento, Manchester, England). These protozoan cultures were prepared in 2 litre flasks using autoclaved wheat seeds in the same laboratory conditions. The predator at a higher trophic level, fourth instar *C. flavicans*, were purchased from a commercial supplier (Northampton Reptile Centre, Northampton, England) and fed *ad libitum* with *Daphnia* sp., obtained from the same supplier. This predator was acclimatised to the insectary conditions for five days prior to experimentation. The prey, newly hatched *C. pipiens* complex mosquitoes, were obtained from the same insectary where a colony had been maintained. Adult mosquitoes were kept in 32.5 × 32.5 × 32.5 cm cages (Bugdorm, Watkins and Doncaster, Leominster, England) and blood fed using defibrinated horse blood (TCS Biosciences, Buckingham, England) through a membrane feeding system (Hemotek Ltd, Accrington, England). Cotton pads soaked in a 10 % sucrose solution were provided for other sustenance. Egg rafts were extracted regularly from cages and placed into 3 litre larval bowls, and hatched larvae were fed *ad libitum* using ground guinea pig pellets until pupation (Pets at Home, Newtownabbey, Northern Ireland), upon which they were transferred into the above cages.

Experimental design

We starved non-ovigerous adult female *M. albidus* and fourth instar *C . flavicans* individually for 24 hours before the experiment to standardise hunger levels. The experiment was undertaken in plastic arenas of 42 mm diameter containing 20 mL of dechlorinated tap water from an aerated source under insectary conditions (see above). To quantify functional responses, we supplied prey densities of 2, 4, 8, 15, 30 or 60 first instar *C. pipiens.* (1.1 – 1.3 mm) per arena to four different predator treatments comprising adult female *M. albidus* (1.6 – 1.8 mm) and fourth instar larval *C. flavicans* (10.0 – 12.0 mm) over a six hour experimental feeding period (*n* = 3 per experimental treatment group). The four predator treatments were: one *M. albidus*; one. *C. flavicans*; three *M. albidus*; or three *M. albidus* alongside one *C. flavicans*. Quantifications of single predator functional responses enabled modelling of the expected multiple predator consumption rates, which could then be compared to those actually observed (see below). In the multiple predator species trials, where a copepod was killed by a chaoborid, it was immediately replaced from a surplus supply (observed once). We did not observe conspecific killing between copepods. Prey were allowed to settle for two hours before the experiment was initiated through the addition of predators. After six hours, the predators were removed and remaining live prey counted to derive those killed. Controls consisted of three replicates at each prey density in the absence of any predator.

Statistical analyses

All statistical analyses were undertaken in R v3.5.1 (R Development Core Team, 2018). We compared overall consumption with respect to the ‘predator’ (4 levels) and ‘prey supply’ (6 levels) factors using a generalised linear model (GLM) assuming a Poisson distribution and log link as residuals were not overdispersed relative to degrees of freedom. An information theoretic approach was followed *via* model averaging to select the model which minimised information loss (Burnham and Anderson, 2002; Bartoń, 2015). The relative importance of each model term was discerned, based on the sum of AICc weights. Models with ΔAICc < 2 were considered interchangeable (Burnham and Anderson, 2002). The significance of factors in the top model was discerned using likelihood ratio tests, with Tukey tests used for *post-hoc* comparisons of significant effects.

Functional response analyses were undertaken using the ‘frair’ package (Pritchard et al. 2017). For observed consumption under each predator treatment, we determined functional response types using logistic regression of prey killed as a function of prey density (continuous predictor). A significantly negative first order term is indicative of a Type II response, whereas a significantly positive first order term followed by a significantly negative second order term indicates a Type III response (Juliano, 2001). To account for prey depletion during the experiment, we fitted Rogers’ random predator equation for conditions without prey replacement (Rogers, 1972; Trexler et al., 1988; Juliano, 2001):

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

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). We non-parametrically bootstrapped (n = 2000) the a and h parameter estimates to produce 95 % confidence intervals around observed functional response curves. This process enabled results to be considered at the population-level, as oppose to the sample-level, negating the additional use of more formal statistical tests (e.g., Barrios O'Neill et al., 2014). Moreover, using the initial *a* and *h* parameter estimates, we calculated the functional response ratio (*a*/*h*) for each predator treatment (see Cuthbert et al. 2019a). This approach balances information from both parameters, with both high values of *a* and low values of *h* conducive to ecological impact.

