



## Cattle dung in aquatic habitats alters mosquito predatory biocontrol dynamics

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### ABSTRACT

Anthropogenic land use changes influence ecosystem functioning and may alter trophic interactions. Intensification of free–range pastoral farming could promote degradation of aquatic habitats, with nutrient inputs adversely affecting water quality and resident communities. Reductions in natural enemies (and potentially efficacy thereof) and dampening of their interaction strength could promote the proliferation of vector mosquitoes, with consequences for disease transmission and nuisance biting. This study examined implications of a cattle dung eutrophication gradient (T0–T4: 0 g L<sup>-1</sup>, 1 g L<sup>-1</sup>, 2 g L<sup>-1</sup>, 4 g L<sup>-1</sup> and 8 g L<sup>-1</sup>) on aquatic habitats for trophic and non–trophic interactions by two larval mosquito (*Culex pipiens*) natural enemies (notonectid: *Anisops sardea*; copepod: *Lovenula falcifera*) using comparative functional responses. Copepods generally exhibited lower interaction strength compared to notonectids, both as individuals and conspecific pairs. Effects of dung pollutants differed among predator groups, with high concentrations dampening interaction strengths being observed for single/paired copepods and paired notonectids, but not single notonectids or heterospecific pairs. Individual predators exhibited Type II functional responses, with feeding rates largely similar across dung concentrations within species. Non–trophic interactions were predominantly negative (i.e., antagonistic) between conspecific pairs and scaled unimodally with prey density. Dung pollution intensified negative non–trophic interactions in notonectid pairs, whereas heterospecific pairs exhibited positive (i.e., synergistic) non–trophic interactions at the highest dung concentration. Physico–chemical properties indicated that turbidity and pH increased with dung treatment concentrations, whereas conductivity and total dissolved solids both peaked at 1 g L<sup>-1</sup> and 2 g L<sup>-1</sup>. These results improve understanding of mosquito regulation in degraded habitats, indicating effects of agricultural pollutants dampen trophic interaction strengths, depending on the taxon.

### 1. Introduction

Over the years, livestock has played a vital and efficient role in strengthening socio–cultural and economic values worldwide (Alary et al. 2011; Bairwa et al. 2013). As such, cattle rearing has significantly improved the economy and livelihoods of communities globally,

especially farmers of small to medium scale production systems (Ali 2007). Due to these benefits, cattle rearing has increased, resulting in ecosystem assemblage changes (Sica et al., 2018) and potentially undesirable environmental disservices, such as overgrazing leading to elevated land degradation and increased greenhouse gas emissions (Lange et al. 1998). Whilst terrestrial land degradation by cattle is

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well-studied (Perkins and Thomas 1993; Assefa and Hans-Rudolf 2016; Sands et al. 2018), pollution inputs to aquatic habitats utilised by livestock are less explored (Buxton et al. 2020a). Communal free range cattle tenure production systems are commonly practiced in arid environments (Jones 2000; Dzavo et al. 2019), whereby animals are kept in unfenced areas without restriction of movement (Bennett and Barrett 2007). As such, cattle can unrestrictedly exploit water sources as drinking points across arid landscapes with patchy aquatic habitats.

Cattle dung eutrophication in aquatic habitats has been found to promote the proliferation of vector species (e.g., mosquitoes) (Buxton et al. 2020a). Mosquitoes are prominent vectors for pathogens and parasites, causing numerous diseases in humans, livestock and wildlife (Dobson and Foufopoulos 2001; Drebot 2015; Vu and LaBeaud 2021). As such, an increase in mosquito populations may be of economic and disease risk concern in diverse environments (LaDeau et al., 2013), given the multi-billion US\$ economic impacts of mosquito invasions worldwide (Cuthbert et al. 2021a). The most common mosquito genera of medical and veterinary importance include *Anopheles*, *Aedes*, *Culex* and *Mansonia* (Becker et al. 2010; Buxton et al., 2021). Efforts to control these vectors through chemical, genetic and biological approaches are increasingly apparent (Jones et al. 2021; Wang et al. 2021). Among these however, biological control using native invertebrate natural enemies has been regarded as an efficacious, sustainable, compatible and environmentally-friendly approach, while simultaneously safeguarding public health and biodiversity integrity (Benelli et al. 2016). For example, biological control through the use of predators is common and efficacious in aquatic systems by targeting the larval stage (Shaalan and Canyon 2009; Batzer and Murray 2018; Mataba et al. 2021), albeit with limitations (Dambach 2020).

Although mosquito natural enemies might persist in polluted wetlands, their trophic interactions have been less explored in polluted aquatic habitats, and particularly in aquatic systems exposed to excess levels of cattle dung, where mosquito larval proliferation is enhanced (Buxton et al. 2020a). Considering shifting environmental conditions due to climate and land use change, interdependent organisms could respond differentially to biotic and abiotic stressors (Harrison et al. 2012; Buxton et al. 2020b). Presently, little is known about how dung eutrophication affects functional responses of mosquito predators in polluted aquatic habitats. These effects might alter both trophic (i.e., consumptive effects, e.g., predation) and non-trophic (i.e., non-consumptive effects, e.g., predator-predator interference) interactions. Quantifications of functional responses (consumer resource use as a function of resource density) (Holling 1959) can be used to determine the efficacy of biological control agents under relevant environmental contexts (Cuthbert et al. 2018a). Further, single predator functional responses can be used to make predictions of expected interaction strengths in the absence of predator interference (i.e., additive effects), then compared to actual observed multiple predators' feeding rates to determine the sign and strength of non-trophic interactions (Sentis and Boukal 2018).

