INTERACTIONS BETWEEN BATS AND MOSQUITOES: A CASE STUDY FROM AN AGRO-FORESTRY LANDSCAPE

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Abstract

Little is known about the trophic interactions between mosquitoes and bats in tropical landscapes that are dominated by human-modified habitats. In this study, we examined the link between insectivorous bats and mosquitoes across 14 cocoa farms and highlights environmental and landscape predictors that could influence the abundance of mosquitoes and insectivorous bats in Cameroon. 1137 individual mosquitoes were collected using CDC light traps and the most abundant belonged to the genus *Culex* (38.6%) followed by *Uranotaenia* (25.1%) and then *Aedes* (4.9%). Insectivorous bats made up 44.5 % of the total bats caught belonging to 16 species and 8 genera. Generalized linear mixed models (GLMM) predicted a significant variation in insectivorous bats across varied cocoa shade ($\chi2 = 22.738$, df = 1, p =1.857e-060), increasing bat abundance from 1.625 at 22% farm canopy to 6.65 at 98% farm canopy. We found no correlation between mosquito abundance and bat abundance however, landscape effects significantly influenced the abundance of bats ($\chi2 = 14.709$, df = 3, p =0.002) and mosquitoes ($\chi2 = 15.718$, df = 3, p =0.001) indicating spatial variability in the change in species abundance. As expected, mosquitoes were highly abundant in the rainy season compared to the dry season due to the availability of breeding sites and suitable conditions for development. This study has contributed to the knowledge of ecological interactions between insectivorous bats and mosquitoes and determined the spatio-temporal effects that predict bat and mosquito abundance in disturbed tropical landscapes. Implications are discussed below.

1. Introduction

Species interactions are core aspects of species-specific biology and ecology that could determine the stability of an ecosystem (Delmas et al., 2019). These interactions form the basis of ecological and evolutionary processes that results in a competition (e.g. parasitism, trophic cascades, resource utilisation) or a symbiosis (e.g. pollination and seed dispersal; Clare, 2014). Characterization of trophic interactions are particularly challenging due to the rate and number of possible interacting species (Clare, 2014). However, recent advancements in molecular methods such as the high-through-put sequencing (HTS) technique, has paved a way for an efficient, precise and cost-effective identification of biodiversity and simultaneous examination of food webs from an extensive array of potentially interacting individuals (Lundgren & Fergen, 2014; Pompanon & Samadi, 2015; Symondson & Harwood, 2014; Tiede et al., 2016; Valentini, Pompanon, & Taberlet, 2009).

Bats are the second most diverse group of mammals known for their widespread consumption of arthropods, including agricultural pests and mosquitoes which are the major vectors of human diseases (Kasso & Balakrishnan, 2013; Kunz et al., 2011; Maas et al., 2016; Wray et al., 2018). Reductions in mosquito populations by bats has mainly been seen in enclosure experiments that do not constitute natural conditions (Griffin, Webster, & Michael, 1960; Reiskind & Wund, 2009). Consequently, questions as to whether bats affect mosquito abundances particularly, major vectors, is largely unknown (Wray et al., 2018). Although some studies have shown the potential of bats as mosquito consumers (Griffin et al., 1960; Reiskind & Wund, 2009), very little is known about the relationship between bats and mosquitoes, particularly, in tropical ecosystems where these species are highly abundant and diverse (Gonsalves et al., 2013; Puig-Montserrat et al., 2020).

The importance of bats and mosquitoes

The greatest diversity and abundance of bats are seen in the tropics (Willig, Kaufman, & Stevens, 2003), with over 100 species identified in Cameroon (ACR, 2018). Bats play key ecological roles in natural and modified ecosystems which are beneficial to animals and humans (Kunz et al., 2011). These ecosystem services includes plant pollination (Bradley & Merrilyn, 1999), seed dispersals (Duncan & Chapman, 1999), recycling and distribution of nutrients (Voigt et al., 2007), and suppression of agricultural pest (Maas, Clough, & Tscharntke, 2013). Insectivorous bats comprise approximately 70% of all bat species and are particularly important in tropical landscapes because of their ability to predate arthropod populations (Maas et al., 2013; Simmons, 2005).

Mosquitoes are major vectors of many infectious diseases and are the primary cause of high morbidity and mortality in humans (Afridi, Afridi, & Saeed, 2017; Medeiros-Sousa, Fernandes, Ceretti-Junior, Wilke, & Marrelli, 2017). Mosquito vectors of medical importance belong to the genera *Anopheles, Culex* and *Aedes* (Tandina et al., 2018). *Anopheles* mosquitoes transmits parasites that causes malaria to humans with over 219 million cases globally, and results in more than 400 000 deaths annually (WHO, 2020). Children under the age of five account for 67% of all malaria deaths with Africa being home to 93% of all malaria cases and 94% of malaria deaths (WHO, 2020).

Highly invasive mosquitoes in the genus Aedes, which are unique in their prominent white markings are vectors of many arboviruses causing diseases such as yellow fever, dengue fever, chikungunya, Zika fever etc (Kamgang et al., 2011; Simard, Nchoutpouen, Toto, & Fontenille, 2005). *Culex* mosquitoes are primary vectors of lymphatic filariasis, which is considered among others as neglected tropical diseases (NTDs) with a mean prevalence of 3.3% in Cameroon (Nchoutpouen et al., 2019). In addition, mosquitoes in the genus *Mansonia,*

Aedeomyia, Uranotaenia and *Coquillettidia* have been implicated in the transmission of avian malaria and other zoonotic and epizootic diseases (Bonneaud et al., 2009; Njabo et al., 2009).

Given the threat posed to public health by mosquitoes, several control strategies have been implemented to curb mosquito populations and reduce risks of outbreaks (Russell & Kay, 2008). In spite of these, mosquitoes still remain one of the biggest veterinary and public health problems of mankind due to their ability to evade control measures using traits such as behavioural avoidance and insecticide resistance (Antonio-Nkondjio et al., 2019). Therefore, an understanding of the role natural predators play in regulating mosquito populations is important to inform current and future mosquito control interventions (Gonsalves et al., 2013).

The relationship between bats and mosquitoes

Dietary studies represent an important step in understanding the relationship between predators and their respective prey (Krauel et al., 2018). Several methods have been used to analyse arthropod predation data, in order to improve taxonomic classification of genetic material in stomach content (Jusino et al., 2019). However, these methods have shown to underestimate the actual number and kind of insects present (Belwood & Fenton, 1976).

Several studies have attempted to estimate the impact of bat predation on mosquito populations (Clare, Barber, Sweeney, Hebert, & Fenton, 2011; Gonsalves, 2012; Griffin et al., 1960). For example, Reiskind & Wund (2009), under enclosed experiments, observed a 32% reduction in *Culex* oviposition rates as a result of predation by the northern long-eared bat (*Myotis septentrionalis*). Gonsalves et al. (2013) detected positive correlations between the abundance of mosquitoes and the small-sized insectivorous bats of the *Vespadelus* genus in open saltmarshes relative to coastal forest habitats. In this study, bat predation was attributed to two effects: high frequency-echolocating calls emitted by bats which enables detection of smaller prey and less vegetation clutter in saltmarshes that optimise prey detectability and availability.