Following McCoy et al. (2012) and Sentis and Boukal (2018), we used a population-dynamic approach to generate predictions of multiple predator functional responses using *a* and *h* paramaeter estimates from single *M. albidus* and *C. flavicans* treatments:

$$\frac{dN}{dt} = -\sum_{i=1}^{n} f_i(N)P_i \tag{2}$$

where N is the prey population density, P_i (i = 1, 2, ..., n) are the population densities of predators i and $f_i(N)$ is the functional response of predator i (i.e., Eqn. 1). This generated a 'null' model of emergent multiple predator effects, which was then compared directly to observed multiple predator functional responses (i.e., 3 × *M. albidus*; 3 × *M. albidus* + 1 × *C. flavicans*). Whilst multiple modelling approaches have been developed for predictions of multiple predator effects, the population dynamic approach has been identifed as more robust and precise than other methods (Sentis and Boukal, 2018). Predictions of prey consumption were generated for all experimental initial prey densities, with Eqn. 2 integrated over the total experimental time (see Sentis and Boukal, 2018). We generated 95 % confidence intervals around predictions using global sensitivity analysis which integrated the 95 % confidence intervals of the *a* and *h* parameter estimates from single predator treatments, alongside their covariance-variance matrix. This generated a number of parameter sets using a Latin hypercube sampling algorithm (Soetaert and Petzoldt, 2010). Equation 2 was integrated for each set over time to generate multiple predator consumption predictions with 95 % confidence intervals derived from the 2.5 % and 97.5 % quantiles of the simulated survival values. Actual and predicted functional responses were then compared on the basis of confidence interval overlaps across prey densities.

Results

Prey survival in controls exceeded 99.5 % and 'natural' prey mortality was thus not corrected for in trials with predators. The 'predator' and 'prey supply' terms were retained in the top model of prey consumption (both relative variable importance = 1). The interaction term between these factors was removed (relative variable importance < 0.01; ΔAICc = 32.70). The predator treatment had a significant effect on consumption of *C. pipiens* ($\chi^2 = 102.69$, $df = 3$, $p < 0.001$) (Fig. 1). Three *M. albidus* consumed significantly more mosquito prey than single *M. albidus* ($p < 0.001$), but consumption by three copepods did not differ significantly from single *C. flavicans* ($p > 0.05$). Single *C. flavicans* consumed significantly more prey than single *M. albidus* ($p < 0.001$), and consumption by individual chaoborids did not differ significantly from that of *C. flavicans* when present alongside three *M. albidus* ($p > 0.05$). However, *C. flavicans* consumption alongside three *M. albidus* was significantly greater than either single or triple *M. albidus* treatment levels (both $p < 0.001$). Prey consumption also increased significantly under greater prey supplies ($\chi^2 = 307.89$, $df = 5$, $p < 0.001$).

All functional responses were categorised as Type II, owing to significantly negative linear coefficients (Table 1). Whilst attack rates were relatively similar between individual *M. albidus* and *C. flavicans*, handling times were considerably reduced for *C. flavicans* (Table 1). Accordingly, individual *C. flavicans* exhibited a substantially higher functional response ratio compared to individual *M. albidus*. Attack rates were also similar between con- and heterospecific multiple predator groups, whilst handling times were considerably lower in the latter group, containing both *M. albidus* and *C. flavicans* (Table 1). Therefore, the functional response ratio was highest under the heterospecific multiple predator group, indicating greater effects on mosquito prey by copepods in the presence of the higher chaoborid predator.

Individually, functional responses of *M. albidus* and *C. flavicans* did not overlap above prey densities of approximately eight (Fig. 2a), and so functional responses (i.e., predation rates) of *C. flavicans* were significantly greater than *M. albidus* at higher densities (i.e., significantly lower handling times and higher maximum feeding rates). The functional response curve of three *M. albidus* was well-predicted from the individual functional response curve (Fig. 2b). Here, predicted confidence intervals overlapped with those observed across all prey densities, indicative of non-significant differences and thus additive multiple predator effects in copepods. Similarly, for three *M. albidus* in the presence of individual *C. flavicans*, predicted and observed functional response confidence intervals overlapped under all prey densities (Fig. 2c). Again, this indicated that multiple predator consumption rates were well-predicted from individual predator treatments.