Little is known on how cattle dung-induced eutrophication affects interdependent organisms, e.g., natural enemies' interactions and efficacy (mosquito consumptive rates). The present study thus investigated mosquito natural enemies' functional responses towards *C. pipiens* larval prey in habitats of varied cattle dung eutrophication to better understand how these systems respond to habitat degradation. We hypothesised that (i) the interaction strength of copepods would be more negatively affected than notonectids across dung enrichment treatments, given that notonectids breathe air at the water surface; (ii) copepods would display lower magnitude functional response than the semi-aquatic notonectids as they are smaller in size; (iii) non-trophic interactions of conspecific pairs would be synergistic, while the heterospecific pairs would show antagonistic multiple predator effects (MPEs); and that (iv) cattle dung eutrophication would reduce predator-predator non-trophic interactions. These findings will be important in informing biological control of vector mosquito species under

changing environments and may assist the conservation of aquatic habitats and their natural enemies. In addition, results from this study may aid in decision and policy making concerning livestock husbandry, movement and their ecosystem disservices in degradation of aquatic systems in arid regions.

## 2. Materials and methods

### 2.1. Animal collection and maintenance

Two sympatric adult predator species (notonectid [*Anisops sardea*] and copepod [*Lovenula falcifera*]) were collected from a pan (022° 52' 16.0S, 027° 47' 42.7E) near Lerala village in the Central District, Botswana. The larval prey (*C. pipiens*) were collected from a water body (22° 35' 05.7S, 27° 06' 58.7E) at the Botswana International University of Science and Technology campus. However, it is common that mosquito predators and larval prey may co-occur in the same habitat (Caillouët et al. 2008). Predator and prey collection were done using a 500 µm mesh and kept in separate netted 5 L aerated containers. *C. pipiens* larval prey were collected as egg rafts and kept in source water and matured tap water (50:50 ratio), fed with crushed rabbit food pellets (Westerman's Premium, Durban, South Africa). Subsequently, both predator and prey rearing were done in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany; Length [92 cm] × Width [78 cm] × Height [126 cm]) set at temperatures of 25 ± 2 °C, and a relative humidity of 75 ± 10% following protocols of Buxton et al. (2020b), reflecting average temperature regimes of aquatic habitats experienced in the specimen collection areas (also see Tladi et al. 2021).

### 2.2. Treatment preparation

To prepare the dung treatments, 50 L of tap water was filled in five large plastic containers (105 cm length × 72 cm width × 40 cm depth) and kept for 48 h to allow maturation (dechlorination). The containers were covered with a 500 µm mesh cloth to avoid colonisation by other aquatic invertebrates. Approximately 10 kg of fresh cow dung was collected randomly from three different cattle paddocks in Palapye village, and used to make a composite dung resource. The fresh dung was homogenised by hand for 5 min in a 50 L plastic container. Five dung treatments of 50 L each, were established with varying concentrations for utilisation in the entire experiment. In the control treatment (T0; 0 g L<sup>-1</sup>), no dung was added, while the remaining four treatment concentrations, (T1–T4; 1 g L<sup>-1</sup>, 2 g L<sup>-1</sup>, 4 g L<sup>-1</sup> and 8 g L<sup>-1</sup>) were established with dung included following Buxton et al. (2020a). The five treatment concentrations were left for a further 48 h maturation period, with contents mixed thoroughly at least every 6 h. The water was then filtered through a 100 µm mesh to remove all debris, allowing for only dissolved nutrients in the water to be retained for the experimental procedures.

### 2.3. Experimental design

Experimental arenas (350 mL glass containers; 23.2 cm circumference × 12.3 cm height) were used to house 250 mL dung concentrations (as established above) with different combinations of mosquito predators and larval prey. Adult individual and predator pairs (both conspecific and heterospecific, i.e., five predator treatments) of *L. falcifera* and *A. sardea* were fasted for 24 h before experimentation to standardise feeding status. They were kept for 24 h of fasting so that they could acclimatise to each degraded aquatic environment before being introduced to the prey. Larval mosquitoes of intermediate size (instars 2–3; 3.0 ± 0.2 mm length) were utilised, as these are preferred by these predators (Buxton et al. 2020c). The larvae were supplied across all 5 dung treatments (i.e., T0–T4) at 6 prey densities (2, 4, 8, 16, 32, 64 individuals) congruent with Buxton et al. (2020d). Overall, there were thus 25 experimental treatments (5 predator groups × 5 dung groups)

supplied with either an individual, conspecific or heterospecific predator grouping at different dung concentrations (0, 1, 2, 4, 8 g L<sup>-1</sup>). Each treatment was, in turn, replicated at least four times across each of six prey densities (2, 4, 8, 16, 32, 64 individuals). Mosquito larvae were first transferred from the rearing habitat water to the allocated treatment concentrations, then filtered with a 200 µm mesh, placed in desired concentrations and subsequently enumerated into experimental arenas to avoid dilution effects. Prior to any experiments, the larval prey were allowed to settle in treatment concentrations (experimental arenas) for 10 min before the introduction of the predators. The experiment was run for 3 h, in keeping with Buxton et al. (2020c), after which mosquito larvae that survived predation were enumerated to derive predatory efficacy. Three predator-free control replicates of each prey density and dung concentration were used to ascertain background prey mortality rates.