In addition, Wray et al. (2018) reported a higher incidence and taxonomic richness of mosquitoes in the faeces of two common bats (little brown bat: *Myotis lucifugus* and big brown bat: *Eptesicus fuscus*) in Wisconsin, USA. Although classification was limited to orders, Mbeng et al. (2019) recently found dipteran insects to be the second most abundant (79.1%) in the diet of four insectivorous bats in the Centre region of Cameroon.

Most studies of bats as predators of mosquitoes have taken place in temperate zones with very little knowledge available from tropical regions. Recently, my research team successfully applied eDNA to analyse the diets of bats in cocoa plantations in Cameroon. Critically, 83% of the 161 insectivorous bats tested consumed *Anopheles* mosquitoes—the genus that transmits malaria (Welch and Powell, unpublished work) (Figure 1). To the best of my knowledge no study has examined the association between mosquitoes and bats in an agroforestry landscape in Cameroon.
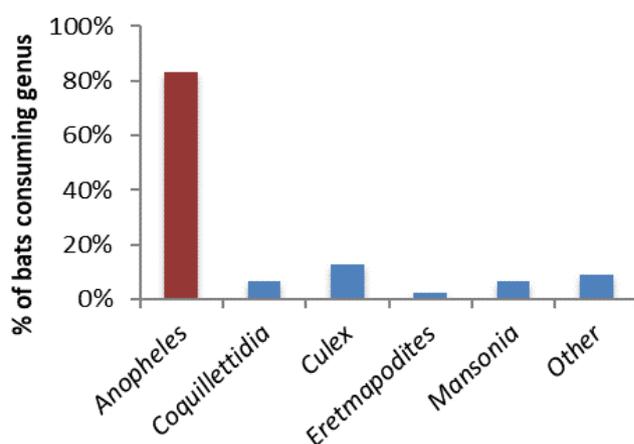

Figure 1: Proportional mosquito consumption by Insectivorous bats in cocoa farms, Cameroon (*Rhinolophus alcyone*, n=80 and *Hipposideros ruber*, n=81).

The widespread existence of *Anopheles* mosquitoes in African bat diets raises the hypothesis that bat predation provides an ecosystem service by regulating vector populations. However, little evidence exists on the relationship between bat activity and mosquito populations

(Gonsalves et al., 2013; Puig-Montserrat et al., 2020). Moreover, biotic and abiotic factors may also modify species interactions, which are often poorly understood (Buxton et al., 2020).

Factors that influence mosquito and bat abundance

The rapid rate of deforestation and forest conversion into agricultural systems is alarming in West African landscapes and this degradable transition results in temporal, spatial and biotic isolation of plant and animal populations (Estrada, Coates-Estrada, & Meritt, 1993; Saunders, Hobbs, & Margules, 1999), leading to the decline in quality of ecological conditions and interactions (Estrada et al., 1993). These fragmented landscapes are dominated by human-modified habitats including secondary forests, tree plantations, and agroforestry (Gardner et al., 2009; Voigt & Kingston, 2015). Cocoa agroforestry is a unique form of agricultural system whereby cocoa trees are planted alongside natural forest trees, providing some level of ecological benefits to biodiversity as well as generating income for the farmers (Rice & Greenberg, 2000).

Cocoa agriculture constitutes one of the predominant agricultural activity in Cameroon and is of great value to the economy given its contribution to the gross domestic product (GDP). Management intensification practices such as removal of shade trees is posing a threat to biodiversity (Daghela Bisseleua, Fotio, Yede, Missoup, & Vidal, 2013), and cocoa pest damage is decreasing cocoa yield, causing losses of between 550 000 to 2.4 million Euros per year (Mahob et al., 2015). These effects are greatest in full-sun management farms where cocoa trees are planted without beneficial shade trees (Daghela Bisseleua et al., 2013). In addition, vector-borne diseases could have negative impacts on the capacity of the working populations resulting in a decrease in cocoa production (Audibert, Brun, Mathonnat, & Henry, 2009).

Climate change (e.g. seasonality, altitude, cold. drought, precipitation and heat) is one important stressor that can influence the abundance and activity of bats and mosquitoes

(Cumming & Bernard, 1997; Jones et al., 2009). The extent to which these factors affects species is highly variable in nature and could be compared to the different types of anthropogenic activities that fragment and alter landscapes across the globe (Vitousek et al., 2008). Insectivorous bats occupy higher trophic levels and are very sensitive to insecticides and toxins. Hence, any variation in arthropod prey abundance could indicate variation in bat abundance (Hutson, Mickleburgh & Racey, 2001; Kasso & Balakrishnan, 2013).

Research objectives and hypothesis

Although insectivorous bats are thought to be one of the most effective consumers of arthropod pests in tropical landscapes, to the best of my knowledge, no research has addressed the ecological relationship between insectivorous bats and mosquitoes in cocoa farms in Cameroon. Additionally, little is known about mosquito communities found in cocoa farms including the presence of disease vector species. This study is significant in that, it is the first step towards understanding trophic interactions in which bats and mosquito vectors are embedded, hence elucidating the potential role if any, that bats play in regulating disease vectors.

My project targeted three specific objectives:

1) Evaluate mosquito abundance between cocoa farms and neighbouring villages and its relationship with environmental factors in Cameroon.

2) Determine association between bat abundance and environmental factors within cocoa farms.

3) Test for association between mosquito and bat abundance in cocoa farms.

Therefore, I hypothesize that (1) The abundance of mosquitoes including disease carrying genera (*Anopheles*) varies between cocoa and village settings, and in association with environmental factors (2) Bat abundance and diversity is also impacted by cocoa farm

management (e.g proportion of farm shade). (3) Mosquito abundance may be correlated with bat abundance either positively, indicating a possible thrive in abundance of both bats and mosquitoes or negatively, owing to a potential suppression of mosquito populations due to bat activity in cocoa farms.

Materials and methods

Study area

Cameroon is situated between western and central Africa and constitutes an important component of the Congo basin due to its huge diversity of biological resources and ecosystems (Sonwa et al., 2007). Like many tropical rainforest regions, Cameroon faces environmental challenges due to deforestation. From the year 2001 to 2015, Cameroon lost over 752, 000 ha of forest and the rate of deforestation has significantly increased over the last five years of this period (Carodenuto, Gromko, & Chia, 2017). Conversion of land for cocoa agriculture is one of the major contributing factors to this forest loss (Ordway, Asner, & Lambin, 2017). Cameroon ranks third among west African countries that produce 73% of the world's cocoa, with production mainly in the South-West, Centre, East, South and Littoral regions respectively (Carodenuto et al., 2017; Sonwa, Coulibaly, Adesina, Weise, & Tchatat, 2004).

This study was carried out in the Central and Eastern regions of Cameroon. Both regions have a subequatorial Guinean climate with an altitude ranging between 500m and 850m, an average temperature of 25°C and an annual rainfall of 1500mm and 2000mm. Vegetation varied from primary forest in the East to a dense semi-deciduous forest around the Centre region (Sonwa et al., 2007). Data collection was executed in four different landscapes (commune: Ayos, Elat, Ngoumou and Somalomo), with study sites (or cocoa farms) varying systematically from high shade (mainly traditional forest associated with cocoa) to medium shade (forest with interspersed fruit and native trees associated with cocoa) and full sun (little or no forest associated with cocoa; Table 1).

Table 1: Summary characteristics of sampling locations in Cameroon, 2019/2020.

