Cattle-induced eutrophication favours disease-vector mosquitoes

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ABSTRACT

Free-range cattle rearing in arid landscapes contribute profoundly to ecosystem degradation. Cattle dung nutrification in aquatic habitats potentially shapes species diversity and abundance due to resource availability. These nutrient-enriched environments may increase oviposition by mosquitoes and influence proliferation of disease vectors. Here, we examined mosquito larval abundance of *Culex pipiens pipiens* (culicine) and an unidentified *Anopheles* (anopheline) species across different concentration treatments of nutrient (cattle dung) loadings (T1-T4; 1 g L^{-1}, 2 g L^{-1}, 4 g L^{-1} and 8 g L^{-1}, respectively) in a randomised outdoor mesocosm experiment. The experiment was run for two weeks post-dung inoculation (Day 7 to 21), with mosquito larvae collected (Day 14 and 21), identified and quantified. Higher dung nutrient concentrations significantly increased mosquito larval abundance relative to dung-free controls. Culicine larvae were 26-times more abundant than anopheline on average. Greater dung concentrations also tended to promote more rapid development in larval mosquitoes. With no colonisation by mosquito larvae in the control treatments, we conclude that the input of dung in aquatic ecosystems promotes vector development and abundance with the potential to increase risk of mosquito-borne infections. We therefore recommend sustainable management policies that tackle likely ecological disservices attributable to free-ranging livestock communities.

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1. Introduction

Livestock, particularly cattle, play a significant global economic and socio-cultural role in the livelihoods of farmers, especially those based in rural settlements (Hebinck and Faku, 2013). Over the years, cattle production has improved human population wellbeing as a source of income, food supply, employment and a sustainable agricultural diversification model (Enahoro et al., 2018). Moreover, livestock is often used as a 'safety net' and buffers farmers in the event of crop production failure (Tolera and Abebe, 2007). However, despite the value of cattle in small-to-large scale farming communities, there is limited consideration for subsequent direct and indirect ecosystem disservices by both free-range (communal) and fenced area cattle production (Lange et al., 1998). Studies that have assessed environmental degradation associated with cattle are largely restricted to terrestrial landscapes, dealing predominantly with overgrazing and its effects (Stavi et al., 2016). However, the implications of cattle on aquatic ecosystem degradation are less well-known.

In many arid regions around the world, aquatic landscapes are characterised by both semi-permanent and temporary wetland systems (Arntzen, 2016). Temporary wetland systems are typically charac-

terised by a unique assemblage of invertebrates (Bird et al., 2019). Within landscapes that contain cattle, however, these systems typically serve as watering points, particularly following intermittent rainfall periods (Scoones, 1991). The known propensity for cattle to urinate and defecate in bodies of water while drinking (Mesa et al., 2015) may have consequences for aquatic faunal groups and nutrient input dynamics in these ecosystems, with concurrent implications for mosquito pest species that utilise these environments as breeding sites (Batzer and Boix, 2016). Although the duration of hydroperiods in these habitats may be a limiting factor to many species, mosquitoes are well adapted to utilise such habitats given their rapid development, particularly under nutrient-rich conditions (Marinho et al., 2016). While climate-mediated factors have been explored within the context of mosquito ecology (Asigau and Parker, 2018), aquatic habitat degradation by animal-induced eutrophication is less explored. Specifically, no work has been done concerning the impacts of cattle dung on mosquito proliferation in temporary wetland systems, despite the ecological significance of these wetlands, and epidemiological importance of mosquitoes (Raj et al., 2014).

Mosquitoes harbour pathogens which cause human, wildlife and livestock diseases, driving high rates of morbidity and mortality worldwide (see Kapesa et al., 2018). For instance, *Anopheles* sp. (malaria-causing *Plasmodium*) and *Culex* sp. (West Nile virus, Rift Valley fever, Usutu virus, encephalitis viruses, filarioid worms, *Haemoproteus*) form an important tripartite ecological component of vector-parasite

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and host disease transmission dynamics (e.g. Schmid et al., 2017; Braack et al., 2018). Importantly, their oviposition site selection can be contingent on patterning of risk and reward among habitat patches (Pintar et al., 2018). For example, many mosquito species detect cues and subsequently disregard breeding sites containing predators (Eveland et al., 2016). Alternatively, some mosquitoes select sites with low larval density to reduce competition (Himeidan et al., 2013), although these sites may further enhance their vectorial capacity (see Juliano et al., 2014). Availability of food may be prioritised over predation risk (Albeny-Simões et al., 2014). In this context, stagnant and slow-moving waters with high organic resources (nutrients) are preferentially selected to sustain the development of mosquito juveniles (Turnipseed et al., 2018). Nutrient-enriched habitats might thus promote mosquito colonisation and proliferation with increased risk of associated diseases. As such, exploring the implications of cattle-induced eutrophication and rapidly increasing human population growth for vector mosquitoes is essential in a public health context.

In the tropics, there is an urgent need for improved understanding of factors facilitating increased mosquito abundance, particularly as human-mediated activities and climate change ensue. Here, we test for such effects using an in situ experimental mesocosm approach in a semi-arid African environment, to investigate the implications of cattle-induced eutrophication in aquatic habitats utilised by mosquitoes. Given the prevalence and contribution to temporary aquatic wetland environments, and their extensive utilisation by cattle and other wildlife, we worked in a rocky outcrop landscape characterised by temporary rockpool habitats. Here, we were particularly interested in medically-important mosquito species given their distribution and prevalence in these diverse landscapes (Kamal et al., 2018). The aim of the study was to investigate the effect of cattle dung concentration loadings on larval mosquito species colonisation and abundance using an in situ mesocosm approach. We hypothesized that: (1) dung-nutrified mesocosms on rocky outcrops would be colonised by vector-mosquito species, and (2) cattle-dung eutrophication will drive similar proliferation rates between culicine and anopheline species.