## 2.4. Physico-chemical properties

The five dung concentrations used in this feeding experiment were assessed to determine their physico-chemical properties (i.e., turbidity, electrical conductivity [EC], total dissolved solids [TDS] and pH). Turbidity was measured using a Hach 2100Q (USA). A multiparameter probe (Aqua read model, model AP-700, Britain) was used to determine EC, TDS and pH. Four sample replicates, each 20 mL, were collected from mesocosms of different concentrations for each parameter.

## 2.5. Statistical analyses

### 2.5.1. Interaction strengths

Predator-prey interaction strength (IS) was calculated and examined as a function of predator treatment (5-level categorical term), dung concentration (5-level categorical term) and prey density (continuous term), as well as the interaction between predator treatment and dung concentration:

$$IS(P, Z) = \frac{N_P - N_{P,Z}}{N_P} \quad (1)$$

where  $N_P$  and  $N_{P,Z}$  are the numbers of live prey at the beginning and end of the experiment, respectively, for each treatment. A generalised linear model with quasi-binomial error distribution and logit link was used to account for overdispersion, because residual deviance was higher than expected based on diagnostic simulations (Hartig 2020). Analysis of deviance with Type III sums of squares was used to compute F-values for effects in the model (Fox and Weisberg 2019). Tukey comparisons were computed *post-hoc* using estimated marginal means (Lenth, 2020).

### 2.5.2. Functional responses

Functional response models were fit for each of the 10 single predator treatments (i.e., copepods and notonectids, each across five dung concentrations). Binomial generalised linear models were used to determine functional response types for each treatment, by modelling proportional prey consumption as a function of prey density (continuous term). Here, a significantly negative linear coefficient indicates a Type II functional response, while a significantly positive linear coefficient and negative quadratic coefficient indicates a Type III functional response (Pritchard 2017). Where a significant Type II functional response was evidenced, we fit Rogers' random predator equation to the data (Rogers 1972):

$$N_e = N_0(1 - \exp(a(N_e h - T))) \quad (2)$$

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 used to fit the model to the data due to the recursive nature of the random predator equation (Bolker 2008). The random predator equation is suitable for non-replacement

experimental designs, such as ours, where total prey depletion is possible. Previous works have found total prey depletion to not compromise the robustness of parameter estimation resulting from the random predator equation (Cuthbert et al. 2020a). To compare functional responses within predator groups, we non-parametrically bootstrapped starting parameter estimates 2000 times (i.e., attack rate and handling time) to produce 95% confidence intervals (Pritchard 2017). Here, visual divergence in confidence intervals evidences statistically clear differences among treatment groups across prey densities, without resorting to more formal statistical comparison.

### 2.5.3. Non-trophic interactions

We employed a population dynamic modelling approach to quantify potential multiple predator effects in conspecific and heterospecific pairs. We used the IS Eq. (1) for each multiple predator treatment to quantify feeding rates in the presence of both trophic (i.e., feeding on prey) and non-trophic (i.e., interference/facilitation among predators) interactions (Sentis et al. 2017). This thus comprised the observed feeding rates from the actual experiment (i.e., including both interaction types).

Next, to distinguish between trophic (IS<sub>T</sub>) and non-trophic (IS<sub>NT</sub>) interactions (which together sum to IS), we used the attack rate and handling time estimates from the single predator functional responses at each dung concentration to predict multiple predator feeding rates. As these multi-predator predictions are based on single predator feeding rates, they represent those in the absence of non-trophic interactions between predators. Estimations of IS<sub>T</sub> were thus calculated following McCoy et al. (2012) and Sentis and Boukal (2018):

$$\frac{dN}{dt} = -\frac{aN}{1 + ahN}P \quad (3)$$

where  $N$  is the prey population density,  $P$  is the predator population density and  $a$  and  $h$  are the attack rate and handling time obtained from the single predator functional response estimates, respectively. Initial values of  $N$  and  $P$  were set at each of the experimental prey densities, with Eq. (3) then integrated over the entire experimental period to obtain predictions of surviving prey (and inversely those killed) at each multiple predator treatment, dung concentration and prey density. To incorporate error into these predictions, a global sensitivity analysis was employed using the 95% confidence intervals of each functional response parameter (covariance was assumed to be zero when unknown). This generated 100 random parameter sets using a Latin hypercube sampling algorithm, from which we used the mean (Soetaert and Petzoldt 2010).