Regions	Landscapes	Cocoa farms	Geographic coordinate (latitude: longitude)	Farm size (hectares)	Cocoa system
Centre	Ayos	NGUI 001*	3.86398: 12.43288	2	Full sun
		NGUI 002*	3.86365: 12.426726	4	Traditional
		EBA 001*	3.90671: 12.510432	2.5	Fruit tree
		EBA 003*	3.91195: 12.50733	3.5	Fruit tree
		EBECK*	4.043473: 12.70014	3	Traditional
	Elat	ELAT S	3.90349: 11.71647	4	Full sun
		ELAT N*	3.89624: 11.71762	1.5	Traditional
	Ngoumou	EBEM*	3.57199: 11.33041	2	Traditional
		NKOL S*	3.58884: 11.32919	3	Fruit tree
		ADZAP*	3.47207: 11.26735	5	Full sun
East	Somalomo	PALM*	3.38741: 12.743	3	Fruit tree
		STADE*	3.38307: 12.74529	1.5	Traditional
		MAMA*	3.35773: 12.73725	5	Fruit tree
		SNAKE*	3.39001: 12.74924	2	Traditional

Study design

Fieldwork was conducted in 14 cocoa farms located across 4 landscapes in the East and Centre regions of Cameroon between August-September of 2019 (rainy season) and January-February of 2020 (dry season). Bats and mosquitoes were sampled once per farm during every field season (wet and dry). In addition to sampling in cocoa farms, mosquitoes were also collected from neighbouring villages (at 13 out of 14 sites as indicated by asterisk in Table 1). These study sites were selected from those being investigated as part of a larger project being conducted in my research group that aims to investigate the ecological role of bats and birds in controlling cocoa pests in West Africa. Cocoa farms varied from 1.3 – 5 ha with each farm not less than 500m apart.

Data collection

Mosquito sampling

Mosquito sampling was carried out by deploying two CDC light traps (model 512, John W. Hock company) in cocoa farms and (where applicable) accompany villages. On each night of sampling, one CDC light trap was deployed in the farm and two in the village. In both settings, each light trap was installed at 1.5m to 2m above ground level and at an approximate distance of 200m apart to prevent overlap. After deployment, light traps were allowed to run for a duration of 12 hours (6:00 pm to 6:00 am). Captured mosquitoes were knocked out by placing mosquito collection cups in a freezer, however, during instances of power outage, smoke was used to immobilize mosquitoes. These mosquitoes were later sorted and stored in silica gel-containing tubes for further identification in the laboratory. In the lab, mosquitoes were examined using a stereomicroscope x 90 (Humascope Stereo, Germany, Ref: 14900) and identified to genus level and to some extent species level using morphological keys and mosquito anatomy reference guides (Edwards, 1941; Gillies & Coetzee, 1987; Harbach & Knight, 1980; Service, 1990). As identification to species level based on morphology was possible on only a few occasions, classification and subsequent analysis was therefore limited to genera.

Bat sampling

Bats were sampled using mist nets (12m x 2.5m). Twenty mist nets were set up in each cocoa farm along established transects from 18:30 pm to 12:30 am each night. Net runs were performed every 15 minutes so as to avoid entanglement and death of bats. Once in the nets, bats were carefully removed and stored in cotton-like holding bags for subsequent processing at the processing station. Captured bats kept in holding bags were then identified, weighted, sexed and measured. Bats were identified to species level based on certain morphological features such as length of forearm, tail length, ear length, nose leaf, teeth, palatal ridges and tragus and also, with the aid of bat identification keys (Happold & Happold 2013; Hayman,

1971; Patterson & Webala, 2012; Rosevear, 1965). Bat wings were punctured before release so as to avoid recaptures. Bat and mosquito sampling in cocoa farms were not conducted simultaneously because the light emitted by CDC light traps could negatively affect bat capture. However, CDC light traps were deployed at an approximate distance of 50m-100m away from bat capturing stations on the day of sampling.

Landscape and climatic measurements

To investigate the effects of landscape factors on the abundance of bats and mosquitoes, we measured farm shade cover (hereafter farm canopy) as well as forest cover at a 5km radius around each cocoa farm. Farm canopy denotes how intensely cocoa farms are managed; with traditional farms (less intensely managed) having higher shade canopy owing to the large proportion of forest trees being retained, whereas the more intensely managed farms (full sun farms) tend to expose cocoa trees to sunlight by cutting most of the shade trees.

To measure farm canopy, 10 photographs at 10 locations (every 24m following mist net transects) were taken in each farm using a camera with a fish-eye lens hung to a pole of about 4m long. Locations were spaced out at 24m from one point to another and approximately 50m from farm edge. Photographs were then converted to black and white using the software ImageJ (Schneider, Rasband, & Eliceiri, 2012) and the percentage of black (vegetation) in each photograph was calculated. Estimates of farm canopy was then calculated as the mean of the 10 pictures.

Forest cover was measured by downloading a percent tree cover layer from MODIS Vegetation Continuous fields (MOD44B; Townshend *et al.*, 2011) and creating a 5km radius buffer around each farm in QGIS 2.18.23 (QGIS Development Team, 2018). Average percentage tree cover was then extracted from pixels within the buffer (processed by Crinan Jarrett, PhD student,

UoG). Values of forest cover varied from 12.9% in a farm next to a town to 63.7% in a farm next to a forest reserve (see appendix 1).

We obtained spatial daily temperature and relative humidity of each cocoa farm during the dry and wet season of data collection by downloading meteorological predictions from the NASA Prediction of Worldwide Energy Resources (POWER) data base (https://power.larc.nasa.gov/data-access-viewer/). The mean daily predictions of temperature and relative humidity were estimated within a 2m resolution of each cocoa farm (appendix 1).

Ethical consideration

Bat and mosquito surveys were performed following regulations put in place by the animal ethics committee of the ministry of livestock, fisheries and animal industries, Cameroon. Also, animal care approval was obtained from Durham University. Authorization to export fieldwork samples was issued by the forestry and wildlife control post of the Yaounde Nsimalen Airport in Cameroon. Informed consent was approved by cocoa farm owners before sampling and this was also, facilitated by research partners from the Congo Basin Institute (CBI) and the International Institute for Tropical Agriculture (IITA).

Data analysis

Statistical analysis was conducted to test for associations between mosquito and bat abundance and how environmental and landscape factors affects the abundance of bats and mosquitoes using the R statistical software package (version 4.0.1; 2020-06-06). A total of three generalized linear mixed effect models (GLMM) were developed, with response and explanatory variables as described in Table 2. All statistical models were fitted using the glmmadmb function from the glmmADMB package. First, the relationship between insectivorous bat abundance, mosquito abundance and other environmental predictors were examined using GLMM with a Poisson distribution (Table 2, Model 1). Here the response

variable was total bat count per capture night per farm, with explanatory variables of season (wet and dry), landscape (commune: Ayos, Elat, Ngoumou and Somalomo), temperature ($^{\circ}$C), relative humidity (%), mosquito abundance (number/trap/night), forest cover at 5km radius (%) and farm canopy (%), and random effect of cocoa farm. Next the relationship between mosquito abundance and environmental variables was examined using GLMM with a negative binomial distribution (Nedelman, 1983). Here I tested how mosquito abundance varied between farm and neighbouring villages ('treatment') and all the same environmental variables defined for the bat model (see Table 2, model 2). Furthermore, we examined variations in individual mosquito genera (*Culex, Uranotaenia, Aedes* and *Anopheles*) between treatments and environmental predictors by fitting a GLMM with a negative binomial distribution as seen in Table 2 (Model 3). Model selection was done using Likelihood Ratio Tests (LRTs) based on backward elimination from the full model for each response variable of interest as defined in Table 2. The most parsimonious model was used to estimate coefficients and predicted values for significant explanatory variables at a threshold of $P<0.05$. I also report percentage count of insectivorous bats and mosquitoes collected during sampling. All statistical plots were derived using the ggplot2 package in R.