Discussion

Robust quantifications of multiple predator interactions are crucial to understanding interaction strengths at the community-level (Sih et al., 1998; Bolker et al., 2003; Wasserman et al., 2016; Sentis and Boukal, 2018). In a natural enemy context, predator-predator interactions may alter the efficacy of biological control agents, with implications for organism population and economic or sanitary risk. In the present study, we first quantified the interaction strengths of two widespread natural enemies towards the disease vector *C.*

pipiens mosquito complex. Larval chaoborids and cyclopoid copepods are known to co-occur and engage in predatory interactions in freshwater environments (Moore et al., 1994), and these groups are well-adapted to temporary aquatic systems which mosquitoes also colonise (Sunahara et al., 2002; Marten and Reid, 2007). Accordingly, both groups have importance as natural enemies for mosquitoes across various aquatic environments. Both the cyclopoid copepod *M. albidus* and larval chaoborid midge *C. flavicans* displayed predatory Type II functional responses towards larval mosquito prey, although the functional response magnitude (i.e., maximum feeding rate) was significantly higher in *C. flavicans*. Predation by conspecific copepod groups was additive, with observed multipe predator consumption well-predicted from simulations based on the functional response of individuals. Similarly, the presence of the predator at a higher trophic level, *C. flavicans*, did not result in significant differences in observed predation compared to expectations based on single predator groups. Accordingly, the predator at a higher trophic level did not result in alterations to copepod-mosquito interaction strengths in the present study, and multiple coexisting copepods did not cause reductions to mosquito prey risk *via* interference. Our results thus indicate that these predator species can be used in combination and that copepod density can be increased without affecting their *per capita* effects on mosquito populations.

Overall consumption rates of chaoborids were significantly higher than cyclopoid copepods in the present study. This is not surprising owing to their larger size and different prey handling strategy. Whilst both focal predator species are ambush consumers that detect prey through hydromechanical cue reception (Riessen et al., 1984; Hwang and Strickler, 2001; Cuthbert et al., 2018a), chaoborids ingest prey whole using prehensile attenae and mandibles, with initial ingestion occurring in the thoracic crop before subsequent regurgitation (Riessen et al., 1984). This enables many individual prey items to be simultaneously packed into the crop, and enables a relatively high predatory effect compared to other trophically analogoues invertebrates (Dodson, 1972; von Ende and Dempsey, 1981; Cuthbert et al., 2019b). On the other hand, *M. albidus* has chewing mouthparts, using mandibles to tear prey items into digestible pieces (Fryer, 1957). Accordingly, it is likely that lower handling time constraints facilitate higher offtake rates towards mosquito prey by chaobiorids as compared to cyclopoid copepods. Nevertheless, despite significant differences in handling times between the two predators, attack rates were more similar between the species, which, combined with Type II functional responses, corresponds to high levels of ecological impact at low prey densities. Indeed, in the context of biological control, Type II functional responses are desireable as they are, at least theoretically, associated with population-destabilising effects on target organisms (Cuthbert et al., 2018d). The functional response ratio has recently been identified as a novel approach to balance information from both functional response parameters in predictions of ecological impact (Cuthbert et al. 2019a). Here, functional response ratios peaked under the heterospecific multiple predator group. Accordingly, greater predator diversities were conducive to the highest ecological impacts in the present study, with the presence of *C. flavicans* bolstering the predatory effects of conspecific copepod groups on larval mosquitoes.

The predictability of multiple copepod functional responses from individual interaction strengths suggests that, at least under densities used in the present study, this group of species does not respond to the presence of conspecifics in a predation context. This lack of predator-predator interference corroborates with the reported efficacy of cyclopoid copepods in mosquito population management, wherein a high numerical response facilitates high predatory effects when copepods reach high abundances (Marten and Reid, 2007; Nam et al., 2012; Cuthbert et al. 2018d). Owing to their sit-and-wait prey capture strategy, this lack of predator-predator interference is not surprising, with multiple predator effects shown to be dependent on the particular behavioural characterstics of species (Wasserman et al., 2016). In a similar vein, the ambush-style capture strategy of chaoborids likely also limited interference between predators in the present study, with intraguild predation only observed in one instance. Furthermore, whilst chaoborids often forage in pelagic water zones, many cyclopoid copepods, including *M. albidus*, are benthic foragers, therefore potentially enabling spatial separation between the two predator types, which further limits antagonistic interactions (see Schmitz, 2007). Applications of natural enemies which occupy different parts of waterbodies could enhance mosquito control *via* reductions in predator free space, yet these effects may be further altered by habitat characteristics such as water volume. Experimental arena size can directly influence the strength of predator-prey interactions (e.g., Uiterwaal and DeLong, 2018), and it is therefore probable that choice of volume can influence experimental quantifications of multiple predator effects in aquatic systems through, for example, modulating predator-predator encounter rates. Given the particular tendency for *C. pipiens* to spend time at the water surface (Yee et al., 2004), effects of different experimental water depths may be especially profound in mediating interaction strengths. Nevertheless, comparative studies which examine feeding rates phenomenologically (see Jeschke et al., 2002) across standardised experimental conditions provide useful insights into predatory interactions, whilst omitting search area as a confounding variable. Further, as the present study used relatively small arena sizes, it is unlikely that larger volumes would alter the additive nature of predator-predator interactions through changes to encounter rates.