Using both the observations (IS) and predictions (IS<sub>T</sub>) per experimental treatment, we determined IS<sub>NT</sub> by subtracting IS<sub>T</sub> (i.e., expected feeding rate in the absence of non-trophic interactions) from IS (i.e., observed feeding rate in the presence of both trophic and non-trophic interactions). Thereby, positive and negative IS<sub>NT</sub> values correspond to prey risk enhancement (i.e., positive non-trophic interaction) and reduction (i.e., negative non-trophic interaction), respectively. A linear model was used to examine IS<sub>NT</sub> as a function of paired predator treatment (3-level categorical term), dung concentration (5-level categorical term) and prey density (continuous term) and the interaction between predator and dung treatments. Homogeneity of variances and residual normality were examined using residual diagnostics. Analysis of deviance was used to compute F-tests with Type III sums of squares (Fox and Weisberg 2019) and Tukey comparisons were used *post-hoc* (Lenth, 2020). Further, as non-trophic interactions have been shown to scale unimodally with prey density (Sentis et al. 2017; Cuthbert et al. 2021b), we fit and compared prey density with and without a quadratic term via AICc, and selected the model that minimised information loss (lower value indicates a better fit). All analyses were computed in R v4.0.2 (R Core Team 2020).

For Physico-chemical properties, data were first checked for

normality using Shapiro–Wilks tests and were found to fulfill assumptions of analysis of variance (ANOVA). One-way ANOVA was used to analyse data, with turbidity, EC, TDS and pH each modelled as a function of dung concentration. Treatment pairs were statistically examined *post-hoc* using Tukey–Kramer’s test. Data on physico–chemical parameters were analysed using STATISTICA 13.2 (Statsoft Inc., Tulsa, Oklahoma).

### 3. Results

#### 3.1. Interaction strengths

All mosquito larvae survived in the predator–free controls; thus, all prey mortality was regarded as being associated with predation by copepods and notonectids. Predator–prey interaction strength (IS) differed significantly among species and species pairs in interaction with dung concentration ( $F_{16,634} = 9.199, p < 0.001$ ). In general, single, or paired copepods exhibited significantly lower IS compared to single/paired notonectids and heterospecific predator pairs (Fig. 1). Species individuals or pairs exhibited differential responses to dung exposure in terms of their IS. Single copepod IS peaked at 2 g L<sup>-1</sup>, and that level was significantly greater than either 4 or 8 g L<sup>-1</sup> (both  $p < 0.05$ ). Single notonectids displayed the reverse, with IS peaking at the highest dung concentration, and significantly lower at 1 g L<sup>-1</sup> than 0 or 2 g L<sup>-1</sup> (both  $p < 0.01$ ), and 4 g L<sup>-1</sup> vs 2 g L<sup>-1</sup> ( $p < 0.01$ ).

For conspecific copepod pairs, IS was again lowest at higher dung concentrations (Fig. 1), with 4 g L<sup>-1</sup> significantly reduced compared to 0, 2 and 8 g L<sup>-1</sup> (all  $p < 0.05$ ). Similarly, paired notonectid IS peaked at 0 and 1 g L<sup>-1</sup>, being significantly higher there than at 4 and 8 g L<sup>-1</sup> (all  $p < 0.001$ ), and 1 vs. 2 g L<sup>-1</sup> ( $p < 0.001$ ). Considering heterospecific pairs of copepods and notonectids, IS was greatest at 8 g L<sup>-1</sup>, and this was significantly greater than 0, 2 and 4 g L<sup>-1</sup> (all  $p < 0.05$ ). Feeding rates at 0 and 1 g L<sup>-1</sup> were also significantly higher than 2 g L<sup>-1</sup> and 4 g L<sup>-1</sup> (all  $p < 0.01$ ). Across predator groups and dung concentrations, feeding rates fell significantly with increasing prey density ( $F_{1,634} = 196.448, p$

< 0.001).

#### 3.2. Functional responses

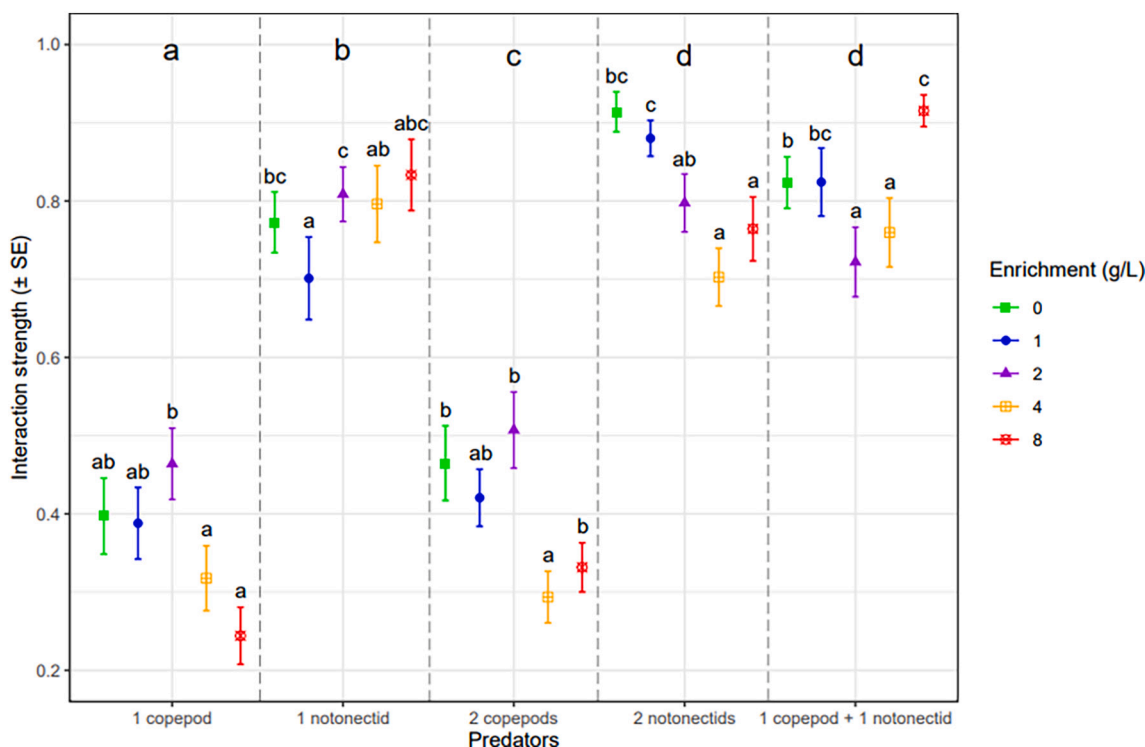
In single predator treatments, both copepods and notonectids displayed Type II functional responses across all dung concentrations, indicated by significantly negative linear coefficients (Table 1). Significant attack rate and handling time estimates were returned across all treatment groups (Table 1). Across all matched concentrations, notonectids consistently displayed higher attack rates and shorter handling times, corroborating higher functional response initial slopes and asymptotes (Table 1; Fig. 2). Both copepods and notonectids generally exhibited highest magnitude functional responses (i.e., greatest maximum feeding rates) at dung concentrations of 2 g L<sup>-1</sup> (Fig. 2).