Table 2: Summary table of generalized linear mixed effect (GLMM) models.

Model	Response variable	Unit	Fixed Effects	Random Effects	Distribution
1	Insectivorous bat Abundance	Number/night/farm	Season + Landscape+ Temperature+ Relative humidity+ Mosquito abundance+ Forest cover at 5km radius+ farm canopy	Cocoa farm	Poisson
2	Total Mosquito Abundance	Number/trap/night	Season + Landscape+ Temperature+ Relative humidity+ Treatment+ Forest cover at 5km radius+ farm canopy	Cocoa farm	Negative binomial
3	Individual mosquito genera abundance i.e. -*Culex* -*Uranotaenia* -*Aedes* -*Anopheles*				

RESULTS

Abundance and community composition of bats and mosquitoes

In total, 1137 and 677 mosquitoes were collected from cocoa farms and neighbouring villages respectively, representing 12 genera. The most abundant genera were *Culex sp.* (38.6%) followed by *Uranotaenia sp.* (25.1%) and then *Aedes sp.* (4.9%). The least abundant genera of mosquitoes were *Lutzia sp.* (0.76%) and *Hodgesia sp.* (0.76%) respectively. Approximately 4.8% of mosquitoes were *Anopheles sp.*—the genera that includes vectors of human malaria (Figure 2).
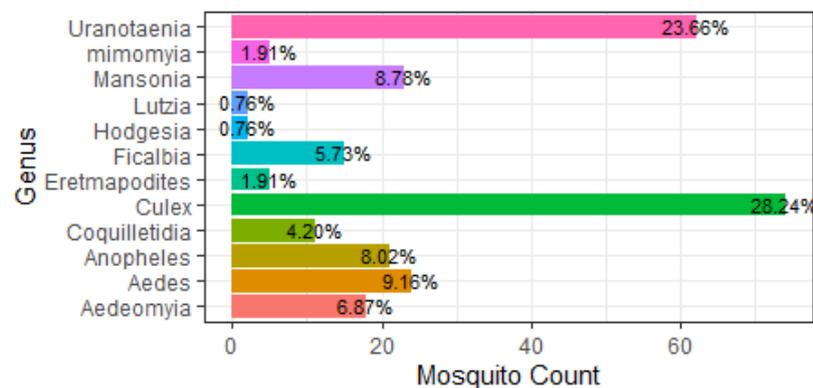

Figure 2: Community composition and abundance of mosquito genera captured at 14 cocoa farms and 13 neighbouring villages in the east and centre regions of Cameroon between August-September 2019 and January-February 2020. Bars indicate the number of captures of each genus, and the figure indicates the percentage of total captures

A total of 283 bats were captured in cocoa farms. Insectivorous bats made up 44.5 % (n=126) of the total bats caught and comprised of 16 species and 8 genera. As shown in Figure 3, *Doryrhina cyclops* (15.1%) was the most abundant species and *Miniupterus inflatus* (0.79%) was the least abundant. Two of the most common insectivores of Cameroon ecosystems were also well represented: *Hipposideros ruber* (14.3%) and *Rhinolophus alcyone* (14.3%; Figure 3).
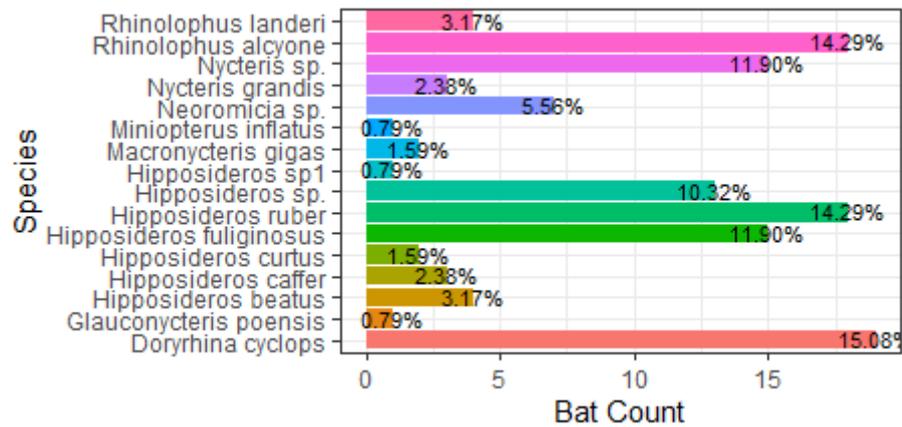
Figure 3: Insectivorous bat abundance and composition in 14 cocoa farms in Cameroon, caught between August-September 2019 and January-February 2020. The bars indicate the number of captures of each species, and the figure indicate the percentage of total captures.

Determinants of mosquito abundance

Total mosquito abundance was significantly associated with season ($\chi2 = 7.52$, df = 1, p = 0.006), relative humidity ($\chi2 = 7.90$, df = 1, p =0.005) and landscape ($\chi2 = 15.72$, df = 3, p = 0.001, Table 3). Specifically, mosquito abundance was significantly higher in the wet (11.9, p = 0.001) than in the dry season (9.3, p = 0.001). After accounting for this seasonal variation, relative humidity had a slight but statistically significant negative association with mosquito abundance (Table 3, Figure 4). As shown in Figure 4, relative humidity was random across the sampling period— with a cluster of points at 70% and 90% respectively. In addition, the abundance of mosquitoes differed significantly across landscapes, with the highest abundance recorded in Ayos (58.0±58.6) and the lowest in Ngoumou (4.41±6.39). Temperature, treatment, farm canopy and forest cover had no significant effect on the abundance of mosquitoes in cocoa farms (Table 3).
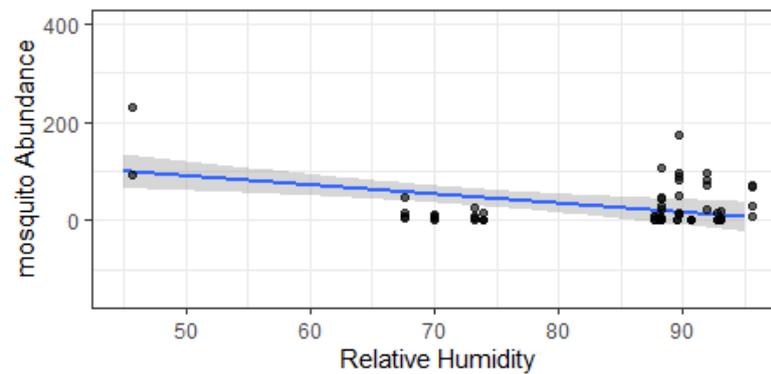
Figure 4: Effects of relative humidity on the abundance of mosquitoes in cocoa farms, Cameroon. The blue line indicates effect size of relative humidity on mosquito abundance and the grey shade demonstrates 95% confidence interval.