2. Materials and methods

2.1. Study area and experimental design

The study was conducted on a rocky outcrop (22°35′46.7S; 27°07′30.3E) on Botswana International University of Science and Technology Palapye campus (Fig. 1a, b) in the austral winter between June and July (2019). The large campus (2500 ha), situated in a semi-arid environment (see Batisani and Yarnal, 2010), is characterised by numerous rocky outcrops, but is fenced and therefore excludes livestock. Outside the campus premises, however, such rocky outcrop landscapes are heavily utilised by free-range cattle, and particularly during the rainy season when rockpools fill up with water (Fig. 1c). Twenty five mesocosms (105 mm length × 72 mm width × 40 mm depth; Fig. S1) were distributed across the selected outcrop on campus, covering an area of approximately 1300 m^2. Mesocosms were placed such that they were at least 3 m away from the tree canopy (to avoid leaf litter infall) and 5 m away from adjacent mesocosms to avoid spatial contagion. Once in place, each mesocosm was filled with 50 L of tap water (Day 1), and left to mature for 7 days. To avoid colonisation by other invertebrates or use by vertebrates during this time, all mesocosms were covered with 500 μm mesh cloth (Fig. S1a). Mesocosms were kept at 50 L by topping up with matured tap water every second day for the duration of the experiment.

After one week of water maturation, treatments were established in the 25 mesocosms (Day 7). Approximately 6 kg of fresh dung was collected from two cows in Palapye village. These two cows were monitored over the course of the day to allow immediate dung collection following defecation. Fresh dung from the two cows was homogenized by hand for 30 mins in a large 100 L plastic container. Five dung treatments were established. In control mesocosms (i.e. C), no dung was in-

oculated. In treatments 1, 2, 3 and 4 (i.e. T1-T4), however, mesocosms were inoculated with dung at 1 g L^{-1}, 2 g L^{-1}, 4 g L^{-1} and 8 g L^{-1}, respectively. Treatment assignment to mesocosms was fully randomised, with 5 replicates assigned to each treatment. Post-dung inoculation, the 500 μm mesh cloth that was placed over each mesocosm for insect exclusion (Fig. S1a), was weighted and submerged at the center within the 50 L of water (Fig. S1b), thus allowing for mosquito colonisation. The experiment was then run for two weeks (allowing for oviposition and larval development) post-dung inoculation (Day 7 to Day 21), with temperature variation in each mesocosm measured at least every 3 days (at mid-day) during this period, using a multi-probe (Aquameter, Aquaread Ltd., Kent, UK).

Mosquito larvae were collected from all mesocosms 7 days after treatment establishment (Day 14) and again on Day 21. On each collection day, all mosquito larvae were removed from each mesocosm using a small (10 cm × 15 cm) 200 μm mesh net. All mosquito larvae were exhaustively collected through repeated scooping of the net through the water column. Following collection, each mesocosm was inspected in random order by three different researchers to ensure that no larvae remained in the mesocosm. All collected mosquito larvae were transferred to 80% ethanol within an hour of collection, for later identification.

2.2. Identification of mosquito larvae

Mosquito larvae were first broadly identified to genus level using morphological features, mainly of the siphon and head, following recommendations by Jupp (1996). The organisms were then sorted according to size as first (1.3 ± 0.2 mm), second (2.5 ± 0.2 mm), third (4.2 ± 0.2 mm) and fourth (5.9 ± 0.3 mm) instars from all the mesocosm treatment collections. Further, a representation of six randomly-selected anopheline and culicine larval specimens (across life-histories) were each subjected to DNA extraction using Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, USA) followed by amplification of the mitochondrial cytochrome oxidase subunit I (COI) using polymerase chain reaction (PCR). This was achieved using species identification universal primers HCO 2198 and LCO 1490 following protocols of Buxton et al. (2019). The PCR products of all amplicons were purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA) and sequenced at Inqaba Biotechnical Industries (Pretoria, South Africa). Obtained raw sequences were trimmed and contigs assembled using CodonCode Aligner 8.0.2 software package and subsequently subjected to the basic local alignment search tool (BLAST) for comparison with already identified species.

2.3. Statistical analyses

Differences in water temperatures were examined according to *treatment* over *time* using linear mixed effects models with individual *mesocosms* included as a random effect. Residual normality and homoscedasticity were assured using diagnostic plotting following log_{10} transformation of temperatures. Generalised linear mixed effects models were used to examine counts of larval mosquitoes with *treatment* (5 levels), *species* (2 levels), *instar stage* (4 levels) as fixed and two-way interacting effects. Observation *day* (2 levels) was also included as an individual term. A random effects structure accounted for repeated measures within each experimental mesocosm. Owing to evidence for zero inflation (number of zeros exceeded those expected from simulations; Hartig, 2019), a zero inflation parameter was applied to all observations (Brooks et al., 2017). There was no evidence for residual overdispersion in the model. The significance of main effects was inferred via analyses of deviance using Wald chi-square statistics with type III sums of squares (Fox and Weisberg, 2019). Selected replicate mesocosms (C3, T1R1, T1R5, T3R5, T4R4) accumulated leaf-litter during the study, with potential to either inhibit or facilitate increased oviposition (Cuthbert et al., 2019). As such, these mesocosms