For copepods, across prey densities, functional response confidence intervals generally overlapped, indicating a lack of significant difference across dung concentrations (Fig. 2a). One exception was at the highest dung concentration (8 g L<sup>-1</sup>), where feeding rates were significantly

**Table 1**

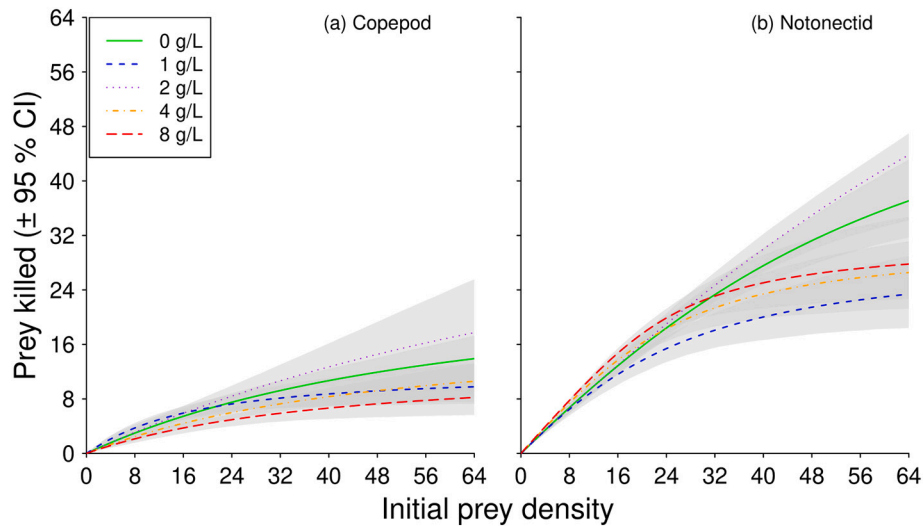
Functional responses of single copepods and notonectids under different dung concentrations. Linear coefficients are shown alongside estimated attack rates and handling times from the random predator equation. Asterisks denote levels of significance ( $p < 0.05 = *$ ;  $p < 0.01 = **$ ;  $p < 0.001 = ***$ ).

Predator	Concentration (g L <sup>-1</sup> )	Linear coefficient	Attack rate	Handling time
Copepod	0	-0.011*	0.528***	0.039**
Copepod	1	-0.030***	0.916***	0.084***
Copepod	2	-0.007*	0.527***	0.022*
Copepod	4	-0.013*	0.427***	0.054**
Copepod	8	-0.014**	0.373***	0.077**
Notonectid	0	-0.024***	2.042***	0.016***
Notonectid	1	-0.031***	2.120***	0.034***
Notonectid	2	-0.013**	1.887***	0.009***
Notonectid	4	-0.040***	3.433***	0.032***
Notonectid	8	-0.049***	4.759***	0.032***



**Fig. 1.** Interaction strength (i.e., proportion of prey killed) of different predator groups under different dung concentrations, pooled across prey densities. Means are shown alongside standard error. Letters denote significant differences within and among predator groups.





**Fig. 2.** Functional response of single copepods (a) and notonectids (b) under different dung concentrations, alongside 95% confidence intervals in grey shades. Curves were fitted from the random predator equation for each separate treatment across prey densities.