Table 3: Summary of best fitted mixed-effect model between mosquito abundance, environmental variables and treatment (cocoa farm versus village) in Cameroon. Estimates and test statistics of significant variables were retrieved from the best fitted model and LRT tests respectively whereas, estimates and chi-square values of non-significant variables were obtained from the most complex model and LRT that excluded the specific explanatory variable.

Covariates	Estimates	SE	df	$\chi 2$ value	P-value
Treatment	-0.0621	0.3147	1	0.0420	0.8376
Shade cover	-0.0097	0.0090	1	1.5100	0.2191
Temperature	-0.0732	0.5118	1	0.0280	0.8671
Relative Humidity	-0.0959	0.0327	1	7.9000	0.0049**
Forest cover	0.0008	0.0155	1	0.0040	0.9496
Season	3.2666	1.0020	1	7.5240	0.0061**
Landscape	-2.2130	0.5655	3	15.718	0.0013**

1 model out of 8; Type of model: GLMM; Predictor distribution: Negative binomial

Further analyses of different mosquito genera indicate some variation between taxonomic groups. *Culex* mosquitoes significantly varied across seasons with a higher abundance recorded in the rainy season than the dry season ($\chi 2 = 10.32$, df = 1, p = 0.001; Figure 5). Also, landscape ($\chi 2 = 11.81$, df = 3, p = 0.008) and forest cover ($\chi 2 = 5.34$, df = 1, p = 0.02) significantly had an effect on the abundance of *Culex* mosquitoes. The Somalomo landscape recorded the highest abundance in *Culex* mosquitoes (25.9, p = 0.48), followed by Ayos (17.3, p = 0.001), Elat (2.8, p = 0.01) and then Ngoumou (1.1, p = 0.001; Figure 6). Forest cover was negatively associated with *Culex* mosquito abundance (Appendix 3). The abundance of *Culex* mosquitoes went from 140 in farms closer to urban areas (95% CI = 18.21,1079) to 0.04 in farms closer to a forest (95% CI = 0.001, 1.52). Temperature ($\chi 2 = 0.008$, df = 1, p = 0.9), treatment ($\chi 2 = 0.53$, df = 1, p = 0.46), farm canopy ($\chi 2 = 0.78$, df = 1, p = 0.37) and relative humidity ($\chi 2 = 0.89$, df = 1, p = 0.34) had no effect on the abundance of *Culex* mosquitoes.
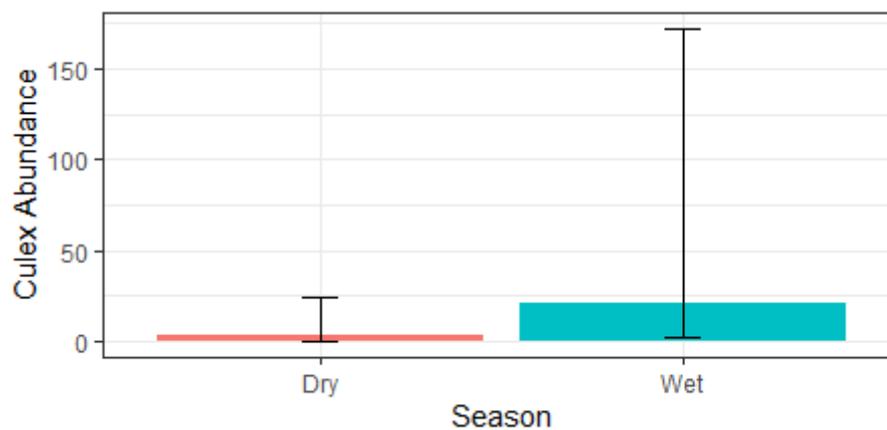

Figure 5: Predicted mean abundance of *Culex* mosquitoes across seasons in the east and centre regions of Cameroon, 2019/2020. Error bars correspond to predicted mean abundance at 95% confidence interval.
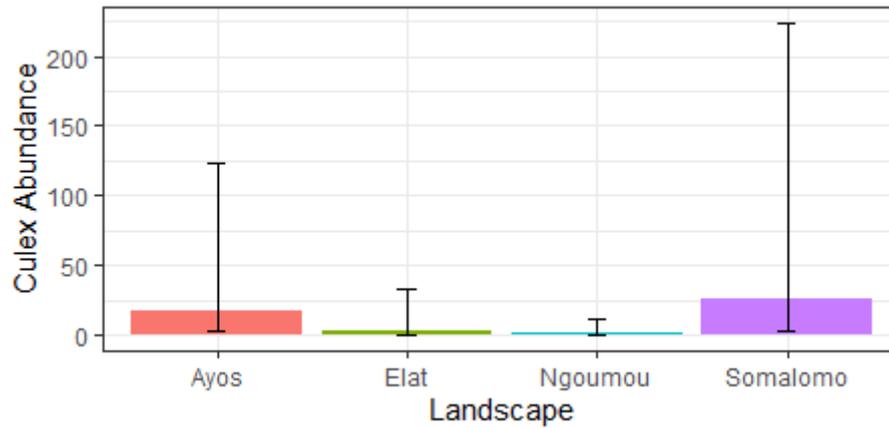
Figure 6: Predicted mean abundance of *Culex* mosquitoes across landscapes in the east and centre regions of Cameroon, 2019/2020. Error bars indicates 95% confidence interval

Uranotaenia mosquitoes significantly varied across landscapes ($\chi2$ = 14.28, df = 3, p =0.003) and treatment ($\chi2$ = 5.89, df = 1, p = 0.01). The abundance of *Uranotaenia* mosquitoes was higher in Ayos (11.6, p = 0.001) and significantly lower in Somalomo (1.7, p = 0.01), Ngoumou (0.4, p = 0.001) and Elat (0.1, p = 0.001) respectively (Figure 7). In addition, cocoa farms recorded higher abundance of *Uranotaenia* mosquitoes than villages (Figure 8).
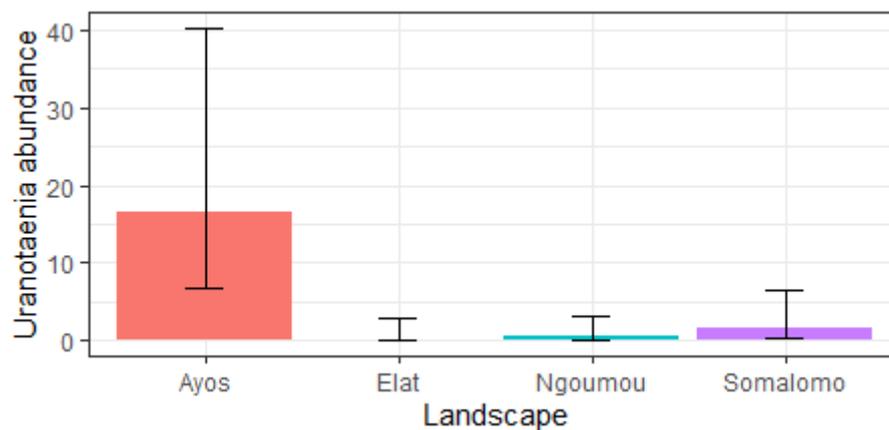