Although not significantly different to single chaoborid treatments, consumption levels towards mosquitoes tended to peak under the combined copepod and chaoborid predator treatment group in the present study. Therefore, both increased abundances and diversity of predators resulted in the greatest interaction strength towards medically important mosquitoes. Alongside general studies on the biological control of crop pests (Snyder et al., 2003, 2006, 2008; Finke and Denno, 2004), a number of studies have examined the combined efficacy of multiple antagonists towards larval mosquitoes (Nilsson and Soderstrom, 1988; Culler and Lamp, 2009; Barry and Roberts, 2014; Brahma et al., 2015; Bofill and Yee, 2019). Yet, there has been a lack of application of functional responses in quantifications of multiple predator effects towards mosquitoes, despite the inherent density-dependence of consumer-resource interactions (Holling, 1959). Whilst antagonistic predator-predator interactions have been reported in previous studies, our results suggest that the promotion of cyclopoids and chaoborids for the control of medically important mosquitoes could further enhance biological control efforts *via* additive effects. However, the *per capita* interaction strength of chaoborids was far higher to that of copepods, with the latter group contributing relatively little to combined interactions. Importantly, both focal predator types in the present study are capable of colonising a broad range of lentic aquatic habitats where vector mosquitoes breed, including ephemeral container-style habitats (Sunahara et al., 2002; Marten and Reid, 2007). In particular, aerial dispersal by chaoborids during their adult stage could enable effective colonisation of discrete habitat patches which other predators cannot reach. Equally, many cyclopoid copepods are able to enter periods of diapause during phases of temporary drying between hydroperiods, reducing the potential for exploitation of predator-free habitats by mosquito colonists (Marten and Reid, 2007). Indeed, diapausing copepods are known to survive for months in the absence of water (Frisch, 2002).

In conclusion, the predatory effect of cyclopoid copepods is not significantly altered by either the presence of conspecifics or predators at a higher trophic level. Both predator types exhibited additive functional responses towards common mosquito prey, conducive to high predictability from individual interaction strengths and a lack of interference among consumers. Accordingly, their combined promotion in aquatic habitats could further assist in mosquito population management strategies through bolstering of predatory effects. Such insights into multiple predator interactions offer improved quantifications of the overall effects of natural enemies upon target species, further informing as to the efficacy of selected biological control agents before their release into empirical systems.

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Contribution of authors

RC conceived the designed the experiment. RC performed the experiment. RC analysed the data. RC and AS did the modelling. AC, AS, AD and JD contributed materials and analysis tools. RC wrote the first draft of the manuscript, and all authors contributed substantially to revisions. The authors declare that they have no conflicts of interest.

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Table 1. First order term results from logistic regression to determine functional response types, alongside rounded parameter estimates and functional response ratios resulting from Rogers' random predator equation.

Predator treatment	First order term, *p*	Attack rate (*a*), *p*	Handling time (*h*), *p*	Functional response ratio (*a*/*h*)
M. albidus (× 1)	-0.03, < 0.001	0.85, < 0.05	0.18, < 0.001	4.67
C. flavicans (× 1)	-0.01, < 0.05	0.95, < 0.001	0.02, < 0.05	57.68
M. albidus (× 3)	-0.03, < 0.001	1.94, < 0.001	0.07, < 0.001	27.82
C. flavicans (× 1) + *M. albidus* (× 3)	-0.03, < 0.001	1.69, < 0.001	0.02, < 0.001	79.99

Fig. 1. Larval *Culex pipiens* consumption under four observed predator treatments across all densities: 1 × *Macrocyclops albidus* (Ma); 1 × *Chaoborus flavicans* (Cf); 3 × *Macrocyclops* albidus; 3 × *Macrocyclops albidus* + 1 × *Chaoborus flavicans*. In the boxplots, 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 the interquartile range.

Fig. 2. Functional responses of *Macrocyclops albidus* (Ma) and *Chaoborus flavicans* (Cf) towards larval *Culex pipiens*, individually (a: solid, *M. albidus*; dotted, *C. flavicans*), or in con/heterospecific predatory units (b: solid, *M. albidus* (× 3) observed; dashed, 3 *M. abidus* (× 3) predicted; c: solid, *M. albidus* (× 3) and *C. flavicans* (× 1) observed; dashed, *M. albidus* (× 3) and *C. flavicans* (× 1) predicted). Shaded areas are 95 % confidence intervals.