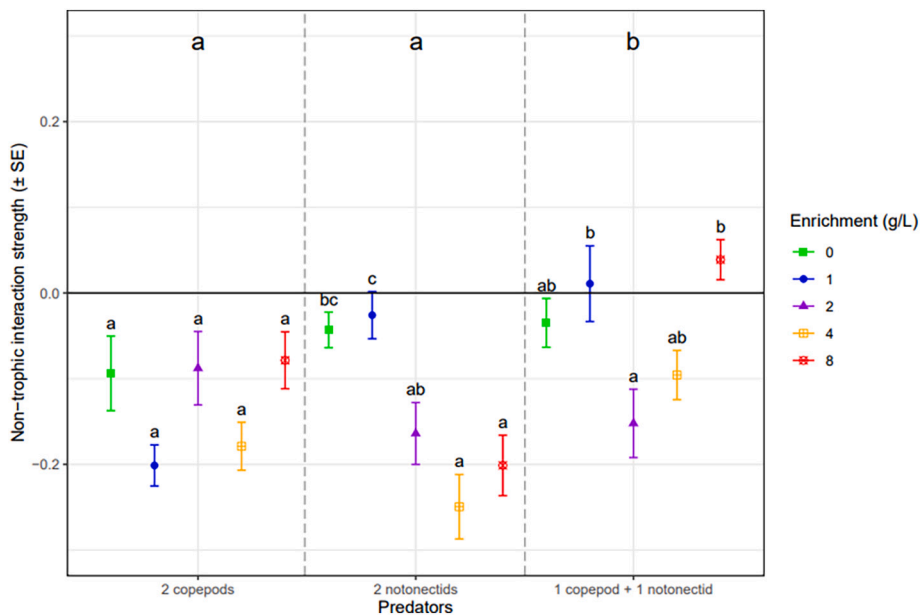
reduced compared to 0, 1 and 2 g L<sup>-1</sup> treatments at low–intermediate prey densities, owing to reduced attack rates (Table 1; Fig. 2a). For notonectids, again there was substantial overlap in confidence intervals among dung treatments (Fig. 2b), however, at high prey densities, 0 g L<sup>-1</sup> and 2 g L<sup>-1</sup> treatments were significantly higher than 1 g L<sup>-1</sup> and 4 g L<sup>-1</sup> given the shorter handling times by the predators (Table 1).

### 3.3. Non-trophic interactions

The strength of non-trophic interactions differed significantly among paired predator treatments in interaction with dung concentration ( $F_{8,391} = 4.845, p < 0.001$ ). For conspecific copepods and notonectids,  $IS_{NT}$  was consistently negative across dung concentrations, while emergent positive  $IS_{NT}$  was exhibited in heterospecific groups (Fig. 3). For copepod pairs, the strength of  $IS_{NT}$  did not differ significantly among dung concentrations and thus antagonisms were

consistently prevalent (all  $p > 0.05$ ). Conversely, for paired notonectids,  $IS_{NT}$  was significantly more negative (i.e., antagonistic) at 4 and 8 g L<sup>-1</sup> compared to 0 and 1 g L<sup>-1</sup> (all  $p < 0.05$ ), and 2 g L<sup>-1</sup> compared to 1 g L<sup>-1</sup> ( $p < 0.05$ ). For heterospecific pairs, conversely, positive  $IS_{NT}$  was exhibited at the highest dung concentration (8 g L<sup>-1</sup>), with  $IS_{NT}$  at 2 g L<sup>-1</sup> significantly more negative (i.e., antagonistic) than 1 or 8 g L<sup>-1</sup> (both  $p < 0.05$ ).

Non-trophic interaction strengths scaled unimodally with prey density across predator pairs and dung concentrations, as the quadratic term inclusion minimised information loss compared to a linear term alone ( $\Delta AICc = 22.925$ ; linear coefficient:  $t = 0.213, p = 0.831$ ; quadratic coefficient:  $t = 4.982, p < 0.001$ ) (Fig. 4). Overall, predator–predator interactions were most antagonistic (i.e., negative) at intermediate prey supplies.



**Fig. 3.** Non-trophic interaction strengths according to paired predator treatments and dung concentrations. The solid zero-line indicates an absence of non-trophic interactions, while negative values indicate antagonisms and positive values synergisms. Means are shown alongside standard errors. Letters denote significant differences within and among predator groups.

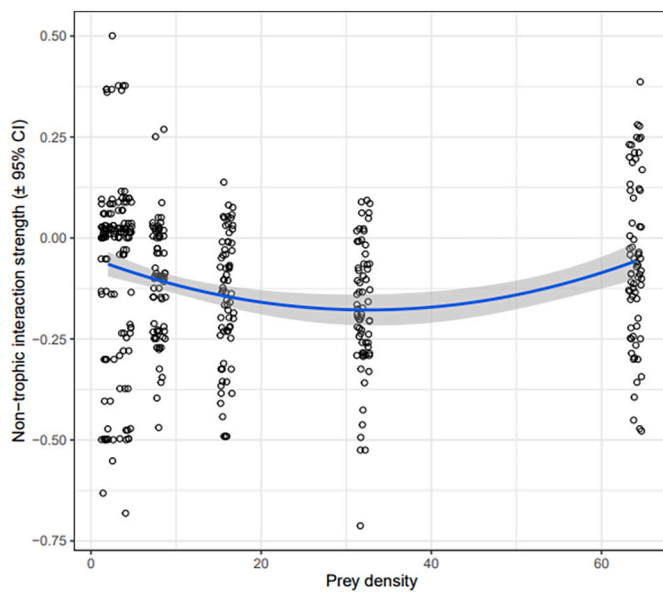


Fig. 4. Unimodal scaling of non-trophic interaction strengths as a function of prey density across predator treatments and dung concentrations. Grey areas are 95% confidence intervals. Nodes represent raw data.