Figure 7: Predicted mean abundance of *Uranotaenia* mosquitoes across landscapes in the east and centre regions of Cameroon. Error bars correspond 95% confidence interval.
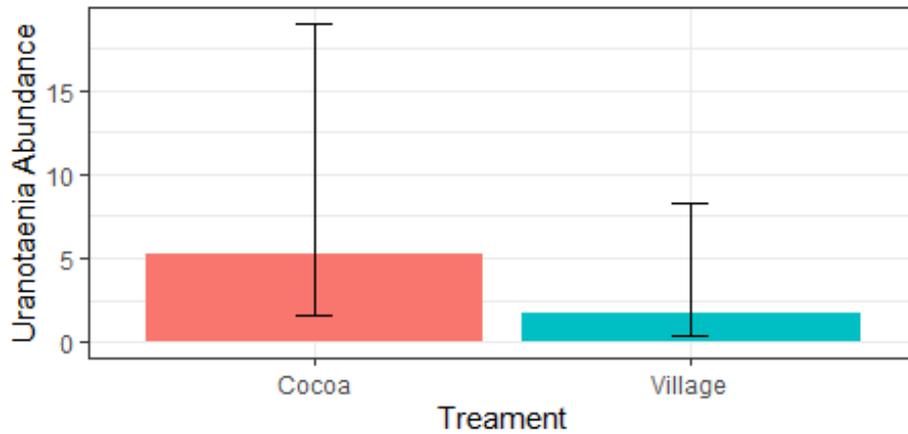
Figure 8: Predicted mean abundance of *Uranotaenia* mosquitoes between treatments (cocoa farms versus villages) in the east and centre regions of Cameroon. Error bars indicate 95% confidence intervals

Treatment was a significant predictor of *Anopheles* mosquito abundance ($\chi2 = 9.98$, df = 1, p = 0.002) with higher abundance recorded in the cocoa farms than the villages (Figure 9). However, temperature, seasonality, relative humidity, farm canopy and forest cover did not predict the abundance of *Anopheles* mosquitoes.
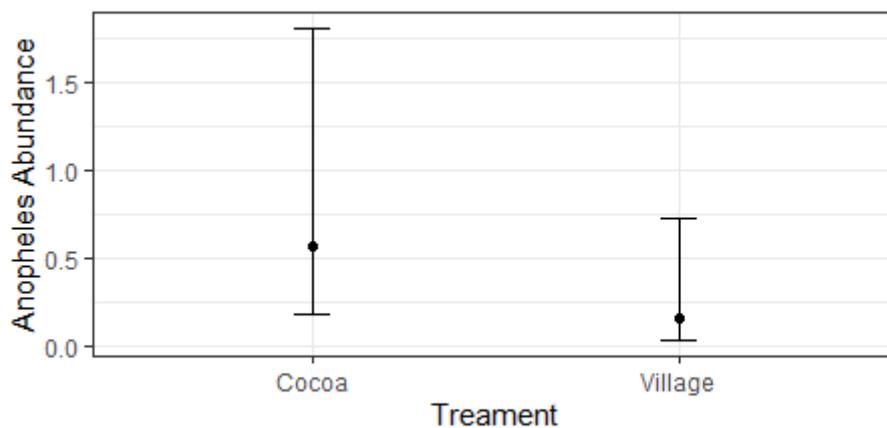

Figure 9: Predicted mean abundance of Anopheles mosquitoes across treatments in the east and centre regions of Cameroon. Error bars indicates 95% confidence interval.

Aedes mosquitoes also identified treatment ($\chi2$ = 9.98, df = 1, p = 0.002), season ($\chi2$ = 9.98, df = 1, p = 0.002) and relative humidity ($\chi2$ = 9.98, df = 1, p = 0.002) as significant predictors of their abundance. Appendix 4 summarises the estimates, significant and non-significant predictors of *Aedes* mosquitoes across sampling sites.

Insectivorous bat association with mosquito abundance and environmental predictors

The effect of farm canopy ($\chi2$ = 22.73, df = 1, p = 0.001; Appendix 2) and landscape variables ($\chi2$ = 14.70, df = 3, p =0.002) was significantly related to the abundance of insectivorous bats (Appendix 2). However, mosquito abundance did not correlate with insectivorous bat abundance ($\chi2$ = 0.01, df = 1, p= 0.9). Farm canopy had a positive effect on Insectivorous bat abundance (Figure 10) with bat abundance increasing per night from a mean of 1.6 at 22% farm canopy (95% CI= 0.8, 3.4) to 6.6 at 98% farm canopy (95% CI= 6.7, 38.4).
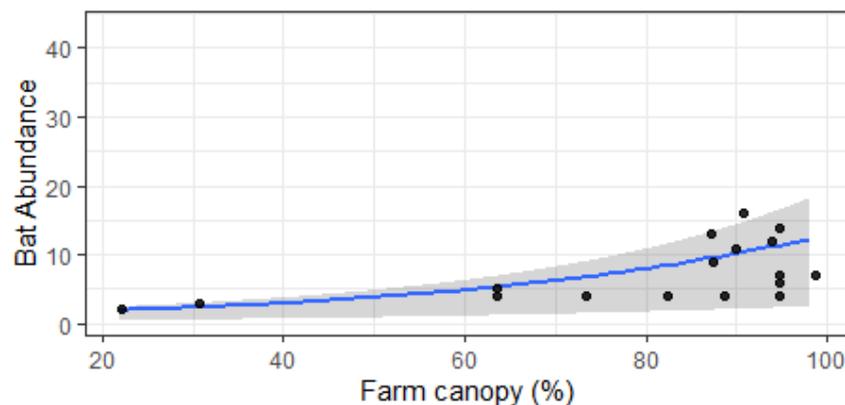

Figure 10: Effects of farm canopy on the abundance of insectivorous bats. The blue line designates the effect size of farm canopy on bat abundance predicted by the best fitted model and the grey shade represents 95% confidence interval.

The abundance of insectivorous bats varied across the four different landscapes in our study area (; Appendix 2, Figure 11). Highest bat abundance was recorded in Elat (7.54, p = 0.03) and Ngoumou (7.16, p = 0.01), followed by Ayos (4.75, p = 0.001) and lastly, Somalomo (3.02, p = 0.005). Temperature, seasonality, relative humidity and forest cover had no significant effect on the abundance of insectivorous bats (see Appendix 2).
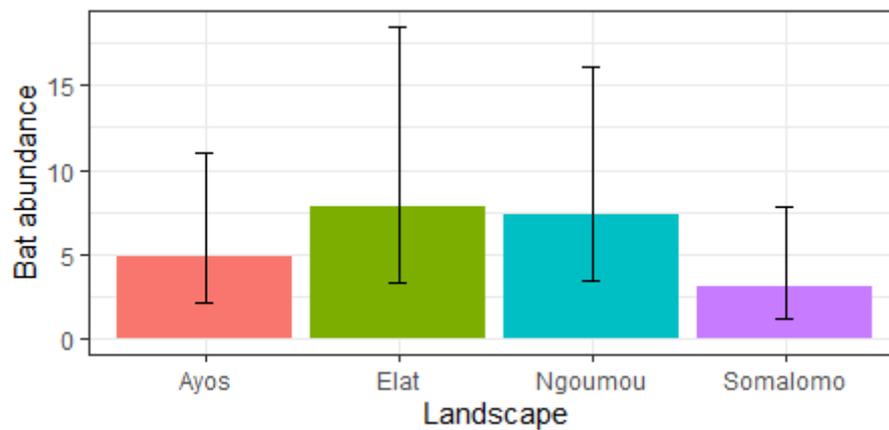

Figure 11: Predicted mean abundance of insectivorous bats across landscapes. Error bars correspond to 95% confidence interval of the predicted mean abundance.

DISCUSSION

In this study, I investigated the environmental determinants of mosquito and insectivorous bat abundance in the east and centre regions of Cameroon and tested for an association between them. Overall, I detected significant spatial variation in both bat and mosquito abundance including individual mosquito genera of *Culex* and *Uranotaenia* abundances. Interestingly, landscapes with significantly higher bat abundance had lower mosquito abundance and landscapes with lower bat abundance recorded higher mosquito abundance. Mosquito populations were seasonal and associated with humidity, whereas the only additional predictor of bat abundance was shade cover. However, there was no significant association between bat and mosquito abundance. Hence, I fail to reject the hypothesis that mosquito populations are regulated by bats.

Determinants of mosquito

The main predictors of mosquito abundance were relative humidity, season and landscape. These findings highlight the importance of spatial and temporal variability in environmental conditions necessary for mosquito growth and survival (Abella-Medrano et al., 2015). Mosquitoes are known to be very sensitive to environmental conditions that could either enhance or hamper their development. In tropical environments, seasonality is often the primary driver of mosquito population dynamics (Mattingly, 1949; Mayi et al., 2020), with numbers exponentially higher in the wet than the dry season due to the increase in aquatic larval habitat (Mwangangi et al., 2012). This observation was confirmed here, with mosquito abundance being significantly higher in the rainy than dry season. These changes in seasonality could also influence the rate of transmission of diseases to humans and animals (Epopa et al., 2019; Okanga & Cumming, 2013). My results are consistent with numerous empirical studies that have found substantial evidence of mosquito seasonality in relation to rainfall (Amaechi et al., 2018; Asigau & Parker, 2018; Mattingly, 1949; Mwangangi et al., 2012). However, few

other studies have observed opposite trends in mosquito abundance with seasonality. For instance, Mayi et al. (2020) recently found no variation in individual mosquito species and abundance across varied habitats and seasons in Western Cameroon, owing to the constant humid condition that occurs throughout the year as a result of raffia palm bushes both in the peri-urban and rural areas. This emphasizes on the importance of environmental changes in relation to vector abundance and disease transmission in an area or locality.

In addition to rainfall, relative humidity and temperature are also known to impact mosquito populations particularly, their rates of development and biting which in turn influence their rates of transmission of pathogens, hence, indirectly affecting the intensity of disease transmission (Ngowo, Kaindoa, Matthiopoulos, Ferguson, & Okumu, 2017). There was no significant effect in temperature on mosquito abundance in this study. However, mosquito abundance was negatively correlated with relative humidity, indicating an opposite trend to the typical increase in mosquito abundance with humidity observed in tropical regions (Clements, 1992). Mosquito sampling during data collection in the rainy season was carried out in the months of August and September which are considered to be the peak rainy season periods in Cameroon. Therefore, high torrential rains during the peak of the rainy season could wash away mosquito breeding sites leading to low abundance and composition in mosquito species as seen in this study (Unpublished data).

Environmental predictors of mosquito abundance were significantly different between genera. Firstly, *Culex* mosquitoes that recorded the highest abundance in this study varied significantly with season, landscape and forest cover. Environmental change and landscape modifications have shown to indirectly affect disease transmission by impacting vector populations, biology, ecology and behavioural patterns (Keesing et al., 2010; Kweka, Kimaro, & Munga, 2016). *Culex* mosquitoes are greatly diverse with varied behaviour patterns that enables them to inhabit a vast range of both aquatic and terrestrial habitats (Edwards 1941; Service 1990; Mayi

et al., 2019). A study conducted in Southwest Cameroon found great diversity and abundance of *Culex* mosquitoes in selectively logged forest compared to the unlogged forest and palm plantation. This findings were attributed to the heterogeneity in landscape composition between habitat types and minimal sunlight in selectively logged forest that could accelerate mosquito growth and development (Mayi et al., 2019). Moreover, the landscapes in our study area (that is, Ayos, Elat, Ngoumou and Somalomo) varied in nature, composition and conditions such as the distance to urban areas, the size of the village, the degree of human-modified effects and population, the distance to forest, the presence of water bodies such as ponds, rivers, streams and lakes, the intensity and rate of accessibility and other environmental factors. I found no variation in mosquito abundance and composition between cocoa farms and villages and this is probably due to the close proximity of cocoa farms to villages and the fact that ecological conditions might have been homogenous within landscapes.

Uranotaenia mosquitoes varied across landscapes and was abundant in cocoa farms than neighbouring villages. This could be due to their penchant for reptile and amphibian blood which are likely more abundant in cocoa farms compared to the villages. Although not attracted to humans, *Uranotaenia* mosquitoes have been implicated in the transmission of zoonotic arboviruses (Harbach, 2016; Kowo unpublished thesis, 2017). Also, the abundance of *Anopheles* mosquitoes significantly varied between treatments with a high abundance in cocoa farms than the villages. This is interesting, given the importance of *Anopheles* mosquitoes in public health as competent vectors of human malaria. The presence of receptables such as discarded pesticide containers in cocoa farms that serve as breeding sites for *Anopheles* mosquitoes could be the reason for my findings. In addition, Anopheles mosquitoes are endophagic, hence, they mainly feed indoors than outdoors (Ngowo et al., 2017). Mosquito sampling in this study was carried out from 6pm-6am outside village houses. Therefore, indoor mosquitoes were not included.

Determinants of Insectivorous bat abundance

This study indicated farm canopy and landscape as the key predictors of insectivorous bat abundance in cocoa farms. The impact of shade cover confirms that the rate and intensity of forest management by cocoa farmers can influence bat communities and abundance. The mean abundance of insectivorous bats increased with farm shade as expected given tree diversity and abundance in cocoa farms influence ecological interactions and conserving individual species (Clough, Dwi Putra, Pitopang, & Tscharntke, 2009; Estrada et al., 1993; Harvey & González Villalobos, 2007). Like birds, bats have adapted to agricultural ecosystems such as cocoa and coffee in addition to forest habitats. These anthropogenic landscapes provide perching, nesting, foraging and roosting sites for forest specialists and forest-agri bat species (Maas et al., 2016). Although relatively less abundant than frugivores and nectarivores, insectivorous bats may thrive in cocoa farms because their floristic and structural complexity support numerous insect populations (Harvey & González Villalobos, 2007).

In addition to local forest management effects, insectivorous bat abundance varied significantly across landscapes indicating significant spatial heterogeneity in bat distribution and abundance. Although confounded with local habitat effects, landscape variables such as proximity to forest do affect bat species richness, abundance and composition (Clough et al., 2009). At the landscape scale, distance to natural forest is key to predicting species richness and composition of both vertebrates and invertebrates in agricultural settings (Clough et al., 2009; Tscharntke et al., 2011). However, we found no effect of forest distance at a 5km radius on the abundance of insectivorous bats, which means the observed spatial variation may be due to another landscape-level-environmental predictor. Other landscape-level determinants of bat abundance could include vegetation indices and degree of urbanisation. One study showed that the amount of forest matrix and wood patches may in fact influence insectivorous bat activity in low intensive management agricultural landscapes as compared to the high intensively managed

ones (Ongole, Sankaran, & Karanth, 2018). Additionally, the 4 landscapes investigated here may have differed in the availability and abundance of insect prey other than mosquitoes (Puig-Montserrat et al., 2020).