### 3.4. Physico-chemical properties

Turbidity differed significantly across all the dung treatment concentrations ( $F_{4,10} = 2329.5$ ,  $p < 0.001$ ). The lowest turbidity was recorded at  $0 \text{ g L}^{-1}$  and increased with dung concentration (Fig. S1a). Electrical conductivity significantly differed among the dung concentrations ( $F_{4,15} = 58.607$ ,  $p < 0.001$ ). The highest EC was recorded at  $1 \text{ g L}^{-1}$  and  $2 \text{ g L}^{-1}$ , whereas the highest concentration ( $8 \text{ g L}^{-1}$ ) had the lowest reading (Fig. S1b). Similarly, TDS was significantly different among treatment concentrations ( $F_{4,15} = 40.750$ ,  $p < 0.001$ ) with the highest concentration ( $8 \text{ g L}^{-1}$ ) exhibiting the lowest TDS. The highest TDS was observed at  $1 \text{ g L}^{-1}$  and  $2 \text{ g L}^{-1}$ , with intermediate values observed for  $0 \text{ g L}^{-1}$  and  $4 \text{ g L}^{-1}$  (Fig. S1c). The pH was also significantly different among dung treatments ( $F_{4,15} = 282.6$ ,  $p < 0.001$ ) with the lowest pH displayed at  $0 \text{ g L}^{-1}$ . An increase in pH was observed across treatment concentrations, with the highest values exhibited at  $4 \text{ g L}^{-1}$  and  $8 \text{ g L}^{-1}$  (Fig. S1d).

## 4. Discussion

This study aimed to unravel how the efficacy of mosquito natural enemies is altered in cattle dung-polluted aquatic habitats. We determined significant interaction effects between predator groups and dung treatments, indicating dung effects differ among predator groupings, yet copepods exhibited significantly lower interaction strength compared to notonectids both as individuals and as conspecifics overall. Without accounting for field abundances, this result suggests that notonectids may offer more efficacious ecological service provisioning under degraded aquatic environments compared to copepods. Highest dung concentrations tended to dampen interaction strength of single/conspecific copepods and conspecific notonectids, but increased feeding rates of single notonectids and heterospecific copepod-notonectid pairs. Nevertheless, functional response types were sustained across predator groups and dung concentrations, and thus dung inputs did not cause shifts from a Type II to Type III functional response. Non-trophic interactions were also significantly affected by dung concentration in interaction with predator groupings, although being consistently negative overall, indicating a predominance of antagonistic effects and thus predator-predator interference. Nevertheless, emergent positive non-trophic interactions were anomalously exhibited in heterospecific pairs

at the highest dung input level. Electrical conductivity and TDS peaked at  $1 \text{ g L}^{-1}$  and  $2 \text{ g L}^{-1}$  while turbidity and pH increased significantly with dung concentrations. This points to a positive correlation between turbidity, pH and dung eutrophication, which may impact the quality of aquatic ecosystems, with cascading effects on predator-prey interaction strengths and mosquito control.

The efficiency of predator-prey interactions in aquatic ecosystems has been shown to depend on numerous biotic and abiotic factors (Juliano 2009; Laws 2017; Buxton et al. 2020c, 2020d). Nevertheless, effects of shifting land use and agricultural intensification have broadly lacked assessment in an aquatic food web context. The results of this study showed that dung-induced eutrophication significantly impacts interaction strengths, especially in copepods. This may be associated with copepods being wholly aquatic organisms, whereas notonectids are semi-aquatic predators (Quiroz-Martínez and Rodríguez-Castro Quiroz-Martínez and Rodríguez-Castro 2007). Thus, notonectids display behavioral differences to copepods, principally in being able to breathe at the surface of the water and having wings which allow access to patchy habitats with preferable conditions (Toth and Chew 1972; Matthews and Seymour 2008; Ferzoco et al. 2019). Therefore, considering single predator interaction strengths in anthropogenically-degraded environments, vector management practitioners using augmentative biological control could be better suited to deploy semi-aquatic predators such as notonectids, for which predation rates are not significantly dampened by the agricultural pollutants tested here. For environments that are relative unpolluted, however, wholly aquatic copepods may be considered. Importantly, both predator functional responses were consistently Type II across treatments, indicating a high capacity to target and consume predators at low prey densities, which could thus lead to the extirpation and effective control of mosquito populations by destabilising effects, irrespective of pollution levels (Cuthbert et al. 2018a).