Association between insectivorous bat abundance and mosquito abundance

This study reports the first analysis of potential trophic interactions between insectivorous bats and mosquitoes in agroforestry landscapes in Cameroon. No significant association between insectivorous bat and mosquito abundance was found in these cocoa farms and found no significant relationship between bats and mosquitoes. This finding does not support the hypothesis that bats are major regulators of mosquito population size in these ecosystems, however there could be other explanations. First of all, I have a relatively small sample size of sites (n=14); and may have required wider sampling to detect an association if present. Moreover, the relative abundance of prey organisms to their predators may not be accurately reflected by their average abundance in the environment (e.g in a mosquito trap); meaning that predators may not consume prey proportional to their apparent abundance (Tiede et al., 2016).

Another explanation for the lack of association between bat and mosquito in this study could be habitat dependency A previous study investigated relationships between bat activity and Aedes mosquito abundance in three different habitat types (forest, salt marsh, and urban areas) and only detected this in saltmarshes (Gonsalves et al., 2013), due to the absence of vegetation clutter that might maximise prey detectability and availability (Gonsalves et al., 2013). Hence, the presence and proportion of forest shade and farm vegetation could limit flexibility in interactions between insectivorous bats and mosquitoes in cocoa farms.

Furthermore, the majority of these insectivorous bat species have been described as feeding primarily on lepidopterans (ACR) although, dipterans constitute some portion of their diet (Mbeng et al., 2019). The energetic demand of insectivorous bats among a wider array of food

preferences could be another justification for our findings. Bats are required to consume food proportional to their weight to meet energy needs and mosquitoes are so tiny that they are considered to constitute just a small portion (~10%) of bat diet (Puig-Montserrat et al., 2020). Thus, lack of association between mosquito and bat abundance may be because mosquitoes are a secondary or relatively minor food source; with bats concentrating most of their foraging elsewhere.

The relationship between mosquito and bat abundance may be obscured by the relative abundance of alternative prey items (e.g other insect taxa) at each site, which was not controlled for in this analysis. Although this study does support the hypothesis that bats play a strong role in regulating mosquito populations, its findings contribute to the knowledge base on the role of bats and mosquitoes in food webs, with implication.

CONCLUSION

Given the relatively small sample sizes and limited number of sites, predictions of mosquito and bat abundance were quite minimal. The identification of mosquitoes to species level was limited, hence, I could not predict whether *Anopheles* mosquitoes were disease vectors or not. Mosquito trapping methods such as those used in this study might also be unreliable indicators of how bats perceive mosquito abundance. Therefore, further investigation that includes other mosquito methods, identification of mosquitoes to species level and large sample size across numerous habitats in Cameroon is required to fully understand the relationship between bats and mosquitoes in tropical landscapes. However, this study is the first to elucidate the potential interactions between insectivorous bats and mosquitoes in an agroforestry landscape in Cameroon. It has unravelled both environmental and landscape factors that determine the abundance of mosquitoes and their predators in these human-dominated landscapes. The use of recent molecular tools such as diet metabarcoding is also required to shed more light on the role of bats in consuming mosquitoes. There is need for sustainable control efforts to prevent the spread of mosquito borne diseases. With Cameroon as the third largest producer of cocoa and one of the highest malaria burden countries, knowledge that contributes to the ecology and ecosystem functioning of insectivorous bats and mosquitoes is vital for the wellbeing of the poor farmers battling between earning a living, developing their economy and preserving biodiversity.

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APPENDICES

Appendix 1: Mean quantities of landscape and environmental parameters in each cocoa farm.

Cocoa farms	Farm canopy (%)	Forest cover at 5km radius (%)	Temperature (ºC)		Relative humidity (%)	
			Wet season	Dry season	Wet season	Dry season
NGUI001	73.4	47.2	21.1	24.3	95.6	60.7
NGUI002	87.5	42.9	22.5	24.3	89.6	60.7
EBA001	94.8	12.9	22.6	26.2	88.2	45.7
EBA003	82.3	16.5	22.1	23.8	92.7	61.5
EBECK	90	53.1	22.9	23.8	89.7	58.3
ELAT S	30.8	37.3	21.9	24.6	89.6	63.1
ELAT N	87.3	35.9	21.6	24.5	92.9	63.1
EBEM	93.9	32.2	23.3	/	90.6	/
NKOL S	90.8	25.7	23.4	25.6	87.7	61.9
ADZAP	22.2	28.8	23.4	25.5	88.3	63.4
PALM	63.5	44.9	22.2	23.9	91.9	73.9
STADE	98.7	43.1	/	24.4	/	73.3
MAMA	94.7	63.7	22.1	24.2	88.1	67.6
SNAKE	88.6	54.5	/	24.6	/	70.0

Appendix 2: Model output of the most parsimonious mixed-effect model of insectivorous bat abundance. Estimates and chi-square values of significant predictors were obtained from the best fit model whereas, estimates and test statistic of insignificant variables were gotten from the most complex model and likelihood ratio tests (LRTs) respectively. SE stands for standard error and df is degree of freedom.

Covariates	Estimates	SE	df	χ2 value	P-value
Mosquito abundance	0.002	0.002	1	0.013	0.909
Shade cover	0.024	0.004	1	22.738	1.8e-06 ***
Temperature	0.485	0.253	1	0.329	0.566
Relative Humidity	0.047	0.024	1	3.572	0.059
Forest cover	-0.005	0.006	1	0.813	0.367
Season	0.295	0.551	1	1.657	0.198
Landscape	0.461	0.164	3	14.709	0.002**

1 model out of 8; Type of model: GLMM; Predictor distribution: Poisson

Appendix 3: Summary of best fitted mixed-effect model of *Culex* mosquito abundance and environmental variables. Estimates and chi-square values of significant predictors were obtained from the best fit model and LRTs respectively whereas, estimates and test statistic of insignificant variables were gotten from the most complex model and likelihood ratio tests (LRTs) that did not contain the said variable. SE stands for standard error and df is the degree of freedom.

Covariates	Estimates	SE	df	χ2 value	P-value
Treatment	0.456	0.411	1	0.532	0.465
Shade cover	-0.009	0.012	1	0.78	0.377
Temperature	-0.060	0.64	1	0.008	0.928
Relative Humidity	-0.051	0.067	1	0.89	0.345
Forest cover	-0.057	0.021	1	5.348	0.021 *
Season	1.923	0.546	1	10.328	0.001**
Landscape	-1.813	0.699	3	11.812	0.01 **

Appendix 4: Summary of best fitted mixed-effect model of Aedes mosquito abundance with environmental variables. Estimates and chi-square values of significant predictors were obtained from the best fit model whereas, estimates and test statistic of insignificant variables were gotten from the most complex model and likelihood ratio tests (LRTs) respectively. SE stands for standard error and df is the degree of freedom.

Covariates	Estimates	SE	df	χ2 value	P-value
Treatment	1.041	0.467	1	4.942	0.026*
Shade cover	-0.0005	0.03	1	0.0004	0.984
Temperature	-0.9	1.52	1	0.523	0.469
Relative Humidity	-0.200	0.059	1	12.003	0.001 ***
Forest cover	0.10	0.04	1	0.094	0.759
Season	4.854	2.086	1	6.635	0.01 **
Landscape	-2.00	2.30	3	5.586	0.133

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