For single predator functional responses, both copepods and notonectids generally exhibited the highest magnitude feeding rates, with shorter handling times at  $2 \text{ g L}^{-1}$  where EC and TDS also peaked. Although predation rates varied under different dung concentrations in this study, water clarity at the highest concentration ( $8 \text{ g L}^{-1}$ ), (i.e., more turbid) did not negatively affect feeding rates, in keeping with Cuthbert et al. (2018b). This suggests a propensity to rely on hydro-mechanical cues for prey detection as opposed to visual recognition at low turbidity levels (Chivers et al. 2013), but could also be an artefact of confinement effects associated with the relatively small-sized experimental arenas that increase prey encounter rates. Equally, these effects could reflect behavioral responses in prey, whereby anti-predator responses may have been dampened to a similar, or greater, extent compared to the reductions in predator efficiency as dung concentration increased (Abrahams and Kattenfeld 1997). Nevertheless, the overall result here is encouraging and points to a low effect on eutrophication on predatory biocontrol considering these study species, with functional responses broadly overlapping. We were, however, unable to assess direct correlations of predator IS and the physico-chemical properties of experimental water. As such, we recommend that future studies should measure physico-chemical properties that may likely affect predator functional responses, e.g., water nutrient and turbidity levels. This could help disentangle species effects as well as physiological and biochemical mechanisms that modulate predator-prey interactions under compromised habitat water quality. Thus, future work should also examine behavioral effects, including costs and benefits of predatory biocontrol across different water conditions, e.g., examining predator versus prey positioning and predator movement, with a view of making inferences on the efficacy of biological control. Furthermore, the efficacy of these predators assessed under field-based degradation scenarios may help confirm the ecological relevance of the results obtained here.

Although the interaction strength of copepods and notonectids is variable as individuals and conspecifics according to dung concentrations, both predators exhibited negative non-trophic interactions across

dung concentrations, indicating a general tendency for interference effects. Negative non-trophic interactions are commonly found in aquatic food webs depending on factors such as prey species (Soluk 1993; Cuthbert et al. 2020b). However, heterospecific pairs may be more likely to exhibit synergisms due to a capacity to display divergent foraging behaviours, for example, by targeting different parts of a water body (Buxton et al. 2020c). In the present study, heterospecific non-trophic interactions were also contingent on the level of dung pollution, with again largely negative non-trophic interactions, but positive non-trophic interactions at the highest dung concentration as an exception to this trend. While the behavioral mechanisms for this effect require further elucidation, it could reflect reduced interference effects between heterospecific predators as pollution levels increased due to increasingly divergent foraging behavioral responses to pollution between species. As the highest dung concentration was associated with the greatest turbidity, there may also be lower interference between predator species due to reduced visibility at this level (Aspbury et al. 2019). Moreover, we identified strong density-dependence in the strength of non-trophic interactions overall, which peaked at intermediate prey supplies, corroborating previous studies (Sentis et al. 2017).

Whereas this study focused on only two predatory species, a rich diversity of natural enemies is available for mosquitoes in nature (Caillouët et al. 2008). Thus, the fate of complementary biological control and overall interaction strength depends on the contributory roles of these other species. More work should thus be done to investigate how other mosquito natural enemy species are affected by eutrophication. Furthermore, this study focused on laboratory-based experiments simulating predator-prey interaction and was short-term by nature. Future experiments may thus need to examine field trials in natural conditions to fully understand implications of such pollution for predator population numbers in the medium to long-term. Further, field-based predator-prey trials under natural conditions should also be considered to assess the efficiency of trophic interactions for mosquito regulation under human-mediated eutrophication and other contexts. Indeed, predators are likely to over-exploit prey under simplified laboratory conditions (Bonsall et al. 2002). In addition, diverse predators of different developmental stages need to be assessed within polluted habitats against a broader range of mosquito species, as well as direct effects of these pollutants on mortality among taxa over time. Furthermore, degraded aquatic habitats need to consider other commonly abundant livestock (e.g., donkey, goat, chicken) and human-induced pollution (e.g., sewage), where mosquito vector species can thrive (Calhoun et al. 2007). Further recommendations are warranted in assessing nutrient and heavy metal content in dung enriched aquatic systems, to further establish their role in degradation and predator-prey interaction. This includes sublethal concentrations of ammonia on aquatic biodiversity, organic matter and the effect of dissolved oxygen, nitrogen and phosphorus on the overall efficacy of natural enemies.

## 5. Conclusions

The results showed that copepods have reduced interaction strengths towards *C. pipiens* as compared to notonectids across dung concentrations, whereas their heterospecific combination pairs had the highest interaction strengths at elevated dung concentrations. In addition, while copepods exhibited lower magnitude functional responses compared to notonectids, functional response form was not affected by dung concentration. These results also showed that non-trophic interactions were largely negative, but emergent positive interactions were displayed in heterospecific groups at the highest dung concentrations. Furthermore, findings of this study showed that turbidity and pH increased with dung concentrations, while EC and TDS peaked at intermediate concentrations; however, their direct correlation with predator-prey interaction is yet to be fully established. These results are significant for applied vector management through the use of context-specific predatory species based on aquatic habitat qualities. Our results help in understanding

ecosystem functioning in degraded aquatic habitats and may inform future biological control under compromised habitat conditions. Given the sustained predatory effects of notonectids and copepods, the promotion and conservation of these diverse predator species is essential by managers of wetland ecosystems. Context-specific selection of biocontrol agents may improve vector control efficacy under anthropogenically-changing environments, reducing vector mosquito populations and associated risk of emerging and re-emerging infections.

## Availability of data and material

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Authors' contributions

Project conceptualisation and management: MB RJW CN.

Data curation: MB PLB.

Formal analysis: MB PLB RC.

Investigation: MB RC PLB RJW CN.

Visualisation and validation: MB PLB RC RJW TD CN.

Writing – original draft: MB PLB RC RJW TD CN.

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## Declaration of Competing Interest

All authors declare no conflict of interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fooweb.2022.e00241>.

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